Free Rad. Res. Comms., Vol. 6, No. 2-3, pp. 83-85
Reprints available directly from the publisher
Photocopying permitted by license only

ESR AND ENDOR STUDIES OF X-IRRADIATED SINGLE CRYSTALS OF GUANINE DERIVATIVES

DAVID M. CLOSE

East Tenn. State University, Johnson City, TN.

WILLIAM H. NELSON

Georgia State University, Atlanta, GA.

EINAR SAGSTUEN and ELI O. HOLE

University of Oslo, Norway

This work is a continuation of an ESR/ENDOR project aimed at understanding the nature of radiation damage to purine derviatives. Much of this work has been on guanine derivatives, because of the role the guanine cation plays in the oriented DNA work.[1,2] The ultimate goal of this work is in understanding the direct effects of ionizing radiation to DNA. A recent review of this work can be found in ref.[3]

The first material studied was a single crystal of Guanine:HCl:H_2O (Gm) x-irradiated at temperatures between 10 K and 77 K. Three radical species were found and characterized by ESR and ENDOR spectroscopy.[4,5] All three are primary products in that they were present immediately following irradiation at T = 10 K. Radical I, which apparently can exist in two slightly different conformations, was identified as the product of electron gain by the parent molecule, and subsequent protonation at O6. Radical I is stable on warming to 250 K, where it decays into a radical formed by net H-abstraction from N9.[6] Radical II, the guanine cation, results from electron loss by the parent molecule, followed by deprotonation at N7.[4] Radical II exhibits a single small nonexchangeable anisotropic hyperfine coupling not correlated with any X-H crystallographic directions. Radical III has been tentatively assigned to a product that results from $^{\cdot}OH$ attack at C8 of the parent molecule, followed by rupture of the C8-N9 bond and ring opeining.[5] The $^{\cdot}OH$ radicals thought to produce Radical III could result from electron loss of the co-crystallized water molecules. The reaction leading to Radical III, unusual in solid-state radiation chemistry, is thought to be mediated by the specific hydrogen bonding network in this crystal. A C8 H-addition is formed by the decay of Radical III at 160 K.[6]

The second set of experiments were on single crystals of Guanine: HCl:$2H_2O$ (Gd) at low temperatures. This crystal contains the same N7 protonated guanine molecule with an additional water of hydration. The results, however, are rather different. At low temperatures we have detected Radical I (the guanine anion, which also decays at 250 K into the N9 H-abstraction radical), a C8 H-addition radical, and a C8 OH-addition radical. No evidence has been found for the guanine cation or Radical III.[7]

Comparisons of the similarities and difference in the radiation chemistry of Gm and Gd have lead to the following speculations about radical production and stabilization. Reduction of the guanine base is rapidly followed by protonation at O6 (to form

Radical I) even at low temperatures in both crystals. Radical I decays at 250 K to yield an N9 H-abstraction radical.

The oxidation pathway is much more involved. It seems reasonable that the base is oxidized in both cases. In Gm the deprotonated cation is stable on warming to 80 K. In Gd the cation is not stabilized because of differences in the H-bonding network around N7-H, or perhaps because of the involvement of radiolysis products from the water molecules. Several possibilities will be discussed including results interpreted in terms of the differences in water coordination around the base.[6,7]

Guanine C8 H-addition products are commonly thought to form as the net results of the protonation of anions at elevated temperatures.[8] This is clearly not the case for these guanine crystals. In Gm the formation of the C8 H-adduct is linked to the decay of Radical III that itself is formed via an oxidative pathway. In Gd the C8 H-adduct is stabilized at very low temperatures. The possibility has been considered that both H-adducts are formed in the same manner, but that only in Gm is the ring-opened radical precursor to the formamido molecule stabilized.[7]

The final difference between the radiation response of these two crystals is the observation of a C8 H-addition radical only in Gd. This is considered to result from ionization of the water molecule directly over the imidazole ring in the native Gd crystal (the only water in Gm is in the ring plane). Therefore, in both crystals there are products that are believed to result from ionization of the water molecules. The observed differences may then result from the direction from which the $^{\cdot}$OH arrives.[7]

It is not likely that studies of only two guanine single crystals will yield much of relevance to the study of the radiation chemistry of DNA. However, when these results are considered along with results of our small but growing number of studies of other purine molecules, the following patterns emerge.

It has been noted that the electronic structure of the guanine cation observed in Gm[4] is in good agreement with the structure of the guanine cation in DNA.[2] A problem arises, however, when one considers the mechanism of formation. In Gm, N7 is protonated in the native molecule. Therefore, to form the observed cation, the molecule must rapidly deprotonate at N7 even at 10 K. However, the guanine cation observed in DNA is observed at 77 K, and is still present on warming to 220 K.[9]

Hüttermann and Voit have recently reported a single crystal study of 5'dGMP.[9,10] Here, as in DNA, the native guanine molecule is not protonated at N7. They observe a guanine cation that decays (above 77 K) by deprotonation at N1, and claim that the same events occur in DNA fibers.[9] These results conflict with our own ENDOR results on 5'dGMP conducted at 10 K.[11] Our results suggest a cation deprotonated at the exocyclic amino group. In our review, oxidation of a guanine molecule results in rapid deprotonation (to maintain charge balance). Therefore, if the molecule is initially protonated at N7, the radiation-induced cation will deprotonate at this site. Otherwise, deprotonation will occur at the amino group. It is reasonable to expect that this will also occur in DNA.

Finally, we have in addition to Gm and Gd a number of situations under study where the decay of a base anion is not directly linked to the formation of an H-addition radical. To test the relevance of these results to the study of DNA, we have recently investigated a single crystal of 1-Methyl Thymine (1MeT). We see the expected thymine anion decay between 45–50 K, but no trace of the thymine H-addition radical until the crystal is warmed to above 200 K.

Acknowledgements

This work was supported in part by NIH grant CA-36810 and by NATO travel grant RG 86/0077.

References

1. Gräslund, A., Ehrenberg, A., Rupprecht, A. and Ström, G. Ionic Base Radicals in γ-irradiated DNA, *Biochim. Biophys. Acta*, **254,** 172 (1971).
2. Hüttermann, J. Solid-State Radiation Chemistry of DNA and its Constituents, *Ultramicroscopy*, **10,** 25, (1982).
3. Close, D.M., Nelson, W.H. and Sagstuen, E. EPR and ENDOR Study of X-Irradiated Single Crystals of Purines at 4.2 K. *Electronic Magnetic Resonance of the Solid State*, Weil, J. (ed.), Canadian Society for Chemistry, Ottawa, Canada, p. 237, (1987).
4. Close, D.M., Sagstuen, E. and Nelson, W.H. ESR Study of the Guanine Cation, *J. Chem. Phys.*, **82,** 4386, (1985).
5. Close, D.M., Nelson, W.H. and Sagstuen, E. Radical Formation in X-Irradiated Single Crystals of Guanine Hydrochloride Monohydrate II. ESR and ENDOR in the Range 10–77 K, *Radiat. Res.*, **112,** 283, (1987).
6. Close, D.M., Sagstuen, E. and Nelson, W.H. Radical Formation in X-Irradiated Single Crystals of Guanine Hydrochloride Monohydrate. Part III. Secondary Radicals and Reaction Mechanisms, *Radiat. Res.* (in press).
7. Nelson, W.H., Hole, E.O., Sagstuen, E. and Close, D.M. ESR/ENDOR Study of Guanine · HCl · $2H_2O$ X-Irradiated at 20 K. *Int. J. Radiat. Biol.* (in press).
8. Gregoli, S., Olast, M. and Bertinchamps, A. Free Radical Formation in Deoxyguanosine-5'-Monophosphate γ-Irradiated in Frozen Solution. A Computer-Assisted Analysis of Temperature-Dependent ESR Spectra, *Rad. Res.*, **72,** 201, (1977).
9. Hüttermann, J. and Voit, K. Free Radicals from Direct Action of Ionizing Radiation in DNA:Structural Assignments from EPR Spectroscopy, *Electronic Magnetic Resonance of the Solid State*, Weil, J. (ed). Canadian Society for Chemistry, Ottawa, Canada, p. 267, (1987).
10. Rakvin, B., Herak, J.N., Voit, K. and Hüttermann, J. Free Radicals from Single Crystals of Deoxyguanosine 5'-Monophosphate (Na Salt) Irradiated at Low Temperatures, *Radiat. Environ, Biophys.* **26,** 1, (1987).
11. Hole, E.O., Nelson, W.H., Close, D.M. and Sagstuen, E. ESR and ENDOR Study of the Guanine cation: Secondary Product in 5'-dGMP, *J. Chem. Phys.*, **86,** 5218, (1987).