

MECHANISMS FOR RADIATION DAMAGE IN DNA

Progress Report

MASTER

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Appendix B. ESR Study of the Anion Radicals of 5-Nitropyrimidines

Appendix C. ESR Study of the N₁-Substituted Thymine π-Cation
Radicals

Appendix D. Electron Reactions with Amino Acid Anhydrides

ABSTRACT

In this project we have proposed a mechanism 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 several investigations have been completed or are nearing completion. These investigations are:

1. An ESR study of the N_1 -substituted thymine π -cation radicals
2. Studies of electron reactions with amino acid anhydrides
3. ESR and pulse radiolytic studies of electron transfer in dinucleoside phosphate anions. Studies which we have made significant progress on in this past year are:
4. Positive ion radicals of the dinucleoside phosphates, and
5. Reactions of the π -cations of thymine derivatives.

In the first study the spin density distribution in the cation radicals of thymidine and thymidine-5¹-monophosphate have been elucidated. Couplings are found to the ribose group in these radicals. It is believed that these results will aid the identification of cation radicals in DNA. In study 2, the results indicate that these cyclic anhydrides can undergo reductive deamination. In study 3, the results show the order of electron affinities of the DNA bases to be Thymine \approx Cytosine $>$ Adenine \approx Guanine.

I. Results This Year

During the past year two articles have been published and two more have been 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.

A. ESR Study of N_1 -Substituted Thymine π -Cation Radicals

π -Cation radicals of DNA bases have been identified in γ -irradiated crystalline thymine, thymidine, cytosine, as well as γ -irradiated DNA. It is our purpose to aid in the characterization of these radicals and to understand their chemistry by producing them individually in aqueous glasses. In our previous studies the π -cation radicals of several DNA bases were identified and their further reactions studied. The present work extends the investigation to several N_1 -substituted thymine derivatives and the nucleotide, thymidine-5¹-monophosphate (TMP).

π -Cation radicals produced by photoionization of thymidine-5¹-monophosphate, thymidine, 1-methylthymine, 1-ethylthymine and thymine in basic 8 M $NaClO_4$ -D₂O glasses at 77⁰K were investigated by ESR spectroscopy. Analysis of the spectrum of each N_1 -substituted thymine shows coupling to the 5-methyl group (21G), to the nitrogen at position 1 ($A_{||} = 13G$, $A_{\perp} < 4G$ with $g_{\perp} - g_{||} = 0.002$) and to β -protons on substituents at N_1 . Analyses were confirmed by computer simulations. The "experimental" spin density distribution was calculated and compared to McLachlan MO predictions. Parameters of the interaction of the 1-position β -protons were determined ($\rho\pi = 0.21$, $B_2 = 79$) and used to evaluate the conformations of the N_1 substituent groups. Results found here are compared to previous results in aqueous glasses and single crystals.

B. Electron Reactions with Amino Acid Anhydrides

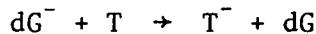
The structure of glycine anhydride (2,5 diketopiperazine) are of interest since they have two peptide bonds and no terminal amine groups. A number of investigations have established that N-terminal amine groups in peptides are readily deaminated after electron attachment. Since the amino acid anhydrides have no terminal amine groups the possibility of secondary amine deamination by electron attachment can be investigated.

In this work we have produced the anions of glycine anhydride (GA) and alanine anhydride (AA) in an aqueous glass and studied their subsequent reactions. Electron attachment to glycine anhydride (GA) at 77°K in 12M LiCl results in an anion which remains stable to 175°K. At 175°K a second radical is found which is suggested to be the species produced by abstraction of a hydrogen atom from a methylene group in GA. This suggestion is verified by the production of this same species by attack of O^- on GA in 8M $NaClO_4$ -D₂O glasses. Electron attachment to D,L alanine anhydride at 77°K results in a stable anion which showed no further reaction on warming. The results found for GA are compared to previous studies of γ -irradiated GA single crystals and a study of γ -irradiated polycrystalline GA reported in this work. A possible mechanism for the production of the second radical from GA anion is discussed

C. Electron Reactions with Dinucleoside Phosphate Anions

During this past summer we completed this work. Most of the details of the investigation were described in our last progress report and are ~~removed~~ included in Appendix A with this report.

In this year's work we completed our investigation of mixtures of the individual nucleosides. We found evidence for electron transfer only in the case of dG and T. In this case there was evidence for the reaction

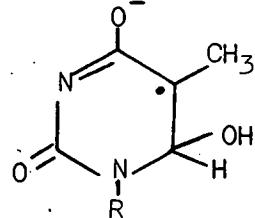


However, results for dA and T did not give clear evidence for a similar transfer reaction.

A joint pulse radiolysis study of electron transfer in TdA anion with R.A. Holroyd and M. Pettei was also completed this past summer (see Appendix). ^{removed} These pulse radiolysis studies show that in the TdA anion electron transfer occurs from adenine to thymine; whereas, no electron transfer is found for mixtures of individual nucleotides. These results correspond nicely to those found for the dinucleoside phosphate anions in aqueous glasses.

D. Reactions of the π -Cations of Thymine Derivatives

We have been investigating the results of annealing samples of π -cations of N-substituted thymines in 8M NaClO₄ glasses. Thus far we have found that the decay product is the same for π -cations of 1-methylthymine or thymidine-5'-monophosphate. The radical formed is of the form R - CH₂• produced by loss of a proton from the 5-methyl group. In each case a third radical species is produced of the form.



This species was thought to arise from 'OH attack on the parent compound, however addition of 0.1M sodium formate has no effect on its yield. Further work will be necessary to determine whether the deprotonated radical could react to form this third species. Results thus far do not show any evidence for a reaction on the ribose group as we proposed last year.

E. Dinucleoside Phosphate Positive Ion Radicals

The cation radicals of a number dinucleoside phosphates (DNP) have been generated. At 77°K for thymidylyl-(3',5')-2-deoxyodensine (TdA) we find the positive ion on dA at pH7 and on both T and dA at pH 12. Warming glasses of TdA cation at pH 12 to 165°K we find the loss of the T⁺ signal. This suggests the possibility of the transfer of positive charge from T to dA in the DNP⁺.

Warming solutions of these DNP cations has of yet provided no evidence for transfer of spin to the ribose group.

II. Co⁶⁰ γ-irradiation Source

During the next year we expect to have a 300 curie Co⁶⁰ γ-irradiation source at Oakland. Professor Larry Kevan at Wayne State University has kindly offered to transfer this source to Oakland. We have accepted this offer and are presently applying to the National Regulatory Commission for a license for this sealed source.

It is expected that this source will greatly aid our research effort. The budget (see proposal) includes the cost of moving the source as well as periodic testing required by the Nuclear Regulatory Commission.

III. Effort of the Principal Investigator

The present term of this contract began August 1, 1975. Since then 20% of his time during the academic year has been spent on this work. The principal investigator will devote 11 weeks of the spring summer sessions to this project. The remaining portion of the summer will be spent on related work funded by the PRF.