"Mechanisms for Radiation Damage in DNA"

Progress Report

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Abstract

In this project we have proposed several mechanisms for radiation damage to DNA and its constituents, and have detailed a series of experiments utilizing electron spin resonance spectroscopy, HPLC, GC-mass spectroscopy and ab initio molecular orbital calculations to test the proposed mechanisms. The results from these various techniques have resulted in an understanding of consequences of radiation damage to DNA from the early ionization event to the production of non-radical lesions (discussed in detail in Comprehensive Report).

In this year's work we have found the hydroxyl radical in DNA's hydration layer. This is an important result which impacts the hole transfer hypothesis and the understanding of the direct vs. indirect effect in DNA. Further we have found the first ESR evidence for sugar radicals as a result of direct radiation damage to DNA nucleotides in an aqueous environment. This is significant as it impacts the biological endpoint of radiation damage to DNA and suggests future work in DNA. Work with DNA-polypeptides show clear evidence for electron transfer to DNA from the polypeptide which we believe is a radioprotective mechanism. Our work with ab initio molecular orbital theory has gain insight into the initial events of radiation damage to DNA. Ab initio calculations have provided an understanding of the energetics involved in anion and cation formation, ion radical transfer in DNA as well as proton transfer with DNA base pair radical ions. This has been extended in this year's work to new, more accurate values for the electron affinities of the DNA bases, understanding of the relative stability of all possible sugar radicals formed by hydrogen abstraction on the deoxyribose group, hydration effects on thiol radioprotectors, and an ongoing study of radical intermediates formed from initial DNA ion radicals. During this fiscal year five articles have been published, three are in press, two are submitted and several more are in preparation. Six papers have been presented at scientific meetings. Also a review article on the "Electron Spin Resonance of Radiation Damage to DNA" was published and another on "Elucidation of Primary Radiation Damage in DNA through Application of Ab Initio Molecular Orbital Theory" prepared.

In the sections below we briefly describe progress made during the fiscal year. In each case the publication status of the work is noted - paper number refers to publication list on pp. 14 and 15.

A. The Influence of Hydration on the Absolute Yields of Primary Ionic Free Radicals in Irradiated DNA at 77K.

Our work on the effect of hydration on DNA radical formation has progressed quite well. In our initial work we established that water of hydration is critical to radical formation and stabilization in DNA; however, the ice surrounding DNA does not contribute to direct DNA damage and is found to have the same properties as bulk ice.

1. Yields of Individual Radicals with dose (paper 1).

In this work an ESR (Electron Spin Resonance) investigation of the yields of the individual free radicals formed in γ-irradiated frozen DNA as a function of hydration and dose at 77K is performed. Analysis of the ESR spectra taken at low hydration shows that the ion radical composition remains nearly constant with dose and that few secondary radicals are formed even at high doses (above ca. 50 kGy). For fully hydrated samples the individual radical composition changes dramatically with dose. Thymine anion radical (T\(^{-}\)) is found in abundance at low doses but nearly disappears at higher doses, with a corresponding increase in the N-3 deuterated cytosine anion radical (C\(_{D}\)^{-}). Guanine cation radical (G\(^{+}\)) decreases at high doses, with a concomitant increase in secondary radical species (S\(^{-}\)). Destruction constants for neutral radicals such as TH\(^{-}\) and Cp\(^{-}\) are found to be substantially smaller than those for ion radicals and provide an indication of the radical charge state. A negative k' value for T\(^{-}\) and a positive k' value for Cp\(^{-}\) are explained in terms of radiation effects that result in the formation of a deuterated cytosine base which greatly increases cytosine's electron affinity.

B. Detection of the Hydroxyl Radical in the DNA hydration layer (paper 10).

DNA in aqueous solution is strongly hydrated by a mass of water approximately equal to the mass of the DNA itself. A variety of investigations have shown that the properties of this hydration layer (about 15–20 waters per nucleotide) are significantly altered from those of bulk water. Low LET irradiation of hydrated DNA deposits approximately an equal amount of energy in this
hydration layer as in the DNA itself. Because the radicals expected from the radiation of water (•OH, e•, H•) have not been detected in the DNA hydration layer by ESR spectroscopy, even at 4 K, it has been assumed that the radiation damage in the hydration layer is rapidly transferred to the DNA in the form of electrons and holes (reactions 1,2). However, the evidence that reaction 2 occurs has been circumstantial; it is believed that deprotonation of H₂O⁺ to form •OH (reaction 3) occurs on the time scale of a molecular vibration (10⁻¹⁴ s) and, as a consequence, may be competitive with hole transfer to the DNA.

\[ \text{e}^- + \text{DNA} \rightarrow \text{DNA}^- \]  \hspace{1cm} (1)

\[ \text{H}_2\text{O}^+ + \text{DNA} \rightarrow \text{H}_2\text{O} + \text{DNA}^+ \]  \hspace{1cm} (2)

\[ \text{H}_2\text{O}^+ + \text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{•OH} \]  \hspace{1cm} (3)

In light of the experimental and theoretical ambiguities, the question of whether •OH is formed in the hydration layer of DNA is still an open one; it is also an important one, since the damage done by •OH will, in many cases, be different from that done by hole transfer. We have undertaken the search for •OH in the DNA hydration layer using two lines of investigation: direct ESR detection of •OH at low temperature and H₂O₂ product analysis. In aqueous glasses, the low field component of the •OH ESR spectrum at X-band frequencies consists of a broad feature extending over ca. 250 G instead of the well-defined \( g \parallel \) component at 2.05 that is found in crystalline ice. The reason for this difference is well understood; in radicals such as •OH, \( g \parallel \) is determined by the extent to which hydrogen bonding lifts the degeneracy of the two oxygen p-orbitals which are degenerate in the gas phase. In a glass, there is a large range of hydrogen bonding strengths to •OH and, therefore, a large range of \( g \parallel \)'s exists. This leads to the broad low field resonance found in glasses. It has been suggested that the hydration layer in DNA forms a glassy structure, thus, in searching for •OH in the DNA hydration layer, we have focused on the ESR spectrum of •OH in a glass as a model for the spectrum expected in DNA.

The second method used as a probe for •OH in the DNA hydration layer is analysis for its diamagnetic product, H₂O₂. When H₂O is γ-irradiated in the absence of oxygen, the only source of H₂O₂ is reaction 4.

\[ \text{HO}^- + \text{HO}^- \rightarrow \text{H}_2\text{O}_2 \]  \hspace{1cm} (4)

We have undertaken a study to detect H₂O₂ in the DNA hydration layer through product analysis of anoxic hydrated DNA irradiated at 77 K. H₂O₂ yields have been determined under ambient conditions in dilute aqueous DNA solution; however, in this early work almost all of the H₂O₂ found originated with the bulk
water, not with the DNA hydration layer.

Previous ESR reports of γ-irradiated DNA at low temperatures have suggested that hydroxyl radicals are not formed in the first hydration layer of DNA. In this report we show that hydroxyl radicals are produced in low yield in DNA's first hydration layer. Due to the glassy nature of this hydration layer at low temperature the hydroxyl radical gives a broad ESR resonance which is not easily detected. Low field ESR spectra of hydroxyl radicals in an irradiated 6M CsF aqueous glass are shown to be nearly identical to those found in DNA; however, the yields in the aqueous glass (G = 0.087 to 0.13 mmole/J) are found to be greater than those in DNA's first hydration layer (G = 0.035±0.02 mmole/J). A large k value for destruction of the •OH in DNA's hydration layer limits the yield of •OH at high doses. The yield of H₂O₂ (which likely results from hydroxyl radical recombinations that occur both during irradiation and upon annealing) is found to be 0.0035 mmole/J in the dose range 65 kGy to 195 kGy. The amount of H₂O₂ formed corresponds to most of that expected from recombination of the •OH trapped at 77K at the equivalent dose. The yield of trapped •OH radicals in the first hydration layer is smaller than the number expected for a glassy water system at low temperatures; this has implications for possible hole transfer to DNA.

In the next grant period we wish to extend this work to firmly establish the breakdown between hole transfer and hydroxyl radical formation. In preliminary work we find no evidence for hydroxyl radical formation below 8 waters/nucleotide.

C. Elucidation of Radiation Damage to DNA through Ab Initio Molecular Orbital Theory.

In this year's work we have completed work on: 1. Higher Level Calculations of IEs and EAs of DNA bases, 2. Sugar Radicals in DNA Model Systems, 3. Thiols Aqueous Phase Ionization Energies, and we are nearing completion on 4. Structures of Products Formed from Primary Radical Ions. Our papers on the Effect of Hydration Water on EAs and IEs (paper 4); Electron Affinities of DNA Bases: Comment (paper 7), have been published.

1. Scaling of Calculated DNA Base Electron Affinities and Ionization Potentials to Experimental Values (paper 12 accepted).

Although experimental gas phase ionizations energies of the DNA bases have been measured by a number of investigators, experimental gas phase electron affinities of the DNA bases have not as yet been reported. A variety of
molecular orbital calculations predict the pyrimidines have higher electron affinities than the purines with recent calculations suggesting the following order; T>C>>A>G. Ab initio calculations reported thus far predict negative electron affinities for all the DNA bases which may seem inappropriate for such polar molecular structures. Since the anion radicals of the DNA bases have been observed by ESR spectroscopy in aqueous solution, one might expect positive electron affinities for the DNA bases. However, it is largely the additional energy of solvation of these species (ca. 3 eV) that results in species stable towards dissociation to the aqueous electron and DNA base. As shown in this work, to a high degree of certainty the vertical electron affinities of all DNA bases are negative and only two of the adiabatic electron affinities are likely to be positive. Although there are no reported EA's for DNA bases in the gas phase, there are many other determinations of gas phase negative electron affinities for similar structures. For example, EA's for benzene, pyridine, pyrimidine, naphthalene, and uracil are found to be all negative (-1.1, -0.7, -0.3, -0.2, and -0.2 (eV)).

The calculation of gas phase electron affinities can be a difficult problem for HF-MO theory. This is even true for small structures for which HF electron affinities are underestimated. In more recent work however, Staley and Strnad have considered a series of hydrocarbons negative ion resonance states, or "negative electron affinities" and have employed HF theory with various basis sets under the Koopmans approximation. While the calculations still underestimate the EA's, they are successfully scaled to experimental electron affinities with excellent fits to experiment found using the D95v basis set.

In this work we perform full calculations of the IE and EA of DNA bases and their ion radicals using the Møller Plesset MP2 technique for electron correlation correction with large basis sets (6-31G* and 6-31+G(d)). In addition Koopmans EA's were calculated at various basis sets, including D95v, 6-31G* and 6-31+G(d). We have compared all these calculations to a variety of experimentally well known vertical EA's and found linear correlation fits to experiment. The best fits for EA are found for the Koopmans values at the D95v level which allows for estimation of the vertical electron affinities of the DNA bases. Calculations at 6-31G* and 6-31+G(d) using both ROHF and ROMP2 theories show a consistent difference between calculated vertical and adiabatic EA's. This allows for a good estimate of DNA base adiabatic EA's, i.e., -0.7; -0.3; 0.2; 0.3; 0.4(eV) from the vertical EA's (-1.23; -0.74; -0.40; -0.32; -0.19 (eV)) for G, A, C, T, and U respectively. While the vertical EAs are all negative the adiabatic values for T, C and U are
positive. While EA's must be scaled, we find that Koopmans IP's calculated at the 3-21G level predict vertical IP's of the DNA bases with only a 0.15 eV average absolute deviation from the experimentally reported values and calculations at MP2/6-31+G(d)//6-31G* for the adiabatic ionization potentials of the DNA bases are all within 0.1 eV of experiment.


In this study, we focus on radicals on the sugar moiety of the DNA molecule for they are known to be precursors in mechanisms leading to strand breakage. There is evidence for a definite role of sugar radicals in the indirect effect of ionizing radiations. Since the C1', C2', C3' and C5' centered radicals have been observed experimentally, theoretical investigation of their structure and relative stability is of importance. In this work, through the use of ab initio molecular orbital calculations performed on a DNA fragment composed of one sugar and two phosphate groups, we present the conformations and properties of the five carbon centered radicals which result from hydrogen abstraction. The effect of phosphate groups on the sugar radical conformation, energetics and electronic properties are evaluated through a comparison of models with and without phosphate groups. Geometry optimization performed at the ROHF/3-21G level reveals the C1' centered radical is the most energetically favored in all the DNA fragments considered in this study while the C2' radical is the least stable, and maintains a near planar configuration (\(^{4}\)T). All energy minima calculated correspond to deoxyribose radicals with a pseudorotation phase angle lying in the South quadrant of the pseudorotation cycle. The phosphate groups significantly affect the puckering mode of the C2' and C3' radicals and energetically destabilize C3' radical relative to the other sugar radicals. Isotropic hyperfine coupling constants significantly differ between models with and without phosphate groups, most particularly in the C3' and C4' radicals. The trend in oxidizing power based on the calculated HOMO energies is predicted to follow the order \(•C1' < •C4' < •C2' < •C3' < •C5'\). Cytosine attachment to the C1' and C4' deoxyribose radicals does not appear to affect the relative energies nor the isotropic hyperfine couplings of these two species. In both deoxyctydine radicals, the base maintains an anti conformation, which therefore does not disrupt the hydrogen bonding pattern in the base pair.
3. Ab Initio Study of Thiol Aqueous Phase Ionization Energies: Methyl Mercaptan and Cysteamine (paper 11).

As part of an ongoing investigation on radioprotective thiols in this work (funded primarily by the NIH), we employ ab initio theory to calculate the ionization energies of two thiols: methyl mercaptan and cysteamine in gas phase and in solution. By comparing these results to the previously calculated ionization energies of the four DNA bases we elucidate the possible mechanisms by which simple thiols protect DNA by electron transfer processes and the influence of hydration on these processes. The ionization energies of two thiol model compounds (methyl mercaptan and cysteamine) are calculated at the ROHF/6-31G* level to aid our understanding of the mechanisms involved in DNA radioprotection. Methyl mercaptan, the thiolate anion and its tri-hydrated form are fully geometry optimized. The resulting gas phase Koopmans ionization energies are 9.68ev, 1.67ev, and 3.63ev respectively. The ionization energy for the solvated methyl thiolate anion, CH₃S⁻(aq), calculated through the use of the SCRF model (ε=78), the Born charge term and a discrete hydration shell, leads to a Koopmans value of 5.6ev. This result is in good agreement with the corrected vertical solution phase ionization energy calculated using the same model (5.4ev), and with experiment (5.7±0.2ev). The gas phase ionization energies of cysteamine, its cation, zwitterion and the penta-hydrated form of the latter are reported. We find the Koopmans ionization energy of the anti configuration of the zwitterion to be ca. 6.0ev. Discrete hydration of the negatively charged sulfur increases the ionization energy by ca. 0.55ev per water while hydration of the amine group decreases it by ca. 0.1ev per water. Subjecting the penta-hydrated zwitterion to the SCRF model leads to a Koopmans solution-ionization energy of 6.42ev. These results predict that both the aqueous thiolate anion and the aqueous cysteamine zwitterion will reduce radiation induced DNA base cation radicals via direct electron transfer as found experimentally. Based on the energetics of the solvent-solute interactions, we propose that the electron transfer process between the cysteamine zwitterion and DNA cations will be influenced by the intervening solvent and suggest that displacement of the primary solvation layer upon ion pair formation will decrease the zwitterion's ionization energy, thereby facilitating the electron transfer.
4. Structures of Products Formed from Primary Radical Ions (work in progress).

Primary anion radicals may undergo rapid irreversible protonation reactions, whereas, the cations may undergo irreversible hydroxide ion addition or reversible deprotonation reactions. For each of the DNA base anion and cation radicals likely to undergo such reactions we are optimizing the structures of the product radical species and determining the total energies.

Calculation of accurate total energies allows for estimation of enthalpies of formation which will be valuable in predicting the relative importance of each product to DNA radiolysis. Various other properties such as spin densities, charge densities, dipole moments, ionization energies, electron affinities for all structures are being computed. The spin densities and charge densities are helpful in predicting the reactivity of the species. The ionization energies and the electron affinities can be of significance in predicting electron disproportionation reactions. This work is approximately 3/4 completed.

D. Reaction of Cysteamine with Individual DNA Base Radicals in γ-irradiated Nucleotides at Low Temperatures.

An ESR investigation of the individual DNA base radicals produced by γ-irradiation of frozen solutions of the nucleotides, TMP, dCMP, dGMP and dAMP and their reactions with cysteamine upon annealing is reported (This work is primarily supported by the NIH). Radicals from the oxidation pathway which include the DNA base one electron oxidized radicals and their successors, G(C8)OH•, A(C8)OH• and thymine dimers (•T_dj) and/or T(C6)OH•, readily react with cysteamine to form RS• and ultimately RSSR••. Reactions of dGMP and dCMP radicals from the oxidation pathway with cysteamine occur at lower temperatures than those of dAMP and TMP, suggesting hole migration. Both T(C6)H• and CH• react with cysteamine to form RS• and diamagnetic products, but G(C8)H• and A(C8)H• do not. Subtraction of the anion radical T• and its proton adduct T(C6)H• from the total radical yield of TMP (with or without cysteamine) suggests that somewhat less than half of the total TMP radicals found are a result of the oxidative pathway. Similar results are found in the other nucleotides. The total spectral intensity derived from the radicals from the oxidative pathway such as G(C8)OH•, A(C8)OH• and •T_dj/T(C6)OH• are somewhat less than that for the protonated anion radicals. Only one non-base radical is identified, a sugar radical at the C1' site on the deoxyribose portion of
dAMP. This species, S(A)•, is also found to react with cysteamine or its disulfide radical anion. This is the first ESR evidence that a sugar radical can be healed by thiols. Analyses performed in the presence and absence of a thiol are found to allow for a clear separation of oxidative and reductive pathways.

E. Product Formation in γ-irradiated DNA as a Function of Hydration (paper 16, full paper in preparation).

In this joint effort with Steven Swarts and Ken Wheeler of Wake Forest University, γ-irradiated DNA hydrated to various levels has been analyzed for 14 types of lesions. These DNA lesions include the release of unaltered bases, and specific DNA base damage. Perhaps the most outstanding finding is that the base damage yields are mainly confined to two products, one from reduction, dihydrothymine, and one from oxidation, 8-hydroxyguanine, properly called 8-oxoguanine, whereas, the base release yields are nonspecific. This product distribution (described further below) and the initial ionizations events have led us to Scheme I which we believe gives an overview of the oxidative radiation chemical damage to DNA. The numbers on the right give the percentage yields for dry and hydrated DNA (which are similar) and those on the left give the percentage of expected ionizations based on the total valence electrons. Clearly, transfer of the hole from other DNA bases to guanine must occur at an early time and is shown in the figure; however, transfer from the sugar to the base is not as efficient and this nicely explains the near uniform yield of unaltered bases. Scheme I therefore delineates the oxidative radiochemical processes from early processes (ionization) through radical intermediates some seen by ESR, to late processes which yield the damaged bases and base release. A similar scheme for the reductive component in DNA should result from our future investigations.

1. Radiation-induced DNA Base Damage as a Function of Hydration.

The induction of base damage products in γ-irradiated DNA, hydrated between 2.5 and 32.8 moles of water per mole nucleotide, was investigated using the gas chromatography/mass spectrometry-selected ion monitoring technique. The yields for 14 individual oxidative base damage products were found to be only slightly dependent on the extent of DNA hydration but greatly dependent on the presence of oxygen. Of the base damage products which were assayed in this study, the predominant base damage products found were those derived from guanine (mainly 8-hydroxyguanine and smaller amounts of 2,6-diamino-4-oxo-5-
formamidopyrimidine) and thymine (chiefly 5,6-dihydrothymine, with lesser amounts of 5-hydroxy-5,6-dihydrothymine, 5,6-dihydroxy-5,6-dihydrothymine, and 5-hydroxy-5-methylhydantoin). When irradiation of hydrated DNA was performed under oxygen, there was an increase in the combined base damage products derived from cytosine (5-hydroxycytosine, 5-hydroxyuracil, 5,6-dihydroxyuracil, and 5-hydroxyhydrantoin) in the DNA. In DNA irradiated at low hydrations (Γ=13), where the direct and quasi-direct effects will have the greatest influence on the induction of damage products, the highest yields were found for 8-hydroxyguanine, 5,6-dihydrothymine, and 2,6-diamino-4-oxo-5-formamidopyrimidine when irradiations were performed under nitrogen. The high yields for 8-hydroxyguanine, 2,6-diamino-4-oxo-5-formamidopyrimidine and 5,6-dihydrothymine compare well with the results of ESR studies which find that the DNA radical precursors which lead to the formation of these nonradical base damage products (e.g. the deprotonated guanine cation radical and the protonated thymine anion) are the major base radicals that are observed in irradiated DNA. We find (1) the yields of all oxidative products is greater than the yields of reductive products, indicating that there are other reductive products which have yet to be identified, (2) constant yields for the combined guanine and thymine products as well as the release of unaltered bases for Γ= 15-20 that implies that the addition of hydration water in causing DNA damage is the same as the DNA itself, and (3) the yields of the individual oxidative base damage products can be explained predominantly based on the reactions that form the initial charged radicals in irradiated DNA and on the intermediate reactions that are involved in the transformation of these initial DNA radical to their respective nonradical endproducts. These results are consistent with the proposal that the radiolysis of the inner-most water molecules of the DNA hydration layer constitutes a quasi-direct effect (e.g. are an extension of the direct effect), due to the fact that these water molecules transfer their radiation-induced damage to DNA through the reaction of charges (e.g. holes and electrons).

F. Formation of Sugar Radicals and Adduct Radicals in DNA and DNA models Systems by Secondary Radical Attack (Work in progress).

In recent efforts we have found that the radical formed by electron attachment to iodoacetamide, •CH₂C(O)NH₂, attacks the deoxyribose portion of nucleotides to form sugar radicals as well as adds to the DNA bases. Sugar attack has also been suggested to occur in DNA itself. Attack of this species thus
provides a method of production of important sugar radical intermediates in the formation of strand breaks. Whereas the hydroxyl radical undergoes addition reactions to the DNA bases in preference to abstraction from the sugar (about 5 to 1 in DNA), we find the the iodoacetamide radical attacks the sugar group of dAMP in greater proportion to addition, making it more facile to follow subsequent sugar radical processes. We find that this radical species also adds to thymine (at C6) and adenine (at C8) DNA bases in their nucleotides.

We also have preliminary evidence that the UCH₂⁺ species in dTMP attacks thymine at C-6 to form the adduct species, UCH₂T⁺. This has been a controversial point in the literature which we hope to resolve in our future efforts.

G. Effect of DNA Complexes with Polypeptides on Radical Ion Distribution.

We have investigated the effect of binding of polycationic species such as spermine, polylysine and polylysine copolymers on the distribution of charge within the DNA strand and between the DNA strand and the polypeptide. A number of DNA-polypeptide and DNA-spermine complexes have been investigated. These include DNA polypeptide complexes with: poly-L-lysine, poly[lysine,phenylalanine] (1:1), poly-[lysine-tyrosine] (1:1), poly[lysine-tryptophan] (4:1), and finally poly[lys,ala,glu,tyr]. In this work we have found that spermine-DNA complexes have nearly the same spectra as DNA alone. This is in part due to the low electron affinity of spermine and the high reactivity of the radical intermediates formed. Spermine is not a major contributor to radical production due to its small wt% in the complex (ca. 20%). It is also likely that spermine radicals which originate from the cationic centers rapidly react with DNA even at low temperatures.

For all polylysine-DNA copolymers, we find that the DNA has a disproportioniate amount of anion radical likely from electron transfer from the polypeptide. Typically our analyses suggest a 40% increase in anion radical for the DNA polypeptide complexes. Hole transfer from the polypeptide is apparently not as facile. In fact with poly[lysine,phenylalanine], poly[lysine,tyrosine] and poly[lysine,tryptophan] complexes with DNA, there is little evidence for anionic charge on the polypeptide but evidence for substantial cationic charge on the aromatic group of the polypeptide. These results have implications for DNA irradiated with histones. The histones likely provide a protective effect from the direct effect of radiation by transfer of negative charge to DNA. Since it has been well established that the electron does not produce strand breaks, it is clear that
increasing the number of anionic radicals on the DNA will likely result in ion recombination and a loss of potential strand break sites. Further work is planned to extend these works to actual histone-DNA complexes before publication.
II. Effort of the Principal Investigator.

The principal investigator spent ca. 2 months of the 15 week of the 1994 spring-summer on this work. Further, a significant fraction of his academic year time (not contractually obligated) is spent on this work. During the upcoming fiscal year the principal investigator will spend ca. 2 months of the 15 week 1995 spring-summer on this work and a significant fraction of his academic year effort.

III. a. Papers Published, in Press or Submitted


2. "Ab Initio Molecular Orbital Calculations of the Structure and Energetics of DNA Deoxyribose Radicals" A. O. Colson and M. D. Sevilla, 42nd Annual meeting of the Radiation Research Society, Nashville, Tennessee, April 29-


