

SPIN TRAPPING OF THE SHORT-LIVED RADICALS FORMED IN γ -IRRADIATED
AQUEOUS SOLUTIONS OF DIPEPTIDES AND NUCLEIC BASES¹⁾

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Short-lived radicals of dipeptides and nucleic bases and additional hydrogen atom formed in γ -irradiated aqueous solutions are trapped with 2-methyl-2-nitrosopropane and structures of these radicals are identified. The line width of spin adduct radical with higher molecular weight possibly gives information about the local dynamic structure around the radical center.

In a previous letter,³⁾ it was reported that a spin trapping technique could be applied successfully to the study of the short-lived organic radicals formed in γ -irradiated aqueous solutions. Our experimental study of the spin trapping has now been extended in the present work to include also the detection and identification of short-lived radicals produced in γ -irradiated aqueous bio-molecule solutions.

2-Methyl-2-nitrosopropane was used as a spin trap reagent. Air-saturated sample solutions were irradiated with ⁶⁰Co γ -rays at a dose rate of 8.8×10^4 rad/hr at room temperature to a total dose of 5×10^4 rad. The short-lived organic radical ($\cdot R$) generated by γ -irradiation reacts with the nitroso-group of 2-methyl-2-nitrosopropane to produce a spin adduct, which is rather stable nitroxide radical. $[\cdot R + (CH_3)_3C-N=O \rightarrow (CH_3)_3C-N(-O\cdot)-R]$ The concentrations of a spin trap and bio-molecules were 0.04 M and 0.2 M, respectively. Experimental procedures for measurements of esr spectra were the same as described previously.³⁾

Figure 1 shows three esr spectra recorded after γ -irradiation of aqueous solutions containing dipeptides and a spin trap. In the cases of γ -irradiated aqueous alanylglycine and glycylalanine solutions, the major components of the esr spectra are closely similar to those obtained from a γ -irradiated aqueous glycylglycine solution.³⁾ They consist of 12 lines due to two nitrogen nuclei and one proton.³⁾ The

hyperfine coupling constants are 15.6 G for one nitrogen nucleus and 2.3 G for one nitrogen and one proton. The strong resemblance of esr spectra of three spin adducts in Fig. 1, suggests that the abstraction of hydrogen atoms occurs from methylene groups of glycine residues and it results in the formation of spin adducts which have a component part, $(\text{CH}_3)_3\text{C-N}(-\text{O}\cdot)-\overset{\cdot}{\text{C}}\text{H-N}$.

Minor components observed on both sides of major quartet groups might be attributable to a hydrogen atom trapped with 2-methyl-2-nitroso-propane. $[(\text{CH}_3)_3\text{C-N=O} + \cdot\text{H} \rightarrow (\text{CH}_3)_3\text{C-N}(-\text{O}\cdot)\text{H} \text{ (I)}]$ The esr spectrum of this spin adduct I consists of a nitrogen triplet and a doublet with hyperfine coupling constants, $a_{\text{N}} = 15.7$ G and $a_{\text{H}} = 18.2$ G. The spin adduct I decays much more rapidly than other spin adducts of dipeptide radicals. The difference in signal intensity of I among three esr spectra in Fig. 1, may come from the instability of I and the concentration of I depends on the time between γ -irradiation and measurement of esr spectra.

An esr spectrum obtained from a γ -irradiated aqueous solution of glycyl-norleucine is shown in Fig. 2. It consists of a nitrogen triplet ($a_{\text{N}} = 16.4$ G) and a doublet ($a_{\text{H}} = 1.5$ G) due to one proton. The structure of a spin adduct has three possibilities according to the hydrogen abstraction from three methylene groups. One of these structures is shown in Fig. 2. Inhomogeneity of the line width of each component in the esr spectrum can be ascribed

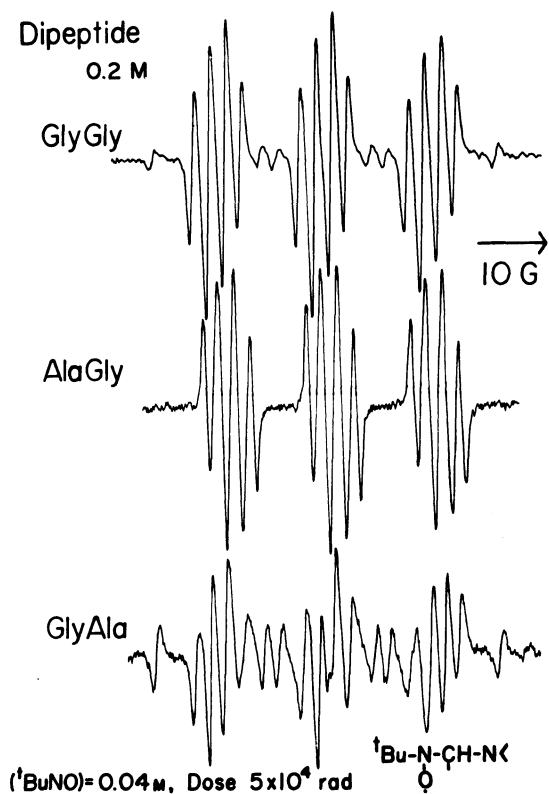


Figure 1. ESR first-derivative spectra of γ -irradiated aqueous solutions of glycylglycine(GlyGly), alanyl-glycine(AlaGly), and glycyl-alanine(GlyAla) at room temperature.

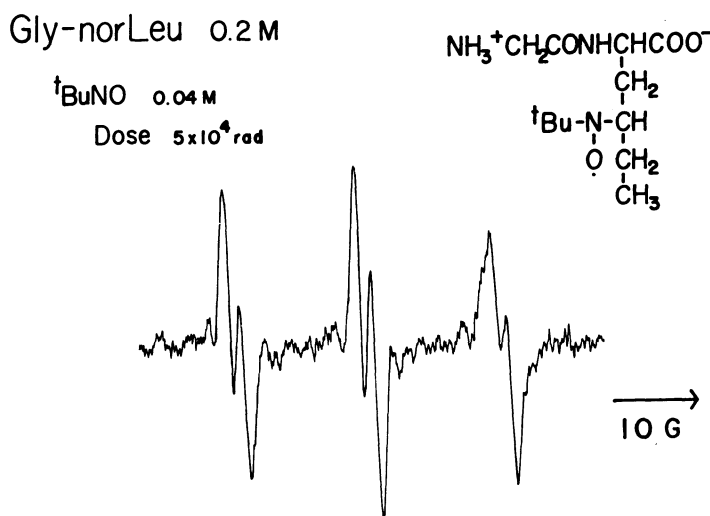


Figure 2. ESR spectrum of a γ -irradiated aqueous glycyl-norleucine solution.

mainly to the different contribution of nitrogen nuclear spin toward the width of each hyperfine line, and it might be related partly to the local dynamic structure around the radical center. Namely, the nitroxide radical obtained by spin trapping of a short-lived radical with higher molecular weight corresponds to a spin labeled compound. Consequently, using a spin trapping technique, it is expected to obtain more direct information about the dynamic structure around the radical center formed in γ -irradiated aqueous biopolymer solutions.

In γ -irradiated aqueous solutions of pyrimidine bases of nucleic acid, it is well known that the hydroxyl radical generated by γ -radiolysis of water adds to the double bond of pyrimidine ring. These resultant radicals were also successfully trapped with 2-methyl-2-nitrosopropane. The esr spectrum obtained from a γ -irradiated aqueous uracil

solution is shown in Fig. 3. The structures of spin adducts can be assigned as shown in Fig. 3. The hydroxyl radical is considered to add to C₅ of pyrimidine ring. The stick diagram in Fig. 3 shows the hyperfine structure. The dominant splitting of a spin adduct of uracil radical is a nitrogen triplet ($a_N = 15.3$ G) and the secondary splittings consist of a nitrogen triplet ($a_N = 3.5$ G) due to N₁ of pyrimidine ring and a doublet ($a_H = 1.8$ G) due to C₆-H proton. Di-tert-butylnitroxide radical $[(CH_3)_3C-N(O\cdot)-C(CH_3)_3]$ is formed additionally, resulting from the decomposition of spin trap by γ -irradiation and consequent trapping of the tert-butyl radical. It consists of a nitrogen triplet ($a_N = 17.2$ G).

The esr spectrum of a spin adduct is also observed in a γ -irradiated aqueous thymine solution. It consists of a triple-triplet due to two nitrogen nuclei with hyperfine coupling constants, $a_N = 15.3$ and 3.5 G, and it is attributed to the spin adduct of a radical formed by the addition of the hydroxyl radical to C₅ of pyrimidine ring as is the case with a γ -irradiated aqueous uracil solution. The hyperfine

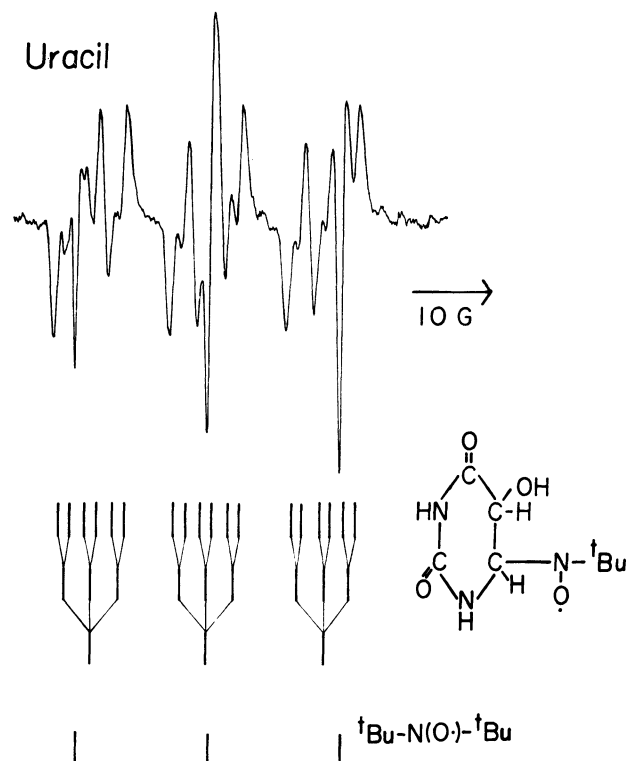


Figure 3. ESR spectrum of a γ -irradiated aqueous uracil solution.

coupling constant due to C₆-H proton is 1.8 G for a spin adduct derived from uracil and it is too small to be observed for a spin adduct derived from thymine. These small hyperfine coupling implies that the dihedral angle between the π orbital of the nitrogen atom of the nitroxide group and C₆-H bond is close to 90°.

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Reference and Footnotes

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