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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 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 irradiated systems of DNA constituents which may shed light on indirect effects. In addition, studies of radiation effects on lipids have been undertaken which will shed light on the only other proposed site for cell kill, the membrane.

Studies which we have completed during this year are:

1. ESR Study of Radicals Produced by one electron loss from 6-Azauracil, 6-Azathymine and 6-Azacytosine: Evidence for both σ and π-Radicals

2. Hydrogen Abstraction Reactions by Amide Electron Adducts: A comparison to Acid, Ester, Aldehyde and Ketone Electron Adducts

Studies which we have made progress on are:

3. An ESR and INDO Study of the π-Cations of 5-Hydroxymethyl-uracil and 5-Hydroxymethyl-cytosine: Evidence for Intramolecular Hydrogen Bonding

4. Studies of the π-Cations of 5-Halouracils and 5-Halocytosines

5. Studies of Radiation Damage to Lipids

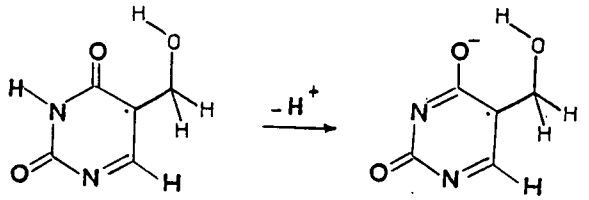
I. Results This Year

Since our last progress report two articles were accepted for publication and two more are in preparation. Below we briefly describe this work and other work which is not yet completed. Papers published and presented on work funded under this contract are listed in Section II.

In our most important work this year we have found the deprotonation from the exocyclic nitrogen in cytosine DNA - base π-Cations is a common occurance. This may have significance for DNA radiolysis. This hypothesis will be tested in next years efforts.

1. An ESR and INDO Study of the π-Cations of 5-Hydroxymethyluracil and 5-Hydroxymethyl-cytosine: Evidence for Intramolecular Hydrogen Bonding.

The π-cation radicals of 5-hydroxymethyluracil and 5-hydroxymethyl-cytosine have been produced by Cl_2^- attack in γ-irradiated basic 12M LiCl and photoionization in basic 8M $NaClO_4$ glasses at low temperatures. Analysis of the ESR spectra found for these radicals shows that each of the π-cation radicals converts to another species probably by a change in protonation state as the temperature is raised. For example 5-hydroxymethyluracil cation shows a 28.5 G splitting for the two hydroxymethyl group β-protons which convert upon annealing to a 36 G splitting, (reaction 1). The splittings and the narrowness of the linewidths found are suggestive of a configuration which is intramolecularly rigid, and stabilized by a intramolecular hydrogen bond from the hydroxyl proton to the 4 position oxygen. The π-cation of 5-hydroxymethylcytosine converts to a radical with substantial spin density on the exocyclic nitrogen which also shows strong evidence for intramolecular hydrogen bonding, (see reaction 2 below).



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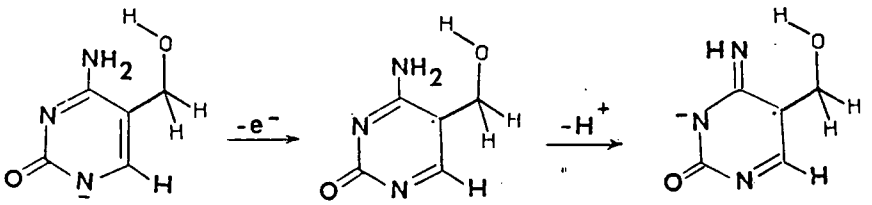
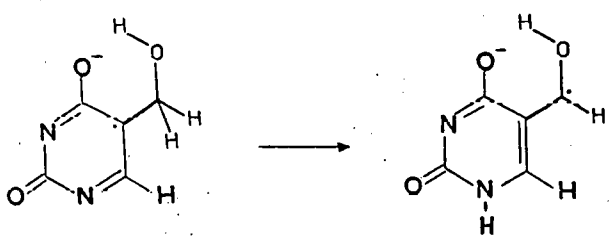


(2)

The deprotonation from the exocyclic nitrogen has been found to occur in 5-methylcytosine, cytosine and 6-azacytosine π-cations. It thus seems a general reaction mechanism for the cytosine moiety and thus may play a part in DNA radiolysis.

INDO calculations for the π-cation of hydroxymethyluracil as a function of orientation of the hydroxyl group show that the hydrogen bond to the 4 position oxygen increases in strength by a factor of three upon deprotonation at the 3-position nitrogen. The hydrogen bond is therefore predicted to substantially stabilize the π-cation radical.

Finally we find that both the π-cations of hydroxymethyl-uracil and hydroxymethylcytosine decompose upon anealing by deprotonation from the hydroxymethyl group as shown below for hydroxymethyl uracil.



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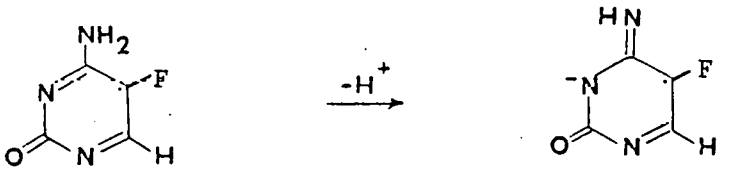
2. Studies of the π-cation Radicals of the 5-Halouracils and 5-Halocytosines.

Due to the enhanced effect of radiation on DNA containing 5-bromouracil in place of thymine, the radiation chemistry of 5-halouracils and their analogs has been investigated extensively in the past. The enhancement has been proposed to be due to electron capture by 5-bromouracil in DNA followed by debromination to form the reactive 5-uracilyl radical. While this reaction clearly remains the most likely explanation of the mechanism of radiation enhancement, still no investigation of another possible reactive species, the π-cation, has been performed in aqueous matrices.

In last years work we were able to produce the π-cation radicals of a number of 5-halopyrimidines including, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, and 5-iodouracil in an aqueous glass at low temperature. The π-cations were produced by attack of Cl_2^- and investigated by ESR spectroscopy. In this years work we have analyzed the spectra of most of the π-cations. This analysis of spectra is being done in collaboration with Dr. Jürgen Hüttermann of the University of Regensburg. This is necessary since the complete analysis of the spectra requires inclusion of nuclear quadrupole terms for Cl, Br and I and Dr. Hüttermann has perhaps the best simulation program in existence for such nuclei.

In addition in this years work we have produced the π-cation of 5-fluorocytosine. This radical shows a large fluorine coupling (A_{1F}=146 G) and a single nitrogen coupling (A_{1I}=13G) at low temperatures; however on warming to 160K the fluorine decreases dramatically to 115 G while two nitrogen splittings are found of nearly equal magnitude (A_{1I}=16.0G). These results are excellent evidence that reaction (4) is taking place.



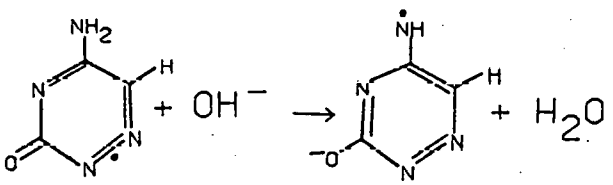
(4)

This is further evidence that cytosine π-cations decay by deprotonation of the exocyclic nitrogen.

3. ESR Study of Radicals Produced by One Electron Loss from 6-Azauracil, 6-Azathymine and 6-Azacytosine: Evidence for both σ and π-Radicals.

π-oxidation radicals of a number of pyrimidine DNA bases have been found to be produced by biphotonic photoionization in aqueous matrices, γ-irradiation of neat solids as well as by attack of OH• and Cl_2^- radicals. Although one σ reduction radical, 5-bromouracil σ* anion, has been reported, no σ-oxidation radicals of DNA bases have yet been reported.

In our last report we reported the first production of a σ-oxidation radical in a nucleic acid base analog, 6-azauracil. This radical is produced in an aqueous glass by attack of the relatively mild oxidizing agent, Cl_2^- Surprisingly, 5-methyl-6-azauracil (6-azathymine) was shown to form a π-oxidation radical under the same conditions. In this years work we have found that Cl_2^- attack on 6-azacytosine results in a σ-radical with nitrogen couplings within 1G of those found for 6-azauracil; however this radical converts to a new radical species whose ESR spectrum is suggestive of a π-radical. It is suggested that a change in the state of protonation (deuteration) in the 6-azacytosine radical results in the change from a σ to π radical (see reaction 5).



(5)

Anisotropic computer simulations are used to verify the analysis of spectra. INDO-MO calculations were performed to aid in the assignment of radicals to structures. A comparison of the INDO calculations of the nitrogen hyperfine splittings with experimental values found for the azauracil σ radical suggests a new three electron N-N bond forms along with concomitant bond shortening after electron loss.

4. Hydrogen Abstraction Reactions by Amide Electron Adducts: A Comparison to Acid, Ester, Aldehyde, and Ketone Electron Adducts.

In the last years work the electron reactions with a number of peptide model compounds (amides and N-acetylamino acids) in aqueous glasses at low temperature have been investigated using ESR spectroscopy. The protonated anion radicals of the amides, RCONHNR', are found to act as hydrogen abstracting agents. For example, the protonated propionamide anion was found to abstract from its parent propionamide. Anions of other amides investigated showed similar behavior except for acetamide anion which did not abstract from its parent compound, but did abstract from propionamide. A reinvestigation of previous work found for acetyl amino acid anions also showed evidence for hydrogen abstraction by the protonated anion from the parent compound. This mechanism was proposed as a likely competing reaction to secondary deamination of the anion reported previously. The relative abstraction tendency of the amide protonated anions with those of carboxylic acids, ester, aldehydes, and ketones was investigated with the tendency for abstraction increasing in the following order:

amides< ketones<carboxylic acids, esters<aldehydes

In this years work the abstraction tendencies of the unprotonated anions were compared to the protonated electron adducts. Without exception it was found that the anions were far weaker abstracting agents than the protonated electron adducts. The reactivity of the protonated anions toward abstraction has not been previously reported. These reactions are considered of signifi-

cance to highly concentrated solutions of biomolecules as in the cell.

5. Studies of Radiation Damage to Lipids.

In work principally funded by the U.S. Department of Agriculture we are investigating free radical reactions induced by the radiolysis of various lipids. We believe that these studies will shed light on the only other proposed site for cell kill, the membrane. Thus far a variety of neat lipids have been investigated. These results will be employed next year to aid our understanding of radiation induced lipid-DNA crosslinking reactions (see proposal).

II. Papers Accepted for Publication.

1. "ESR Study of Radicals Produced by One Electron Loss from 6-Azauracil, and 6-Azacytosine: Evidence for Both σ and π Radicals" M.D. Sevilla and S. Swarts, J. Phys. and Chem., in press.
2. "Hydrogen Abstraction Reactions by Amide Electron Adducts: A Comparison to Acid, Ester, Aldehyde and Ketone Electron Adducts" M. D. Sevilla, C. L. Sevilla, S. Swartz, Radiation Phys. and Chem., in press.

III. Papers Presented at Scientific Meetings.

1. "An ESR and INDO Study of the π-Cations of 5-Hydroxymethyluracil, 5-Hydroxymethylcytosine, and 5-Fluorocytosine: Evidence for Intramolecular Hydrogen Bonding" M. D. Sevilla, M. McGlashen, presented at the 30th Annual Meeting of The Radiation Research Society, Salt Lake City, April, 1982.
2. "An ESR Study of σ and π Radicals Produced in 6-Aza DNA Bases by One Electron Loss" M. D. Sevilla and S. Swartz, presented at the 183rd National Meeting of The American Chemical Society, Las Vegas, March, 1982.

IV. Effort of the Principal Investigator.

The present term of this contract began January 1, 1982. Since then fifteen percent of the principal investigator's time during the academic year has been spent on this work. The principal investigator will devote 10 weeks of the 15-week spring-summer sessions to this project.