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DOE Office of Energy Research**

Part 4: Physical Sciences

L.A. Braby and Staff

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Preface

This 1993 Annual Report from Pacific Northwest Laboratory (PNL) to the U.S. Department of Energy (DOE) describes research in environment and health conducted during fiscal year (FY) 1993. This year, the report consists of four parts, each in a separate volume.

The four parts of the report are oriented to particular segments of the PNL program, describing research performed for the DOE Office of Health and Environmental Research (OHER) in the Office of Energy Research. In some instances, the volumes report on research funded by other DOE components or by other governmental entities under interagency agreements. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the multidisciplinary nature of the research effort.

The parts of the 1993 Annual Report are as follows:

Part 1: Biomedical Sciences	J.F. Park, Program Manager A.L. Brooks, Report Coordinator C.C. Lumetta, Editor
Part 2: Environmental Sciences	R.E. Wildung, Program Manager L.K. Grove, Editor
Part 3: Atmospheric Sciences	W.R. Barchet, Program Manager B.V. Johnston, Editor
Part 4: Physical Sciences	L.A. Braby, Program Manager S.L. Downs, Editor

Activities of the scientists whose work is described in this annual report are broader in scope than the articles indicate. PNL staff have responded to numerous requests from DOE during the year for planning, for service on various task groups, and for special assistance.

Credit for this annual report goes to the many scientists who performed the research and wrote the individual project reports, to the program managers who directed the research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the four parts, and to Ray Baalman, editor in chief, who directed the total effort.

T. S. Tenforde
Manager, Health and Environmental Research Program

Previous reports in this series:

Annual Report for

1951	HW-25021, HW-25709
1952	HW-27814, HW-28636
1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
1955	HW-39558, HW-41315, HW-41500
1956	HW-47500
1957	HW-53500
1958	HW-59500
1959	HW-63824, HW-65500
1960	HW-69500, HW-70050
1961	HW-72500, HW-73337
1962	HW-76000, HW-77609
1963	HW-80500, HW-81746
1964	BNWL-122
1965	BNWL-280, BNWL-235, Vol. 1-4; BNWL-361
1966	BNWL-480, Vol. 1; BNWL-481, Vol. 2, Pt. 1-4
1967	BNWL-714, Vol. 1; BNWL-715, Vol. 2, Pt. 1-4
1968	BNWL-1050, Vol. 1, Pt. 1-2; BNWL-1051, Vol. 2, Pt. 1-3
1969	BNWL-1306, Vol. 1, Pt. 1-2; BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2; BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2; BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2; BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4
1975	BNWL-2000, Pt. 1-4
1976	BNWL-2100, Pt. 1-5
1977	PNL-2500, Pt. 1-5
1978	PNL-2850, Pt. 1-5
1979	PNL-3300, Pt. 1-5
1980	PNL-3700, Pt. 1-5
1981	PNL-4100, Pt. 1-5
1982	PNL-4600, Pt. 1-5
1983	PNL-5000, Pt. 1-5
1984	PNL-5500, Pt. 1-5
1985	PNL-5750, Pt. 1-5
1986	PNL-6100, Pt. 1-5
1987	PNL-6500, Pt. 1-5
1988	PNL-6800, Pt. 1-5
1989	PNL-7200, Pt. 1-5
1990	PNL-7600, Pt. 1-5
1991	PNL-8000, Pt. 1-5
1992	PNL-8500, Pt. 1-4
1993	PNL-9000, Pt. 1-4

Foreword

Part 4 of the Pacific Northwest Laboratory Annual Report for 1993 to the DOE Office of Energy Research includes those programs funded under the title "Physical and Technological Research." The Field Task Program Studies reported in this document are grouped by budget category and each Field Task proposal/agreement is introduced by an abstract that describes the projects reported in that sections. These reports only briefly indicate progress made during 1993. The reader should contact the principal investigators named or examine the publications cited for more details.

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Dosimetry
Research

Biological Effectiveness of Radon Alpha Particles

Radiation exposures due to radon in indoor air result in a maximum of one or two alpha particles to a typical lung cell. The consequences of these exposures, relative to the effects of the large numbers of electron tracks required to produce the same dose, are a major concern in establishing exposure limits for radon. Determining these effects is complicated because normal experimental techniques result in a Poisson distribution of the number of tracks through the individual cells making up the experimental population. Since the mean number of tracks per cell is very small at the dose of interest, the relative variance is so large that the results of such experiments do not adequately test theories about the consequences of exposure. An accelerator based single-particle irradiation facility, which will irradiate large populations of cells with specific numbers of charged particles, has been placed in operation. Initial experiments, designed to test the response of cells as a function of the number of alpha particle tracks, are underway. Both the reproductive survival of cells, and the frequency of micronuclei produced in the first division after irradiation, are being correlated with the number of charged particle tracks. The effectiveness of alpha particle tracks, with their high LET and variation of energy deposition through the cell nucleus, is being compared to that of 3 MeV proton tracks which have a relatively low LET and which deposit a constant amount of energy per unit track length throughout the cell nucleus.

Single-Particle Irradiation System Development

L.A. Braby, M.K. Lien, M.E. Middendorf, and J.M. Nelson

Our original design of a system for single particle irradiation of individual cells has been previously described (Braby 1992). This design relies on a parallel beam of particles from a tandem accelerator in order to be able to use an adjustable collimator to produce a range of beam spot sizes. During testing, this system has been made to work for short periods of time, but it places extreme requirements on the adjustment of the accelerator focus and alignment (Braby and Nelson 1993). A program to upgrade the PNL tandem accelerator to improve beam stability has been initiated, but it is anticipated that a substantial amount of development will be required to achieve the stability required for single cell irradiation with an adjustable collimator. However, most of the biological experiments that have been planned can be achieved with a fixed

5 μm diameter collimator. The primary requirement of these experiments is the need for single particle detection, and this portion of the design is unaffected by the collimator type. The fixed collimator was installed this year, and biological experiments were begun on a routine basis.

Figure 1 illustrates the irradiation system in the configuration using a fixed final aperture and adjustable primary aperture. This differs from the previous design only in the details of the beam exit window and the way that the adjustable collimators are tuned. In this configuration, the exit window consists of a 50 μm thick copper foil (gold plated) with a 5 μm diameter laser drilled hole centered on the end of the beam line. This copper foil is covered by a 1.5 μm plastic film which provides the vacuum seal, and a 7 μm plastic scintillator. The scintillator is made to be removable since it shows the effects of radiation damage before the vacuum window does and it can be rotated to distribute the damage over a larger area. Again, the mylar bottom of the

Figure 1. Schematic of the Single Particle Irradiation System Using a Fixed 5 μm Aperture at the End of the Evacuated Beam Line

special petri dish is in contact with the scintillator, placing the cell nucleus approximately 10 μm from the end of the aperture. The primary purpose of this configuration is to eliminate the need for a precisely parallel beam of particles by eliminating the distance between the final aperture and the target, but it has two additional advantages relative to the adjustable collimator design. The aperture is visible in the cell positioning microscope, so the position of the collimated particle beam relative to the coordinate system of the video image processing system, can be determined directly, without the need to expose a radiachromic film sample. This reduces the time required to set up the beam. Also, the gold plated copper foil provides a uniform background against which the cells can be viewed. This has produced a noticeable improvement in the video image, and also

increased the image brightness, eliminating the need for the image intensifier which further improved image contrast.

The primary difficulty with the fixed aperture at the end of the vacuum beam line is in mounting it with its axis parallel to the beam without it becoming plugged with components of the material used to mount it. Because the foil must be 50 μm thick to stop some of the proton beams we use for LET standards, and the hole is only 5 μm in diameter, if it is off axis by only 5 degrees, the projection of the entrance end will not overlap the exit end and only scattered particles will pass through it. A special jig has been built to hold the beam line end piece and the copper foil during mounting to ensure accurate alignment, but surface roughness on the small machined parts has apparently resulted in misalignment in some cases. A more frequent problem is

plugging of the hole by a small amount of glue or a component of the glue used. Capillary action in the gap between parts is enough to lead the glue to the hole if there is any excess. Furthermore, the most successful material in terms of making a vacuum tight seal, an epoxy formulated for use on vacuum systems, appears to contain an additive that can continue to move, and sometimes plug the hole even days after the epoxy has hardened. As a result, the assembly of exit apertures is something of an art requires a trial and error approach. Fortunately, the apertures are quite rugged once completed and do not have to be replaced frequently.

With the fixed aperture only a few micrometers from the target, the effects of beam alignment and slit edge scatter are substantially reduced, but they are not eliminated. To control the energy deposition in targets, it is important to minimize slit edge effects. This is done by using the adjustable aperture to limit the beam current and minimize the primary beam hitting the final aperture foil. The adjustment of this aperture is done most easily with the aid of a solid state detector and multi channel analyzer. Starting with the adjustable aperture set much larger than the fixed aperture, a high count rate is detected, and the spectrum contains a significant number of particles with energy less than the accelerator energy. These are the result of edge scatter in the final aperture. As the size of the adjustable aperture is reduced, provided it is aligned with the beam through the final aperture, the count rate is reduced and the scattered component is eliminated. The desired count rate through the system, about 500 particles per second, is easily obtained by adjusting the aperture, assuming the acceleration beam current is a few nanoamps.

The most effective diagnostic test for overall system performance is the positioning of single tracks adjacent to targets on a track etch plastic detector. As targets, we use etch pits produced by a prior irradiation with single particles placed at fixed grid positions. The irradiation and etch

pit geometry is illustrated in Figure 2. Although this system provides a good test of the number of particles delivered, there is some uncertainty in the evaluation of the actual position of the track through a cell nucleus since approximately 50 μm of the plastic surface must be etched away to produce a visible etch pit. This means that the position of the track is measured at 50 μm depth, several times the depth of the cell nuclei in the irradiation configuration. Thus multiple scatter adds to the uncertainty of the track positioning and exaggerated the apparent targeting errors. Figure 3 shows a portion of a track etch experiment of this type.

The single particle irradiation system, in this configuration, has been used for a number of cell irradiation experiments (see next report - Braby, Nelson, and Brooks). However, there are several improvements that can be made to the system in order to increase the range of biology experiments that are practical. One of the primary limitations of the current system is the time required to irradiate each cell. Initially, while

Figure 2. Schematic of the Final Aperture, Scintillator, and a Track Etch Detector Target. If a track etch pit is used as a target and positioned a fixed distance to the right of the collimator, the pit produced by a single particle will indicate the position of that particle after it has passed through the window assembly and approximately 50 μm of the track etch material.

Figure 3. Photograph of a Portion of an Experiment to Test the Performance of the Single Particle Irradiation System. Previously produced track etch pits were used as targets and the beam was positioned at a fixed position to the side of the target.

using the Grinnell image processor and VAX 750 computer to determine the positions of targets, it required approximately 1 min per cell. Much of this time was because of the cumbersome nature of the Grinnell cursor positioning system. The time was cut almost in half, to an average rate of over 100 cells per hour, by replacing the external image processor with a single board system within the DOS based computer that controls the irradiation procedure. This allows use of a mouse to position the cursor over targets to be irradiated, and is a much faster process. It also eliminates many sources of down time which

were caused by the communication between the DOS machine and the VAX. This conversion has been planned for some time but proved to be difficult because of incomparability of different portions of the software required for video processing and cell positioning. Eventually we found a brand of Pascal which provided the tools for a smooth interface to the C language based video system drivers.

The next largest time saving would result from eliminating the need to replace the microscope lens with the photomultiplier for each cell irradiation. A scintillation detector that

surrounds the microscope objective would also eliminate the concern about the accuracy of repositioning the lens for each cell, a problem because it could lead to a gradual change in the actual position of the beam relative to selected targets. The problem is that reliable detection of single particles requires a detector with a numerical aperture of about 2. Furthermore, detection of the cells requires a microscope lens with a magnification of at least 10 and a working distance of at least 3/8 in. The available lenses are necessarily large, and, in their standard mounts, preclude any system for gathering enough scintillation light to detect single particle events. However, we discovered that the optical elements of one suitable lens were small enough that they would fit in the central opening when four 1 in. photomultipliers were arranged on a tight circle. By removing the lenses from this commercial microscope objective and reassembling them in a specially tapered tube, a lens that could be nested with four photomultipliers was assembled. A test with a single photomultiplier positioned to the side of the scintillator indicated that the light gathering efficiency would be satisfactory, so we have had the necessary components manufactured. The four tube detector assembly has been assembled and tested for image quality and light gathering efficiency. It seems to work well, but we have not had an opportunity to test it in a cell irradiation experiment. If it works as expected, we expect it to increase the number of cells irradiated per hour to about 150.

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Braby, L.A. and J.M. Nelson. 1993. "Single-Particle Irradiation of Mammalian Cells". In *Physical Sciences*, Part 4, of Pacific Northwest Laboratory Annual Report for 1992 to DOE office of Energy Research, PNL-8500. Pacific Northwest Laboratory, Richland, Washington.

Cell Survival and Micronucleus Formation Following Interaction with Small Numbers of Alpha Particles

L.A. Braby, J.M. Nelson, and A.L. Brooks

When mammalian cells are exposed to alpha particles, either from collimated radioactive sources or from a charged particle accelerator, the mean number of tracks through the cell nucleus required to produce cell lethality is generally significantly more than one, and typically between 5 and 10. This large number is difficult to reconcile with the large amount of DNA damage that an alpha particle track is expected to produce. Several possible mechanisms which could account for this discrepancy have been proposed. One suggestion is that the DNA is packaged within the interphase cell nucleus in a way that prevents most charged particle tracks from interacting with the DNA. Another possibility is that, in spite of the exponential dose response relationship, the majority of the damage produced by high LET radiation is repaired, independent of the dose rate or post irradiation culture conditions. Finally, it may be that a large fraction of the genetic information, and thus the integrity of much of the DNA, is not essential for the reproductive survival of the cell in culture.

One way to begin testing these alternative ideas about the effects of individual high LET particle tracks is to compare cell lethality with a measure of DNA damage such as micronucleus production. If each alpha particle track produces unrepaired DNA damage, one would expect

there to be a high probability for each alpha particle event to result in a large DNA deletion and therefore a micronucleus. If some of these deletions include only DNA that is not essential for cell survival, one would observe a higher frequency of micronuclei than cell inactivation, and thus the high number of particle events per lethal event. If, however, most large deletions are lethal but the DNA is packaged so that there is a significant chance that an alpha particle will not produce a large deletion (micronucleus), the number of cells with micronuclei, and the fraction not surviving should be the same. This would also be true if all alpha particle tracks produce damage, but most of the damage is repaired, independent of irradiation schedule and culture conditions. In careful experiments where micronucleus production and reproductive survival were determined for individual cells following low LET irradiation (Revell) it was shown that there was a nearly perfect correlation between micronucleus production and loss of reproductive integrity, suggesting that all deletions large enough to be detected as a micronucleus are lethal. However these low LET experiments involved a very large number of charged particle tracks per cell nucleus, and the resulting damage was distributed nearly uniformly through the nucleus. This does not help in the effort to understand how high LET particles can fail to produce lethal damage.

In an effort to understand the effects of small numbers of high LET particles, we have used the single particle irradiation system to determine the probability of cell inactivation and micronucleus production as a function of the number of alpha particles crossing the cell nucleus. For reference, the equivalent measurements were made using 3 MeV protons. For the initial measurements, 4.5 MeV alpha particles, with a stopping power of $\text{keV}/\mu\text{m}$, were used because there is relatively little change in stopping power through the cell nucleus (Figure 4).

Exponentially grown CHO cells were plated on the special thin bottomed petri dishes designed for this irradiation system, and were incubated at 37°F for about 4 hours to allow the cells to attach at the plastic film, and begin to flatten. The dish was then transferred to the irradiation system, which operates at room temperature. The first irradiations were conducted in normal air, and a significant effect on the growth rate of unirradiated control cells was observed if the dish was held in the irradiation chamber for more than about 3 hours. More recent experiments have been conducted in a controlled atmosphere with 5% CO_2 in air. Experiments indicate that this prevents any significant change in growth rate, but holding times in the irradiation system are limited to no more than 3 hours to avoid any possible conflicts. The surface of the dish is divided into 9 squares, and the particle fluence per cell nucleus is specified separately for each area. This allows sham irradiated controls, and several different irradiations on each dish, thus avoiding differences due to the characteristics of the dish of how it was processed. Following irradiation dishes for survival assay are returned to the incubator and held for 4 days. Following this growth period, they are fixed and stained with crystal violet. The stained dish is placed on the computer controlled stage of a second video microscope, and the data file from the irradiation system is entered in the computer. The dish is automatically positioned to the video fields which contained cells which were irradiated, and the resulting clones are evaluated for survival on the bases of the number of cells in each clone and their morphology.

The results of survival experiments with small numbers of 4.5 MeV alpha particles is shown in Figure 5. The results of these early experiments still show substantial uncertainty because of the limited number of cells which have been tested, but the number of alpha particle events per cell nucleus to leave 37% survival, about 10, is

Figure 4. Stopping Power in Muscle as a Function of Residual Range for the Alpha Particles Available in the Single Particle Irradiation System. The alpha particle energy is marked at representative points, and the positions of the plastic scintillator and cell nucleus along the track of a 4.5 MeV alpha particle are marked.

consistent with the range of values obtained from conventional track segment irradiations. For comparison, it takes approximately 150 3 MeV proton tracks to produce the same level of effect (Figure 6).

For the micronucleus assay, cells were prepared and irradiated the same way as for the survival assay. However after irradiation cytochalasin-b was added to the dish before it was returned to the incubator. It was then held for 24 hours before the cells were fixed, stained, and examined using a fluorescence microscope. Figure 7 shows the micronucleus assay results for 4.5 MeV alpha particles. At a fluence of 5 particles per cell nucleus, approximately 0.2 micronuclei were detected per binucleated cell. We assume that this indicated that 0.2 micronuclei were produced per irradiated

cell,, in close agreement with the approximately 20% of the irradiated population which were rendered reproductively dead.

Although there is significant uncertainty in the results of these early experiments, the results seem to be consistent with the model that the DNA is packaged so that individual charged particle tracks have only a small probability of producing a large deletion, or that most high LET radiation induced damage is repaired, even in these experiments which do not provide any delay of cell proliferation. These results do not appear to be compatible with the assumption that all charged particle tracks produce damage, but that most of the damage involves DNA which is not essential for cell survival. However, it is possible that essential segments of DNA are distributed throughout the entire genome in a way

Figure 5. Survival of CHO Cells Exposed to Small Numbers of 4.5 MeV Alpha Particles. The large error bars result from the relatively small number of cells exposed in these early experiments.

Figure 6. Survival of CHO Cells Exposed to 3 MeV Protons Through the Cell Nucleus

Figure 7. Frequency of Micronuclei in CHO Cells Exposed to Small Numbers of 4.5 MeV Alpha Particles. The number of binucleate cells counted at each point is indicated in parentheses.

which results in only deletions larger than a specific size being lethal. If this size happens to correspond to the minimum size detectable in micronuclei experiments this result could also be observed. To improve the reliability of this conclusion, we will test this observation at additional alpha particle energies, and will also improve the precision of the results at all energies by increasing the numbers of cells exposed.

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Measurement
Science

Lasers in Analytical Chemistry

Principal Investigator: J. E. Murphy^(a)

Other Investigator: R. J. Miller

The research conducted under this project emphasizes obtainment of basic, new scientific knowledge needed for development of laser analytical methodologies possessing the attributes of general applicability to both atoms and molecules, high selectivity, extreme sensitivity, and true reliability. In the case of atoms, these efforts have culminated in the technique of continuous-wave double resonance ionization mass spectrometry, or cw-DRIMS (Bushaw 1989). The concept utilizes two, sequential, resonant optical excitation steps to populate a chosen high Rydberg state of the analyte atom of interest; this state population is subsequently photoionized and the resultant atomic ions are detected by conventional mass spectrometry methods. Selectivity is achieved through both optical bound-bound transitions by exploiting physical properties unique to the excited states of the analyte atom such as isotope shifts and hyperfine structure splittings. Sensitivity can be limited by spontaneous decay of excited state populations and by the efficiency of the photoionization process. Identification of optimum analytical schemes therefore requires detailed knowledge of the structural and dynamical properties of atomic and molecular excited states. Knowledge of factors that influence the ion production efficiency, such as the location of Cooper minima, that is zeroes in the photoionization cross section, is also needed. While criteria for selection of favorable atomic bound-bound transitions have been elucidated, the dynamics of the photoionization event remain poorly characterized.

The excited states of molecules typically lie at higher energies than those of heavy atoms, and molecular transition probabilities tend to be significantly smaller than those of atoms. The existence of vibrational and rotational degrees of freedom, the occurrence of more diverse decay mechanisms, and the general propensity for interactions between electronic states combine to complicate the behavior of molecules over those of atoms. Basic questions therefore arise. Can ultrahigh resolution cw lasers be applied successfully to molecular multiphoton excitation schemes and to molecular multiple resonance sequences? Can ultrahigh resolution studies decipher the richness of molecular phenomena? Initial investigations of the molecule nitric oxide (NO) have been undertaken to address these basic questions.

Thus, the current emphasis of this research project is directed to systematic investigations of the structural and dynamical properties of selected small molecules, and to studies of photoionization dynamics through photoelectron angular distribution measurements. Experimental and theoretical investigations are combined to yield maximum insight into general physical phenomena. The principal objectives of these endeavors are (i) to examine the potential of cw-DRIMS type techniques for analytical applications to molecules, and (ii) to obtain a more comprehensive understanding of the photoionization process thereby contributing to improved sensitivity and reliability in all photoionization based analytical methods.

(a) NORCUS Postdoctoral Research Associate

The complexities of the structures and of the dynamics of selected Rydberg states of nitric oxide provide a stringent test of the utility of cw laser sources in investigations of highly excited molecular electronic states. The results summarized below, fortified by prior studies of the (3ss)A 2S+ Rydberg state of NO (Miller 1989a; Murphy 1993) and continuing investigations of spontaneous molecular decay mechanisms, are serving to establish the power of such investigations. Both multiphoton excitation schemes and multiple resonance sequences can be exploited to advantage, and the information content of such investigations is clearly sufficient to decipher the rich phenomena of molecular structure and dynamics. Knowledge of term energies serves to identify those states that can provide maximum selectivity in applications of measurement science. Knowledge of the mechanisms and rates of spontaneous decay processes serves to identify those states that can provide maximum sensitivity and reliability in applications of measurement science.

The potential rewards resulting from these developments in laser based measurement science are vast indeed. Applications relevant to current and future health and environmental concerns include contributions to chemical speciation of environmental contaminants; to identification and quantification of atmospheric constituents; to definition of the mechanisms of formation and destruction of reactive intermediates within the atmosphere; to design of synthetic enzymes for immobilization and treatment of hazardous wastes; and to elucidation of structure-function relationships associated with newly discovered enzymatic processes within the brain.

The Mechanism of the F 2D(u=3) K 2P(u=2) NO Perturbation

Ultrahigh resolution, (2+1 ϵ) laser induced fluorescence (LIF) dip spectroscopy, resonantly enhanced through the A 2S+(u=1) state, has been used to determine the term energies and natural linewidths of individual rovibronic levels of the (4pp)K 2P(u=2) and the (3dd)F 2D(u=3) Rydberg states of ¹⁴N¹⁶O that lie just above the second dissociation threshold near 8.5 eV. A plot of the reduced term energies as a function of total angular momentum exclusive of spin N clearly indicates crossings of the K(u=2) and F(u=3) diabatic potential curves providing evidence for local perturbations. Indeed, the DL=2, F 2D(u=3) $\frac{1}{4}$ A 2S+(u=1) transitions are nominally forbidden in one-photon and they are observed only because of intensity borrowing that results from the perturbation with the K(u=2) state. Since the L-doubling within K(u=2) is large, this perturbation occurs at much lower N (N=11) with the P+ component of K(u=2) than with the P- component (N>16). The F(u=3) K(u=2) interaction is

therefore parity dependent, manifesting itself as an observable L-doubling within the F(u=3) levels. Nonperturbed levels of F(u=3) have also been probed via (1+1 ϵ) resonantly enhanced multiphoton ionization (REMPI) experiments utilizing vacuum ultraviolet (VUV) excitation.

Figure 1, a portion of the reduced term energy plot, illustrates the region of the F(u=3) K(u=2) perturbation. It is noteworthy that the observed interactions are quite weak, with magnitudes of only ~ 0.75 cm⁻¹. Two potential interaction mechanisms can be invoked to explain these observations. One potential mechanism, schematically depicted in Figure 2, represents an indirect coupling mechanism involving two, homogeneous Rydberg-valence interactions, namely F 2D(u=3) B ϵ 2D(u=9) and K 2P(u=2) B 2P(u=29). Since B ϵ 2D(u=9) and B 2P(u=29) are coupled through spin-orbit interactions, F 2D(u=3) and K 2P(u=2) become indirectly coupled, that is F 2D(u=3) B ϵ 2D(u=9) B 2P(u=29) K 2P(u=2). The other potential mechanism involves a direct, heterogeneous, interseries

Figure 1. Reduced term energies as a function of total angular momentum exclusive of spin N for rovibronic levels of the $(4pp)K\ 2P(u=2)$ and $(3dd)F\ 2D(u=3)$ Rydberg states of $14N16O$ in the immediate region of the $F(u=3)$ $K(u=2)$ perturbation. Data sets are identified by electronic state (spin component, symmetry component). The $F\ 2D(F_1, f + F_2, e)$ data set represents the VUV observations.

Figure 2. Schematic representation of the indirect $F\ 2D(u=3) B\ 2D(u=9) B\ 2P(u=29) K\ 2P(u=2)$ coupling mechanism.

Rydberg-Rydberg interaction that is mediated through the molecular rotation. In this case the coupling matrix element is given explicitly by Eq. (1) where , , and specify respectively the

operators characterizing the total angular momentum exclusive of spin, the total electronic orbital angular momentum, and the orbital angular momentum of the Rydberg electron. The $\pm$ right superscripts denote raising and lowering operators. Inspection of Eq. (1) indicates that the coupling matrix elements contain fundamental physical information: the term contributes to characterization of the electronic wave function while the factor serves to further describe the molecular potential. Matrix elements of the form couple Rydberg series described by different nominal values because, in the case of molecules, is not a good quantum number. Scrutiny of such perturbations therefore provides a sensitive indicator of additional electron partial waves within the interacting Rydberg series.

A thorough, multistate analysis of the experimental observations demonstrates that the direct, heterogeneous Rydberg-Rydberg interaction is the main contributing mechanism responsible for the $F(u=3) \sim K(u=2)$ perturbation. This mechanism dominates because the nominal $4pp$ electron configuration contains a 5% d-partial wave character. The spin splittings observed within $F(u=3)$ (see Fig. 1) result in part from the indirect $F\ 2D(u=3) B\ 2D(u=9) B\ 2P(u=29) K\ 2P(u=2)$ coupling mechanism, and in part from the 1.5 cm^{-1} spin-orbit interaction within $K\ 2P(u=2)$ through the direct $F(u=3) K(u=2)$ coupling mechanism.

The significance of these results to an understanding of the dynamics of the $K(u=2)$ and $F(u=3)$ states is of particular interest. Knowledge of the partial wave decompositions of a given Rydberg state allows a quantitative description of the dynamics of photoionization of that state through predictions of the partial wave components of the continuum that can be accessed upon photoionization, and thereby of photoelectron angular distributions (Dubs 1985; Dubs 1988). It is also noteworthy that, compared to nonperturbed F state lifetimes (Hart

1987), the weak $F(u=3) K(u=2)$ interaction shortens the F state lifetimes by as much as a factor of 30 via couplings to rapid predissociation channels (Miller 1989b).

Theoretical Predictions of Magnetic Hyperfine Structure

D.F. Feller, E.D. Glendenning,^(a) and R.J. Miller

Development of theoretical methods capable of providing accurate predictions of atomic and molecular hyperfine structure parameters has begun in collaboration with Drs. D. F. Feller and E. D. Glendenning of the Computational Technology Group, Molecular Science Research Center here at PNL. Direct comparisons of the results of such theoretical investigations to experimental determinations of these same structural parameters (Miller 1989a) yields enhanced insights into the origins and physical significance of the intrinsic properties of atoms and molecules. Additionally, accurate theoretical predictions of these basic properties offers important guidance to the experimentalist, greatly reducing the efforts required for identification of selective and sensitive laser analytical excitation schemes.

The initial phase of this research has concentrated on calculations of properties of the X 2P ground state of nitric oxide (NO). The objectives of this work were (1) to determine an appropriate basis set for evaluation of magnetic hyperfine structure parameters and (2) to determine the quality of wave function required for convergence to the isotropic hyperfine parameter. For example, the quality of wave function has been examined by reducing the threshold for configuration selection and by extending the list of reference configurations: both factors increase the length of the configuration interaction expansion.

The magnetic hyperfine structure parameters of X 2P NO have been determined through a variety of abinitio theoretical methods based upon restricted and unrestricted Hartree-Fock zeroth order wave functions (Feller 1993). Examples of the former include singles configuration interaction (CI), multireference CI, and averaged coupled pair functional theory. Examples of the latter include Møller-Plesset perturbation theory (through fifth order, with estimates to infinite order), coupled cluster methods, and quadratic CI with approximate inclusion of triple and quadratic excitations. The performance of the various methods in reproducing the difficult-to-describe 14N and 17O isotropic hyperfine interactions is judged in light of available experimental data and estimated full CI values. The full CI limit was approached through a systematic sequence of increasingly extensive, selected multireference CI wave functions that in principle include the full CI limit as its final element. While the isotropic coupling constants were found to converge very slowly (by comparison to other one-electron properties) along this sequence, the selected CI approach was sufficiently efficient in its recovery of correlation effects to be used with large basis sets. The largest calculation in the sequence of CI wave functions included over two million configurations. Computed energies and molecular properties exhibited sufficient regularity to allow extrapolated fittings via simple functional forms. The error arising from the lack of basis set completeness was estimated by comparison to fully numerical, partial-wave self-consistent field (SCF) and singles CI results. Effects associated with the vibrational motion of the nuclei were accounted for by numerical integration of the one-dimensional Schrödinger equation.

(a) NORCUS Postdoctoral Research Associate

Figure 3 illustrates the dependence of the calculated ^{14}N hyperfine parameter on the CI expansion for different configuration selection thresholds T_E . The quantity is a measure of the contribution from the reference configurations to the total wave function. This value tends towards unity as the full CI limit is approached. Thus it is expected that the calculated property will asymptotically approach the limiting value as. Inspection of Figure 3 indicates that the best extrapolated estimates of the full CI limits for the ^{14}N isotropic hyperfine structure parameter A_{iso} are 20 ± 1 MHz. These predictions are in excellent agreement with the experimentally determined values of 22.2 MHz (Meerts 1972) and 22.5 MHz (Kristiansen 1977).

Additional, basic molecular properties of ground state NO were computed utilizing the

Figure 3. Variation in the ^{14}N isotropic hyperfine structure parameter as a function of the weight of the reference space, Σc_i^2 , for different configuration selection thresholds T_E . The dashed lines represent extrapolations to the full CI limits as.

multireference singles and doubles CI (MRSD-CI) wave functions employed for prediction of the magnetic hyperfine parameters. Table I summarizes the theoretically predicted values of these properties and compares them directly to available experimental results.

These investigations are currently being extended to the $(3s\sigma)A\ 2S+$ Rydberg state of nitric oxide. There is particular interest in predicting variations in the fine structure and hyperfine structure parameters characterizing interacting vibronic levels of selected Rydberg and valence states in the region of the first dissociation threshold. Codes are also being developed to enable calculations on the Cray, thereby allowing use of more effective basis sets and multireference treatments.

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Capillary Electrophoresis and Mass Spectrometry

The analysis of environmental, hazardous mixed waste or biological mixtures is best addressed by combined separation-mass spectrometry techniques. The development of these improved analytical methods rests upon the speed, selectivity, and efficiency of the separation combined with the sensitivity and flexibility of mass spectrometric analysis methods. This program is developing methods that are widely applicable to nonvolatile or highly polar compounds, interactable with more conventional methods such as gas chromatography-mass spectrometry. Currently, new methods based upon capillary electrophoresis-mass spectrometry (CE-MS) are being investigated. The goal of this research is to develop ultra sensitive CE-MS methods applicable at the attomole level for environmental and health-related problems.

Development of Capillary Electrophoresis-Mass Spectrometry for DNA Adduct Research

R.D. Smith, H.R. Udseth, J.H. Wahl, and D.G. Gale

The history of analytical advances in mass spectrometry (MS) has highlighted the special importance of the combination of separation methods having high selectivity and resolving power in conjunction with the high sensitivity and specificity of mass spectrometric detection. "Real world" samples are invariably mixtures and are often very complex. Any useful analytical methods must accommodate contributions from the sample matrix, interfering substances, etc. The dynamic combination of capillary electrophoresis (CE), a separation method of high efficiency, speed, and flexibility, with electrospray ionization (ESI)-MS, is particularly advantageous.

The on-line combination of capillary zone electrophoresis (CZE), one form of CE, with ESI-MS (Olivares et al. 1987; Smith et al. 1988b) was developed at PNL. Subsequently, a liquid sheath-electrode interface was developed from which the solvent composition and flow rate of the electrosprayed liquid could be controlled independently of the CZE buffer (which is desirable since high-percentage

aqueous and high-ionic strength buffers that are useful in CZE are not well tolerated by conventional ESI sources) (Smith et al. 1988a). The interface provides greatly improved interface stability and performance and is adaptable to other forms of CE. Because CE relies on analyte charge in solution and the ESI process is nearly universal for charged species, and the CE/ESI-MS combination is highly complementary. Thus, the CZE/MS approach offers previously unobtainable separation efficiencies (for the combination with MS) as well as detection limits that can greatly surpass existing methods (Edmonds et al. 1989; Loo et al. 1989a; Loo et al. 1989b; Smith et al. 1989b; Smith et al. 1990a; Smith et al. 1990d; Smith et al. 1991; Goodlett et al. 1993; Wahl et al. 1992; Smith et al. 1993, 1993b).

One reason for the growing interest in CE-MS techniques is for identification and analysis of DNA adducts. This is an important but formidable analytical challenge due to the extremely low concentrations at environmental levels of exposure. Ideally, we desire not only the ability to detect "known" compounds, but to determine the structure of unknown DNA adducts with sample sizes far too small to be addressed by other analytical methods. This capability would provide the essential tool to understand the origin of

health effects upon exposure at the molecular level. These desires lead to our interest in CE, ESI-MS, and ESI-MS/MS. In fact, it can be argued that the CE-MS/MS combination obtained using the ESI interface should provide a near-ideal analytical approach for the characterization, identification and detection of DNA adducts.

To realize the full potential of this new analytical marriage, several problems remain to be addressed:

- The utilization and transmission of ESI-produced ions must be increased. Currently, ESI losses in the interface and during transmission reduce potential sensitivity by 10^4 (Smith et al. 1990a).
- High resolution separations utilizing an analyte enrichment scheme are required to both deal with the complexity of "real" samples and to obtain sufficient sensitivity with the small volumes utilized in CE.
- Improved MS and MS/MS sensitivity, resolution, and mass measurement accuracy are necessary to enhance detection and allow interpretation of mass spectra containing multiply-charged molecules.

In the last year we have made several significant advances related to the CE-MS interfacing technology and its application. These advances include a) the development of a new sheathless electrospray source compatible with the extremely low CE flow rate, b) the development of methods for CE-MS of noncovalent complexes and c) the first on-line combination with high resolution Fourier transform ion cyclotron resonance mass spectrometry.

While the coaxial sheath flow electrospray interface has facilitated progress in CE-MS, it has some drawbacks. It contributes electrolyte to the ESI source that can decrease sensitivity (but less than might be expected, presumably due to poor mixing with the CE effluent) and also gives rise to chemical noise. Our laboratory has

developed a sheath-less CE-MS interface approach using a contoured metalized capillary terminus. Figure 1 shows separations and mass spectra for tryptic digests obtained for three cytochrome *c* proteins (bovine, candida Krusei, and equine, with molecular weights of ~ 12 kDa). A total of approximately 30 femtomole of the starting protein (before digestion) was consumed for each separation. By rapidly scanning (0.6 s/scan), mass spectra could be acquired for each separation in less than 6 minutes, allowing each of the tryptic fragments to be identified, as well as mixture components due to incomplete digestion. The routine implementation of such capabilities can have enormous impact for more rapid and sensitive protein mapping, and when used in conjunction with tandem mass spectrometry (i.e., MS/MS), biopolymer sequencing.

Recent work with CE-MS using quadrupole mass spectrometers has demonstrated the potential for extraordinary sensitivity and the capability to accommodate extremely small sample sizes. For example, sample volumes with 5 to 10 μm i.d. capillaries are generally on the order of 10 picoliters. Handling of femtomole level samples of this volume is generally impractical, and integrated or on-line methods of sample handling is essential. Combinations with micro-column methods for protein digestion (using immobilized enzymes) and other front-end sample work-up strategies must necessarily be optimized for specific applications. Such CE methods and the ancillary "picoscale" manipulation techniques are anticipated to provide an essential tool box for biochemical analysis on the femtomole and subfemtomole levels.

The gentle nature of the electrospray ionization process has also created new opportunities to directly probe biologically important noncovalent associations in solution (Smith and Wahl, 1993), and even qualitative aspects of 10 polymer higher order structure. While the gentle

Figure 1. Comparison of Three Total Ion Electropherograms Obtained for CE-MS Separations of Tryptic Digests of Three Cytochrome c Species. The separation used a 10 μ m i.d. capillary and a new sheathless CE-MS interface developed at PNL to allow mass spectra to be obtained from 10 femtomole injections using a conventional quadrupole mass spectrometer. Mass spectra are shown for representative regions and generally indicate elution of more than one component.

interface conditions necessary to preserve such interactions by ESI-MS can result in a substantial decrease in sensitivity, CE-MS is still feasible, as demonstrated in Figure 2. Shown are separations of Ribonuclease S (RNase S) using two different pH buffers.^(a) RNase S is a noncovalent complex consisting of a larger 13.7 kDa S-protein and a smaller 2166 Da S-peptide. The complex dissociates under acidic buffer conditions, resulting in two peaks in the CE separation (Figure 2). The mass spectrum shown was obtained from a CE-MS separation using an ammonium bicarbonate buffer. The mass spectrum is dominated by peaks arising from intact RNase S (i.e., the S-peptide - S-protein complex). The ability to use CE-MS to probe noncovalent DNA protein, protein-ligand, DNA-drug and other solution interactions is an exciting prospect. The practicality of such applications will benefit greatly from improved MS performance.

The prospects are excellent that this improved performance should soon be realized. The sensitivity of the ESI-MS detector can be attributed to two factors, (a) the overall ion sampling and transmission efficiency of the ESI-MS interface (typically only 10^{-3} to 10^{-5} with present instrumentation) and (b) the ion utilization efficiency, which derives from the type of mass spectrometer used and its mode of operation. For example, a quadrupole mass spectrometer efficiency of 10^{-1} or better. If 100 ions must be detected to give a S/N ratio of 2 during elution with an ion sampling efficiency of 10^{-3} operated in a scanning mode would typically have an ion utilization efficiency of about 10^{-3} , while in a SIM mode it might have an ion utilization of an analyte zone, the best achievable sensitivity

(a) Goodlett, D.R.; R.R. Ogorzalek Loo, J.A. Loo, J.H. Wahl, R.D. Smith, unpublished work.

Figure 2. Application of CE-MS to the Study of Ribonuclease S, a Noncovalent Complex Consisting of a S-protein and a Smaller S-peptide. The CE separation in an acetic buffer (pH 3.4) gives two peaks with mass spectra corresponding to the dissociated S-protein and S-peptide species. The separation in an ammonium bicarbonate buffer (pH 8.0) gives one broad peak. The mass spectrum obtained during elution of this peak is dominated by the intact Ribonuclease S complex, although a smaller contribution due to the S-protein formed by dissociation of the complex in the ESI interface is also observed.

would correspond to approximately 0.6 and 60 attomoles during scanning and SIM operation respectively—even if ESI efficiency were 100%. In fact, our best results are close to these levels of performance. Future gains will derive from improving ESI-MS sampling and transmission efficiency.

The CE-FTICR combination offers exciting opportunities where both high MS resolution and accurate mass measurement are necessary in addition to high sensitivity. We have recently developed a new FT-ICR instrumentation and have obtained ultra high resolution mass spectra (up to 10^6), and good sensitivity ($< \text{femtomole}$ detection limits for small proteins) for off-line ESI-MS. Figure 3 shows an initial example that demonstrates the practicality of on-line CE-FTICR). The left side of the figure shows the total ion electropherogram obtained for a separation of a polypeptide and five proteins. Although the duty cycle (ion injection time divided by the total time between spectra) obtained in these initial experiments is low (2-10%), a mass spectrum could be acquired every 6 s due to data storage limitations, 6 femtomole injections of individual components with 20 μm i.d. capillaries provided excellent quality spectra with resolutions of up to 50,000, as shown in Figure 3, the FTICR provides resolution of the 1 dalton spacing of the isotopic envelope associated with each protein charge state. Significantly, such high MS resolution was obtained even for the largest protein examined (carbonic anhydrase, 29,021 Da). The combination of sensitivity and MS resolution demonstrated by these initial results represent a breakthrough in CE-MS performance. Because CE provides the capability to slow or completely stop a separation nearly instantaneously, the potential exists to fully exploit the sensitivity and high resolution capability of FTICR in conjunction with MS^n experiments for structural characterization of biopolymers. Future work is aimed at obtaining further substantial gains in the duty cycle, sensitivity, and MS resolution of CE-FTICR.

It is believed that the realization of such advanced CE-MS instrumentation will provide an enabling capability across broad fields of

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DNA Sequencing

New approaches to DNA sequencing are needed to provide massive increase in speed and cost reduction that is essential for the success of the Human Genome Program. This project is aimed at the development of a novel concept for high speed DNA sequencing based upon sequentially degrading a single DNA molecular ion in an Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. This approach would eliminate the need for the preparation and electrophoretic ordering of mixtures of nested oligonucleotides. If successful, this approach would also make possible important studies of site-specific DNA modifications, adduction, damage, and repair at the single molecule level that are now precluded. This new approach, termed single molecule analysis by mass spectrometry (SMAMS), is utilizing new FTICR instrumentation developed at PNL.

Rapid Sequencing Techniques for DNA

*R.D. Smith, C.G. Edmonds, X. Cheng,
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ICR-MS of DNA Molecular Ions

Generating oligonucleotide mixtures, separating them by electrophoresis, and reading and verifying them presently constitutes in excess of 80% of the total effort in DNA sequencing. Limitations of these methods arise from the behavior of the enzymes employed for chain extension, the chemistry and spectroscopy of the fluorescently labeled residues, and the resolution and speed of the gel separations. Presently, overall raw sequencing rates of 600 residues per hour with accuracies in the range of 95 to 99% can be achieved. Most new approaches for reducing the cost and time required for these sequencing activities involve automation and/or multiplexing of currently practiced methods. As an example, electrophoresis in gel-filled capillaries, or very thin gel layers, is being investigated as an approach for speeding the electrophoretic separation and facilitating its automation. These approaches should improve efficiency, but still fall short of the massive increase in speed and cost reduction desired for large scale DNA sequencing.

In the search for methods to increase sequencing speed, a number of new approaches are being investigated, but no clearly superior technology has yet emerged. This project is aimed at greatly speeding DNA sequencing by sequentially degrading a single trapped DNA molecular ion. Such methods could have inherently high rates of sequence determination, and could significantly accelerate the overall process of DNA sequence determination by eliminating the preparation and electrophoretic ordering of mixtures of nested sets of oligonucleotides. Successfully developing this approach would not only be relevant to DNA sequencing, but perhaps even more importantly, would make possible important studies of site-specific DNA modifications, adduction, damage, and repair, which are presently precluded.

New developments in combined Fourier transform ion cyclotron resonance (FTICR) mass spectrometry and electrospray ionization (ESI) provide the basis for the formation, isolation, and extended trapping of single highly charged DNA molecular ions with molecular weights well into the mega-dalton range. This new approach, termed single molecule analysis by mass spectrometry (SMAMS), is being explored utilizing new ESI-FTICR instrumentation

developed at PNL. Figure 1 gives a schematic illustration of the FTICR instrumentation.

In the first full year of this project, success has been obtained in four areas vital to the project. These developments include (a) the demonstration that large molecules can be trapped for extended periods ($> \text{hours}$), (b) large molecules can be measured with extremely high resolution ($> 10^6$), (c) large segments of plasmid DNA can be electrosprayed intact without dissociation, and (d) the first example of single molecule detection and remeasurement for a large multiply charged molecule was obtained using FTICR.

The FTICR instrumentation has continued to be refined over the last year (Winger et al. 1993), and we have been able to demonstrate that molecular ions ranging from $< 1 \text{ kD}$ to $> 1 \text{ MD}$ can be trapped for periods of many hours. In addition, it is possible to make numerous remeasurements of the same ion population during this period and we have introduced the

use of rf-quadrupole cooling to accomplish the m/z -selective accumulation of ions in the FTICR cell. We have shown also that with proper collisional cooling of large molecules, extremely high resolution mass spectra can be obtained. As shown, in Figure 2, we have achieved a resolution of $> 2.5 \times 10^6$ for a small protein, approximately three orders of magnitude better than typical of conventional mass spectrometers.

An essential component of this project is the ability to electrospray large segments of DNA intact, without inducing dissociation of the gas phase species. In FY 1993, we have conducted experiments that demonstrate conclusively that this can be done. The conventional electrospray ionization mass spectrum for a plasmid DNA sample (pGem-5S) of molecular weight 1.946 MD is shown as essentially an unresolved broad peak due to the limitations of sample size, probable heterogeneity due to residual adduction, and insufficient resolution. Ancillary

Figure 1. Schematic of the Electrospray Ionization-FTICR Instrumentation Developed at PNL

Figure 2. Ultra-high Resolution ($>2.5 \times 10^6$) Mass Spectrum for Small Portion of a Mass Spectrum for Bovine Insulin Obtained by ESI-FTICR

Individual Ion Detection by FTICR

Figure 3. FTICR Mass Spectra in Small m/z Region for Individual Ions from (A) a nominal 5 MDa PEG; (B) a nominal 1.06 MDa PSS; (C) a nominal 600 kDa PEG; (D) a nominal 177 kDa PSS; (E) bovine albumin dimer; (F) bovine albumin; and (G) the background in the corresponding m/z region for (F). The PSS spectra were obtained for negatively charged ions, while the other species were all examined using positive ion ESI. Background spectra obtained in other m/z regions were similar.

experiments in which gel electrophoresis was conducted on plasmid samples before and after electrospray ionization showed that the DNA remained intact.

In order to pursue our approach to DNA sequencing, we must be able to trap and remeasure many time **single** (i.e., individual) large ions in the FTICR cell during the course of the step-wise degradation of the large molecular ion. We have recently demonstrated that this can be accomplished. Figure 3 shows the mass spectra in small m/z ranges for **individual** ions of MWs spanning nearly two orders of magnitude and the typical background noise. For acquisition of the data shown in Figures 3A-E, a single ion from each sample was isolated and detected. For Figure 3F, several discrete albumin molecular ions, the smallest compound studied, were trapped and detected with a S/N of ~ 2 . The S/N of ~ 2 obtained for bovine albumin with 30 charges essentially defined the lower limit for

detection if **individual** multiply charged ions in this m/z range with the conditions employed. It is envisioned that extension to lower MWs could be obtained by formation and detection of higher charge state ions at lower m/z . For example, a 30 kDa protein at an m/z of 1000 will carry 30 charges and should be detectable. The present detection limit of 30 charges may also be lowered by further improvement in preamplifier design, noise reduction, the acquisition of longer time domain signal, or a higher magnetic field strength.

Finally we have made initial measurement of large intact plasmid DNA molecules. Figure 4 shows a time-resolved mass spectrum for an individual ion from a pGEM-5S plasmid DNA sample. By inducing a charge transfer reaction in the gas phase and observing the discrete changes in charge states, the charge state of the ion can be assigned. This then allows the mass to be precisely calculated to be 1.952 ± 0.002 MDa,

Figure 4. A time resolved Electrospray Ionization FTICR-Mass Spectrum for obtained from a single molecule from a Sample of Closed Circular Supercoiled DNA (p-GEM-5S) of Molecular Weight 1,945,664 (free acid)

only slightly larger than calculated based upon the expected sequence (1.946 MDa). An additional advantage of this approach is that a number of molecules can be studied simultaneously. These results demonstrate a number of new capabilities and also extend the useful range of mass spectrometry for DNA by nearly two orders of magnitude, providing a technical foundation for our sequencing concept.

These results demonstrate many of the essential features of our high-speed DNA sequencing concept. While we continue to advance and refine these methods, we will increasingly focus our attention on the crucial remaining issue; i.e., the chemical processes necessary for the sequential stepwise degradation of large multiply charged oligonucleotides in the gas phase.

Radiological
and Chemical
Physics

Radiation Dosimetry

The primary goal of the Radiation Dosimetry program is to understand the connections between the physical events produced by ionizing radiation and the biological consequences of those events. These consequences result from complex sequences of reactions involving a variety of initial radiochemical products, the products of later chemical reactions, and biological responses. However, differences in consequences occur when only the type of charged particle that deposits the energy is changed. This indicates that the spatial and temporal relationships of those initial products profoundly influence the subsequent reactions.

Dosimetry research attempts to provide realistic models of the physical processes and to combine them with information on likely chemical and biological reactions to produce models of response at the cellular level. The models are designed to be testable by experiments at each level of the system. Models of energy deposition along individual charged particle tracks have been used to calculate energy deposition in small volumes and the fraction of the energy deposited by track ends. Volumes a few nanometers in diameter, as well as those a few micrometers in diameter, appear to be directly relevant to the biological damage. Energy deposition in the larger volumes can be measured experimentally and used to test the validity of the calculations.

In order to determine characteristics of the energy deposition patterns that are relevant to the biological response, it is necessary to develop and test both phenomenological and mechanistic models that can be related to specific assumptions about the effects of energy deposition patterns and initial chemical products. As models are developed, data from the literature or from specific experiments conducted in the Radiation Biophysics program are used to determine if the model is applicable to a specific biological system. As inappropriate models are rejected, those that remain converge to form an acceptable description of the relevant biological processes. The phenomenological models also can be applied more directly to calculate risk of environmental radiation exposure since they give direct predictions of effects which may be related to changes in the health of an organism. Although the phenomenological and mechanistic models often emphasize different properties of the irradiation, it is important to assure that the two types of models are consistent in the areas where they overlap.

Biological Effectiveness of High Energy Ions and Their Delta Rays

L.A. Braby

The tracks of high energy ions such as protons or alpha particles passing through a biological system can be visualized as a primary track with occasional delta rays (energetic electrons) branching off of it. The primary track consists of the ionizations and excitations produced by the primary ion itself, plus those produced by low energy secondary electrons in the immediate vicinity of the point where the primary

interaction occurred. The stochastic nature of the primary ionization processes leads to random clustering of ionizations along the primary track. Furthermore, the delta rays can produce additional clusters of radiation damage, both where their highly scattered paths cross the primary track, and where they slow down and their stopping power increases, near the ends of their paths. It appears likely that these clusters of damage, which can cause combinations of strand breaks with other types of DNA damage and probably render the strand breaks difficult of

even impossible to repair, are the origin of serious forms of DNA damage such as deletions and interchanges. However, it is difficult to estimate the fraction of the total amount of chromosome aberrations or other serious damage which is produced by delta rays. For most radiations present in the environment, the range of the delta rays is not sufficient to separate the energy they deposit from that deposited by primary particles. However, for high energy heavy ions, the delta rays are energetic enough that their energy deposition patterns can be measured separately from those of the primaries (Metting, et al. 1988). Furthermore, it is possible to estimate the biological effectiveness of the primary track and the delta rays separately using the hit size effectiveness concept (Varma and Bond 1982). The hit size effectiveness function gives the probability that a single charged particle event will produce a specific biological effect, as a function of the energy deposited in that event. Investigation of different endpoints and different site sizes (for the evaluation of the lineal energy) have shown that the correlation does not depend critically on the site size used, and that a $1\text{ }\mu\text{m}$ diameter site makes a good "phantom" for whatever structures within a cell are responsible for the biological effect.

The exact form of the hit size effectiveness function is difficult to determine because it can not be measured directly. At this time it can only be determined by deconvolution from the available data on the response of various biological systems to low doses of different types of radiation, and the lineal energy spectra for those radiations. Two problems, the limited number of experiments and the overlap in the lineal energy spectra of the different radiation fields, result in a great deal of "noise" in the resulting hit size effectiveness functions. However, the basic shape of the function is remarkably similar for a wide range of endpoints. The two smooth curves in Figure 1 represent the extremes of the functions which have been determined for

Figure 1. Smoothed Hit Size Effectiveness Functions Representative of the Extremes of Those Found Experimentally

various experimental systems. The striking features of these functions is that there is some response even at rather low values of lineal energy, in the range that is characteristic of relatively high energy x ray spectra, and a peak in the response around $100\text{ keV}/\mu\text{m}$.

The single event lineal energy spectrum for a broad beam of $600\text{ MeV}/\text{amu}$ iron ions is illustrated in Figure 2. Clearly the majority, approximately 85%, of the sites are effected only by delta rays, with lineal energy between 0.001 and $10\text{ keV}/\mu\text{m}$. The remaining 15% of the sites are

Figure 2. Lined Energy Spectrum for a Broad Beam of 600 MeV/amu Ions, Constructed from Data Measured with a Wall-Less Proportional Counter

hit directly by the primary track, with energy deposition in the range of 20 to several hundred keV/ μm . However, these primary ion events are from 400 to 660 times as effective in producing the biological effect (depending on which of the two extreme hit size effectiveness functions is used) as are the delta ray events. As a result, between 0.8 and 1.3% of the hits with a biological effect will have been initiated by a delta ray only.

Even in the case of these high energy heavy ions, where the maximum range of the delta rays is large compared to the size of a typical cell, it is clear that the delta rays are seldom the sole cause of a specific type of biological damage, but they may frequently add to the damage produced by the primary track. The role of delta rays in carcinogenesis is potentially more complicated. In this case there could be a competition between two biological effects, cell killing and malignant transformation (or the initial step in transformation). If the forms of the hit size effectiveness functions are significantly different for these two endpoints, there will be a shift in the ratio of the

response to primary tracks and delta rays. For example, if the effectiveness function for cell killing was more heavily weighted for high lineal energy events than the effectiveness function for transformation, then a primary track would be more likely to kill the cell, and a higher fraction of the transformed cells would be due to delta rays. However, at the doses of interest, only a single charged particle event will occur in each cell. If the effectiveness functions are the same shape, even though the probability of transformation is much lower than the probability of killing, the fraction of the cells transformed by delta rays will be unaffected by the competition with cell killing. Currently, the noise in the hit size effectiveness data prevents a perfect answer to this question, but it appears that there is not a large difference in the shapes of the functions for different endpoints, so it is unlikely that delta rays are responsible for a significant fraction of malignant transformation.

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The Stochastics of Track-Ends

W.E. Wilson, J. Song, and L.A. Braby

It has been speculated that the high density of ionization and energy deposition at the end of secondary electron tracks (a so-called 'track-end') may constitute a significant radiation of high RBE. In an attempt to shed some light

were simulated, using Monte Carlo methods, and the production of track-ends and their energy content was statistically analyzed. The results indicate that electrons of 5 keV or less produce very few branch tracks and thus few track-ends. Electron scattering theory provides quantitative support for this conclusion. Instead of being divided among several branch track-ends, the energy is deposited in localized clusters randomly spaced along the meandering path of the primary electron.

Goodhead has emphasized that a significant portion (30 to 50%) of the energy deposition by all low-LET radiations is imparted by electrons of ≤ 5 keV and that a substantial part of it is in localized clusters occurring predominately near the terminal 'track-end' of the electron path (Goodhead 1989). Also, Pimblott et al. (1990) have calculated that approximately 70% of the energy lost by MeV electrons in simulated water is through events of less than 100 eV.

Because of the similarities between track ends and ultra-soft x-rays which have been shown to have high biological effectiveness (Goodhead et al. 1981), and because of their possible involvement in producing locally multiply damaged sites (Brenner and Ward 1992), we have determined the frequency of occurrence of track-ends by computing low-energy electron tracks via Monte Carlo methods (Paretzke 1987). Mono-energetic electron tracks of 1 to 10 keV initial energy were simulated and the number of track-ends per primary determined along with the fraction of the total energy deposited in them. Sample sizes were typically greater than 10^4 tracks at each primary energy.

For each primary electron energy, E_0 , the stochastics of the energy deposition were studied as a function of two variable energy thresholds. One energy threshold, E_t , defines the 'track-end.' Whenever an electron's residual energy drops below E_t all subsequent inelastic collisions then constitute a 'track-end.' During slowing down, but while the electron has energy greater

than E_t , energy depositions are considered to fall outside the track end. The second energy parameter, E_s , defines a 'branch track,' that is, a secondary electron generated with energy greater than E_s is assumed to be a separate track and is scored accordingly. When the residual energy of the electron in a branch track degrades in energy to less than E_t , there is produced a corresponding track-end. For each primary electron, the number of track-ends is thus one greater than the number of branch tracks. Obviously, the branch track threshold must be larger than the track-end threshold.

Our usage is similar to that of Mozumder and Magee, wherein they categorize electron energy deposition into the fixed entities 'spurs,' 'blobs,' and 'short tracks,' however their focus is for the most part on electrons of some tens of keV and higher (Mozumder and Magee 1966); here, we define just two tracks entities, and consider the partition of deposited energy between the two *as functions of* the variable energy parameters defining the entities.

The fraction of energy deposited in track-ends was found to be a linear function of the energy threshold E_t with a slope weakly dependent on E_s ; for increasing primary energy, the results show an increasing dependence of the slope on E_s .

The computed average number of branch-tracks per primary produced by primary electrons of 1 to 10 keV is a function of the threshold energy, E_s , defining a branch track. Generally, less than one branch track is produced on average, by each primary electron. Only for primary energies above 5 keV and a branch-track threshold of 300 eV or less does the number of branch tracks exceed one per track. These results indicate that electrons of 5 keV or less produce very few branch tracks and thus few track-ends.

The results shown are readily understood. The number of track-ends produced per primary is just the number of branch tracks plus one for

the primary itself, thus we can express the fraction, f , of the primary energy that appears in track-ends as,

$$f = (N(E_s) + 1) \frac{E_t}{E_0}$$

where $N(E_s)$ is the average number of branch tracks produced by a primary of energy E_0 . $N(E_s)$ will in general depend on E_s , the assumed threshold for branch track production as shown in Figure 3. From the observations exhibited in Figure 4 for 1 keV primaries $f \approx E_t/E_0$, and hence $N(E_s) \ll 1$ which is confirmed by Figure 3. The slopes of the curves in this 1 keV case are essentially $1/E_0$ and hence a value for f of $1/2$ at $E_t = 500$ eV is predicted. The reason f is slightly less than 0.5 at $E_t = 500$ eV in the top panel of Figure 4 arises from the way the last inelastic interaction was scored as the primary's energy crosses the E_t boundary. That last quantum of energy deposition was scored pre-track-end and hence on average the track end will contain less than E_t of energy. The variation

Figure 3. Average Number of Branch-Tracks Produced Per Primary Electron. For primary energies less than 5 keV and a branch-track thresholds greater than 300 eV, the number of branch-tracks does not exceed one per primary.

Figure 4. Fraction of the Total Energy Deposited in 'Track-Ends' by Low Energy Electrons. The energy threshold for defining a track-end is E_t , whereas E_s defines the threshold for a 'branch-track' that will produce its own track-end when it slows. E_0 indicates the initial primary electron energy. The weak dependence of the slopes of the lines on E_s implies that production of 'branch-tracks' is infrequent for low-energy primary electrons.

of the slopes of the lines in the other panels of Figure 4 are understood in terms of the increase of $N(E_s)$ with decreasing branch track threshold and increasing primary energy as exhibited in Figure 3.

The results of this Monte Carlo study did not confirm our preconceived mental image of a low-energy electron track as a "many-branched-tree."

Reference to simple scattering theory, however, supports the conclusions reached above. The production of energetic secondaries by swift electrons in ionizing collisions is described by the Mott cross section. The non-relativistic formulation is sufficient for the low-electron energies considered in this note; see for example Evans 1955. The energy spectrum of secondary electrons produced by primaries of energy E_0 is proportional to the respective differential cross section shown in Figure 5. For such low primary energies the secondary spectra are very "Rutherford-like;" they vary essentially as w^{-2} up to $E_0/2$, where w is the secondary electron energy. From the differential cross sections one can estimate the mean free path for producing energetic secondaries. Consider,

$$1/\lambda = n \int_{\Delta}^{E_0/2} \sigma(w) dw$$

where $\sigma(w)$ is the differential Mott cross section for ejecting ionization electrons of energy

Figure 5. Differential Mott Cross Section for Producing Secondary Electrons with Energy Between w and $w + dw$. The cross section varies basically as w^{-2} at the low energies considered in this note.

between w and $w + dw$, $E_0/2$ is the kinematic maximum energy that a primary electron can transfer to a secondary and n is the number of target electrons per unit volume. λ_{Δ} is then the mean free path for the production of secondary electrons with energy w greater than or equal to Δ .

By numerical integration of the Mott cross section formulas, we obtain λ_{Δ} as a function of Δ as shown in Figure 6 for 1 to 10 keV primary electrons. If we associate Δ with E_s , for the range of values of E_s discussed in this note, we obtain mean free paths that are in the range of 100 nm to a few microns (unit density water). Such path lengths are comparable to the total range of the respective primary. The arrow on the ordinate indicates the range of a 1 keV electron, ca. 58 nm (ICRU 1970); in this instance, a 1 keV electron at a constant velocity would produce an average of one secondary of energy 100 eV or greater approximately every 85 nm.

Figure 6. Mean Free Path for Production of Secondary Electrons with Energy Equal to or Greater than Δ . The curves are obtained by numerical integration of the differential cross sections shown in Figure 5. The predicted mean free paths are generally longer than the entire range of the primary thus supporting the observation that production of energetic branch-tracks and their track-ends is infrequent.

But its total range is significantly less than this distance, so we can expect that production of branch tracks of 100 eV or more will be infrequent, as the Monte Carlo simulations indicate.

Production of branch tracks is infrequent for primary electrons of 5 keV and less. There is generally only one track end, produced by the primary itself, and a conceptual image of such a low energy electron track as a many branched tree is incorrect. Inelastic collisions of such low energy electrons with atoms and molecules in the media predominately transfer less than 200 eV of energy and, therefore, the structure of a low-energy delta ray is perhaps more reasonably viewed as a sequence of random-sized clusters strung along the meandering path of the primary electron.

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Clustering in Positive Ion Track Structure

W.E. Wilson

To objectively and quantitatively measure output sensitivity to input parameter variations and model assumptions in track structure simulations, we used cluster analysis methods evaluating the stochastic features of charged particle tracks. The specific algorithm and method discussed here is the K-MEANS algorithm (Hartigan 1975; Späth 1980), a general computational tool used to look for correlation among diverse data in a systematic fashion, where correlated means "close" by some measure. Applying this algorithm to track structure features is simple because we use it to discover items that are literally spatially close.

The algorithm's input requirements include an initial an initial partition of the ensemble of data into K groupings or clusters. Each datum must be in only one cluster; no cluster may be empty. In the sample algorithm discussed here, the cluster number K is conserved, i.e., the algorithm neither adds nor deletes clusters. Within the algorithm, the centroid of each initial cluster is evaluated, along with a measure of the discordance of the data within each cluster. The algorithm seeks to minimize the global discordance

by attempting, systematically, to reassign a datum to every other cluster. The algorithm stops when no acceptable reassignment is found for one complete pass through the data ensemble. In this way, the algorithm achieves a local minimum, as opposed to a global minimum that would require testing all possible permutations of the data, a problem much too large even for the most powerful of hardware.

As the measure of discordance of the data within a cluster, we use the sum over the transfer points in the cluster of their squared distances from the centroid of the cluster, this is a quantity similar to χ^2 . By taking the square root of the quantity returned by the algorithm, we have an rms measure of the physical radius of each cluster. Also, summing the energy associated with each transfer point in the final clusters defining the total energy deposited within a cluster, and dividing the energy by the rms radius expresses the cluster energy density in terms of a radiation-quality quantity similar to light energy transfer (LET).

In the following examples, we analyzed 100 nm segments of 1 MeV proton tracks simulated with PITS, using a modified version of MOCA8 for transporting the delta rays. Only the ion track core was sorted in the sense of energy restricted LET by ignoring delta rays produced with more than 200 eV of energy. For the initial input partition to the K-MEANS algorithm, each track was sorted into ascending order along the path of the ion, then the first N transfer points were placed into the first cluster, the next N into the second, etc., until all transfer points were assigned and K, the number of clusters, was adjusted accordingly for each track, as generated. Values for N tried were 4, 5, 6, 8, and 10. No striking variation in results stood out, so we focused on a mean cluster size for N of 6; all results discussed here were obtained using this value. To test only the sensitivity to the localization phenomenology, the other input physical model parameters were held fixed.

The probability distribution in cluster size, as measured by the number of transfer points in the cluster, is shown in Figure 7 for the three levels of dispersion described above (i.e., metal, tissue, and none). Straight lines connect the data points to help identify trends, even though the variable on the abscissa can take on only integer values. Introducing a dispersion feature restricted to short ranges, as might be typical of tissue-like matter, gives rise to only minor differences in the cluster distribution when compared to 'none.' The numbers of pairs and triples are only slightly reduced, the numbers of "fours, fives and sixes" are enhanced. However, for the conductor case, the number of medium sized clusters is significantly reduced and the number of large clusters, number of transfer points ≥ 11 , is enhanced.

The sensitivity of the physical size of the clusters to the range of the dispersion function is illustrated by the frequency density distributions for the rms radius (Figure 8). The change in the clustering radii found by the algorithm as the localization model is varied is just what one would expect; for no dispersion the radius is the

Figure 7. Cluster Size Distributions for All Clusters. Three cases of dispersion are compared; one appropriate to a tissue-like absorber, one for a metal (conductor) and for comparison, no dispersion function used (#TP = number of transfer points).

Figure 8. Probability Density Distributions for rms Radius of the Clusters Returned by the K-MEANS Algorithm. Three cases of dispersion are compared.

smallest. The most probable radius is about 0.5 nm. As the range of the dispersion function is increased, the observed cluster radii are systematically larger, with the most probable radius increasing to about 0.8 and 1.5 nm for the tissue-like and metal cases, respectively. What is, perhaps at first thought surprising, is that the radii for a given case do not depend on the number of transfer points in the cluster, all cluster sizes exhibit the same most probable value. However, most likely no physical significance to this result

exists because this is exactly the quantity that the clustering algorithm seeks to minimize for each cluster and, therefore, it tends to make them equal without regard to how many transfer points are in any given cluster.

Given the final assignment of each transfer point to a designated cluster, we can now ask about the total energy deposition associated with each cluster. For this purpose, we use the LET-like quantity defined above. Figure 9 presents

Figure 9. Probability Density Distributions for the Ratio of Total Cluster Energy to rms Radius. Three cases of dispersion are compared.

the frequency density distributions for the ratio of energy deposited to rms radius of the cluster. The total energy quite obviously will depend on the number of transfer points in the cluster, and because the rms radius of the cluster is independent of the number of transfer points, one sees a monotonic increase in the energy density. For the three cases of dispersion, the energy density is least on average for the metal-like case because the mean rms radius is greatest for it. There is not much difference between the short range tissue-like case and none. The most probable energy density is approximately 200 eV/nm for a cluster size of 14 transfer points for the none case, and only slightly less for the short range tissue-like case.

The preceding tests that addressed the question of the sensitivity of track-structure clustering to assumptions about the localization of energy transferred to the medium used water vapor as input model parameters. We can ask the same question with regard to the absorber phase. To gain some insight into how condensed phase input parameters affect track structure clustering, we have generated and analyzed track segments simulated with Positive Ion Track Structure (PITS) using liquid water parameters to compare with water vapor results. The comparison uses the short-range tissue-like dispersion for both liquid and vapor tracks throughout. Figure 10 shows the distribution of all cluster sizes as measured by the number of transfer points with the appropriate water vapor case from Figure 7.

The obvious striking feature is the large increase in "triples." The harder primary delta-ray spectrum afforded by the shift in oscillator strength toward larger energy losses (Heller et al. 1974; Berkowitz 1979) undoubtedly gives rise to this significant difference.

The comparison of the frequency distributions for the rms radius and for the energy density in water vapor and liquid are shown in Figure 11

Figure 10. Cluster Size Distributions for All Clusters. Input physical parameters for liquid water (Heller et al. 1974) and for water vapor (Paretzke 1987) were used for the two cases.

and 12, respectively. The curves for water vapor are repeated from Figures 8 and 9 with the data for cluster size equal to 3 added since this feature is a prominent difference between the two cases. The most probable rms radius is smaller for liquid water at about 0.6 nm compared to about 0.8 nm for the vapor simulation. This presumably has to do with the large number of ionizations produced in the liquid example; a larger number of transfer points in a given length of track segment simply implies their mean distance apart will be less.

The most probable energy density for large clusters (i.e., those with greater than ten transfer points) does not appear to change significantly by going to liquid phase.

Cluster Distance Distributions

The K-MEANS algorithm returns the coordinates of the centroids of the final clusters as well as the number of transfer points in each. It is a simple matter to calculate and score the distance between clusters (i.e., between cluster *i* and *j*); furthermore, because the number of transfer

Figure 11. Probability Density Distributions for rms Radius of the Clusters Returned by the K-MEANS Algorithm. PITS output for liquid and vapor water are compared.

points in each is known, one can score the distance between them as a function of their respective size (number of transfer points in each). We therefore define $f(d)_{m,n}$ as the differential distance distribution for cluster pairs of size m and n . The symmetry of the problem results in data structure that is an N by N symmetric matrix of distributions $f(d)$, (actually, the distributions are arrays or vectors, because discrete bins are used in scoring) where N is the maximum cluster size allowed. The distributions on the diagonal are the differential distance distributions for clusters of equal size, those close to but off the diagonal are for clusters of similar size, and distributions for dissimilar clusters are far from the diagonal. Visualization of such a complex of data on the standard printed page is limited; one possibility

Figure 12. Probability Density Distributions for the Ratio of the Total Clusters Energy to rms Radius. Results for liquid and vapor water are compared.

is to select a row of the matrix and display the *conditional* probability or distance distribution for cluster pairs of which one is of that selected size (row).

In Figure 13, we selected row size, and the conditional probability densities for the distance between a cluster containing six transfer points and clusters of all other sizes. This result is for 1 MeV proton track segments of 100 nm length using water vapor input parameters and tissue-like dispersion. A logarithmic scale is employed for the distance axis to expand the onset region of the distributions so the differences between curves is more evident. Panel A shows the distributions for the second cluster of the pair being of size 6 or smaller and Panel B shows them for size 6 or larger.

Because the ion path segments were 100 nm, inter-cluster distances greater than 100 nm are naturally infrequent. The initial rise of the distributions beginning below 1 nm to their most probable value around 2 to 3 nm is related to the finite physical size of the clusters and the associated distributions in their rms radius (Figures 8 and 11). Distance to the nearest cluster neighbor of a given size (transfer points) is indicated by

Figure 13. Differential distance distributions for cluster pairs, conditional on one of the pair containing 6 transfer points. The size of the other cluster of the pair is designated in the legend. This example is for 100 nm segments of 1 MeV proton tracks simulated in unit density water vapor and using tissue-like dispersion. For clarity some distribution are omitted.

the most probable value. The distributions for cluster sizes of 1, 2, and 3 transfer points have a lesser initial slope and reach their respective maximum at a slightly greater distance than do the distributions for larger clusters. A possible explanation for this is that the larger clusters will preferentially be near the ion path where the ionization density is on average highest; conversely, small clusters will tend to be found in the periphery of the ion track and hence nearest small-cluster neighbors will be farther away.

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Radiation Physics

Ionizing radiation interacting with and depositing energy in an absorbing medium initiates a chain of events producing long term chemical and biological responses. The initial spatial and temporal patterns of energy deposition result in the production of secondary electrons and free radicals followed by the slowing of the secondary electrons that define the structure of charged particle tracks; hence, the initial biological damage. Thus, experimental and theoretical investigations of the differential cross sections for secondary electrons produced by charged particles representative of neutron-induced recoils in tissue or radon alpha particles comprise the core of radiation physics studies. These quantitative data provide insight into energy deposition processes by high-LET radiation and form the basis of many energy degradation and transport models used to describe energy deposition in representative biological media. Since energy deposition is a stochastic process, the emphasis at PNL is on using Monte Carlo based models to understand energy deposition in heterogeneous cellular environments.

Detailed information about individual energy deposition processes is obtained from differential cross section measurements of electrons emitted in fast ion-gas phase target interactions. Recent experimental studies have focused on ionization induced by ions that possess bound electrons because these ions form an important part of the total energy deposition. For information about electron production and transport in condensed-phase media, differential electron emission from thin foil targets following passage of fast protons is studied. These experimental data compliment and supplement collaborative theoretical work which investigates the role of bound projectile electrons in collisional ionization of atomic and molecular targets. Monte Carlo based energy transport studies combine these experimental and theoretical results with literature data for studying the systematics of local energy deposition in molecular, cellular, and heterogeneous condensed phase systems. This approach uses knowledge of basic energy deposition processes and models of energy transport to contribute to the ability to extrapolate observed biological effects as a function of dose, dose rate, and radiation quality.

DNA Fragmentation

Interactions between ionizing radiation and biological material result in energy deposition within individual molecules of DNA. This initial step in radiation damage leads to subsequent chemical and biological changes; changes that can ultimately result in cell mutation or death. Chemical and biological changes occur when the ionized molecules or their dissociation fragments attempt to re-establish their local charge neutrality, either via geminate recombination or through chemical interactions with the surrounding media. Thus, knowing what ions or fragments are formed, and with what probabilities is

critically important for modeling radiation damage in biological media.

DNA is the critical target that, when damaged, results in biologically relevant effects. Thus, studies of radiation induced damage of DNA, specifically those processes leading to DNA fragmentation, can provide vital information for modeling purposes. However, in biological media, DNA damage occurs via direct interactions between ionizing radiation and the DNA molecule or via indirect processes where the radiation induces physical or chemical changes in the surrounding media and these changes then lead to DNA damage via secondary

interactions. Therefore, it is important to investigate the direct processes and to study how the surrounding media moderates or effects these processes.

Our approach to answering these questions is to use electrospray ionization and mass spectroscopic techniques to transfer well-defined intact segments of biomacromolecules from the liquid- to gas-phase where they are bombarded with an energetic electron beam. The charged fragment and parent ions are then mass analyzed and identified. Using this method, qualitative information about the fragmentation spectra are obtained and quantitative information about specific ionization probabilities with additional effort. The electrospray ionization technique also permits some control over the degree of solvation of the macromolecule that permits us to study how the surrounding media influences the fragmentation spectra and probabilities.

DNA Fragmentation by Direct Interactions with Ionizing Radiation

R.D. DuBois and A.L. Rockwood

A new project was initiated to experimentally investigate "direct radiation-induced damage to biomacromolecules." Specifically, the ionic fragments produced when specific DNA segments are bombarded with energetic electrons will be studied. These studies will be performed as a function of DNA segment size, electron energy, and degree of solvation of the DNA segment. The goals are to determine the primary fragment ions produced via direct, as opposed to media-related, interactions between DNA and ionizing radiation and to study how the surrounding media modifies these interactions.

The experimental method used for this work consists of using electrospray ionization technology to transfer intact segments of DNA from the liquid- to the gaseous-phase, isolate specific, well-defined, segments via mass analysis, and allow these segments to interact with energetic electrons. Fragment ions produced in these

interactions will be detected. A schematic diagram of the apparatus used for these studies is shown in Figure 1. In this apparatus, parent DNA ions of interest will be stored in an ion cyclotron resonance (ICR) cell and electrons will be injected into the cell. Interactions will be detected by a decrease in the parent ion signal, accompanied by increases in the daughter products signals.

Initially, parent ion signal intensities were investigated for several short DNA segments. A good prospect for initial fragmentation studies appears to be the four component DNA segment d(AGCT) because it primarily produces a +4 ion. Therefore, likely daughter ions are the singly charged constituents that would be relatively easy to distinguish using m/z analysis techniques.

Ionization by Neutral Projectiles

R.D. DuBois and S.T. Manson

Because no long-range coulomb forces exist for neutral atoms, neutral projectiles are assumed to produce little, or no, target ionization. However, previous studies of total (DuBois and Köver 1989) and differential (Heil et al. 1992) ionization studies have shown that fast hydrogen atoms induce ionization nearly as efficiently as isotachic protons.

The reason for this is that, for dressed ion impact, electrons bound to the projectile participate in the collision in both an active and a passive role. In their passive role, they interact indirectly with the target electron(s) via their partial screening of the nuclear charge. This reduces the target ionization cross section with respect to that for the bare ion. This is the situation commonly assumed to dominate. However, in their active role, they interact directly with target electron(s). This increases the ionization cross section with respect to that for the screened ion.

In 1992 (Manson and DuBois 1992), we used the first Born approximation to predict that the

Figure 1. Schematic Diagram of electrospray ionization source and ion cyclotron resonance cell used to store and detect ions.

active role will dominate for distant collisions between energetic atoms. Likewise, it will dominate the total target ionization cross sections.

Total cross sections for ionizing helium by various neutral projectiles (Figure 2) appear to confirm the latter expectation. In addition, double-differential electron emission cross sections were measured for hydrogen and helium particles, neutral and charged, impacting on a helium target. Some of these data for hydrogen atom and proton impact are shown in Figure 3. For hydrogen atom impact, the passive role described above is reflected by a decrease in the cross section, relative to that measured for proton impact, in the 10 to 100 eV region. Above 100 eV, electron emission from the projectile is responsible for the large peak centered at approximately 250 eV. As the emission energy decreases below 10 eV, i.e., as the impact parameter of the collision becomes large, the cross section for hydrogen atom impact increases. This results from direct interactions between a projectile and target electron in which a target

electron is ionized and the projectile electron is excited, primarily to the continuum. Thus, the active role predominantly results in ionizing a projectile and a target electron.

Figure 2. Total cross sections for ionization of helium by different neutral projectiles. The dotted curves indicate linear and quadratic dependences on the projectile nuclear charge Z .

Figure 3. Doubly differential electron emission cross sections for hydrogen and helium ions and atoms colliding with helium.

From a dosimetry viewpoint, this active role has two important consequences. The first is that neutral atoms induce ionization nearly as effectively as ions, and hence, they should not be ignored in track structure models. The second is that the energy deposited via the active role is different than that deposited via the passive role. A third ramification is that the active role also produces a slow electron in the projectile frame which, due to the kinematic transformation, is an energetic electron in the laboratory frame. This electron is predominantly emitted in the forward direction in the laboratory frame and has the same velocity as the projectile, i.e., it and the projectile ion are spatially and temporally correlated. Hence, they arrive at the next target at the same time and could produce correlated ionizations leading to double strand breaks in biological tissue.

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Secondary Electron Emission from Thin Foils

C. Drexler and R.D. DuBois

Stochastic track structure models of energy deposition by energetic charged particle impact are often based on ionization cross sections obtained from gas-phase targets. Suitable approximations and modifications are then added to simulate condensed-phase conditions. A major question then arises concerning the validity of this method. To test the validity of this type of model, we are investigating differential electron emission from thin foils. These data, which provide experimental information about electron production and transport in condensed-phase media, can then be compared to model predictions and used to test the validity of various assumptions that were made.

A year ago we described preliminary results obtained for 1 and 2 MeV protons interacting with a thin carbon foil positioned perpendicular to the beam direction. The proton beam was pulsed, thus time-of-flight (TOF) electron spectroscopy was used to investigate the emitted electron energy spectra at various angles with respect to the beam direction. Because scattered protons were also detected by our TOF analyzer, for thick foils (e.g., 40 $\mu\text{g}/\text{cm}^2$) our measurements were restricted to electron emission angles greater than 90°.

Our initial results confirmed our expectations that surface contamination by absorbed gases seriously effected the low energy electron emission. We attempted to "clean" the surface by baking the foils at 200 to 300°C for 24 hours. After baking, time-elapse spectra were collected. A continual increase in the low energy electron yields was interpreted as arising from the gradual buildup of surface contamination of oxygen from the residual gas in the vacuum system (Drexler 1993).

Therefore, our emphasis during the past year was on improving the base vacuum of the apparatus, installing a low-energy ion gun for sputter cleaning of the targets, and designing and constructing a new analyzer. Considerable progress has been made in all three areas. Our base vacuum, under operating conditions, is now better than 5×10^{-10} Torr (uncorrected ion gauge pressures). Mass analysis indicates that the oxygen (and water) components are smaller than 0.1% of the pressure. At these pressures, assuming unit sticking probabilities, a monolayer of oxygen requires approximately 2 weeks to form. Sputter cleaning the foils now provides reproducible surface conditions. The sputtering process typically increases the pressure to the $0.9 - 2 \times 10^{-9}$ Torr range during data collection. However, the sputter gas is argon and argon atoms do not effectively adsorb onto the surface.

With these improvements, the spectra shown in Figure 4 were collected. Figure 5 shows data collected a year ago using elevated temperatures to clean the surface. By comparing these data, we see that the initial spectra in both cases are nearly identical; however, cleaning the foils by baking clearly influences the spectra at later times. We initially interpreted this change as arising from a buildup of oxygen surface contamination, but the time-elapsed spectra in

Figure 4 do not confirm this interpretation. Also, the similarity of the "time zero" spectra indicate that it is not a temperature effect. A possible explanation may be that the effects we observe in Figure 5 arise from a buildup of hydrogen surface contamination and that this buildup requires elevated temperatures.

Our previous indications that the buildup of surface contamination was a serious problem, the difficulty previously discussed about scattered projectile ions restricting our measurements to the backward direction, and the inability of using TOF energy analysis for higher emission energies led us to design and construct a new electron analyzer. This analyzer is a combination TOF and electrostatic analyzer capable of investigating electron emission at several angles simultaneously. Construction is nearly complete and testing will begin next year.

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Pits: A Code Set for Positive Ion Track Structure

W.E. Wilson,^(a) J.H. Miller,^(a) and H. Nikjoo^(b)

Positive Ion Track Structure (PITS) is a code set for simulating positive ion tracks in a variety of media, with and without secondary electron transport. Experimental results suitable for helping to develop computer simulations of charged particle track structures and for verifying the written code itself are scarce. One reason for this is that most of the focus has been on water as the absorber and water, in any phase, is not a tractable experimental target. The primary reason for introducing PITS to rectify this

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Figure 4. Yield of electrons from a $5\mu g/cm^2$ carbon foil that are emitted at 135° with respect to incoming 0.5 MeV proton beam. Spectra demonstrate changes due to sputter cleaning the foil surface.

Figure 5. Yield of electrons from a $5\mu g/cm^2$ carbon foil that are emitted at 135° with respect to incoming 2 MeV proton beam. Spectra demonstrate changes due to buildup of surface contamination over a period of time.

situation. It does not represent "new code" because it draws heavily from the MOCA series of detailed-histories charged particle transport codes.

Considerable evolution has occurred in computer hardware and software since MOCA was introduced two decades ago (Paretzke 1973). Modernizing the code, to bring it into conformance with current standard coding practices gives additional motivation by making to maintaining (updating) and documenting considerably easier. Measured against current professional software engineering standards, the extant codes that are the subject of this workshop are poorly documented, many are not documented at all.

The basic concept is schematically described in Figure 6; segmentation is emphasized with each code module written symbolically to the maximum extent possible. Model parameter values, i.e., the physical data specifying the projectile and the absorber, are gathered together and entered via data file(s) at the beginning. This makes it possible to see the effect of specific additions and changes in the physical data

Figure 6. Schematic Diagram of the PITS Code Modules. Modularization provides easier maintenance and documentation, and also the opportunity to use any of several existing codes for delta ray transport.

and model. Knowing that codes A and B give different results is not sufficient if we do not know why. Knowing how the physical model assumptions in a given code effect the predictions made by that code is more important. Doing this in an efficient manner requires a carefully designed code and good documentation.

Modularization of the code provides a hidden benefit because different computer languages may then be used for different modules. For example, the C computer language offers features that most current FORTRAN implementations do not provide and that can be used to advantage, especially in scoring tracks.

By carefully defining the interface requirements, the secondary electrons (delta-rays) can be transported by any of several existing codes. We have successfully used MOCA8, KURBUC and CPT(100) with a preliminary version of PITS.

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Dispersion of Energy Transfer

W.E. Wilson and J.H. Miller

In anticipation of introducing an impact parameter representation of the inelastic cross sections in PITS, a phenomenological model to disperse energy transfer points dispersed away from the trajectory of the particle has been added for both ion and secondary electron interactions. We use a functional form described by Paretzke et al. (1991) for the probability density $P(b)$ for interaction at a lateral distance b from the path of the charged particle:

$$P(b) = C \frac{b}{b_0^2 + b^2} e^{-\omega b / v \gamma}$$

where v is the electron speed, $\hbar\omega/2\pi$ is the energy transferred to the absorber, γ and b_0 are constants, and C is an arbitrary normalization factor b_0 is very nearly the most probable impact parameter value, i.e., $P(b)$ is a maximum at essentially b_0 . For preliminary sensitivity tests, a constant average energy loss was assumed where the coefficients in the exponential factor were held constant at $v\gamma/\omega = 20$ and transfer points off the track axis were localized only to energy loss events of less than 50 eV. Three cases were tested, one representative of a metal (conductor) such as carbon, a second more representative of tissue, and the last case for comparison is without dispersion of transfer points.

The probability density $P(b)$ for the first two cases are shown in Figure 7. For the carbon case, $b_0 = 0.1$ nm and because the functional form of $P(b)$ goes to infinite values of impact parameter b , localization was restricted to impact parameters of $b \leq b_{\max} = 10$ nm. This case is depicted in the upper right panel of Figure 7. For the tissue-like case, $b_0 = 0.025$ nm and $b_{\max} = 2$ nm; $P(b)$ is plotted in the lower left of Figure 7 for this case.

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Figure 7. Probability Density $P(b)$ for Impact Parameter b . Truncating the function at $b_{\max}$ restricts the allowable impact parameters to $0 \leq b \leq b_{\max}$. The two cases shown are representative of a conductor, upper panel, and tissue-like matter, lower panel.

Radiation Biophysics Program

The Radiation Biophysics Program provides experimental molecular and cellular data to test currently evolving theoretical models of radiobiological effects evolving within the Radiation Dosimetry and Modeling Cellular Effects programs. These experimental studies address predictions of mechanistic models that relate endpoints, such as changes in the kinetics of production and removal of molecular damage, structural alteration of DNA, mutation, transformation, and cell inactivation to their initiating energy-deposition events. Experiments are designed specifically to provide information on radiation effects in areas where modeling studies suggest such measurements will facilitate distinctions between different basic concepts of damage production and expression in macromolecules. Plasmid and genomic DNA are irradiated under specific conditions of dose, dose rate, radiation quality, and target environment. The damage produced is then identified, quantified, and evaluated to determine the relative sensitivity of the DNA molecule, of specific DNA sequences, and of macromolecular structures to its formation. Data so derived provides information relating lesion formation, distribution, and type to physical energy deposition and the structure, orientation, and environment of the affected DNA molecule.

Studies of initial damage production and the effects of biological processing of that damage are performed in carefully defined cultured mammalian cells irradiated under controlled conditions and allowed different opportunities for damage alteration or removal. Study findings provide a means of evaluating the cellular mechanisms that cellular-effects models are based. Different techniques provide the detail and precision required to distinguish between mechanisms predicted by different mathematical models of cellular effects. Novel concepts based on these experimental data are integrated into evolving biophysical and biochemical models, that help focus attention on new or alternative mechanisms to be tested.

Chemical Adducts and Radiation Sensitivity of Supercoiled DNA

E.W. Fleck and J.M. Nelson

DNA supercoiling is introduced by varying degrees of normal biological processes of transcription, replication, and gene recombination. Studies with DNA plasmids show increased negative superhelicity enhances the sensitivity of DNA to strand breakage by ionizing radiation (Miller et al. 1991). We intend to investigate the effect of additional perturbations of DNA structure introduced by aberrant structures (e.g., base mismatches, hairpin- or cruciform-forming structures, and chemical adducts on this radiation induced damage). To investigate these effects, we must construct DNA plasmids that contain

structural anomalies at specific locations and that have specific degrees of superhelicity. These plasmid constructs will enable mechanistic studies of the effects of perturbed structure on the radiation sensitivity of these physiologically stressed molecules.

Earlier work by Miller et al. (1991) demonstrates the increased sensitivity of pIBI-30 plasmid DNA to its first single-strand break induced by x-rays. These observations were made by irradiating the plasmid DNA at 2°C in T₁E₁ (TE) buffer (10 mM Tris, 1 mM EDTA; pH 7.2). We quantified the levels of Form I, Form II, and Form III plasmids using densitometry of ethidium bromide stained gels following UV treatment. UV light is very

efficient at nicking DNA. This treatment fragmented the DNA on the gel, thereby permitting equivalent ethidium bromide binding regardless of which form or what degree of supercoiling the DNA molecule had been in prior to UV exposure.

Milligan et al. (1992) re-examined our study (Miller et al. 1991) and found no change in the x-ray sensitivity as a function of superhelicity. However, there were several differences between their investigation and ours; in their studies they used the pUC-18 plasmid, irradiated at 2°C in tris buffer. They also quantified the plasmid DNA levels using densitometry of ethidium-bromide stained gels after determination of the binding constants of ethidium bromide to covalently closed circular pUC 18 in Forms I, II, and III, and at several different levels of negative superhelicity.

Swenberg et al. (1990) repeated our work using pIBI 30, but at room temperature using γ rays finding that the radiation sensitivity of the first strand break increased only when the negative superhelicity was greater than $\Delta L_k = -2.5$. However, they also found that the pIBI-30 plasmid demonstrated its lowest sensitivity at negative superhelical densities around $\Delta L_k -2.5$ with the sensitivity increasing again as the DNA becomes less negatively supercoiled. They irradiated the plasmid in a low-scavenging capacity phosphate buffer (pH 7.2), using the method of Milligan et al. (1992) to determine concentrations of the various forms of plasmid.

Clearly, our observations have led to some controversy. Consequently, we have spent considerable effort to repeat these studies and re-affirm our earlier findings and verify that the phenomena reported by Miller et al. (1991) and Swenberg et al. (1990) was indeed real. However, we needed an alternative method of determining single-strand break induction which

would not involve quantifying fluorescence of ethidium bromide. Because we wanted a completely different technique, we chose to use Southern blot methods with radio-labelled probes, followed by densitometric determinations of the concentrations of undamaged and damaged plasmid species, as the preferred method. This way, we employed an unambiguous method of quantifying plasmid DNA Forms I and II (the products arising from a radiation-induced single-strand break in Form I plasmid). Although technically far more demanding than the ethidium-bromide technique in common use, most investigators consider it superior.

During the past year we developed, calibrated, and demonstrated that we could generate high precision standard curves of DNA concentration versus Southern blot band intensity. After perfecting the methods, we determined the x-ray sensitivity of plasmid DNA as a function of negative superhelicity, ionic strength of the buffer in which it had been irradiated, and temperature during irradiation. We decided to use the pBR-322 plasmid as a target because it is readily available commercially, and it contains exactly the same sensitive regions (which leads to only one or at most a few stress-induced single-strand regions) as found in pIBI 30. We prepared pBR 322 at six topoisomeric conformations $\Delta L_k = -4, -9, -14, -19, -22$, and -26 native or wild-type plasmid) and in two phosphate buffer concentrations (10 mM and 50 mM). Each of the topoisomers of plasmid DNA was irradiated (in duplicate) at five temperatures (10°, 20°, 30°, 40°, and 50°C). We have only completed about two thirds of the analyses of these irradiation products. However, a lower radiation sensitivity of pBR-322 plasmid DNA at $\Delta L_k = -4$ and -9 , compared to the higher superhelical densities, can already be demonstrated.

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The Role of Salvage Pathways in Repair of Split-Dose Damage

B.S. Jacobson and J.M. Nelson

Double mutants of Chinese hamster ovary (CHO) cells, deficient in both the HPRT gene and the TK gene, have been isolated from strain AT3-2 to investigate their radiation response. These mutants are resistant to guanine and thymidine analogues which are lethal to normal cells because they are unable to recycle either guanine (HPRT gene) or thymine (TK gene) through the salvage pathways of DNA synthesis. All of the double mutants grow more slowly than either the original parent AT3-2 cells or the HPRT single mutant from which the double mutants were isolated.

We hypothesized that these salvage pathways play an important role in the repair of radiation damage. This hypothesis was tested by irradiating cultures of mutant and wild-type cells using split-dose methods and the determining the effect on reproductive integrity using clonogenic cell

survival methods. Cells irradiated with single doses of 250 KV x-rays (given at 1.0 ~ Gy/min) showed relatively small differences in sensitivity between the double mutants and their parent AT3-2 cells. However, split-dose studies indicated significant differences between the phenotypical wild-type AT3-2 and double-mutant cells. Following two equal doses (2.0 Gy) separated by intervals of up to two hours, the AT3-2 cells showed the expected increase in survival (with time between exposures) while the double mutant, DM12, did not. This observation is consistent with the hypothesis that the DNA salvage pathway is involved in split-dose repair.

However, there are other possible explanations for this phenomena. In these irradiation experiments, cells were released, them from the polystyrene substrate upon which they had been growing, by trypsinization, and resuspended and diluted in fresh medium prior to irradiation. Studies elsewhere, mainly with Chinese hamster V79 cells, have shown that as the cells reattach to their new substrate following trypsinization, their radiosensitivity decreases. This effect could mimic split-dose repair. However, single-exposure experiments, in which the irradiation was delayed for up to 7 hours after trypsinization, showed no change in radiosensitivity of the wild-type AT3-2 cells, while the DM12 cells actually became more sensitive, as indicated by the preliminary data shown in Table 1. This effect could cancel the effect of split-dose repair.

Table 1. Preliminary Survival Data for Irradiated DM12 Cells

Time After Trypsinization (hours)	Surviving Fraction
1.45	0.025
2.7	0.0206
4.6	0.0110
5.9	0.0104
7.5	0.008

At present, we are not certain if this change is rapid enough to account for the difference in split-dose response. These studies will be repeated.

We have also obtained growth rate data for these mutant cell lines, as requested by the editors of *Mutation Research*, for a paper submitted by B. S. Jacobson and T. L. Morgan. Unlike CHO-K1 cells which we have studied extensively, the growth rate of AT3-2 cells decreases with increasing mutation load, but not as sharply as expected. Part of this apparent slow growth may be due to lower plating efficiencies and longer post-transfer lag times.

These findings are summarized in a manuscript entitled *Optimum Inactivation Dose and Indices of Radiation Response Based on the Linear-Quadratic Survival Equation*, by B. J. Jacobson and T. L. Morgan, prepared and submitted to *Radiation and Environmental Biophysics*. This paper has been accepted for publication and is currently in press. In addition, these data were also presented in a paper entitled *Growth Rates and Radiosensitivity of X-Ray-Induced and Spontaneous Nucleoside Salvage Mutants in CHO Cells* at the annual meeting of the Radiation Research Society, in Dallas this last spring.

Modeling Cellular Responses to Genetic Damage

This project contributes to the understanding of health risks from exposure to ionizing radiation through studies of interactions of swiftly moving ions and electrons with the genetic material of mammalian cells. Several types of indirect evidence (Ward 1985) point to locally multiply-damaged sites (LMDS) as the critical lesions in DNA that determine cellular responses to radiation exposure. Deoxyribonucleic acid (DNA) double strand breaks are a special case of this type of genetic damage. Work in FY1993 focused on two aspects of the production of LMDS: 1) cross sections, differential in the energy of secondary electrons, for ionization of DNA in solution and 2) electron migration along DNA chains. The energy spectrum of secondary electrons determines their potential to produce additional ionization. Electron transfer determines the probability that energy given to secondary electrons in ionizing collisions will be deposited in the neighborhood of the less-mobile positively-charged molecular ions. Hence, these two components of the information are needed for computer simulation of clusters of ionizations that are precursors to LMDS in DNA.

Ionization of DNA in Solution

J. Miller, W. Wilson, and R. Ritchie

Yields of DNA double-strand breaks induced by sparsely-ionizing and high-linear energy transfer (LET) radiations suggest that clusters of ionization containing 2 to 6 on pairs in a volume with characteristic dimensions of 1 to 4 nm are the most likely precursors of LMDS (Brenner and Ward 1992; Michalik 1993). Because the size of these clusters is comparable to the diameter of the DNA double helix, both direct ionization of DNA and free-radical attack resulting from ionization of the aqueous medium are probably involved in the production of LMDS.

Computer simulation of the direct component of LMDS in DNA requires cross sections that are differential in the energy of the electrons ejected from the various chemical groups (bases, sugar, and phosphate linkage) of a DNA polymer. The kinetic energy of secondary electrons will be a key factor in determining the probability for additional ionization within 1 to 4 nm of a parent positive ion. The dielectric response of an aqueous medium is very effective in screening the projectile charge in glancing collisions that give rise to low-energy secondary

electrons (Ritchie et al. 1978). This shift in the secondary-electron energy spectrum means that more electrons are produced with the potential for generating ion pairs; hence this phase effect increases the density of ionization (Paretzke et al. 1986).

This effect of phase on the energy spectrum of secondary electrons should impact the production of LMDS by clusters of ionization in chromatin. To model this phenomenon, the dielectric response theory applied to liquid water (Ritchie et al. 1978) must be extended to include heterogeneous materials. We have accomplished this objective in the approximation that the target molecule (DNA) is present in sufficiently low concentration that it does not influence the dielectric response function of the bulk medium (treated as liquid water). This approximation may not be strictly valid for DNA in cells but it will allow us to include the enhancement of ionization density observed in liquid water in modeling LMDS in DNA.

Figure 1 shows our results for the energy spectrum of secondary electrons ejected from DNA in liquid water by a 122.5 eV primary electron. The single differential cross section

Figure 1. Differential Cross Sections for Ionization of DNA by 122.5 eV Electrons with (Solid) and without (Open) Screening by an Aqueous Medium.

(in atomic units) per target electron has been multiplied by the primary electron energy to yield an ordinate with the units of square Bohr (1 Bohr = 0.0529 nm). The abscissa is the energy transferred to DNA in the collision which is the sum of the ionization potential (about 8 eV) and the kinetic energy of the ejected electron. Screening of the charge on the primary electron by electronic polarization of the water medium dramatically reduces the cross section for ejection of electrons from DNA with energies less than about 20 eV.

The probability dP/db that collisions with impact parameters (distance of closest approach between projectile and target) between b and $b + db$ will transfer a specified amount of energy to the target can be obtained by a Fourier transform of the quantum theory of collisional energy transfer (Ritchie et al. 1989). Three examples of impact-parameter probability distributions (IPPDs) are shown in Figure 2 for ionization of

DNA in liquid water by 122.5 eV electrons. The difference between the IPPDs for transfer of 54 and 22 eV in ionizing collisions with DNA is expected from Bohr's semiclassical theory of stopping power (Bohr 1913) which showed that, at a given projectile velocity, smaller impact parameters are required for larger energy transfer. The results shown in figure 2 for transfer of 11 eV to the target do not conform to the expectations of classical collision theory. Although the classical theory predicts that the smaller energy transfer occurs in more glancing collisions, IPPDs for transfer of 11 and 22 eV are similar in our model. This is another manifestation of the effective screening of the projectile charge in collisions with large impact parameter.

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Modeling Electron Transfer in DNA

J. Miller, A. Fuciarelli, and K. Miaskiewicz
Energy and charge transfer along DNA chains are crucial issues in the theory of LMDS in DNA (Miller et al. 1992). The biological effectiveness of LMDS is linked to the concept that clusters of lesions in DNA have a lower probability of being accurately repaired than isolated lesions. Enzymatic DNA repair operates on a much longer time scale than the physical and chemical processes that produce DNA lesions; hence, dispersion of the initial pattern on energy deposition

by energy and charge migration must be considered in modeling the characteristics of LMDS.

Experimental results by Fuciarelli et al. (in press) indicate that electrons adducts formed by the interaction of double-stranded DNA with hydrated electrons can migrate up to 7 base pairs depending upon the base sequence. Models of electron migration on DNA chains focus on the competition between electron transfer and trapping. Protonation of base radical anions at nitrogen atoms is only a temporary electron trap; however, stabilization by protonation of carbon atoms is usually irreversible (Steenken 1989). In single-stranded adenine sequences, formation of C-protonated radical anions is not rapid enough to prevent electrons from migrating to 5-bromouracil (5-BrU) where they are detected by conversion of 5-BrU to uracil. Steenken has proposed that rapid proton transfer in A⁻:T base pairs catalyzes the formation of A(C2)H[•] and A(C8)H[•]. Since these C-protonated radical anions block electron transfer, the absence of detectable electron migration in dA:dT oligomers supports Steenken's hypothesis.

The mechanism proposed by Steenken for irreversible trapping of electrons at A:T base pairs does not appear to function in G:C base pairs since electron migration in the dG:dC oligomers has been detected by Fuciarelli et al. (in press). They also find a greater yield of uracil when 5-BrU is placed on the 3' end of these oligomers than when 5-BrU is present on

the 5' end. A mechanism for this effect is suggested by Figure 3 that compares stacking of 5-BrU and G in two B-DNA double-stranded oligonucleotides. When 5-BrU is on the 3' side of G (right-hand panel), the overlap of π -electron orbitals is much greater than when 5-BrU is on the 5' side of G (left-hand panel). Under the reasonable assumption that this overlap determines the rate of electron transfer between stacked bases, more electrons should be able to reach 5-BrU when it is on the 3' side of G.

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Figure 3. Stacking of Guanine and 5-bromouracil (5-BrU) in B-DNA when 5-BrU is on the 5' (Left Panel) and the 3' (Right Panel) Side of Guanine.

Perturbations of the Conformation and Dynamics of DNA by Defined Radiation-Induced Lesions

This project uses computer-based molecular modeling to investigate changes in DNA structure due to the presence of radiation-induced lesions. It developed out of Laboratory Directed Research and Development (LDRD) supported under the Perturbed Molecular Structures Initiative. The first external funding on the project was received at midyear FY-1993; hence, the work described below was partially supported by LDRD.

DNA Intrastrand Cross Links

J. Miller and K. Miaskiewicz

Ionizing radiation in the absence of oxygen produces intramolecular cross links between the base and sugar moieties of nucleic acids. Formation of 8,5'-cyclopurines begins by abstraction of hydrogen from the C(5') position of sugar followed by radical addition to the C(8) position of the base with loss of the hydrogen atom that is normally at that position. Schematic diagrams of the two possible stereoisomers of 8,5 ft-cyclopurines are shown in Figure 1. For the R isomer, the torsion angle O(5 ft)-C(5 ft)-C(4 ft)-C(3 ft), denoted by τ , is about 200° . The standard IUPAC-IUB notation for this conformation is *ap*. For the S isomer, τ is about 300° , which is a conformation denoted by *-sc*. B-DNA and isolated nucleotides are most often observed in the *+sc* conformation that corresponds to a τ near 60° . Hence, formation of intrastrand cross links usually involves rotation about the exocyclic C(4 ft)-C(5 ft) bond.

Since 8,5 ft-cyclopurines are not observed when DNA is irradiated in the presence of oxygen, this decay mode of C(5 ft) radicals must be slower than their reaction with oxygen. We investigated preferences for R or S isomer of 8,5 ft-cycloadenosine (Fuciarelli et al. 1987) under the assumption that addition of C(5 ft) radicals to C(8) of the purine base is much slower than their conformational fluctuations about the C(4 ft)-C(5 ft) bond. If this is the

case, then a thermodynamic equilibrium will be established among the conformational states of the C(5 ft) radical and the relative energy of *ap* and *-sc* conformers will determine the relative abundance of R and S isomers among the final cycloproducts. Some *ab initio* quantum-chemical results for C(5 ft) radicals formed by abstraction of hydrogen from adenosine are shown in Figure 2. A favorable interaction between the 5 ft terminal OH and O(1 ft) of the sugar provides an energetic preference for the *ap* conformation. These results are consistent the observation by Fuciarelli et al. (1987) that R isomers are about 2 times more abundant when adenosine is irradiated at neutral pH.

In contrast to their results for irradiation of adenosine, earlier experiments Raleigh and Fuciarelli (1985) revealed a 3-fold preference for the S isomer when adenosine 5 ft monophosphate (5ft-AMP) was irradiated at neutral pH. The ratio S to R isomers decreased to unity as the pH was lowered from 7 to 5. Calculations similar to those illustrated by Figure 2 show that the *-sc* conformation is preferred of radicals formed by abstraction of hydrogen from the C(5 ft) position of 5 ft-AMP due to interaction of the phosphate group with the 3 terminal OH. These results are consistent with the observations of Raleigh and Fuciarelli (1985) because the interaction that causes an energetic preference for S isomers will be eliminated at low pH due to protonation of the 5 ft phosphate group.

Figure 1. Schematic Diagrams DNA Intrastrand Cross Links. View is along the exocyclic C(4 ft)-C(5 ft) bond from 5 ft to 4 ft. τ denotes the torsion angle O(5 ft)-C(5 ft)-C(4 ft)-C(3 ft).

Figure 2. Relative Energy of Radical Precursors to 8,5 ft-cycloadenosine. The -sc conformation of the C(5 ft) radical leads to the S isomer. The more stable ap conformer leads to the R isomer.

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