TK
C00-2364-1

MECHANISMS FOR RADIATION DAMAGE IN DNA

Progress Report

Michael D. Sevilla
Oakland University
Rochester, Michigan

June 1, 1973 - May 31, 1974

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

Prepared for the Division of Biomedical and Environmental Research
of the U.S. Atomic Energy Commission under Contract No. AT(11-1)-2364

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

TABLE OF CONTENTS

	Page
Abstract.....	1
I. Introduction	2
II. Results to Date.....	3
A. Negative Ions of the DNA Bases.....	3
B. Positive Ions of the DNA Bases.....	4
C. Proposed DNA Studies.....	4
D. Related Work,.....	5
1. Reactions of Electrons with 5-Halouracils	5
2. Reactions of Electrons with Amino Acids and Peptides.....	5
Appendix A. An ESR Study of Pyrimidine, Pyrimidine Nucleoside and Halopyrimidine Anions	
Appendix B. An ESR Study of Several DNA Base Cation Radicals Produced by Photoionization	
Appendix C. Reactions of The Cation and Anion Radicals of Several DNA Bases	
Appendix D. Radicals Formed by the Reaction of Electrons with Amino Acids and Peptides in a Neutral Aqueous Glass	
Appendix E. Radicals Formed After Electron Attachment to 5-Halouracils in Aqueous Glasses	
Appendix F. The Purine DNA Base Cation and Anion Radicals: An ESR Study	

Reprint removed
Reprint removed
Reprint removed
Reprint removed
Reprint
Reprint

The work for appendices A, B and C was completed before funding of this investigation by the Atomic Energy Commission.

App A Photochemistry and Photobiology, 1972, Vol 15, pp 407-409. Pergamon Press Printed in Great Britain

App B [Reprinted from the Journal of Physical Chemistry, 76, 3571 (1972).]
Copyright 1972, by the American Chemical Society and reprinted by permission of the copyright owner.

App C [Reprinted from the Journal of Physical Chemistry, 76, 3577 (1972).]
Copyright 1972, by the American Chemical Society and reprinted by permission of the copyright owner.

App D [Reprinted from the Journal of Physical Chemistry, 77, 2954 (1973).]
Copyright 1973 by the American Chemical Society and reprinted by permission of the copyright owner.

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 mechanism. Thus far we have produced and investigated the positive and negative ions of a number of purine and pyrimidine DNA bases in aqueous glasses. The g values and hyperfine couplings, both anisotropic and isotropic, have been determined when possible. It has been found that 5-methyl substituted pyrimidine cations (thymine, thymidine, 5-methylcytosine and 5-methylcytidine) react in these aqueous systems by deprotonation of the 5-methyl group. The thymine anion has been found to protonate in neutral aqueous glasses. Investigations of the cation and anion radicals of the other DNA bases have shown a lessened tendency toward further reaction. In related work we have studied the reactions of electrons with 5-halouracils as well as amino acids and peptides. In our work with 5-halouracils we have isolated the anions found their relative stability and followed their further reactions. Our results were found to be in agreement with those of other experimental methods.

Introduction

This project proposes a mechanism for radiation damage to DNA and suggests a series of experiments to test the proposed mechanism.

Briefly described the mechanism is that positive and negative ions formed with the DNA strand migrate through the stacked DNA bases and subsequently react with the DNA base most likely to react towards protonation of the anion and decomposition of the cation. It is suggested that the thymine anion and thymine cation are the DNA base ions which most probably will react in this manner. Thus ions formed within the DNA strand would localize the radiation damage predominantly on the thymine base.

In our previous proposal we suggested work along three lines to test the proposed mechanism for radiation damage to DNA. First the anions of the DNA bases would be produced and their further reactions studied. Second the positive ions of the DNA bases were to be produced and their further reactions investigated. Finally experiments on γ -irradiated DNA were to be performed.

II. Results to Date

We have made good progress on the studies of the positive and negative ions of the DNA bases. These results are described briefly below and in more detail in our publications and preprints which are included. It should be noted that some of the work described was performed during the period between the approval of the proposal (September, 1971) and its funding (June, 1973). This work was generously supported by Oakland University.

A. Negative Ions of the DNA Bases.

In an initial report (Appendix A) we produced the anions of a variety of pyrimidine DNA bases. We showed in this work that the 6 position carbon which is known to be the site of protonation in the thymine anion is also the site of high unpaired spin density. We later studied the protonation reactions of thymine anion and other pyrimidines (Appendix C). We found that thymine protonates most readily among the pyrimidines.

In work performed during the past year we have studied the purine anion radicals for possible protonation reactions and to determine their g values for possible identification of these species in single crystals. (Appendix F). We are presently investigating these possible reactions in nucleoside and nucleotide anions in both neutral (12M LiCl) and alkaline (5M K_2CO_3) glasses.

B. Positive Ions of the DNA Bases

We have thus far investigated the positive ions of thymine, thymidine, 5-methylcytosine, and 5-methylcytidine (Appendices B, C). We have found that the principal reaction for these 5-methyl substituted pyrimidines is deprotonation of the methyl group.

In the past year we have produced the positive ions of the purine DNA bases and found their g values for possible identification of these species in single crystals (Appendix F).

At present we are investigating the positive ions of purine and pyrimidine nucleosides and nucleotides to determine whether possible deprotonation reactions occur at the ribose group (see proposal page 18). This would be a possible reaction mechanism in DNA radiolysis and is therefore of interest.

C. Proposed DNA Studies

We have not yet begun our investigations of γ -irradiated DNA. Since we plan to perform experiments on DNA in conjunction with our studies of the DNA subunits, we will perform experiments this next year as outlined in the proposal. It is of course most important to the final analysis of the ESR spectrum of γ -irradiated DNA that the reactions of the individual subunits be known. Since it is our thesis that most of the damage in DNA should at some intermediate state be localized on thymine and since we

investigated the likely reactions of the thymine ion precursors, we believe these preliminary investigations of DNA are justified at this time.

D. Related Work

1. Reactions of Electrons with 5-Halouracils

The proposed mechanism for radiation damage to DNA is applicable to 5-bromouracil containing DNA as well. It is well known that 5-bromouracil undergoes reductive debromination to form a uracilyl radical. In DNA it has been proposed that the uracilyl radical formed abstracts from a rebase group and this ultimately results in strand breakage (J. E. Zimbrick, J. F. Ward and L. S. Myers, Jr. Int. J. Radiat. Biol., 16, 505 (1969)). We would simply add that electron addition to bromouracil comes by migration of the electron through the stacked DNA bases in the DNA strand. In our work on 5-halouracils we have been the first in ESR experiments to isolate the anions of the 5-halouracils and follow their reactions (Appendix E).

Our results were found to be in agreement with other techniques. Of special interest was the observation that the uracilyl radical was found to hydrate to form a somewhat more stable radical species. This is suggested as a possible competing mechanism with abstraction in DNA.

2. Reactions of Electrons with Amino Acids and Peptides

Our work on electron reactions with amino acids and peptides is the latest in a series of papers dealing with

this subject. (Appendix D). In this work we have studied the influence of pH on subsequent reactions after electron attachment. We found that the relative stabilities of the various radicals produced after electron attachment were changed; however, new radical species were in general not produced due to the change in pH. Although this work is not directly related with the proposed work, eventually such work should have significance to the radiolysis of DNA on chromosomes. In such systems DNA is surrounded by protein (histones) and can be attacked by radicals generated in the histones.

RADICALS FORMED AFTER ELECTRON ATTACHMENT TO
5-HALOURACILS IN AQUEOUS GLASSES

M. D. Sevilla, R. Failor and G. Zorman
Department of Chemistry
Oakland University
Rochester, Michigan 48063

ACCEPTED FOR PUBLICATION IN THE JOURNAL OF PHYSICAL CHEMISTRY

Abstract

Radicals produced after electron attachment to 5-bromo-6-methyluracil and the 5-halouracils at 77°K in neutral (12M LiCl) and alkaline (8M NaOH) aqueous glasses have been investigated by ESR spectroscopy. Electron attachment to 5-bromo-6-methyluracil in 12M LiCl at 77°K results in an anion radical. The anion dehalogenates upon warming to 150°K to form the 6-methyluracil-5-yl radical. ESR spectra indicate that upon further warming this second species undergoes hydrolysis to form the 5-hydroxy-6-methyl-(5,6)-dihydrouracil-6-yl radical. The results for the halouracils in 12M LiCl suggest stable anions for bromo-, chloro- and fluorouracil at 77°K. Warming the bromouracil anion to 155°K results in a spectrum attributed to the uracilyl radical. The chloro- and fluorouracil anions are stable at this temperature. Warming to 165°K results in the hydrolysis of the uracilyl radical to form a radical analogous to that found for 5-bromo-6-methyluracil. Some hydrolysis is noted for chlorouracil indicating partial dehalogenation of the anion. The fluorouracil anion remained stable at this temperature. In 8M NaOH the π -anions were found to be somewhat less stable. The π -anions of 5-bromouracil and 5-bromo-6-methyluracil were not observed at 77 K; however, those of chloro- and fluorouracil were observed. The difference in stability of the anions in the two glasses is attributed to the fact that the nitrogens are protonated in the neutral glass whereas they are not in the alkaline glass. In addition protonation of the anion at an oxygen in the neutral glass is also likely. This would produce a neutral radical and further stabilize the radical.

Introduction

The radiation sensitivity of 5-bromouracil containing DNA has produced considerable interest in the radiation chemistry of 5-halouracils.² This sensitivity has been attributed to the dehalogenation of bromine after attack by radiolytic intermediates.² A number of studies employing differing techniques have shown that one of these radiolytic intermediates, the electron, induces reductive dehalogenation in 5-halouracils.³⁻⁵ The primary intermediates, the anion radicals, have been observed in pulse radiolytic experiments in the cases of fluorouracil and chlorouracil.⁴ The bromouracil anion has not been observed in pulse radiolytic experiments.

Several esr studies have been performed on the halouracils. In an esr investigation employing steady state radiolysis to generate electrons in aqueous solution it was found that the lifetimes of the halouracil anions were too short to be observed.⁵ Esr studies on γ -irradiated single crystals of 5-halouracils have given evidence for radicals which could be interpreted as protonated anions; however no evidence for dehalogenation was found.^{6,7}

Esr studies in alkaline aqueous glasses at low temperature have resulted in reports that the anion radicals of the 5-halouracils are stable at 77°K⁸ and that they are unstable toward dehalogenation at this temperature.⁹ In order to resolve this apparent discrepancy as well as to further elucidate the mechanisms of reaction after electron attachment to 5-halouracils, we have investigated the reaction of electrons with these compounds in neutral and alkaline aqueous glasses.

Experimental Section

5-Halouracils were obtained from Calbiochem (A grade) and used without further purification. 5-Bromo-6-methyluracil was synthesized from 6-methyluracil by the method of Sasaki and Ando.¹⁰ The product was recrystallized from ethanol.

The experimental procedure was essentially that used in our previous work in 8M NaOD and 12M LiCl.^{9,11,12} In this technique a solution of 12M LiCl/H₂O, 12M LiCl/D₂O (98% D) or 8M NaOD (ca. 92% D) containing 10 mM K₄Fe(CN)₆ and ca. 1 mM of the halouracil is cooled to 77°K. The glass formed is photolized with 254 nm light at 77°K for times usually less than one minute. Trapped electrons formed in the photolysis are photobleached with filtered light from an incandescent lamp. The filter used (a mixed solution of K₄Fe(CN)₆ and K₃Fe(CN)₆) filtered uv and blue light. This was necessary as several of the anion radicals formed dehalogenated when exposed to unfiltered light.

The g values and hyperfine splittings in this work are measured relative to potassium peroxyamine disulfonate ($A_N = 13.0$ G and $g = 2.0056$). The g values are measured from the center of the spectrum and are reproducible to ± 0.0003 .

Results and Discussion

1. 5-Bromo-6-Methyluracil

5-Bromo-6-methyluracil in a neutral glass: 5-Bromo-6-methyluracil (BrMeU) was investigated to aid our interpretation of the results found for the halouracils which follow. The substitution of a methyl group at position six allows for a clear distinction between the various radicals formed after electron attachment.

The reaction of electrons with BrMeU in 12M LiCl/D₂O at 77°K resulted in the spectrum in Figure 1A. Analysis of the ca. 1:3:3:1 quartet yields a 16.8 G methyl group splitting with $g = 2.0026$. This initial spectrum could be due to the anion radical or the 6-methyluracil-5-yl radical (radical II) produced by debromination of the anion. The following evidence strongly supports the anion as the initial species. First, the methyl group splitting is nearly identical to that found for the methyl group in the 6-methyluracil anion.⁸ Next resolution of the methyl proton splittings would not be expected for the 6-methyluracil-5-yl radical due to the nature of the interaction in σ -radicals. Finally the observed splitting is in agreement with π -electron spin density calculations which indicate a large spin density ($\rho \approx 0.5$) at position 6 in all uracil and halouracil anions.^{8,13,14} Warming the anion to 140°K or irradiating with unfiltered light from an incandescent lamp gave the 24 G singlet shown in Figure 1B. This spectrum is attributed to radical III produced by debromination as shown in reaction 1. Resolution of the methyl proton splittings is not found for this radical as expected.

Further warming of radical II in LiCl/H₂O to 160°K resulted in the spectrum shown in Figure 1C. The spectrum consists of 5 lines in the approximate ratio of intensities 1:3:4:4:3:1. Interpretation of the spectrum yields a 19.5 G splitting due to three equivalent protons and a 42 G splitting due to a single proton. The only radical consistent with such splittings is a radical with one β -proton at the 5-position and a radical site at position 6. This strongly suggests that the hydration reaction (reaction 2) takes place on warming to form the 5-hydroxy-6-methyl-(5,6)-dihydrouracil-6-yl radical (III).

This is confirmed by the fact that a quartet of 20 G is observed in LiCl/D₂O after warming to 170°K. Due to the wide linewidths the β -deuteron splitting is not resolved. To eliminate the possibility that a bimolecular reaction of the radical with the parent compound was occurring, experiments were performed where the original concentration of BrMeU was varied over 10 fold in LiCl/H₂O. The results showed no change in the rate of production of the third radical species with concentration as would be expected for a hydration reaction.

5-Bromo-6-Methyluracil in an Alkaline Glass: Electron attachment to BrMeU in 8M NaOD at 77°K resulted in an esr spectrum consisting of a 8 G

wide singlet at $g = 2.0022$. Since a quartet spectrum is expected for the π -anion,¹⁵ the results suggest the π -anion radical is not stable in 8M NaOD. The observed spectrum is associated with the 6-methyluracil-5-yl radical (radical IV).

Although radical IV shows no more resolution than the very similar radical II, the linewidth for radical IV is considerably less than that found for II. This may be due to the structural differences in the two radicals. Warming radical IV to 160°K resulted in a second radical perhaps due to a hydration reaction. However this spectrum has not yet yielded to interpretation.

The greater stability of the anion in the neutral glass over that found in the alkaline glass may be due in part to the fact that the nitrogens are protonated in the neutral glass. In addition the production of a neutral radical by protonation of an oxygen is also likely. This would further stabilize the radical. Such oxygen protonated radicals have been suggested from studies of the γ -irradiation of 5-halouracil single crystals,⁶ and the pulse radiolysis of thymine in aqueous solution.¹⁶

2. 5-Halouracils in a Neutral Glass

5-Bromouracil: Electron attachment to 5-bromouracil (BrU) at 77°K in 12M LiCl/D₂O or /H₂O results in a 13 G doublet spectrum at $g = 2.0025$ as shown in Figure 2A. Warming to 155°K results in an irreversible conversion to a broad 31 G singlet in D₂O (Figure 2B). In H₂O the singlet is somewhat more broad. In light of the results found for MeBrU, where the well resolved anion converts to a broad singlet, a reasonable explanation of these results

Is that the initial spectrum is due to the anion or protonated anion and the second broadened spectrum is due to the uracil-5-yl radical (V). We therefore tentatively assign the initial species to the anion radical and the second species to the uracilyl radical. Upon warming the second species to 160°K in LiCl/H₂O a quartet spectrum shown in Figure 2C is found. Interpretation of this spectrum results in a 42 G (β -proton) splitting and a 20.5 G (α -proton) coupling. In D₂O only a 19 G doublet is observed. These results are excellent evidence for the hydration of the uracilyl radical to produce radical VI as in reaction 3.

If samples are warmed directly to 165°K where the glass softens to allow radical migration the quartet spectrum appears in much lessened intensity. This is attributed to radical-radical recombination which competes with the hydration reaction at these temperatures.

5-Chlorouracil: Electron attachment to 5-chlorouracil (ClU) in 12M LiCl/D₂O or /H₂O results in an esr spectrum consisting of a 15 G doublet splitting at $g = 2.0029$ (Figure 2D). The radical remained stable to 165°K where the glass softens. The g value, hyperfine splitting and temperature stability of this radical strongly suggest that it is the anion or protonated anion. No evidence is found for a broad singlet spectrum expected for a uracilyl radical; however, a partial conversion to a quartet identical to that found for Br-U is found to appear at 165°K in some experiments. This may be due to partial dehalogenation of the anion caused by the warming or more likely by light during photobleaching and transfer.

5-Fluorouracil: Electron attachment to 5-fluorouracil (FU) at 77°K results in evidence for an exceptionally stable anion. Over the temperature range 77°K to 170°K a spectrum is found consisting of a 13 G doublet further split by a second doublet of 5.3 G with $g = 2.0033$ (Figure 2E). This second splitting is likely due to interaction with the fluorine atom in the anion. This is reasonable in light of the nuclear spin of 1/2 for fluorine and the fact that even a small spin density on a fluorine can result in a relatively large hyperfine splitting.¹⁷ No temperature dependence in hyperfine splitting or g value was observed.

3. 5-Halouracils in an Alkaline Glass:

Reaction of electrons with the 5-halouracils in 8M NaOD resulted in stable π -anions in two cases. Electron attachment to FU at 77°K resulted in an esr spectrum whose analysis yielded a 12 G splitting at $g = 2.0033$. The g value is the same as found in LiCl and is good evidence for a π -anion radical. No further reaction was noted upon photobleaching with unfiltered light or warming. At 77°K electron attachment to ClU resulted in an initial spectrum indicative of the π -anion. The spectrum consists of a 14 G doublet at $g = 2.0028$ (Figure 3A). The π -anion was light sensitive and upon irradiation with unfiltered light from an incandescent lamp for 10 minutes converted to a much more well resolved 13.1 G doublet splitting at $g = 2.0022$ (Figure 3B). Irradiation for intermediate time periods resulted in spectra which showed an overlap of both the π -anion and the second radical. Electron attachment to BrU at 77°K resulted in a spectrum identical in hyperfine splitting, g value and lineshape to the second radical found for ClU. In addition both the second radical from ClU and the BrU radical power saturated readily; thus, low microwave power levels were utilized in the recording of their spectra. These spectra are likely due to the uracil-5-yl radical (radical VII) produced

by dehalogenation of the π -anions of ClU and BrU. Several findings support this conclusion. The low g value is indicative of a sigma radical.¹⁸ The 13 G splitting observed for this radical is in the range expected for sigma radicals of aromatic systems. For example the phenyl radical has an ortho splitting of 18 G whereas the 2-pyridyl radical has an ortho hydrogen splitting of 8 G.¹⁹ The fact that BrU and ClU give identical spectra is also good evidence for dehalogenation. Finally the improved resolution of the uracilyl radical in NaOD over that found in LiCl/D₂O is in agreement with the results found for 6-methyluracil-5-yl in the two media.

In light of these results we must correct our previous work where we reported stable anions for all halouracils in 8M NaOD at -160°C.⁸ In our present work we have shown that the BrU π -anion is unstable in 8M NaOD even at 77°K; whereas, the FU and ClU π -anions are found to be stable at 77°K. Since in the previous work samples were photobleached with unfiltered light the ClU π -anion was likely predominantly converted to the uracilyl radical. The report by Simpson and Zimbrick that all halouracil anions are unstable toward dehalogenation at 77°K in 8M NaOD is also in disagreement with the results of this work. These workers do not report photobleaching with filtered light. This would explain the lack of observance of the ClU π -anion. Since the BrU π -anion is unstable it would also not be observed; however, the FU π -anion is stable to visible light and should be observed.

An explanation for the difficulty in the identification of the radicals observed in 8M NaOD in our work and other work is that the halouracil π -anions and the uracilyl radical have very similar spectra in the alkaline glass.

However as we have indicated these radicals can be distinguished by differences in g value, lineshape and power saturation behavior.

Comparison to Other Work and Conclusions

A comparison of our results with some recent work is of interest. Bansal, Patterson and Schuler in work employing conductometric pulse radiolysis report that 15%, 50% and 80% of the hydrated electrons in aqueous solution react to form F^- , Cl^- and Br^- from the respective halouracil.³ The yields of halide ion found are not directly comparable to our results; however, they are reasonable in light of the relative stabilities of the anions found in our work. Patterson and Bansal further report in a separate pulse radiolysis study that the stabilities of the halouracil anions are in the order: $FU > ClU > BrU$.⁴ We find the same order of stabilities in the neutral and alkaline glasses. We also find that the anions of the halouracils are more stable in the neutral glass than in the alkaline glass. This is likely due to protonation of the anion at one of the oxygens to produce a neutral radical as was suggested to explain the similar results found for the $BrMeU$ anion in the two media. Patterson and Bansal also report that the uracilyl radical gave a hydrolysis intermediate. This finding is in good agreement with the results found here. However, we have been able to identify the hydrolysis intermediate as radical VI.

Zimbrick, Ward and Myers have hypothesized a mechanism for radiation sensitization of BrU -DNA.² They suggest that electron attachment to BrU in BrU -DNA results in the uracilyl radical and that this species subsequently abstracts from an adjacent deoxyribose group. The final step leads to single strand breakage. This mechanism is quite plausible. However, we can suggest

that the hydration of the uracilyl radical is a possible competing reaction with the abstraction reaction. This is reasonable in light of the fact that the uracilyl radical would have restricted movement in the DNA structure. The hydrated radical if formed could of course also abstract from the ribose group; although, this would be less favored energetically than abstraction by the uracilyl radical.

Acknowledgment

The authors would like to thank Virginia Brooks for synthesis of 5-bromo-6-methyluracil.

References and Footnotes

1. This research was supported in part by the Division of Biomedical and Environmental Research of the U.S. Atomic Energy Commission.
2. J. E. Zimbrick, J. F. Ward and L. S. Myers, Jr., Int. J. Radiat. Biol., 16, 505 (1969); ibid., 16, 525 (1969).
3. K. M. Bansal, L. K. Patterson and R. H. Schuler, J. Phys. Chem., 76, 2386 (1972).
4. L. K. Patterson and K. M. Bansal, ibid., 76, 2392 (1972).
5. P. Neta, ibid., 76, 2399 (1972).
6. J. Hüttermann and A. Müller, Int. J. Radiat. Biol., 15, 297 (1969).
7. E. G. Nice and D. Rorke, ibid., 15, 197 (1969).
8. M. D. Sevilla and C. Van Paemel, Photochem. Photobiol., 15, 407 (1972).
9. L. D. Simpson and J. D. Zimbrick, Radiat. Res., 51, 459 (1972).
10. T. Sasaki and M. Ando, Bull. Chem. Soc. Jap., 41, 2215 (1968).
11. M. D. Sevilla, J. Phys. Chem., 74, 669 (1970).
12. M. D. Sevilla, C. Van Paemel and G. Zorman, ibid., 76, 3577 (1972).
13. J. Baudet, G. Berthier and B. Pullman, Compt. Rend., 254, 762 (1962).
14. M. D. Sevilla, J. Phys. Chem., 75, 626 (1971).
15. Due to the fact that the nitrogens of the parent compound are deprotonated in this alkaline matrix, this species is likely a trianion radical. To emphasize the fact that it is the π -electron system which has gained the electron the radical is designated a π -anion radical.
16. L. M. Theard, F. C. Peterson and L. S. Myers, Jr., J. Amer. Chem. Soc., 75, 3815 (1971).
17. A. Carrington and A. D. McLachlan, "Introduction to Magnetic Resonance" Harper and Row, New York, 1967, p. 112.
18. R. W. Fessenden and R. H. Schuler, J. Chem. Phys., 39, 2147 (1963).
19. J. E. Bennett and B. Mile, J. Phys. Chem., 75, 3432 (1971).

Figure Legends

- Figure 1 ESR spectra of radicals formed by electron attachment to 5-bromo-6-methyluracil in 12M LiCl. A. The anion radical(I) or its protonated analogue in D_2O at 77°K. B. The 6-methyluracil-5-yl radical (II) in D_2O after warming to 150°K. C. The 5-hydroxy-6-methyl-(5,6)-dihydrouracil-6-yl radical (III) after further warming to 165°K in H_2O .
- Figure 2 ESR spectra of radicals formed by electron attachment to 5-halouracils in 12M LiCl. A. The 5-bromouracil anion or its protonated analogue at 77°K in D_2O . B. The uracil-5-yl radical (V) produced by warming the bromouracil anion to 155°K in D_2O . C. The 5-hydroxy-(5,6)-dihydrouracil-6-yl radical (VI) produced by warming V to 160°K. D. The chlorouracil anion or protonated analogue at 77°K in D_2O . E. The fluorouracil anion or protonated analogue at 140°K in D_2O .
- Figure 3 ESR spectra of radicals formed by electron attachment to 5-chlorouracil in 8M NaOD. A. The δ -anion radical at 77°K. B. The uracil-5-yl radical at 110°K formed by photolyzing the radical in A with unfiltered visible light.

The Purine DNA Base Cation and
Anion Radicals: An ESR Study

by M. D. Sevilla and P. Mohan

Department of Chemistry
Oakland University
Rochester, Michigan

Running Title: Purine Ion Radicals

Introduction

The radical ions of the DNA bases have recently been reported as the primary intermediates in the radiolysis of DNA. Graslund et al. report that the anionic radical in γ -irradiated DNA is likely that of thymine (or cytosine) and the cationic radical is that of guanine (or cytosine). For these results it is clear that investigations of the ion radicals of the individual DNA bases are of importance to the understanding of the effect of radiation on DNA.

The cation and anion radicals of the pyrimidines, thymine and cytosine, have been investigated and their further reactions elucidated in a number of ESR studies employing aqueous glasses (Holroyd and Glass 1968, Srinivasan, Singh and Gopal-Agengar 1969, Lion and van De Vorst 1971, Sevilla and Van Paemel 1971, Sevilla, Van Paemel and Nichols 1972, Sevilla, Van Paemel and Zorman 1972). The ion radicals of the purines have not been as thoroughly investigated partially due to the poor resolution found for their ESR spectra. However Lion and van De Vorst report studies of the anion radicals in alkaline aqueous glasses at low temperatures. The anions are reported to undergo protonation reactions upon UV irradiation. Both the anions and cations of the purines, adenine and guanine, have been reported in ESR studies of γ -irradiated polycrystalline purines (Dertinger and Hartig 1972). Only the g values are reported by Dertinger and Hartig due to poor resolution of the ESR spectra.

In this work we have investigated the cation and anion radicals of the purine DNA bases in neutral aqueous glasses. The purpose of this investigation was to determine the g values of the purine ion radicals for comparison to those found in polycrystalline purines and to investigate possible protonation reactions of the purine anions in a neutral aqueous medium.

Materials and Methods

Several techniques were employed to generate ion radicals of adenine and guanine. The positive ions were produced by photoionization (254 nm) at 77°K of 10^{-3} M purine in 12M LiCl (98% D₂O). The photoionization process has been previously described and suggested to be a biphotonic process (Sevilla, Van Paemel and Nichols 1972, Helene, Santus and Douzou 1966). To produce the cation without interference from the photoejected electron

$K_3Fe(CN)_6$ ($\leq 10^{-2}M$) was added as an electron scavenger. To produce the anions of the purines without interference from the cations $10^{-2}M K_4Fe(CN)_6$ in $12M LiCl(D_2O)$ was photoionized in the presence of $10^{-3}M$ purine.

The purines were obtained from Schwarz. The g values were measured vs. peroxyamine disulfonate, $g = 2.0056$, utilizing a Varian V-4500-10A ESR spectrometer.

Results and Discussion

The ESR spectra after photolysis of samples containing adenine or guanine in $12M LiCl(D_2O)$ consist of unresolved singlets which are considered to be the overlap of the spectra of the purine cation, trapped electron and a small amount of purine anion. Photobleaching ($\lambda \geq 4300\text{\AA}$) mobilizes the electron and results in a combined ESR spectrum of the positive and negative ions. Some reduction in total intensity results in this step from the reaction of the electron and cation. The spectra at this point show no resolution and have linewidths in the range 11 to 13G (Table 1). The g values for the combined spectra of the purines (Table 1) are considered to approximate the average of the cation and anion radicals. This would only be exactly so if each radical's spectrum were of the same lineshape and linewidth.

Samples which contained $K_3Fe(CN)_6$ as an electron scavenger resulted after photoionization and photobleaching in ESR spectra which are considered to be due to cations. These spectra are also unresolved singlets. Their g values and linewidths are greater in magnitude than found for the combined spectra (Table 1).

The purine anion radicals were produced by the photoionization of $K_4Fe(CN)_6$ technique. The ESR spectra of the adenine and guanine anions were virtually unresolved. The g value reported in the table for the anions are less than those found for the cations. The overall results are self consistent since the anion and cation g values average to about the mixed anion-cation values.

Lion and van De Vorst report the g values for the purine anion radicals in $2M NaOH$ at $77^\circ K$ to be 2.0028 (adenine) and 2.0035 (guanine). The results for adenine differ somewhat from those found in $12M LiCl$. This may be due to the alkaline medium which removes the 9 position nitrogen proton ($pK = 9.$); although large differences in g value with matrix are unusual. The g values reported by Dartinger and Hartig for the ion radicals

in irradiated dry polycrystalline adenine and guanine are: anions $g = 2.0032$ (adenine), $g = 2.0043$ (guanine); cations $g = 2.0040$ (adenine), $g = 2.0055$ (guanine). The g values for adenine are within experimental error of those found here. However the results for guanine differ significantly. Since g values found for the guanine anion in this work and by Lion and van De Vorst are considerably lower than reported by Dertinger and Hartig it is possible that the radicals attributed by Dertinger and Hartig to the guanine anion and cation radicals are due to other species. For example, that value attributed to the guanine anion is nearly identical to the cation g value found in this work. Only in γ -irradiated oriented DNA has a resolved spectrum attributed to the guanine cation been reported (Graslund et al, 1971). Unfortunately no g value was reported for this radical.

We have performed experiments attempting to induce protonation reactions at carbon sites in the purine anion radicals in 12M LiCl(H_2O). The anions were generated either by photoionization of the purine or $K_4Fe(CN)_6$ with subsequent photobleaching of the trapped electrons. The anion radicals were then warmed to 180°K for varying periods of time. No evidence for a protonation reaction was found. A very small concentration of secondary radical was noted; however this was due to the reaction of hydrogen atoms with purine after warming. (A small concentration of hydrogen atoms is found at 77°K. The hydrogen atoms are likely produced by electron reaction with H_3O^+). Lion and van De Vorst (1971) have noted that protonation reactions can be induced in purine anions in 8M NaOH glasses by UV light ($>3200\text{\AA}$). We have found the same result for guanine anion in 12M LiCl. Lion and van De Vorst found in earlier work (1970) utilizing the ferrocyanide method for generating anions in an alkaline aqueous glass that no protonation occurred on warming. However in their more recent work (1971) in which γ -irradiation was employed they suggest protonation occurs on warming. In view of our results which show no protonation and their earlier results we would suggest that the radicals produced by warming γ -irradiated aqueous glasses are a result of attack by hydrogen atoms and hydroxyl radicals which are produced during γ -irradiation and mobilized upon warming.

The results of this present work and previous work in both alkaline (Holroyd and Glass 1968, Srinivasan et al, 1969, Lion and van De Vorst 1970) and neutral (Sevilla, Van Paemel and Zorman 1972) glasses have shown that

4.

of the four DNA bases only the thymine anion readily protonates. We believe these results give the probable explanation for the fact that only the thymine anion has been found to protonate in γ -irradiated DNA (Omerod 1965).

Table 1
ESR Spectral Parameters of the
Purine Ion Radicals

	<u>Adenine</u>		<u>Guanine</u>	
Radical	g value ^a	linewidth	g value ^a	linewidth
Anion and Cation	2.0038	11G	2.0039	13G
Cation	2.0043	12G	2.0042	14G
Anion	2.0034	9G	3.0034	9G

a. The uncertainty on the g value is estimated to be ± 0.0003 .

References

- Dertinger, H. and Hartig G., 1972, Intern. J. Radiation Biol., 21, 279.
- Graslund, A., Ehrenberg A., Rupprecht A., and Strom, G., 1971, Biochem. Biophys. Acta 254, 172.
- Helene, C., Santus, R., and Douzou, P., 1966, Photochem. Photobiol., 5, 127.
- Holroyd, R. A. and Glass, J., 1968, Intern. J. Radiation Biol., 14, 445.
- Lion, Y. and van De Vorst, A., 1971, Intern. J. Radiation Phys. Chem., 513 and 521.
- Lion, Y. and van De Vorst, A., 1970, Intern. J. Radiation Phys. Chem., 2, 51.
- Ormerod, M. G., 1965, Intern. J. Radiation Biol., 9, 291.
- Sevilla, M. D., Van Paemel, C., 1972, Photochem. Photobiol., 15, 407.
- Sevilla, M. D., Van Paemel, C., and Nichols, C., 1972, J. Phys. Chem., 76, 3571.
- Sevilla, M. D., Van Paemel, C., and Zorman, G., 1972, J. Phys. Chem., 76, 3577.
- Srinivasan, V. T., Singh B. B., and Gopal-Ayengar, A. R., 1969, Intern. J. Radiation Biol., 15, 89.