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MECHANISMS FOR RADIATION DAMAGE IN DNA

Progress Report

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ABSTRACT

In this project we have proposed 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 this past year several investigations have been completed. These investigations are:

1. Reactions of the π-cations of thymidine TMP and 1-methylthymine.
2. Charge transfer in aromatic peptide π-cation radicals.
3. Electron reactions with amino acid anhydrides.

A study in which we have made significant progress in this past year is

4. π-Cation radicals in DNA and dinucleoside phosphates.

In the first study the cations of thymine derivatives were found to decay by two paths, hydroxyl ion addition and methyl group deprotonation. In the second it was found that π-cations of aromatic amino acid residues transferred the charge to the carboxyl group in dipeptides. This resulted in decarboxylation. In the last study it was found that the positive charge in DNA localizes mainly on the purine base guanine.

I. Results This Year

In the past year, two 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 and seminars presented on work funded under this contract are included in Section II.

a. Reactions of the π-Cations of Thymidine TMP and 1-Methylthymine

Recent e.s.r. investigations have proposed a number of radiation mechanisms in DNA. Results with γ-irradiated DNA at low temperatures have indicated that anion radicals are formed predominantly on thymine and the cation is formed on guanine. Other work by Gregoli, Olast, and Bertinchamps has suggested that the positive ions of purine and pyrimidine nucleotides react by OH^- addition to form a neutral radical.

In previous publications from this laboratory we reported that deprotonation of the methyl group was a mechanism for the reaction of the cation radicals of a number of 5-methylpyrimidine DNA bases in aqueous glasses. In this work we found evidence for both the above mechanisms in N_1-substituted thymine π-cations. The relative rate of the reactions is found to be dependent on the substituent at position 1 and the concentration of base. In 8M NaOD (NaOH) spectra suggestive of OD^- (OH^-) addition to position 6 in the π-cations of 1-methylthymine, and thymidine were found immediately after u.v. photolysis at 77K. Production of the same radicals by electron attachment to 1-methyl-5-bromo-6-hydroxythymine, and 5-bromo-6-hydroxythymidine in 8M NaOD confirms the OD^- addition mechanism. Results found for these brominated compounds in 12M LiCl(D_2O) after electron attachment show that the a_6(H)

splitting was sensitive to changes in substituents at position 1 as well as changes in environment. This variation in splitting is shown to be accounted for by small conformational changes in the radicals. In 8M $NaClO_4$ π-cations of the substituted thymines gave evidence for both deprotonation and OD^- addition.

This work is in press and will be published in Faraday Discussions Chemical Society, 1977. It is found as Appendix A in this report.

b. π-Cations of DNA and Dinucleoside Phosphates

In our most recent work we are performing a study of various dinucleoside phosphate and DNA π-cations. Photolysis of these molecules in 8M $NaClO_4$ at 77K has resulted in the finding that principally the guanine π-cation is stabilized in DNA or in DNP's containing guanine.

We believe there are two possible interpretations of the results found for DNA. First the observation of the guanine π-cation may simply be due to its selective photoionization. In agreement with interpretation it is found that guanine is somewhat more easily photoionized than adenine whereas the pyrimidines can not be photoionized in neutral glasses. However, under basic conditions where thymine can be photoionized still only the π-cation of guanine is observed. Interestingly, the signal due to the π-cation is much more intense in basic solutions. The second possibility is that photoionization from guanine, adenine, and thymine is followed by hole transfer to the DNA base with the lowest ionization potential (guanine). Since hole transfer through the stacked DNA bases is likely to occur and since on the average a guanine base should be only a few DNA bases from the original hole site, this mechanism has the most appeal.

This work is considered to be quite significant and should be completed during this upcoming year. The initial results reported above will be published as part of a chapter entitled "Mechanisms for Radiation Damage in DNA Constituents and DNA" in "Excited States in Organic and Biochemistry," B. Pullman and N. Goldblum, Eds., Reidel Press, 1977. This manuscript is included as Appendix B.

c. Charge Transfer in Aromatic Dipeptide Cations

In this work, which is jointly sponsored by the U.S. Army Natick Laboratory, we have found that decarboxylated radicals are produced when di- and tri-peptides with an aromatic amino acid residue next to the C-terminal are photoionized. The process of radical production is found to be biphotonic and intramolecular in nature. The radical produced is equal in concentration to O^- which originates from the photoejected electron.

The mechanism we find most consistent with these results is the intramolecular transfer of an electron from the carboxyl group to the photoionized aromatic ring in the peptide, followed by decarboxylation.[25] Since the process is occurring at 77K in a rigid aqueous glass, the peptides must be in a conformation which places the carboxyl group near the aromatic ring. The fact that the tripeptide phe-gly-gly and the individual aromatic amino acids show no decarboxylation supports the suggestion that the transfer process is strongly conformationally dependent. The incomplete transfer which is found for tyr and trp peptides in 8M $NaClO_4$ and the complete lack of transfer which is found for phe and trp peptides in 8M NaOD is likely due to conformations which lower the probability of hole transfer to the carboxyl group, and/or, in the case of tyrosine containing peptides, a competing mechanism,

i.e., the deprotonation of the π-cation of the phenol ring to form the phenoxyl radical. Presumably the stability of the phenoxyl radical would not allow for hole transfer.

This work has been submitted for publication and is included here as Appendix C.

d. Electron Reactions with Amino Acid Anhydrides

This work was completed during this past year and was very recently (June) published in Journal of Physical Chemistry. It is included as Appendix E.

II. Papers Presented and Seminars

Invited Papers:

"Mechanisms for Radiation Damage in DNA Constituents and DNA" 10th Jerusalem Conference on Quantum Chemistry and Biochemistry (Hebrew University) March 27-31, 1977.*

"Reactions of the N_1-Substituted Thymine π-Cations" the Faraday Division of the Chemical Society (University of Leicester) March 23-25, 1977.*

"ESR Studies of Radiation Induced Radical Ions in Biological Molecules" presented at the Radical Ions Gordon Research Conference, July 5, 1976.

Seminars:

"Charge Transfer on π-Cation Radicals of Aromatic Amino Acid Containing Dipeptides" presented at the Radiation Laboratory, Notre Dame, May, 1977.

"π-Cation Radicals of DNA Bases" presented at Wayne State University, November, 1976.

*See Appendix F for trip report.

III. Facilities

In the area of equipment upgrading we have had one success and one setback. We have now interfaced a Tektronics 4051 computer with 32K of memory and a digital plotter to the V-4500 ESR spectrometer. We have written the programs necessary for spectrum recording on tape, addition and subtraction of spectra, double integration, g value determination, and spectrum simulation. Since the 4051 can also be used as a terminal, we have access to the University's computer facilities to simulate complex spectra.

The acquisition of the Co-60 γ-irradiation source from Wayne State has run into several problems. The Nuclear Regulatory Commission requirements for safe transport and operation of the device at Oakland will necessitate extensive efforts due to the device's antiquated nature and a radiation leak through the top of the irradiator. The time required to design and fabricate the safety devices required (interlocks, warning alarms, fire sprinkler systems, increased shielding, enclosed room for source) does not make the device's acquisition very desirable at this time. Consequently, radiations will be performed at Wayne State for the near future. This will actually result in the most efficient use of our research time.

IV. Effort of the Principal Investigator

The present term of this contract began August 1, 1976. Since then 20% of his time during the academic year has been spent on this work. The principal investigator is devoting 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 U.S. Army Natick Development Laboratory.