DOE/ER/60455--T4

DE93 003715

"Mechanisms for Radiation Damage in DNA"

Progress Report

Office of Health and Environmental Research
of the
Department of Energy

Grant No. DE-FG02-86ER60455.A007

Period: June 1, 1992 - May 31, 1993

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December 1992

Table of Contents

Page

Abstract

In this project we have proposed several mechanisms for radiation damage and recently radiation protection 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.

In this years work we have performed experiments which elucidate the role of hydration water on DNA radiation damage, continued the investigation of the localization of the initial charges on DNA and employed ab initio molecular orbital theory to gain insight into the initial events of radiation damage to DNA. Ab initio calculations have provided an understanding of the energetics envolved in anion and cation formation, ion radical transfer in DNA as well as proton transfer with DNA base pair radical ions. This information has aided the formation of new radiation models for the effect of radiation on DNA.

During this fiscal year four articles have been published, two are in press, two are submitted and several more are in preparation. Six papers have been presented at scientific meetings. This years effort includes a review article on the "Chemical Consequences of Radiation Damage to DNA". This review presents an overview of this field at this time.

I. Progress Report 1992-1993.

In the sections below we briefly describe progress made during the fiscal year.

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

1. Total Radical yields and the role of water. Elucidation of free radical mechanisms in DNA induced by high energy radiation is important to the understanding of the ultimate effects of radiation on DNA. Experimental determination of the low temperature DNA radical composition and the total number of radicals present, usually expressed in terms of G values, combined with models for energy deposition are of aid in the elucidation of the initial events in the radiation damage to DNA. However, it has become clear that the yields and distributions of radicals and their subsequent molecular products depend on a number of factors, including the extent of hydration, the strandedness and conformation of the DNA, the DNA base sequence as well as the temperature and dose (*vide infra*).

It is well known that DNA structure and conformation, DNA base stacking as well as hydrogen bonding between base pairs are all influenced the degree of hydration. Previous IR and NMR investigations of DNA hydration have shown that the first 12 to 15 hydration water molecules (H_2O) per mononucelotide are bound most tightly to the DNA and do not form an ice-like structure on cooling to low temperatures. The next 6-9 water molecules complete what is called the primary hydration layer (20 to 21 water molecules/nucleotide) and are distinct from the bulk water phase although intermediate in properties. Gregoli was the first to show that the primary hydration layer (ca. 20 waters) acted so as to approximately double the ion radical formation on DNA. Huettermann has recently shown that relative radical yields in DNA increase over twofold from dry to hydrated DNA confirming Gregoli's earlier work. Swarts et al have reported results on base release as a function DNA hydration which suggest ionizations in the first 15 waters act to transfer dry charge to DNA whereas subsequent waters are suggested to form hydroxyl radicals.

In this work, we present an investigation of the effect of hydration water on the production of ion radicals in γ-irradiated DNA at 77 K. We present the first study of absolute yields of radicals in DNA and its associated water-ice phase as a function of hydration. The total radical yield is found to increase by over two fold after the primary hydration layer (20 water molecules/nucleotide) is added to DNA. At hydrations greater than ca. 20 waters/nucleotide the excess water freezes into a separate and radiologically independent bulk ice phase. Ice formation steals ca. 5 waters from the hydration layer which causes a proportionate (by weight) drop in the total DNA radical ion yield (G). Hydroxyl radicals are not observed even at great signal amplification in the primary hydration layer but are found only in the ice phase which suggest efficient transfer of holes and electrons to the DNA. Thus DNA and its associated hydration water form the target mass for radiation damage. These results show 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. At 18 waters per nucleotide (Γ=18) about 1/4 of all ion radicals produced by the radiation are trapped at 77 K; whereas at Γ=2.5 only 1/10 are trapped. The k value for destruction is found to vary only slightly with hydration with the exception of a lower value for dry DNA. A simple model is employed to estimate the cluster radius and volume for a single radiation event in DNA. For hydrated DNA the cluster radius is found to be ca. 36 Å with a slightly smaller value for the dry DNA as expected from it's smaller k value. About 130 base pairs are contained within the cluster volume for both dry and hydrated DNA. For hydrated DNA the average ion pair separation (46 Å) found suggests an average migration distance of trapped electrons from the hole of 14±3 bases (for migration along the DNA strand).

2. Yields of Individual Radicals with dose.

An EPR (Electron Paramagnetic Resonance) investigation of the influence of hydration and dose on the absolute yield of the individual free radicals formed in γ-irradiated frozen DNA has been performed. Analysis of the EPR spectra as a function of hydration shows that the primary ion composition at low hydration (0% and 14.3% wt. D_2O), the radical composition is 15% $C^{-\bullet}$, 15% $A^{-\bullet}$, 30% $T^{-\bullet}$ and 40% $G^{+\bullet}$. Further the radical composition varies little with dose and few secondary radicals (S• and TH•/TD•) are formed at high doses (beyond ca. 120 kGy). For the samples at higher hydrations, from 37.5% to 86.6% D_2O, the radical composition varies little with hydration but greatly with dose. At very low doses (1.1 kGy) the composition is ca. 25% C^{-}•, 35% T^{-}• and 40% G^{+}•. These values change with dose until at 240 kGy, we find ca. 48% C^{-}•, 5% T^{-}•, 30% G^{+}•, 16% S• and 1% TH•/TD•. Computer fits by means of a global least squares to the yield vs. dose data result in the G values for radical formation and K values for radical destruction for each of the ion radicals formed at 77K. At high doses a new constant, K', is employed to account for the change is G value with dose resulting from radiation induced changes in DNA. The G values for each of the base radicals increase with the hydration level, but the K values are relatively constant. A stochastic model of the process of radiation damage suggests that certain secondary radicals probably associated with the sugar phosphate backbone likely arise from a second hit on the guanine cation which is suggested to result in a excited and reactive cation.

These results are believed to provide evidence for a new mode of formation of strand breaks in DNA, i.e., excitation reactions of DNA cations radicals. This work is considered significant and we plan to follow this up in the next fiscal year with further experiments which elucidate excitation and sugar radical formation.

b. Ab Initio Molecular Orbital Calculations of DNA Base Pairs and Their Radical Ions .

In our previous work in our last progress report we reported *ab initio* molecular orbital calculations of radical ions of DNA bases and DNA bases, stacked four base DNA subsystems. The results of these calculations combined with previous work lead to a revised model for the initial localization of charge in irradiated DNA. Our results predicted that thymine has the highest electron affinity and guanine the lowest ionization potential of the four DNA bases. As a consequence, the electron in irradiated DNA is more likely to be found on thymine and the hole on guanine. Results obtained with base pairs and four base stacked systems again suggest that the electron will localize on thymine. However a simple proton shift from guanine to cytosine also shifts the electron from thymine to cytosine. No such shift from A to T is predicted to be energetically favorable. For the hole, proton shifts are not predicted to alter the hole site due to the fact that guanine hole in the G-C base pair is calculated to be lower in energy than the adenine hole in the A-T base pair either before or after proton shift within the A-T base pair. These results therefore suggest that electron attachment will initially favor thymine and subsequent proton transfer reactions between G-C base pairs will shift the electron to cytosine. Previous results in our laboratory have shown that in irradiated double stranded DNA at 100 K, the anion is mainly on cytosine and the hole on guanine. However, very recent results have shown that in single stranded DNA where proton transfer between base pairs is not possible, the thymine anion is found to increase its contribution as expected from the results of this work.

In this years work we are investigating: 1. Effect of Hydration water on EA's and IE's . 2. Electron Correlation Effects on IE and EA in DNA bases. 3. Cation Radical Sites on the DNA Sugar PhosphateBackbone. 4. Structures of Products Formed from Primary Radical Ions.

1. Effect of Hydration water on EAs and IEs . In this work we have performed calculations DNA base pairs and their ion radicals at the 3-21G level which include waters of hydrations at probable sites of hydration in DNA. We have found that the waters of hydration have a small effect on both the ionization potentials and electron affinities. Both the EAs and IEs are slightly increased by the waters of hydration but the relative order for both is unchanged from the order without waters of hydration, i.e., C>T>A>G. The evidence also shows that the waters of hydration stabilize the anion radicals more than cations.

2. Electron Correlation Effects on IEs and EAs of DNA bases. Simple Hartree Fock level calculations do not correct for electron correlation. With large bases sets the electron correlation correction becomes the major source of error in the simple HF calculations. In our work we found that our calculated values for the IE were lower the the experimental by about 1 ev. Koopman's IE energies which use the energy of the orbitals of the neutral molecule to predict the IE energy. Koopman's values actually make compensating errors which mimic the IE quite well. As a consequence this simpler approach was employed in previous work.

In this work we perform full calculations of the IE and EA of DNA bases and their ion radicals using Møller Plesset second order perturbation theory for electron correlation corrections. The large basis set (6-31+G(d)) was employed in this work for electron correlation corrections. All structures were optimized at the 6-31G* HF level. Preliminary results found thus far show that the electron correlation per electron is found to be 0.02 AU or 0.56 ev as an average over all electrons. The values for the IE are corrected by nearly 0.7 ev to values near those found experimentally. Further the electron correlation correction's increase the electron affinities by about 0.5 ev. However the corrections vary only slightly from one DNA base to another so that the relative IEs and EAs are not affected.

3. Cation Radical Sites on the DNA Sugar PhosphateBackbone. Up to this time ESR spectroscopy of irradiated DNA at temperatures from 4K to 77K has not show any radicals other than those on DNA bases. DNA is composed of 55% by weight sugar phosphate backbone and at least an equal weight to the DNA in hydration water. Thus although they make up less than 25% of the target mass DNA bases apparently have all the free radicals formed in irradiated DNA. An explanation for this is that ionization in the hydration layer and sugar phosphate backbone transfer spin and charge to the DNA bases. Another explanation is that ionization on the deoxyribose phosphate backbone results in very broad radical species which are not easily observed by ESR. We find no solid evidence for the latter in our ESR spectra. Since the most damaging effect on radiation is the DNA double strand break it is critical to understand the possible mechanisms of production of radiation lesions on the DNA deoxyribose phosphate backbone. To this end we are performing ab initio mo calculations on sections of the DNA dexyribose phosphate backbone to determine sites for localization of the hole. Thus far we have investigated the components, phosphate, deoxyribose and several combinations such as phosphate sugar phosphate and sugar phosphate sugar. We have optimized structures which include sodium cation with up to four water molecules hydrating the sodium phosphate group. The calculations show that there are two sites which the cation or hole can localize before subsequent reaction or transfer to the DNA base. These are: 1. localization to the ring ether oxygen in the ribose group or 2. localization to one of the singly bonded oxygens on the phosphate group. The site of localization varies with the backbone structure and conformation and result from the fact the the phosphate and deoxyribose portions of the backbone have nearly the same ionization energy. Most calculations favor the deoxyribose portion of the backbone.

The IE of the sugar phosphate backbone is found to be about 2 ev higher than the bases. This explains the transfer of the charge to the bases and the lack of observation of a large fraction of sugar phosphate radicals in DNA. It has become clear in recent pulse radiolysis work that excited DNA base cations may result in DNA strand breaks. It is clear that a DNA base excited state could undergo a charge transfer to the sugar which could then react to form the strand break. Such mechanisms are now under investigation.

4. Structures of Products Formed from Primary Radical Ions.

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 determined the total energies.

Calculation of accurate total energies allows for estimation of enthalpies energies 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 computed 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 one half completed.

c. Radiation-induced DNA Damage as a Function of Hydration.

1. Release of Unaltered Bases. 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 several types of lesions. These DNA lesions include the release of unaltered bases, specific DNA base damage, and nucleoside damage. These studies are reviewed briefly below.

The release of unaltered bases from irradiated DNA, hydrated between 2 and 33 moles of water per mole of nucleotide (Gamma) was investigated by HPLC. The objective of this study was to elucidate the yield of the four DNA bases as a function of dose, extent of hydration, and the presence or absence of oxygen. The increase in the yield of radiation-induced free bases was not constant with Gamma over the range of hydration examined. For DNA with Gamma between 2 and 15 the yield of free bases was nearly constant. However for DNA with Gamma > 15 the yield increased rapidly. We suggest that the release of bases originating from irradiation of the hydration water is obtained predominantly by charge transfer from the direct ionization of first 12-15 water molecules and predominantly by the attack of hydroxyl radicals generated in the outer more loosely bound water molecules. This work is now published.

2. Formation of Specific DNA Base Damage Products.

In this joint study we have examined the levels of radiation induced base damage produced in double stranded salmon sperm DNA hydrated between 2 and 33 moles of water per mole of nucleotide (Gamma). The base damage products are assayed using gas chromatography/mass selective detection techniques following formic acid hydrolysis of the DNA.

The DNA base damage products that are likely formed from the dry electron and positive hole, i.e. dihydrothymine and 8-hydroxyguanine, predominate at low DNA hydrations. For higher levels of DNA hydration (Gamma>13) the base damage products formed by reaction with hydroxyl radical (i.e., 5-hydromethyl uracil, thymine glycol, FAPyAdenine, and 8-hydroxyadenine are the major species observed in addition to 8-hydroxyguanine. In the presence of oxygen greatly reduced levels of dihydrothymine are found at low hydration levels. This is likely due to scavenging of electrons by oxygen or the addition of oxygen to the thymine electron adduct preventing the formation of the dihydrocompound.

This work is in preparation for publication.

II. Effort of the Principal Investigator· The principal investigator spent two months of the 15 week of the 1992 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 2 months of the 15 week 1993 spring-summer on this work and a significant fraction of his academic year effort.

III. a. Papers Published, Papers in Press or to be Submitted

1. "Radiation-induced DNA Damage as a Function of Hydration I. Release of Unaltered Bases," S. G. Swarts, M. D. Sevilla, D. Becker, C. J. Tokar, and K. J. Wheeler, Radiation Res., 129, 333-334 (1992).
2. "Relative Abundances and Reactivity of Primary Ion Radicals in Irradiated DNA at Low Temperatures: II. Single vs. Double Stranded DNA" Mengyao Yan, David Becker, Steven Summerfield, Paul Renke and Michael D. Sevilla, J. Phys. Chem., 96, 1983-1989 (1992)
3. "Ab Initio Molecular Orbital Calculations of DNA Bases and Their Radical Ions in Various Protonation States: Evidence for Proton Transfer in GC Base-Pair Anions" Anny-Odile Colson, Brent Besler, David M. Close and Michael D. Sevilla, J. Phys. Chem. 96, 661-668 (1992).
4. "Ab Initio Molecular Orbital Calculations on DNA Base Pair Radical Ions: Effect of Base Pairing on Proton Transfer Energies, Electron Affinities and Ionization Potentials" Anny-Odile Colson, Brent Besler and Michael D. Sevilla, J. Phys. Chem. 96, Nov. (1992).
5. "Superoxide Dismustase Mimics Protect Cultured Rabbit Lens Epithelial Cells from Hydrogen Peroxide Insult" J. R. Reddan, M. D. Sevilla, F. J. Giblin, V. Padgaonkar, D. C. Dziedzic, V. Leverenz, I. C. Misra, and J. L. Peters, Experimental Eye Research, accepted for publication (1993).
6. "The Chemical Consequences of Radiation Damage to DNA" D. Becker and M. D. Sevilla, invited review for Advances in Radiation Biology, Academic Press (1993).
7. "The Influence of Hydration on the Absolute Yields of Ionic Free Radicals in γ-irradiated DNA at 77K", Wending Wang, David Becker, and Michael Sevilla, submitted for publication.
8. "Absolute Yields of Individual Ion Radicals and Secondary Products in γ-irradiated DNA at 77K" Wending Wang, David Becker, and Michael Sevilla, to be submitted for publication.
9. "ESR Study of the Fate of Primary Ion Radical Intermediates in Frozen DNA-Thiol Solutions: Electron Transfer from Thiols to DNA Cation" M. D. Sevilla, D. Becker, S. R. Summerfield and M. Yan, in preparation.
10. "Reactions of DNA Primary and Secondary Radicals with Thiols: Effects of Oxygen", M. D. Sevilla, D. Becker, S. R. Summerfield and M. Yan in preparation.

11."Specific DNA Base Damage Produced by Irradiation of DNA Hydrated to Various Levels" S. G. Swarts, G.S.Smith, M. D. Sevilla, D. Becker, and K.T. Wheeler, in preparation.

b. Papers Presented at Scientific Meetings

1. "Relative Abundance and Reactivity of Primary Ion Radicals in Irradiated DNA at Low Temperatures: Single vs. Double Stranded DNA, M.D. Sevilla, D. Becker, M. Yan, S.R. Summerfield and P. Renke, Fortieth Annual Meeting of the Radiation Research Society, Salt Lake City, Utah, March 14-18, 1992.

2. "Ab Initio Molecular Orbital Calculations of DNA bases and their Radiacl Ions in Various Protonation States: Evidence for Proton Transfer in GC Base Pair Radical Anions" A. Colson, B. Besler, M. D. Sevilla and D. M. Close, presented at the Pathways to Radiation Damage in DNA Conference at Oakland University, June 14-18, 1992.

3. "Ab Initio Molecular Orbital Calculations of Proton Transfer Profiles in DNA Base Pair Radical Ions" A. Colson, B. Besler, and M. D. Sevilla, presented at the Pathways to Radiation Damage in DNA Conference, Meadow Brook Hall, Oakland University, June 14-18, 1992.

4. "Relative Abundance and Reactivity of Primary Ion Radicals in gamma Irradiated DNA at Low Temperatures: Single vs. Double Stranded DNA, M. Yan, D. Becker, S.R. Summerfield, P. Renke and M. D. Sevilla, presented at the Pathways to Radiation Damage in DNA Conference, Meadow Brook Hall, Oakland University, June 14-18, 1992.

5. "The Influence of Hydration on Yields and Distribution of Primary Ionic Free Radicals in Low Temperature γ-irradiated DNA", Wending Wang, David Becker, and Michael Sevilla, presented at the Pathways to Radiation Damage in DNA Conference, Meadow Brook Hall, Oakland University, June 14-18, 1992.

6. "Ab Initio Molecular Orbital Calculations of Proton Transfer Profiles in DNA Base Pair Radical Ions" A. Colson, B. Besler, and M. D. Sevilla, presented at Gordon Research Conference on Radiation Chemistry, Salve Regina College, Providence, Rhode Island, July 6-10, 1992.

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