Free Rad. Res. Comms., Vol. 6, No. 2–3, pp. 97–98
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Printed in Great Britain

SPIN TRAPPING OF SUGAR RADICALS IN SOLID AND AQUEOUS PHASES

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Ionizing radiation directly and indirectly produces free radicals in DNA. Many studies concerning free radicals produced in DNA usually employ its constituents because of their structural simplicity. Information from nucleosides and nucleotides is especially important because free radicals induced at the sugar moiety are expected to give information about the mechanisms of strand breaks.

While free radicals produced in the solid phase can be studied by a method combining a single crystal and ENDOR,[1] it is necessary to design a method to make it possible to identify the short-lived free radicals in the aqueous phase. The spin trapping method is most favorable in this respect because such short-lived free radicals can be transformed to stable nitroxide free radicals.[2,3]

We applied this method to the study of sugar radicals produced in nucleosides and nucleotides. In the present paper we mainly describe results obtained from 5'-TMP. 2-Methyl-2-nitrosopropane (MNP) was used as a spin trapping reagent. An N_2O-saturated aqueous solution containing 5'-TMP and MNP (5, 10 and 27 mM for 5'-TMP, MNP and N_2O, respectively) was X-irradiated at a dose of 5.4 kGy. Irradiation produced several spin-adducts between free radicals in 5'-TMP and MNP. These spin-adducts were separated by reverse-phase HPLC in the ion suppression mode. Each of the separated spin-adducts was subsequently examined by UV-absorbance spectrometry. As a temporary measure, the spin-adducts having a maximum absorbance at 260 nm were regarded as those of the sugar radicals.[4] The sugar radicals are known to be linked to the release of an unaltered base after several radical reactions.[5] Less release of the base was observed when 5'-TMP coexisted with MNP. Furthermore, release of the unaltered base from the separated spin-adduct was observed after 20 h. These results proved that the spin trapping undoubtedly occurred at the sugar moiety.

Three sugar radicals were identified as spin-adducts with MNP.

Sugar radical 1. The ESR spectrum showed a hyperfine structure consisting of a primary triplet and a secondary doublet, indicating the interaction of a spin with a proton at the β position. A radical at the C5' of the sugar induced by the H-abstraction was assigned to this spectrum.

Sugar radical 2. The ESR spectrum showed a hyperfine structure consisting of only a primary triplet. A radical at the C4' of the sugar by the H-abstraction was assigned to this spectrum.

Sugar radical 3. The ESR spectrum showed a hyperfine structure consisting of a primary triplet and a secondary triplet, indicating the interaction of a spin with a

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nitrogen at the β position. A radical at the C1' of the sugar by the H-abstraction was assigned to this spectrum.

In the case of solid free radicals, the γ-irradiated nucleoside or nucleotide powder was dissolved in an aqueous solution containing MNP under anaerobic conditions. In general, it is known that the secondary free radicals, as well as the primary free radicals, are generated in the solid phase. Therefore, it is expected that some of the free radicals will be identical with those in the aqueous phase, and others will be different from those in the aqueous phase. For pyrimidine nucleotides, the primary H-abstraction radicals at C1' and C5' were observed as the common radicals in both aqueous and solid phases, and the secondary radicals at C4' and C5' positions were identified as radicals observed only in the solid phase.

The present results will be useful for better understanding of the mechanisms of raidation-induced damage formation at the sugar moiety of DNA.

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