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MECHANISMS FOR RADIATION DAMAGE IN DNA

Progress Report

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NOTICE

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ABSTRACT

In this project we have proposed several mechanisms for radiation damage to DNA constituents and DNA, and have detailed a series of experiments utilizing electron spin resonance spectrometry to test the proposed mechanisms. In the past we have concentrated chiefly on the direct affect of radiation on DNA. We are currently investigating systems of DNA constituents and peptides which may shed light on indirect effects.

Studies which we have completed during the past year are:

1. Studies of γ-Irradiated N-Acetyl Amino Acids and Peptide Solutions at 77^{o}K.
2. Studies of Barriers to Hindered Rotation in Peptide Radicals

Studies in which we have made progress in this past year are:

3. π Cations Produced in DNA Bases by OH$^{\cdot}$ Attack
4. Studies of spin transfer in γ-Irradiated Nucleoside - Peptide Solutions

These studies have shown that:(1)Frozen aqueous solutions provide a suitable matrix for γ-irradiation studies.

(2) γ-irradiated peptides in frozen aqueous solutions follow chemistry expected from previous studies.

(3) π-Cations of DNA base can be produced by OH$^{\cdot}$ attack.

I. Results This Year

In the past year, three articles were published and three more were prepared for publication. The articles published and the papers prepared are attached as appendices. Below we briefly describe this work and other work which is not yet completed. Papers presented on work funded under this contract are included in Section II.

a. DNA Base π-Cations Produced by OH· Radical Attack

In our most recent work we have investigated OH· radical attack on thymine and other DNA bases in γ-irradiated $NaClO_4$ glasses. Originally Neta reported OH· attack on several DNA bases at pH 11 in aqueous solution at room temperature produced ring opened radicals. However, the hyperfine splittings of three species were within experimental error of those reported in our work for DNA base π-cation radicals in glassy matrices. In a joint effort with Dr. P. Neta of University of Notre Dame, Radiation Laboratory, the futher investigation of these systems was begun this year. We studied the OH· attack on DNA bases in glassy matrices while Dr. Neta investigated OH· attack on certain 1,3 methyl substituted uracils. These compounds were employed to distinguish between ring openings and π-cation radicals. Unfortunately, in Dr. Neta's experiments the signal level was not sufficient for observation of the radical intermediates.

In our experiments we have found that ·OH radicals produced in γ-irradiated 8M $NaClO_4$ glasses attack thymine to produce the thymine π-cation. Hyperfine splittings measured from thymine π-cation produced by photoionization are identical to that produced by OH· attack. It is therefore clear that at least at low temperatures OH· attack will produce DNA base cations. This work is continuing.

b. Studies of γ-Irradiated N-Acetylamino Acids and Peptides in Frozen Aqueous Solutions

We have completed studies of γ-irradiated frozen aqueous solutions of N-acetylamino acids and peptides. This work is presented in detail in Appendices E and F. This work was jointly sponsored by the U.S. Army Natick Development Center. Below we briefly summarize this effort. We believe this work is essential to the understanding of the indirect effect of radiation on DNA, where radicals produced in the surrounding histones may attack DNA.

1. N-Acetylamino Acids

The γ-irradiation of a number of N-acetylamino acids at 77 K in frozen D_2O and H_2O solutions was studied. The radicals produced by the irradiation, their relative amounts and their stability, were studied as a function of temperature. The results found for ices of N-acetylalanine illustrate the reaction mechanisms found. At low temperatures (120 K) the peptide anion and decarboxylated species dominate the spectrum. Upon warming to 190 K the anion converts to the amide and fatty acid radical by secondary deamination.

$$CH_3\dot{C}O^{-}\text{-}NDCH(CH_3)CO_2^{-} \xrightarrow{D+} CH_3COND_2 + \cdot CH(CH_3)CO_2^{-}$$

Further warming to 220 K results in abstraction of the intermediate radicals from the parent compound to form the α-carbon radical.

$$CH_3\dot{C}HCO_2^- + CH_3CONDCH(CH_3)CO_2^- \rightarrow CH_3CH_2CO_2^- + CH_3COND\dot{C}CH_3CO_2^-$$

Results found for N-acetylglutamic acid show similar reactions and also show evidence for electron attachment to the two carboxyl groups as well as the peptide linkage. An investigation as a function of pD shows that the site of electron attachment is strongly pD dependent with the carboxyl groups being favored at low pD and the peptide linkage favored at high pD.

2. Peptides

The γ-irradiation at 77 K of frozen aqueous solutions of over 14 peptides were investigated by ESR (electron spin resonance) spectroscopy. The investigation was composed of three parts. The first part consists of a study of simple dipeptides of glycine and alanine, i.e., gly-ala, ala-gly and ala-ala. Detailed analyses were made of the radicals produced by the irradiation, their relative concentrations and their stability as a function of temperature. The results show evidence for anion formation, primary deamination and decarboxylation initially. This is followed by abstraction from the parent compound to form the α-carbon radical at the C-terminal residue. In the case of ala-ala there is evidence for "hole" stabilization at low temperature. In the case of gly-ala some secondary deamination is noted. In the second part the analysis of the final spectra for 11 dipeptides are reported. The final radicals were all found to be a result of abstraction from the α-carbon of the C-terminal residue to produce the radical shown below.

$$NH_3^+CHRCONH\dot{C}R'CO_2^-$$

Radicals with R' = H or $-CH_2R''$ produced a hyperfine coupling due to one proton of 18 to 19 G. Since 15 of 20 common amino acid residues have a structure with R' = H or $-CH_2R''$, the dominant "final" radical in proteins should produce a doublet spectrum. In the final portion, three dipeptides with aromatic side groups were studied in frozen H_2O solutions. It was found that electron attachment to the aromatic ring effectively competed with deamination of the primary amine group. The electron attachment to the aromatic ring was followed by protonation at a carbon site on the ring. The results for gly-his were typical. Here we found 25% protonation, 30% deamination and 45% of a spectrum indicative of decarboxylation. For gly-tyr, phenoxyl radical formation was found to dominate decarboxylation as a decomposition pathway for the hole.

c. Studies of Spin Transfer in γ-Irradiated Nucleoside-Peptide Solutions

In this work, the possible transfer of spin between nucleosides and peptides in frozen aqueous solutions has been investigated. A number of mixed systems were examined; deoxyguanosine, deoxyadenosine, thymidine and glycylglycine or N-acetylalanine mixtures were employed. The results show no evidence for transfer at low temperatures. Spins are generated in the two components independently. On warming, there is evidence for electron transfer from

N-acetylalanine to thymidine, however, other systems do not show clear evidence for transfer.

d. Activation Barriers in Peptide Anion Radicals

Activation energies for methyl group rotation, in the radicals of type $H_3C\text{-}\dot{C}<$, produced from acetic acid, acetamide and peptides, have been determined. Reaction of electrons at 77 K, with acetic acid and acetamide in 12M LiCl (D_2O) as well as in 8M NaOD glasses, have produced the acetic acid anion, the acetate dianion and the acetamide anion. ESR spectra of these have revealed a doublet (ca. 32 G) at 90 K reversibly interconverting to a 1:3:3:1 quartet, of ca. 15 G hyperfine splitting, at higher temperatures (170 K). This interconversion has been attributed to the hindered internal rotation of the methyl group about the $H_3C\text{-}C<$ bond. The ESR spectra are analysed using modified Bloch equations for three-jump process. The mean-life time (τ) at each temperature (T) has been estimated by a comparison of experimental and simulated ESR spectra. The activation energy (E_a) for the six-fold barrier to the rotation is found to be 5.0 ± 0.5 kcal/mole in acetate dianion and is about 3.0 kcal/mole for the acetate and acetamide as well as N-acetyl amino acids. ESR spectra characteristic of the tunneling methyl group were observed at low temperature ($20\ K < T < 100\ K$) for the radicals produced in γ-irradiated polycrystalline samples of glycyl-L-alanine and L-alanyl-L-alanine.

II. Papers Presented

"ESR Study of Conformational Affects on Radicals Produced by Electron Attachment to Amino Acids and Peptides," presented at the 176th Annual Meeting of the American Chemical Society, September 1978.

III. Effort of the Principal Investigator

The present term of this contract began November 1, 1978. Since then 20% of his time during the academic year has been spent on this work. The principal investigator is devoting 7 weeks of the spring-summer sessions to this project. The remaining portion of the summer will be spent on related work funded by the U.S. Army Natick Development Laboratory.