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MASTER

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 the past year are:

1. π Cations Produced in DNA Bases by Attack of Oxidizing Radicals
2. INDO Studies of Radicals Produced in Peptides and Carboxylic Acid Model Compounds
3. Electron Reactions with Carboxylic Acids, Ketones and Aldehydes
4. γ-Irradiation of Esters and Triglycerides

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

5. Study of Radicals Generated in Model Compounds for the Sugar-Phosphate Backbone

The first study is of special significance since in it we show that $OH^{\bullet}$ attack on DNA bases can lead to π-cation

formation.

I. Results This Year

In the past year, five articles were published and three more were prepared for publication. 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.

1. DNA Base π-Cations Produced by OH· Attack

We have had good success this year in the study of π-cations of DNA bases produced by the indirect effect, i.e., the attack by OH· radical. In last year's work, we investigated OH· radical attack on thymine in 8M$NaClO_4$ glasses and found the thymine π-cation was produced. In this year's work, we have extended the study to over eleven DNA bases and analogs. We found that oxidizing intermediates OH·/Cl_2^- produced in γ-irradiated 12M LiCl produce the π-cations of all the DNA bases and uracil without a troublesome background signal found in $NaClO_4$ glasses. Compounds which produce π-cations include thymine, uracil, 3-methyluracil, 6-methyluracil, orotic acid, isoorotic acid, guanine, and adenine. Compounds which are not found to produce π-cations are 1-methylthymine, thymidine, and 1-methyluracil. The mechanism suggested for formation of the π-cations is direct electron transfer.

$$\text{Base} + \text{OH}\cdot\ (Cl_2^-) \longrightarrow OH^-\ (2Cl^-) + \text{Base Cation}$$

A pH and substituent dependence is also noted for the pyrimidines. At pH's where the nitrogen at position 1 is protonated, π-cation radicals are not formed. In addition, when there are

substituents at position 1, π-cations are not found. Analysis of the ESR spectra of the DNA base π-cations for hyperfine splittings and g values were performed by aid of computer simulations. The analyses are in agreement with values found for several DNA base cations produced by photolysis and by direct γ-irradiation of single crystals.

2. Computer Simulations of Anisotropic Spectra of DNA Bases

In recent years quite a number of single crystal studies of DNA constituents have been reported including complete A and g tensors with direction cosines. Since in most biological samples there is no crystalline order, it is important to be able to simulate the ESR spectra of DNA base radicals in a polycrystalline or randomly oriented matrix. Currently we have two anisotropic simulation programs functioning. One is a simple first order simulation program which includes the nuclear Zeeman term and variable linewidth. The second is the more sophisticated Maruani program which has been updated by Sagstuen and ourselves. This program can simulate polycrystalline spectra to the second order including the nuclear zeeman terms. It can handle g and A tensors in different axis systems and computes forbidden transitions. We have employed this program with good success in our recent work on DNA base π-cations (see section A and appendix).

In order to make effective use of the single crystal data now known we will continue to employ this program in our work.

3. Studies of Deoxyribose Phosphate Model Compounds

A number of model compounds for the deoxyribose-phosphate moiety in DNA are being investigated. The planned lines of investigation fall into three categories, studies of electron attachment reactions, studies of hydrogen atom and OH· attack, and photolytic damage. The model compounds chosen include dimethyl phosphate, dimethyl acid pyrophosphate, trimethyl phosphate, acetylphosphate, as well as ribose-phosphate molecules. This portion of our work is just beginning but we have produced the $\cdot CH_3$ and $\cdot CH_2OPO_3^{-2}$ radicals by photolysis and $\cdot CH_2OPO_3R^{-2}$ by hydrogen atom attack. The phosphorous coupling is clearly observed in these spectra. We should gain sufficient expertize in recognizing such couplings in our future planned work to elucidate mechanisms which involve the phosphate moiety.

4. INDO Studies of Radicals Produced in Peptides and Carboxylic Acids Model Compounds

Detail molecular orbital (INDO) calculations were performed for two model compounds of the peptide anion (acetamide anion) and carboxylic acid anion (acetic acid anion). Two important results were found. The first is that the anions are predicted to be quite nonplanar. Second, the relationship for the β-proton splitting as a function of orientation is found to be altered by this nonplanarity (see attached manuscript). We believe this result will have bearing on the interpretation of many radical ESR spectra with β-proton couplings.

5. Allied Work on Lipids

In work funded partly by the U.S. Army Natick Development Center, we have investigated radiation damage to lipids and model compounds. Since theories of cell damage and cell kill include the membrane as a possible site for such action, it is of importance to understand the mechanisms of radiation damage to membrane constituents. Our work has progressed from the simple carboxylic acids, to ketones, aldehydes, asters and finally triglycerides. We believe significant new mechanisms have been elucidated in each of these studies. This work is described in more detail below.

A. Electron Reactions with Carboxylic Acids, Ketones and Aldehydes

Electron reactions with a number of carboxylic acids, ketones and aldehydes were investigated in a deuterated neutral aqueous glass at 77K by ESR spectroscopy. Free radical reactions which took place upon warming the glasses to temperatures at which the electron adducts become mobile were studied. For carboxylic acids ($C_nH_{2n+1}CO_2D$, n=2,3) the deuterated anion radicals ($RCH_2\dot{C}(OD)_2$) give results which suggest abstraction of a hydrogen from the parent acid to produce radicals of structure $R\dot{C}HCO_2D$. The formic acid deuterated anion is found to undergo an analogous mechanism. The acetic acid deuterated anion is not found to abstract from acetic acid, but is found to abstract from isopropyl alcohol. Ketone electron adducts are also found to abstract from their parent compounds to yield radicals of structure $R\dot{C}HC(O)R'$ with R and R'= CH_3, C_2H_5.

Electron reactions with 5 aldehydes (RCHO with R=H, CH_3, C_2H_5, C_3H_7 and i-C_3H_7) gave their corresponding alcohol radicals ($R\dot{C}HOD$) after deuteration at 77K. Radicals with R=C_2H_5, C_3H_7, and i-C_3H_7 were found to abstract from the parent aldehyde to form radicals of structure $RR'\dot{C}CHO$. The methanol radical ($\dot{C}H_2OD$) showed no further reaction while the ethanol radical ($CH_3\dot{C}HOD$) resulted in the production of the acetoin anion. The results for acids, aldehydes and ketones show that radicals of structure $R\dot{C}(OD)R'$ with R'= OD, H, or an alkyl group can act as hydrogen atom abstracting agents.

B. Mechanisms of Radiation Damage to Esters and Triglycerides

Reactions which occurred after electron attachment at 77K to a number of small carboxylic acid esters and triglycerides in a aqueous glass are reported. Most ester anions are found to decay on warming to form alkyl radicals by β-scission.

$$RC(O^-)OR' \longrightarrow RCO_2^- + R'\cdot$$

The alkyl radical (R'·) produced by annealing is found to hydrogen abstract from the parent ester at an π-carbon site

$$R'\cdot + R''CH_2CO_2R' \longrightarrow R''-\dot{C}H-CO_2R'$$

or in the case of ethylformate from the formate hydrogen

$$CH_3CH_2\cdot + HCO_2C_2H_6 \rightarrow C_2H_6 + \cdot CO_2C_2H_6$$

Results found for methyl formate anion suggest hydrogen abstraction by the anion itself may compete with alkyl radical formation. The anion of the triglyceride triacetin, is found

to undergo an analogous mechanism to the ester anions producing the propane diol diester radical, $\cdot CH_2CH(OAc)CH_2(OAc)$, Ac=acetate. This species subsequently hydrogen abstracts from the parent compound to produce the α-carbon radical, $\cdot CH_2CO_2-$. Results found after annealing tripropionin radical anion give evidence for abstraction from the α-carbon in the propionate side groups producing, $CH_3\dot{C}HCO_2-$. Studies of a γ-irradiated ester (ethylmyristate) and two triglycerides (tripalmitin and tristearin) yield results which suggest that the mechanism of ester anion decay found in aqueous glasses applies to γ-irradiated neat long chain esters and triglycerides. Results found in this work are compared to the results of product analysis.

II. Papers Published and Submitted for Publication

1. "An ESR Study of DNA Base Cation Radicals Produced by Attack of Oxidizing Radicals." M.D. Sevilla, D. Suryanarayana and K.M. Morehouse, submitted for publication.

2. "An Electron Spin Resonance Study of γ-Irradiated Frozen Aqueous Solutions Containing N-Acetylamino Acids." M.D. Sevilla, J.B. D'Acry and K.M. Morehouse. J. Phys. Chem., 83 2893(1979).

3. "An Electron Spin Resonance Study of γ-Irradiated Frozen Aqueous Solutions Containing Dipeptides. Mechanisms of Radical Reaction." Michael D. Sevilla, James B. D'Arcy, and Kim M. Morehouse. J. Phys. Chem., 83, 2887(1979).

4. "ESR Studies of Barriers of Ring Inversion in Cyclic Monocarboxylic Acid Radicals." D. Suryanarayana and Michael D. Sevilla, J. Chem. Phys., 72, 1325(1980).

5. "An INDO Study of the Anion Radicals of Acetic Acid and Acetamide: Non Planarity and Barriers to Methyl Group Rotation." Michael D. Sevilla and D. Suryanarayana, J. Phys. Chem., accepted for publication.

6. "Electron Spin Resonance of Radiation Produced Free Radicals." Michael D. Sevilla, J. Chem. Ed., accepted for publication.

7. "An ESR Study of Electron Reactions with Carboxylic Acids, Ketones and Aldehydes in Aqueous Glasses." M.D. Sevilla, S. Swarts, R. Bearden, K.M. Morehouse, and T. Vartanian, submitted for publication.

8. "An ESR Study of Electron Reactions with Esters and Triglycerides." M.D. Sevilla, K.M. Morehouse and S. Swarts, submitted for publication.

III. Papers Presented at Scientific Meetings

1. "An ESR Study of γ-Irradiated Frozen Aqueous Solutions of N-Acetylamino Acids and Dipeptides." M.D. Sevilla, J.B. D'Arcy and K.M. Morehouse, presented at the 178th Annual Meeting of the American Chemical Society, September, 1979.

2. "Electron Spin Resonance Studies of Barriers to Ring Inversions in Cyclic Monocarboxylic Acids." D. Suryanarayana and M.D. Sevilla, presented at the 178th Meeting of the American Chemical Society, September, 1979.

3. "Electron Reactions with Carboxylic Acids Esters, Ketones and Triglycerides." M.D. Sevilla, presented as an invited lecture in the Symposium on "ESR Studies of Transients in Irradiated Systems" at the 28th Annual Meeting of the Radiation Research Society, June, 1980.

4. "DNA Base Cation Radicals Produced by Hydroxyl Radical Attack: An ESR Study." M.D. Sevilla and K. Morehouse, presented at the 28th Annual Meeting of the Radiation Research Society, June, 1980.

5. "ESR and INDO Studies of Anion Radicals in Acetic Acid and Acetamide." D. Suryanarayana and M.D. Sevilla, presented at the 22nd Rocky Mountain Conference of Analytical Chemistry, Denver, August, 1980.

6. "Electron Spin Resonance of Radiation Produced Free Radicals." M.D. Sevilla, presented at 180th Meeting of the American Chemical Society, August, 1980.

7. "An ESR Study of DNA Base Cation Radicals Produced by Hydroxyl Radical Attack." Michael D. Sevilla and K.M. Morehouse, Second Chemical Congress of the North American Congress, Las Vegas, August, 1980.

IV. Effort of the Principal Investigator

The present term of this contract began January 1, 1980. Since then, fifteen percent of the principal investigator's time during the academic year has been spent on this work. The principal investigator devoted seven weeks of the spring-summer sessions to this project. The remaining portion of the summer was spent on related work funded by the U.S. Army Natick Development Laboratory.