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The identity and structure of free radicals in γ-irradiated amino acid derivatives

M. Aydin

Faculty of Education, Adiyaman University, TR 02030-Adiyaman, Turkey

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The technique of electron paramagnetic resonance spectroscopy was used to determine the identity and structure of free radicals produced at room temperature by γ-irradiated powders of some biologically important compounds, namely, N-acetyl-L-glutamine, glycine ethyl ester hydrochloride and L-threonine. The paramagnetic species observed in these compounds were identified as $H_2NCOCH_2CH_2\dot{C}(NHCOCH_3)COOH$, $\dot{C}H_2COOCH_2CH_3$ and $CH_3CH(OH)\dot{C}HCOOH$, respectively. The g values and the hyperfine structure constants of the unpaired electron with the environmental protons and the ^{14}N nucleus were determined. The free radicals were stable at room temperature for more than three months. Some spectroscopic properties and suggestions concerning the possible structure of the radicals were also discussed in this study.

Key words*:* EPR; Free Radicals; γ-Irradiation; Amino Acid Derivatives.

1. INTRODUCTION

Free radicals play an essential role in a variety of chemical and biological processes [1–3]. Electron paramagnetic resonance (EPR) is a highly sensitive spectroscopic technique for examining free radicals [4]. The EPR study of the radicals produced in organic compounds by X-ray and γ-ray irradiation has led to valuable information on the structures of various paramagnetic species [5–12]. The EPR spectrum of γ-irradiated powders of glycyl-L-glutamine has been investigated by Başkan at room temperature and at 150 K [13]. The radiation damage center was attributed to the $H_2NCOCH_2CH_2\dot{C}(NHCOCH_2NH_2)COOH$ radical. Furthermore, in the single crystal of N_α-acetyl-L-glutamine γ-irradiated at room temperature, the free radicals have been identified as $NH_2COCH_2CH_2\dot{C}(NHCOCH_3)COOH$ and $NH_2COCH_2CH_2CH(NHCOCH_3)\dot{C}\bar{O}OH$ [14]. The irradiated single crystals of glycine have been studied by several groups of workers [15-20]. The observed species in these studies were found to be $NH_3^+\dot{C}HCO\bar{O}$ and $\dot{C}H_2COOH$.

N-acetyl-L-glutamine (NALG), glycine ethyl ester hydrochloride (GEHCl) and L-threonine (LT) are biologically very important amino acid derivatives which play an essential role in metabolic processes. To our knowledge, NALG, GEHCl and LT powders have not been investigated at room temperature. Therefore it is the goal of this work to identify and check the stabilities of the radical species produced in these amino acid derivatives by the EPR technique.

* To whom all correspondence should be sent: E-mail: maydin@adiyaman.edu.tr

2. EXPERIMENTAL

The samples used in this study were purchased from Aldrich. Powder samples of the compounds were irradiated with a ^{60}Co γ-ray source (Nordion-Canada model JS 9600) at a rate of 2 kGy/h for a total of 10 h at room temperature. For the EPR measurements of the samples quartz tubes were used. The paramagnetic species generated by γ-irradiation were studied in a Varian model X-band E-109C EPR spectrometer at room temperature. The modulation amplitude was below 5.10^{-2} mT and the microwave power was 2 mW. The g factors were found by comparison with a diphenylpicrylhydrazyl (DPPH) sample with g = 2.0036 [21, 22]. The spectrum simulations were made using McKelvey's programs [23].

3. RESULTS AND DISCUSSION

The NALG powder γ-irradiated at room temperature gives the spectrum shown in Fig. 1a. The line resolution of the spectrum is poor. Its thorough examination reveals that it consists of lines of approximate intensity ratio 1:1:3:3:4:4:3:3:1:1. This spectrum exhibits intensity distribution 1:3:4:3:1 because of two β-protons and a ^{14}N nucleus (I=1) with approximately equal coupling constants (a_β = 1.68 mT and a_N = 1.46 mT). Then each of these five lines is subdivided

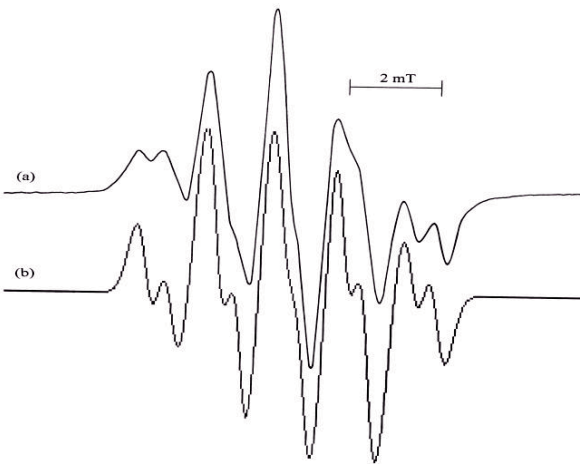

Fig.1. (a) The EPR spectrum of NALG powder γ-irradiated at room temperature, (b) simulation form of the spectrum using a_β = 1.68 mT, a_N = 1.46 mT, a_{NH} = 0.58 mT and linewidth 0.30 mT.

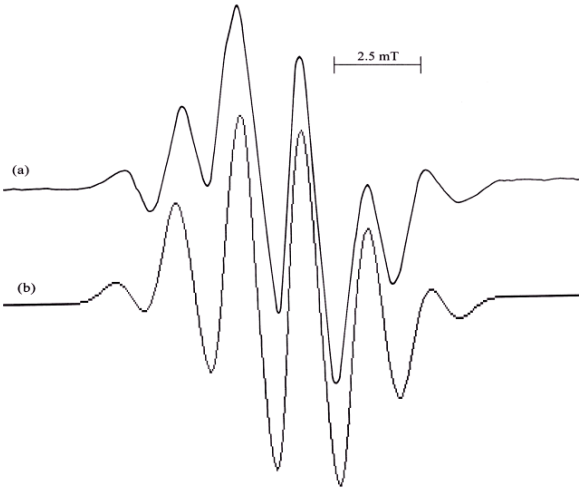

Fig.3. (a) The EPR spectrum of LT powder γ-irradiated at room temperature, (b) simulation form of the spectrum using $a_\alpha = a_{CH} = a_{CH3}$ = 1.80 mT and linewidth 0.58 mT.

into doublets owing to the proton near the adjacent ^{14}N nucleus which is of a splitting of 0.58 mT. According to these findings, the paramagnetic species can be identified as the $H_2NCOCH_2CH_2\dot{C}(NHCOCH_3)COOH$ radical. The measured g value of this radical is g = 2.0027 ± 0.0005. The spectrum simulated with the above given values agrees with the experiment (Fig.1b). The radical with the obtained hyperfine constants and g value is similar to the radicals observed in the γ-irradiated glycyl-L-glutamine, glycyl-L-glutamine monohydrate and alanyl-L-glutamine powders [13, 24, 25]. It can be stated that γ-irradiation produced free radicals in NALG by loss of the bond of hydrogen atom from the carbon atom bond to the $HCOCH_3$ and COOH group and the unpaired

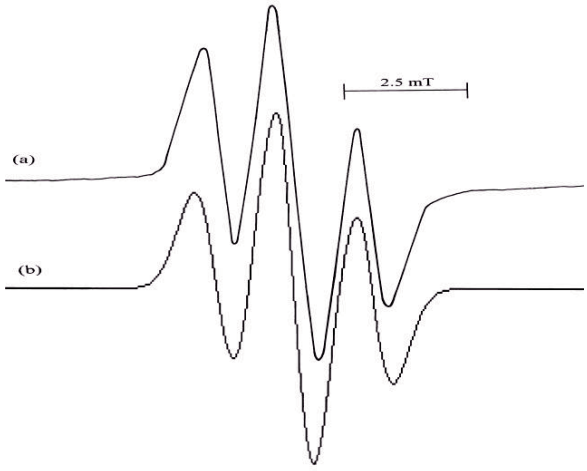

Fig.2. (a) The EPR spectrum of GEHCl powder γ-irradiated at room temperature, (b) simulation form of the spectrum using a_{CH2} = 1.78 mT, and linewidth 0.68 mT.

electron interacts with two β-protons, one NH proton and the ^{14}N nucleus.

The characteristic stable EPR spectrum of the γ-irradiated GEHCl is shown in Fig. 2a. The spectrum consists of three equally spaced (1.78 mT) lines with a relative intensity distribution of 1:2:1. This spectrum belongs to the $\dot{C}H_2COOCH_2CH_3$ radical, hence we suppose that the 1:2:1 ratio of the triplets is due to the interactions of two α-protons which are all magnetically equivalent. The g value of the radical is g = 2.0033 ± 0.0005. A simulation of the GEHCl spectrum is shown in Fig. 2b. A similar EPR spectrum was observed for the γ–irradiated glycine in an aqueous solution at 195 K [26]. The reported hyperfine coupling constant of the two α-protons is 2.10 mT. This value is similar to the hyperfine coupling constant value measured in this study. The measured EPR parameters of the irradiated polycrystalline glycine at room temperature are also in agreement with our results [27, 28]. The identity of the radicals in γ–irradiated glycine has been the object of much debate [15, 29, 30]. It is generally accepted that both $\dot{C}H(NH_2)COOH$ and $\dot{C}H_2COOH$ radicals are present at room temperature in γ–irradiated glycine [31]. According to these results, we can say that the $\dot{C}H_2COOCH_2CH_3$ radical is obtained by the removal of a NH_2 group from the central carbon atom.

Fig.3a presents the EPR spectrum of LT powder γ–irradiated and recorded at room temperature. The spectrum consists of six lines with intensity distribution 1:6:10:10:6:1 with 1.80 mT spacing. The unpaired electron interacts with one α-proton, one CH proton and three CH_3 protons, which are all

magnetically equivalent. According to these results, the radical should be $CH_3CH(OH)\dot{C}HCOOH$. The determined g value is $g = 2.0035 \pm 0.0005$. A spectrum simulated with these values agrees with the experiment and is shown in Fig. 3b. A similar radical was observed for the irradiated single crystal of L-threonine at room temperature and therefore the above interpretations are valid for this compound as well [32]. We can state that this radical produced by γ–irradiation is due to the breakage of a C-NH_2 bond leading to unpaired electron spatially extending through CH_3 and CH groups.

4. CONCLUSION

The γ-irradiated NALG, GEHCl and LT powder samples indicated the inducement of the $H_2NCOCH_2CH_2\dot{C}(NHCOCH_3)COOH$, $\dot{C}H_2COOCH_2CH_3$ and $CH_3CH(OH)\dot{C}HCOOH$ stable radicals, respectively. The identity and the structure of the radicals were determined. It was observed that the EPR parameters of the radicals discussed here are consistent with the literature data.

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ИДЕНТИЧНОСТ И СТРУКТУРА НА СВОБОДНИ РАДИКАЛИ В ГАМА-ОБЛЪЧЕНИ ПРОИЗВОДНИ НА АМИНО КИСЕЛИНИ

М. Айдин

Факултет по педагогика, Университет Адияман, TR 02030- Адияман, Турция

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(Резюме)

Електронно-парамагнитна резонансна спектроскопия е използвана за определяне на идентичността и структурата на свободните радикали, произведени при стайна температура от гама-облъчени прахове на някои биологично важни съединения, а именно, N-ацетил-L-глутамин, глицин етилов естер хидрохлорид и L-треонин. Парамагнитните частици, наблюдавани в тези съединения са идентифицирани съответно като $H_2NCOCH_2CH_2\dot{C}(NHCOCH_3)COOH$, $\dot{C}H_2COOCH_2CH_3$ и $CH_3CH(OH)\dot{C}HCOOH$. Определени са *g*-стойностите и константите на свръхфина структура на несдвоения електрон с обкръжаващите протони и ядрото ^{14}N. Свободните радикали са стабилни при стайна температура за повече от три месеца. Някои спектроскопски свойства и предложения относно възможната структура на радикалите също са обсъдени в това изследване.