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GAMMA RADIOLYSIS OF RNA:

AN ESR AND SPIN-TRAPPING STUDY

Alasdair J. Carmichael, Carmen M. Arroyo, and
Thomas L. Walden

Radiation Biochemistry Department
Armed Forces Radiobiology Research Institute
Bethesda, Maryland 20814, U.S.A.

INTRODUCTION

It is well known that the radiolytic cleavage of water produces hydroxyl radicals ($^{\bullet}OH$), hydrogen atoms ($^{\bullet}H$) and hydrated electrons (e^{-}_{aq}).[1] Although these species react rapidly with the individual nucleic acid bases,[2-4] it is generally accepted that when nucleic acid (DNA, RNA) solutions are exposed to ionizing radiation, the principal reaction leading to nucleic acid damage is caused by $^{\bullet}OH$. Besides direct formation, $^{\bullet}OH$ is formed from H_2O_2, also a product of the radiolysis of water, in the presence of trace amounts of divalent metal ions.[5] The study reported here deals with the reaction of RNA with $^{\bullet}OH$, produced in aqueous solutions by gamma radiolysis. Of concern are the processes that occur following this reaction. For instance, are the precursors to the nucleic acid damage localized at specific base sites or do they occur indiscriminately throughout the nucleic acid molecule? Recently Kuwabara et al.[6] using HPLC, spin trapping and ESR identified the precursors of thymine damage in X-irradiated DNA solutions. For the present study, poly A, poly C, poly G and poly U were used as standards for RNA, and RNA was used as a model system for similar reactions that may occur in DNA. The nucleic acid free radical intermediates were detected and identified by ESR and spin trapping following hydrolysis of the spin-trapped nucleic acid molecules.

MATERIALS AND METHODS

To eliminate possible small-molecular-weight contaminants, solutions of RNA, poly A, poly C, poly G and poly U were dialyzed against several changes of doubly deionized distilled water and then lyophylized. The spin trap 2-methyl-2-nitrosopropane (MNP) was prepared by dissolving 20-30 mg of MNP in 10 ml of water and stirring in a water bath at 45°C for approximately 2 h. Solutions of RNA and of the various polynucleotides were prepared by dissolving the lyophylized powders (5×10^{-4} to 1×10^{-3} M) directly in the MNP solution and adjusting the pH to 8. These solutions were ^{60}Co gamma-irradiated at a dose rate of 45 Gy/min to a total dose of 700 Gy. ESR spectra of the spin-trapped RNA and polynucleotides were immediately recorded following irradiation. The spin-trapped RNA samples were hydrolyzed with base (NaOH, pH 12.6) or enzymatically (RNAase). The spin-trapped polynucleotides were hydrolyzed with base. ESR spectra were recorded at

various times during hydrolysis until the hydrolysis was complete as judged by the resolution of the ESR spectra. ESR spectra were recorded on a Varian E-109 X-band spectrometer at 100 KHz magnetic field modulation. The spectra were analyzed by comparison with computer-generated ESR spectra.

RESULTS AND DISCUSSION

The ESR spectrum obtained for the spin-trapped RNA is shown in Figure 1a. This spectrum consisting of three broad lines is characteristic of nitroxide groups bound to slowly tumbling large molecules in solution. Figure 1b shows the ESR spectrum obtained following base or enzymatic hydrolysis of the spin-trapped RNA. This spectrum consists of a triplet of sextets indicating that the unpaired nitroxide electron is interacting with the nuclei of a β nitrogen and β hydrogen. The hyperfine coupling constants for this spectrum are a_N=1.48 mT, a_N^β= 0.25 mT and a_H^β= 0.15 mT. Following base hydrolysis of the spin-trapped polynucleotides, only poly C (Figure 2a) and poly U (Figure 2B) yielded ESR spectra consisting of a triplet of sextets. The hyperfine coupling constants for the ESR spectrum corresponding to the hydrolyzed poly C are a_N= 1.49 mT, a_N^β= 0.27 mT and a_H^β= 0.16 mT. For the hydrolyzed poly U, a_N= 1.48 mT, a_N^β= 0.25 mT and a_H^β= 0.15 mT.

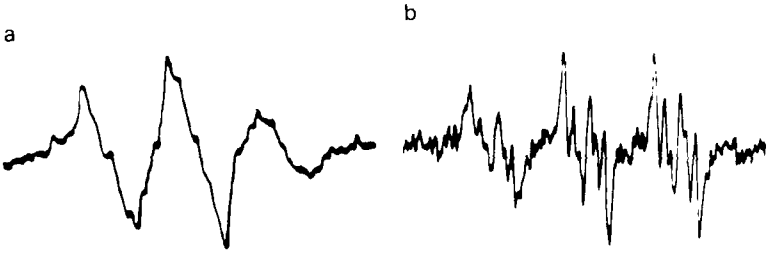

Figure 1.

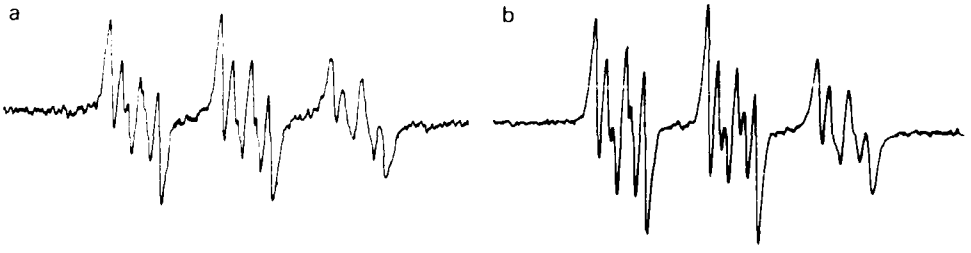

Figure 2.

The results suggest that in RNA only uracil radicals were spin trapped. Furthermore, due to the β nitrogen and β hydrogen interaction with the unpaired nitroxide electron, the results indicate that MNP reacted with uracil-C6-carbon-centered radicals. Because hydroxyl radicals react with all bases at approximately the same rate,[2] two possible explanations for the

observed results are immediately obvious: 1) The spin-trapping efficiency is not equal for radicals formed on each base, and 2) the stability of the various spin adducts is not equal. Although still being investigated, the first possibility appears unlikely because [MNP]>[RNA]>[$^{\bullet}$OH] which should permit MNP to react with any base radical formed in RNA. The second possibility is unlikely because the ESR spectra of the polynucleotide spin adducts and of their hydrolyzed products persist for 48 h or longer. Another explanation for the observed results is that the free radical spin density migrates via an intramolecular mechanism, still unclear at this time, to the uracil bases. Radiolysis of poly (A,U) and poly (C,U) in the presence of MNP support this explanation. The results of these experiments also show specific uracil spin trapping.

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