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ESR STUDIES OF THE SHORT-LIVED ORGANIC RADICALS FORMED IN γ-IRRADIATED AQUEOUS SOLUTIONS. SPIN TRAPPING WITH 2-METHYL-2-NITROSOPROPANE

Hitoshi TANIGUCHI and Hiroyuki HATANO
Department of Chemistry, Faculty of Science, Kyoto University,
Kitashirakawa, Sakyo-ku, Kyoto 606

A spin trapping technique was applied successfully to the detection and identification of short-lived radicals formed in γ-irradiated aqueous solutions of organic compounds. From an aqueous methanol solution, $\cdot CH_2OH$ radical is trapped by 2-methyl-2-nitrosopropane. From glycylglycine and glycyl-α-alanine solutions, it is confirmed that hydrogen atoms are abstracted from glycine residues.

A spin trapping technique1) has been utilized recently to identify the radicals formed in the radiolysis of organic liquids.2,3) However, its application to the radiolysis of aqueous solutions seems to be limited to the *in situ* radiolysis systems using the *aci* anion of nitromethane ($CH_2=NO_2^-$) and fumarate ion as spin traps.4)

In this letter, it is reported that the spin trapping technique is successfully applied to the study of the short-lived organic radicals produced in γ-irradiated aqueous solutions. 2-Methyl-2-nitrosopropane was used as a spin trap and it was prepared by the oxidation of *t*-butylamine with *m*-chloroperoxybenzoic acid.5) Glycylglycine and glycyl-DL-α-alanine were purchased from the Foundation for the Promotion of Protein Research in the Institute for Protein Research, Osaka University. Other materials used were of the purest grade commercially available.

The concentrations of 2-methyl-2-nitrosopropane and organic substrates were 0.05 *M* and 0.2 to 0.5 *M*, respectively. The spin trap was dissolved in aqueous solutions of organic substrates immediately before γ-irradiation. Air-saturated sample solutions were irradiated with ^{60}Co γ-rays at a dose rate of 8.8×10^4 rad/hr at room temperature to a dose of 5×10^4 rad. Esr spectra were obtained at room temperature with an X-band spectrometer (JEOL, Model PE-3X). Hyperfine coupling constants were measured using Mn^{2+} in MgO as a reference (splitting between the two central peaks, 86.9 ± 0.1 G).

Figure 1 shows an esr spectrum recorded after γ-irradiation of an aqueous solution containing methanol and 2-methyl-2-nitrosopropane. It consists of ^{14}N triplet of 1:2:1 triplets with hyperfine coupling constants, a_N = 15.5 G and a_H = 6.1 G; these values are in good agreement with those recently reported for the hydroxymethyl tert-butyl nitroxide radical, $(CH_3)_3C\text{-}N(O\cdot)\text{-}CH_2OH$, formed in the irradiated methanol itself,[2] taking into account an appreciable solvent effect on the nitrogen hyperfine coupling constant of nitroxide radicals.[6] In the present work, the hydroxymethyl radical derived from methanol is considered to be formed in the reaction with the hydroxyl radical generated by the radiolysis of water, while in the irradiated methanol, the hydroxymethyl radical is formed in the reaction with the positive ion ($CH_3OH\cdot^+$) generated by the radiolysis of methanol.[2,3]

The esr spectrum obtained with a γ-irradiated aqueous glycylglycine solution is shown in Figure 2. The dominant splitting is a ^{14}N triplet (a_N = 15.6 G) and the secondary splittings consist of a quartet with the intensity ratio close to 1:2:2:1. The quartet is believed to originate from the combined interaction with one ^{14}N nucleus and one proton with nearly identical coupling constants, $a_N = a_H$ = 2.3 G. The abstraction of a hydrogen atom by the hydroxyl radical would occur from methylene groups and give rise to the primary radicals $NH_3^+\dot{C}HCONHCH_2COO^-$ and/or $NH_3^+CH_2CO\text{-}NH\dot{C}HCOO^-$ and to the corresponding spin adducts with 2-methyl-2-nitrosopropane. In view of the structure assigned to the free-radical intermediate in the reaction of glycylglycine with the hydroxyl radical formed in a $Ti^{3+}\text{-}H_2O_2$ system,[7] the structure of a spin adduct, $NH_3^+CH_2CONHCH(COO^-)\text{-}N(O\cdot)\text{-}C(CH_3)_3$, seems to be the most probable of the two alternatives.

In the case of a γ-irradiated aqueous glycyl-α-alanine solution, the major component of the esr spectrum is closely similar to that obtained from a γ-irradiated aqueous glycylglycine solution, suggesting the hydrogen abstraction from the glycine moiety and the resulting formation of a $NH_3^+\dot{C}HCONHCH(CH_3)COO^-$ radical. Perhaps this is the first information about the structure of a glycyl-α-alanine radical in an aqueous solution.

Further studies on the spin trapping of short-lived radicals formed in γ-irradiated aqueous solutions of other peptides and proteins are now in progress to elucidate the mechanism of γ-radiolysis of aqueous biomolecule solutions.

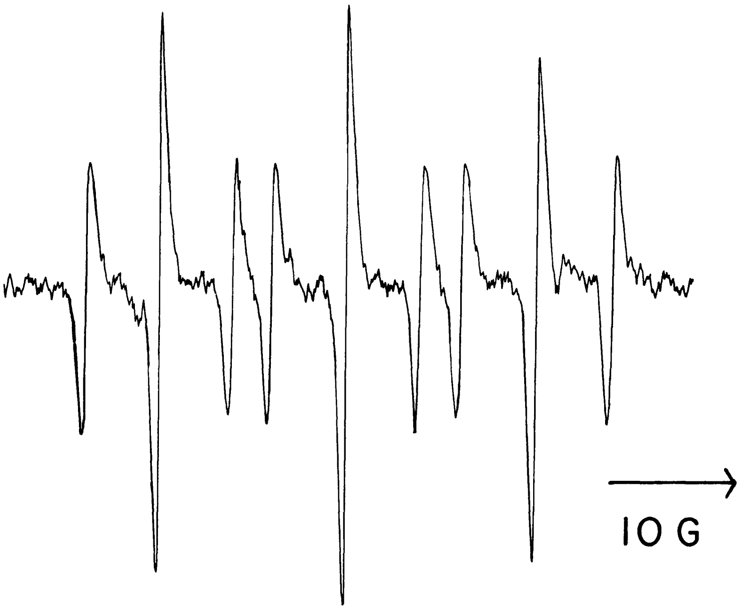

Figure 1. Esr first-derivative spectrum of a γ-irradiated aqueous methanol solution at room temperature. The concentrations of methanol and 2-methyl-2-nitrosopropane were 0.5 M and 0.05 M, respectively.

Figure 2. Esr spectrum of a γ-irradiated aqueous glycylglycine solution. The concentrations of glycylglycine and 2-methyl-2-nitrosopropane were 0.2 M and 0.05 M, respectively.

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