MECHANISMS FOR RADIATION DAMAGE IN DNA

Progress Report

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Appendices A and B are reprints of work performed
in the preceding Fiscal year.

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ABSTRACT

In this project we have proposed a mechanisms for radiation damage to DNA and detailed a series of experiments utilizing electron spin resonance spectrometry to test the proposed mechanisms. In this past year three investigations have been completed or are nearing completion. These investigations are: 1. Studies of electron transfer reactions in dinucleoside phosphates 2. Studies of the anions of 5-nitropyrimidines and their reactions, and 3. Studies of protonation reactions at carbon sites in anion radicals of certain model compounds and aromatic amino acids. In the first study, the relative electron affinities of the DNA bases were determined in a model system of the DNA strand. In addition, the study of the reactions of these anions showed that the thymine anion is the most reactive of the DNA bases in this model system. In the second study anisotropic and isotropic spectra of the anion radicals of 5-nitropyrimidines were characterized by newly developed computer simulation programs. Several of the anions were found to react to form iminoxy radicals. In the third study showed that protonation reactions at carbon sites in anions are reactions which are general for molecules with unsaturated linkages. Thus, this mechanism is of significance to the radiolysis of many biological molecules including DNA.

1. RESULTS THIS YEAR

During the past year we have made significant progress in several areas. We believe that perhaps the most significant work is described in section A below. The results not only give evidence for the role of the electron in DNA radiolysis, but they yield the relative electron affinities of the DNA bases within a model system of the DNA strand. General descriptions of the other work performed during this year are also given below. Detailed descriptions are given in appendices C, D, and E.

A. Electron Reactions with Dinucleoside Phosphate Anions. Electron transfer reactions within various dinucleoside phosphate radical anions were investigated by ESR spectroscopy to determine the relative electron affinity of the various DNA bases within a model system of the DNA strand. The reactions of the dinucleoside phosphate anions were also investigated in order to determine the mechanisms of reaction within this model system. Electrons are produced by photolysis of $K_4Fe(CN)_6$ in a neutral aqueous glass at 77°K. Upon photobleaching the electrons reacted with the dinucleoside phosphate and formed the anion radical. The anions of the four DNA-nucleosides were also produced and their ESR spectra were appropriately weighted and summed by computer to simulate the spectra found for the dinucleoside phosphate anions. From the analysis the relative amounts of each of the nucleoside anions in the dinucleoside phosphate anion were determined. For example in thymidylyl-(3' 5')-2-deoxyadenosine (TdA), where the bases are likely stacked so as to allow electron transfer to the base with the greatest electron affinity, the electron is found to localize on thymine.

Whereas in equal mixtures of T and dA in which the molecules are isolated in the aqueous medium anions of both nucleosides are found in approximately equal amounts. These results and those found for the other dinucleosides studied (TdC, TdG, dAdC, TT) clearly suggest the electron affinity of the pyrimidine bases are greater than the purine bases, however, the results are not sufficient to distinguish between the individual purine or pyrimidine bases. Upon warming thymidine containing dinucleoside phosphate anions protonation occurs at C-6 on thymine to produce the well known "thymyl" spectrum. No reactions on the other DNA bases in the dinucleoside phosphate anions were found.

The report given in appendix C is a more detailed description of this work. It is, however, a first draft of this work. Several more experiments are to be performed to clarify the relative electron affinity of cytosine.

B. Negative Ions of the 5-Nitropyrimidines. Mechanisms of radiation damage to 5-nitropyrimidines have been the subject of a number of recent papers. These studies have been motivated in part by the fact that the nitropyrimidines may be efficient radiation sensitizers. Several seemingly contradictory reports have occurred in the literature as to the stable initial radicals formed in the radiolysis of these compounds. It was our purpose in this work to elucidate the reactions induced by the electron in 5-nitropyrimidines. We also desired to test new expanded computer programs which simulate anisotropic spectra. (see section C)

The experimental results are described in detail in Appendix D. Briefly we found that the anion radicals were precursors to iminoxy radicals. Iminoxy radicals have been found at room temperature in single crystal work.

In addition the computer simulations of anisotropic spectra gave detailed information in terms of g values and hyperfine splittings which are normally available only through studies in single crystals or in solution. It is our belief that the simulation of anisotropic spectra will continue to be an invaluable technique in the characterization of radicals formed in DNA subunits and DNA itself.

C. Protonation Reactions at Carbon Sites in Anion Radicals of Certain Model Compounds and Aromatic Amino Acids. This study was funded in part by the Petroleum Research Fund. It was undertaken because protonation reactions at carbon sites in anions are significant to the radiation chemistry of all unsaturated compounds. As we have pointed out previously the thymine and thymidine anions have been shown to undergo a reaction of this type. This is also the most generally accepted mechanism for the formation of the "thymyl" (5,6 dihydrothymine-5-yl) radical in DNA. Our results with the purine anions show that reactions of this type can occur for these species as well (Appendix A). The fact that the dinucleoside phosphate anions protonate on thymine is further evidence of the significance of this reaction mechanism.

The study began with a series of model compounds in order to determine the type of unsaturated molecules which readily protonate in aqueous media. Studies of aromatic amino acids and their analogs were performed to determine if protonation occurs and at which sites they occurred. Molecular orbital calculations of the spin density and free valency showed that protonation occurred at the site of high spin density and at sites where the free valency was greater than 0.63. These calculations show that the free valency at position 6 (the site of protonation) in thymine anion is 0.77. The anions of the purine DNA bases which have less of a

tendency to protonate have free valencies under 0.65.

The results of this work suggest that free valency calculations are able to predict the site of protonation if a reaction does occur; however, they are not sufficient to predict whether or not rapid protonation at carbon sites will occur.

D. Positive Ions of Dinucleoside Phosphates. This investigation has just begun and will perhaps constitute the major effort for the next year. Thus far we have been able to generate the positive ion of thymidylyl-(3'-5')-thymidine in a number of aqueous matrices. The results found suggest a reaction may occur with the ribose portion of the molecule. If this is so it suggests a mechanism for possible strand breakage in DNA. Hopefully the experiments suggested in the proposal will elucidate the mechanism of reaction.

E. On Line Computer Capabilities. In this past year we have interfaced the Varian ESR spectrometer with an IBM 1130 computer. The analog digital conversion is done by an EA1-693 converter. Through this interfacing and a number of computer programs we have developed the following capabilities:

1. To sum multiple scans of the same spectrum to decrease the noise level (the noise level decreases by the square root of the number of scans taken).
2. To store spectra from up to 100 different radicals.
3. To add and subtract these spectra to simulate spectra in complex systems as was done in our work with dinucleoside phosphate anions.

4. To simulate anisotropic or isotropic spectra from g and A tensors for comparison to experiment. These programs are capable of performing either rhombic or axial calculations assuming co-axial g and A tensors. Thus far these simulation programs have been very helpful in the interpretation of complex anisotropic spectra. (see appendices D and E)

The graphic output of the computer is plotted either on a scope or a Calcomp plotter.

11. EFFORT OF THE PRINCIPAL INVESTIGATOR

The present term of this contract began August 1, 1974. The principal investigator spent the first two weeks of August on this project. Since then 20% of his time during the academic year has been spent on this work. The principal investigator will devote 9 weeks of the spring summer sessions to this project. The remaining portion of the summer will be spent on related work at Lawrence Berkeley Laboratory, University of California, Berkeley.

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April 29, 1975

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CONTRACT NO. AT(11-1)-2364, OAKLAND UNIVERSITY

We are transmitting copies of documents submitted in accordance with our requirements covering the subject contract.

The Contractor has included a 50-word proposed technical program which will be incorporated as the contractual scope of work. Please advise me if you do not agree.

Harold N. Miller, Director
Contracts Management Office

CMO: JS

Enclosures:

1. Proposal (4 cys.)
2. Budget (4 cys.)
3. S&E Forms (3 cys.)
4. Financial Statement (2 cys.)
5. Progress Report No. C00-2364-2 (4 cys.)
6. Report Nos. C00-2364-3 thru -8 (4 cys. ea.)

cc: CH Patent Division -w/encs. 5 & 6
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