Free Rad. Res. Comms., Vol. 6, No. 2-3, pp. 91-92
Reprints available directly from the publisher
Photocopying permitted by license only

FREE RADICAL FORMATION IN NUCLEOSIDES AND NUCLEOTIDES OF GUANINE: ESR AND ENDOR OF GUANOSINE 5'-MONOPHOSPHATE AND GUANOSINE: DIMETHYLFORMAMIDE X-IRRADIATED AT 10 K

EINAR SAGSTUEN and ELI O. HOLE

University of Oslo, Norway

WILLIAM H. NELSON

Georgia State University, Atlanta, GA, U.S.A.

DAVID M. CLOSE

East Tennessee State University, Johnson City, TN, U.S.A.

Current interest in the radiation-induced free radical processes in the DNA purine-constituent guanine has been stimulated by recent reports on radiation effects on DNA itself.[1-4] Two main conclusions emerge from these studies. Firstly, the radiation damage to DNA may primarily be due to the direct action of radiation and not to indirect effects due to the attack of hydroxyl radicals produced by primary ionization of surrounding water molecules. Secondly, the primary damage reportedly results solely in thymine reduction and guanine oxidation. Hence, the manifold of secondary products observed after irradiation of DNA supposedly are due to these two species and their secondary reactions. It is particularly notable that no sugar radical has been detected in DNA, despite the fact that electron loss products from sugar often are major oxidation products in mononucleotides. As a part of a program devoted to attain understanding of radiaton-induced free-radical processes in guanine derivatives,[5-11] the current study includes two guanine nucleosides, guanosine 5'-monophosphate (in its free acid form), abbreviated 5'-GMP, and the complex guanosine: dimethylformamide, abbreviated Gr:DMF. In these two compounds, the guanine base exists in different forms. Thus, in 5'-GMP the N7-position is protonated.

Single crystals of the two compounds were X-irradiated at 10 K and studied at this, and at higher temperatures, using X- and K-band ESR, ENDOR and FSE spectroscopic techniques as previously reported.[4]

In 5'-GMP the following products were detected at 10 K: R1: The C8 H-adduct to the guanine base; R2: The O6 protonated guanine anion; R3: A weak 2 mT doublet probably due to a C8 OH-adduct[4] or a sugar centered radical. Upon thermal annealing R2 decays at 250 K with no apparent successor. Also the resonance assigned to R3 disappeared at this temperature. At room temperature only the H-adduct remained. Reaction mechanisms proposing that R1 is a part of the oxidation sequence of reactions will be discussed.

Preliminary results for Gr:DMF indicate the presence of at least four different radical species at 10 K. One of these is the N10 deprotonated cation of the guanine base, exhibiting about 31% spin density at the nitrogen atom. In addition a coupling to HC8 was observed with about 18% spin density at C8. These data are virtually identical to those observed for a similar radical in 5'-dGMP.[12] A second base-centered radical, believed to be the N7 protonated anion radical, exhibits unpaired spin density at C8 (22%), N7 (12%) and N1 (14%). ENDOR lines due to two almost equal, non-exchangeable α-proton couplings were detected at a few orientations, but could not be analyzed in detail. Most probably these lines are due to a sugar-centered radical, or a radical in the DMF moiety. Finally, weaker features due to the C8 H-addition radical could be observed.

Upon thermal annealing at 40 K the anion radical transforms into a sharp doublet resonance. This doublet may be described as an anomalous β-proton coupling which does not seem to be associated with the guanine base. The isotropic value of the coupling is about 3 mT. This radical decays between 100 and 150 K with no apparent successor radical. In the same temperature region also the N10 deprotonated cation decays with no successor.

At room temperature only the C8 H-addition radical remains, in the same yield as initially at 10 K. Several weak proton couplings of this radical were anlayzed. Most informative was an almost pure dipolar interaction between about 40% spin at N7 and a nearby proton. Theoretical calculations[12] showed that the N . . H distance is 1.76 A, typical for a strong N . . . H–O hydrogen bond, probably to the ribose sugar of a neighbour guanosine molecule.

Structural details of the different radicals formed in these two crystals will be given, and possible reaction sequences will be discussed in light of the knowledge gained from other guanine derivatives.[5-11]

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., Voit, K., Oloff, H., Gräslund, A. and Rupprecht, A. Specific formation of electron gain and loss centres in X-irradiated oriented fibres of DNA at low temperature. *Discussion of the Chemical Society*, **78**, 135, (1984).
3. Cullis, P.M. and Symons, M.C.R. in: *Mechanisms of DNA damage and repair* Simic, M.G., Grossman, L. and Upton, A.C. (eds.), Plenum Press, New York, (1986).
4. Gregoli, S., Olast, M. and Bertinchamps, A.J. Radiolytic pathways in γ-irradiated DNA: influence of chemical and conformational factors. *Radiat. Res.*, **89**, 238, (1982).
5. Close, D.M., Sagstuen, E. and Nelson, W.H. ESR study of the guanine cation. *J. Chem. Phys.*, **82**, 4386, (1986).
6. 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 10–11 K. *Radiat. Res.*, **112**, 283, (1987).
7. Close, D.M., Sagstuen, E. and Nelson, W.H. Radical formation in X-irradiated single crystals of guanine hydrochloride monohydrate. III. Secondary radicals and reaction mechanisms. *Radiat. Res.*, in press, (1988).
8. Strand, P., Sagstuen, E., Lehner, T. and Hüttermann, J. Free radical formation in crystals of guanine hydrochloride dihydrate: an ESR and ENDOR study. *Internat. J. Radiat. Biol.*, **51**, 303, (1987).
9. 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, (1988).
10. Hole, E.O. and Sagstuen, E. Free radical formation in crystals of 2'-deoxyguanosine 5'-monophosphate irradiated at 15 K. *Radiat. Res.*, **109**,190, (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).
12. Sagstuen, E., Awadelkarim, O., Lund, A. and Masiakowski, J. Trapping site geometry of $N_2H_4^+$ radical ion in X-irradiated single crystals of $N_2H_5HC_2O_4$: an ENDOR study. *J. Chem. Phys.*, **85**, 3223, (1986).