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FREE RADICAL FORMATION IN SINGLE CRYSTALS OF 2'-DEOXYGUANOSINE 5'-MONOPHOSPHATE, AND GUANINE HYDROBROMIDE MONOHYDRATE AFTER X-IRRADIATION AT 10 AND 65 K: AN ESR, ENDOR AND FSE STUDY

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In DNA it has been proposed that the direct action of ionizing radiation produces mainly two species, the guanine cation and the thymine anion. No sugar radicals were reported.[1-4] Several different guanine derivatives have recently been studied in order to gain more information about the radiation chemistry of guanine.[5-17] The present work includes single crystals of guanine hydrobromide monohydrate (GmHBr) and the disodium salt of the nucleotide 2'-deoxyguanosine 5'-monophosphate tetrahydrate (5'-dGMP). In GmHBr the base is protonated at N7. Some of the results obtained question the guanine cation/thymine anion theory. The effect of different electrostatic surroundings on the radical production and stability, and also the interaction between base, sugar, and phosphate groups, have been of special interest.

Single crystals of 5'-dGMP exposed to high doses (10–20 Mrad) of X-radiation at 10 and 65 K, were studied at temperatures between 10 and 295 K, using X- and K-band ESR/ENDOR and FSE techniques. There were no clear differences between the results obtained after irradiation at 10 K and 65 K. In addition, low-dose studies (0.45–0.9 Mrad) were performed at 10 K. All the resonances which were observed after low-dose exposure were also observed at high doses. In the latter case however, additional resonances were present.

Four different base centered radicals were observed after high-dose X-irradiation at 10 K; a N10-deprotonated cation (R1), a species believed to be the charged anion (R2), a C8 H-addition radical (R3), and an unidentified base radical (R4). Two of these radicals, R2 and R4, were also observed after low-dose irradiation at 10 K. R1 has previously been reported and characterized.[12] This resonance disappeared at about 200 K with no apparent successor. R2 was observed after both high and low doses of x-radiation. The resonance was very unstable and decayed almost immediately upon warming or upon uv-bleaching.[11] No successor was observed. The resonance due to R3 was weak, but observable, at 10 K. Upon warming, the intensity increased slowly until about 220 K. At this temperature the radical quickly grew in concomitant with the decay of a sugar centered radical (see below). R4 was observed after both high and low doses. The relative and absolute intensity of the ENDOR lines were much stronger after low doses X-irradiation than after high doses. The radical was characterized by one carbon centered and two nitrogen centered α-couplings. R4 decayed between 160 K and 180 K with no observable successor. These data disagree with the results previously published.[14]

After high radiation doses at 10 K there were evidences for at least six different sugar centered radicals, R5-R10. Upon warming R11 appeared. Some of these radicals were hardly observable in the ESR spectra, but the ENDOR spectra left no doubt about their presence. R5 was also observed after low irradiation doses. R5, the O_3'-centered alkoxy radical, has previously been observed and characterized.[10,11] R6, a C_3'-centered radical, is probably formed by a net hydrogen abstraction at C_3'. At 200 K the R6 resonance dominated the ESR pattern. As previously reported[11] the radical started to decay at 220 K concomitant with a increase in the R3 resonance, thus indicating a radical transformation from the sugar to the base. R7, a C_2'-centered π-radical is probably also formed by a net hydrogen abstraction. R8 is characterised by only one unusually large β-coupling (151.4 MHz). The resonance decayed at about 200 K with no apparent successor. R9 appeared in four slightly different conformations. FSE spectra suggested that mainly one α-coupling, due to about 75% π-spin density on carbon, contributed to the resonance pattern observed. R10 is characterised by two carbon centered α-coupling. The radical decayed at about 50 K concomitant with the appearance of two new α-couplings ascribed to R11.

X-irradiation of GmHBr at 10 K yielded evidence for at least five different base radicals; an O6-protonated anion (RI), a C8 H-addition radical (RII), a radical which is believed to result from OH attack at C8 resulting in C8-N9 bond rupture (RIII), and at least two unidentified base-radicals (RIV and RV). At least one additional radical, RVI, appeared upon warming.

RI was still present after warming to 265 K. This radical was also observed in the mono and dihydrates of guanine hydrochloride, GmHCl,[6] and GdHCl,[9] and in the nuceotide guanosine 5'-monophosphate, 5'-GMP.[15] The well known RII was stable at room temperature. RIII, which was also observed in GmHCl,[6] decayed between 130 K and 150 K with no apparent successor. RIV is characterized by at least one nitrogen α-coupling (16% spin density, probably on N1). The resonance decayed between 65 K and 200 K. RV seemed to be associated with four different α-couplings due to 13% π-spin density on C8, 11% on N7, and 12% on N10. RV decayed between 200 K and 265 K concomitant with the appearance of at least three new resonance lines ascribed to RVI.

The present as well as previous results indicate that the electrostatic surroundings affect the radical production in the guanine base. If the guanine molecule initally is protonated at N7, as is the case in GmHBr, GmHCl, GdHCl, and 5'-GMP, the electron loss products tend to deprotonate at N7.[5] If the native guanine base is neutral however, as it is in DNA, 5'-dGMP and the co-crystal guanosine: dimethyl-formamide (GrDMF), the deprotonation occurs at the exocyclic nitrogen, N10.[12,17] Since the electrostatic surroundings in DNA and in 5'-dGMP are very similar, it seems reasonable to expect that this also could occur in irradiated DNA. The fate of the guanine anion does also seem to be determined by the surroundings. In the N7-protonated cases, the anion protonates at O6,[6,7,15,16] while in 5'-dGMP it decays rapidly with no apparent successor. None of the guanine compounds so far studied yields evidence for the anion as precursor for the C8 H-addition radical.

It has been proposed[13,14] that no sugar radicals are prodcued after low-dose irradiation of 5'-dGMP, and that the radical formation is different from that at high doses. In the present data three radicals were observed after low-dose X-irradiation at 10 K, two base-centered and one sugar-centered. The observation of the sugar-centered oxidation product (R5) may to some extent account for the fact that the cation was absent at low doses. Furthermore, there was no evidence for any low-dose radical which was not also produced after high doses of X-irradiation.

Even though both GmHBr and GmHCl are protonated at N7 there are considerable differences in the radical production. In GmHCl three radicals were observed at 10 K, the O6-protonated anion R1, the N7-deprotonated cation, and the ring opening radical RIII.[5-7] In GmHBr, however, at least five different radicals were present. It has so far not been possible to verify whether any of the unidentified radicals could be the cation. In GmHCl, RIII transformed into RII at about 150 K. In GmHBr no such transformation was observed. RII was present already at 4 K. and RIII decayed between 130 K and 150 K without any apparent successor.

The difference between the two isomorphous guanine crystals must be due to different effects from the Cl and Br molecules.

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