EPR of Gamma-irradiated L-Glutamine Hydrochloride and *N*-Carbamoyl-L-glutamic Acid

Şemsettin Osmanoğlu[a], Murat Aydın[b], and M. Halim Başkan[a]

[a] Department of Physics, Faculty of Arts and Sciences, Dicle University, TR 21280-Diyarbakır, Turkey
[b] Faculty of Education, Dicle University, TR 21280-Diyarbakır, Turkey

Reprint requests to Prof. S. O.; Fax: (+90) 412-2488039; E-mail: sems@dicle.edu.tr

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The electron paramagnetic resonance spectra of γ-irradiated L-glutamine hydrochloride and *N*-carbamoyl-L-glutamic acid single crystals have been investigated at room temperature. Radiation damage centres are attributed to $\dot{C}H$, $\dot{N}H_2$ and $CH_2\dot{C}(NH_2)COOH$ radicals.

Key words: EPR; Gamma Irradiation; Free Radicals; Amino Acid Derivatives.

1. Introduction

L-Glutamine is a biologically important amino acid which plays an essential role in metabolic processes. It occurs in the free state and as part of the protein structure in plants and animals, and therefore it was the aim of this work to give some information about the activities of selected compounds. There have been many studies on EPR of free radicals of amino acids and derivatives or γ-irradiated ones at low and room temperatures [1 – 6]. *N*-Acetyl-L-glutamic acid, L-glutamic acid and D,L-glutamic acid hydrochloride were γ-irradiated and studied at 100 and 350 K with EPR [7]. To our knowledge, no further EPR studies have been carried out on amino acid derivatives. Therefore it was the purpose of this study to investigate analogues of the above mentioned compounds, L-glutamine·HCl (LGHCl) and *N*-carbamoyl-L-glutamic acid (NCLGA), in the hope of determining their radical structure after γ-irradiation.

2. Experimental

L-Glutamine·HCl and *N*-carbamoyl-L-glutamic acid single crystals were grown from concentrated aqueous solutions. L-Glutamine·HCl crystals are orthorhombic, space group $P2_12_12_1$, with $a = 13.32$ Å, $b = 11.75$ Å, $c = 5.16$ Å, the unit cell containing four molecules [8]. Single crystal data of *N*-carbamoyl-L-glutamic acid were not available. The single crystals and powder were irradiated at room temperature by a ^{60}Co γ-ray source of 0.3 Mrad/h for 5 h. The EPR spectra were recorded with a Varian model X-band E-109 C EPR spectrometer using 2 mW microwave power. The crystals were rotated on a Lucite Piller about their crystallographic axes, and the angle of rotation was read on a scale graduated in degrees. The experiments were carried out with several single crystals at several times. The g factors were calibrated by comparison with a DPPH sample ($g = 2.0036$).

3. Result and Discussion

Free radicals, produced by gamma-irradiation of single crystals of L-glutamine·HCl, have been investigated at room temperature with EPR. The spectra in Figs. 1 – 3 exhibit 5, 11 and 9 lines, respectively. These spectra were observed when the magnetic field was parallel to the *c*-, *b*- and *a*-axes, respectively. The radicals in LGHCl were identified as $\dot{C}H$ (π electron radical) and $\dot{N}H_2$ (σ electron radical). The EPR spectra of the radicals were unchanged and undiminished at room temperature for more than three days after irradiation. The a_{CH} hyperfine coupling values, when the crystal is rotated around the *a*-, *b*-, and *c*-axes, did show that it is isotropic within experimental error; its measured average is $a_{CH} = 8.60$ mT. However, the g values are slightly anisotropic, and their average value is $g = (2.0037 \pm 0.0005)$.

The characteristic EPR spectrum of the γ-irradiated LGHCl in Fig. 1 exhibits an intensity distribution of 1:2:2:2:1 with 11.87 mT spacing. This spectrum was obtained with $B_0 // c$-axis. In the spectrum, the outer doublet is due to a π electron radical of type $\dot{C}H$, the

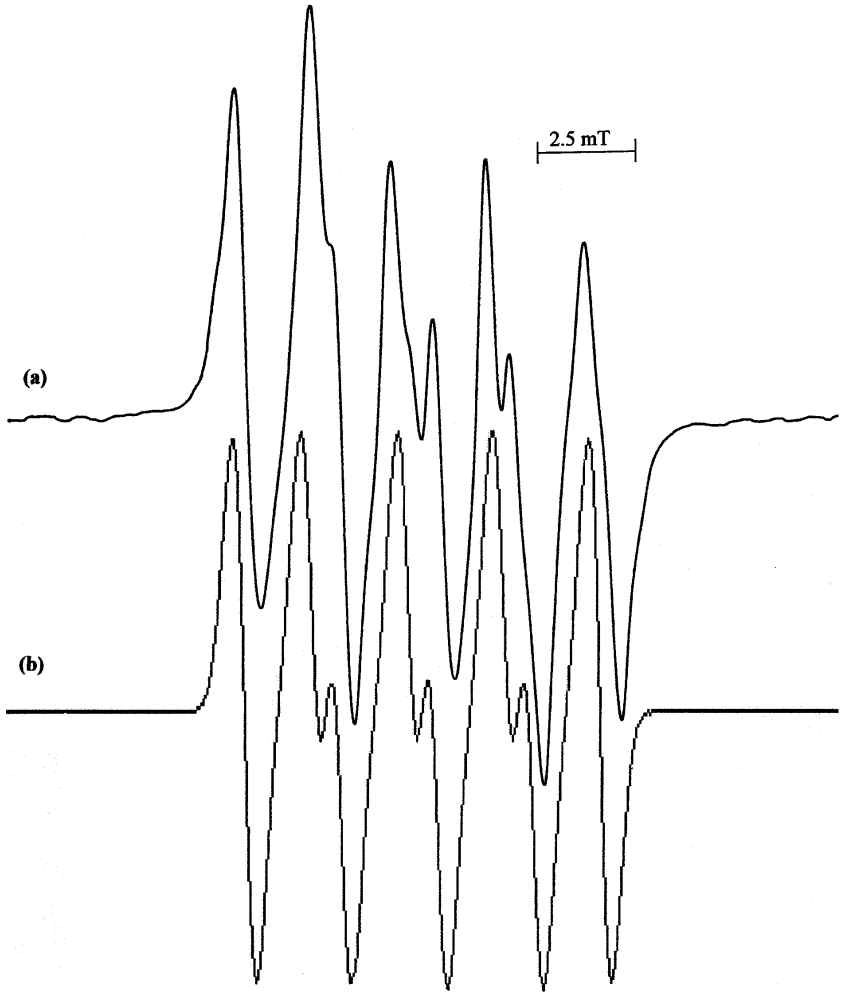

Fig. 1. (a) The EPR spectrum of γ-irradiated L-glutamine hydrochloride obtained along the $B_0//c$-axis, (b) simulated form of the spectrum using $\dot{C}H$, $a_{CH} = 8.10$ mT; $\dot{N}H_2$, $a_N = 2.04$ mT, $a_H = 0.65$ mT and linewidth 0.35 mT.

hyperfine coupling constant of the unpaired electron with the proton directly attached to the carbon atom varies from 7.80 mT to 9.80 mT, depending on the orientation of magnetic field. This value of the hyperfine coupling constant agrees with some other literature data [3, 7, 9, 10]. The $\dot{N}H_2$ radical belongs to the central triplet. We clearly see triplet splitting in the central part of the spectrum of LGHCl in Fig. 1a due to the ^{14}N nucleus. The central doublets are due to one proton to the adjacent ^{14}N nucleus, and their isotropic hyperfine constant is 0.65 mT. The g value is slightly anisotropic and $g = (2.0037 \pm 0.0005)$. The spectrum simulated with the hyperfine parameters is presented in Figure 1b. The experimental and simulated EPR spectra were found to agree well with each other. The hyperfine constants are similar to R-$\dot{N}$H and NH_2-R-$\dot{N}$H radicals [7, 9].

The EPR spectrum of γ-irradiated LGHCl single crystal was obtained with the magnetic field parallel to the b-axis as shown in Fig. 2, at room temperature. It can be interpret in terms of eleven lines with the relative intensities 1:1:2:1:1:2:1:1:2:1:1. The outer doublet is due to the $\dot{C}H$ radical, the average value of the proton splitting is about 8.60 mT. The inner nine lines of the spectrum are due to the $\dot{N}H_2$ radical. This radical exhibits a three line spectrum because of the nitrogen atom. Each line also splits into three lines owing to the two protons. The simulation of the spectrum, using the EPR simulation program of McKelvey [11], is shown in Figure 2b. Our average value of the nitrogen splitting is about 2.04 mT, and the proton splittings are approximately 0.82 mT. The measured value of the g factor is $g = (2.0037 \pm 0.0005)$. These values agree well with those derived from the $\dot{N}H_2$ radi-

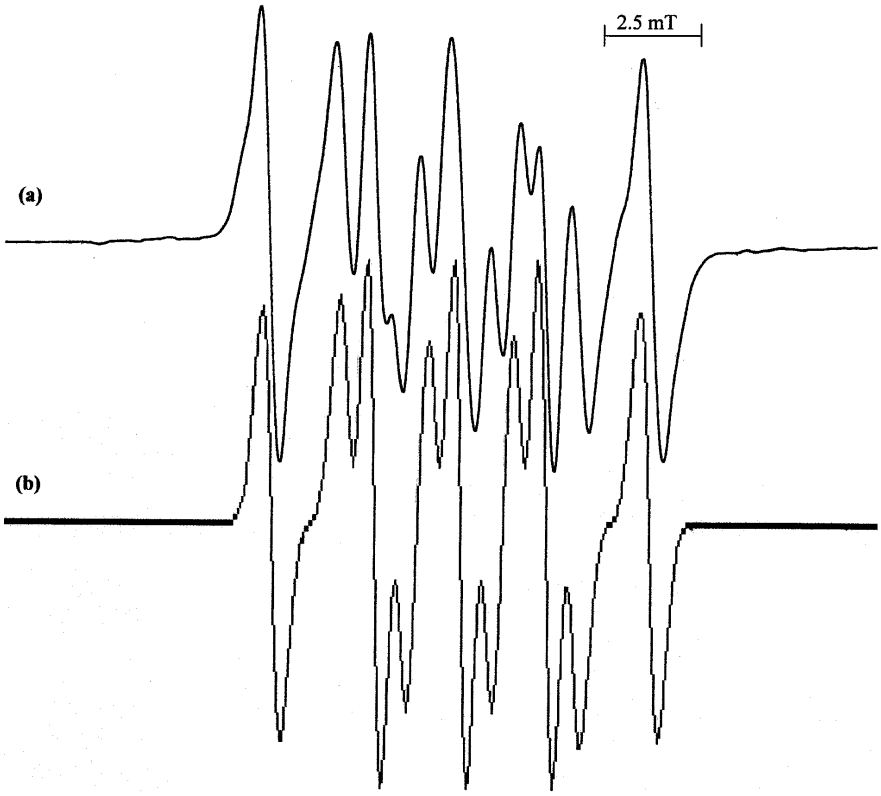

Fig. 2. (a) The EPR spectrum of γ-irradiated L-glutamine hydrochloride obtained along the $B_0//b$-axis, (b) simulated form of the spectrum using $\dot{C}H$, $a_{CH} = 9.60$ mT; $\dot{N}H_2$, $a_N = 2.10$ mT, $a_{H_2} = 0.64$ mT and linewidth 0.30 mT.

cal in D,L-glutamic acid hydrochloride and hydroxylammonium chloride, hydroxylammonium sulfat, hydrazinimum sulphate [7, 12]. Zengin et al.'s spectra were obviously similar to that of γ-irradiated LGHCl. The present values of g and the hyperfine constants for the $\dot{C}H$ and $\dot{N}H_2$ radicals obtained in LGHCl are given in Table 1.

Figure 4a shows the EPR spectrum of a single crystal of *N*-carbamoyl-L-glutamic acid irradiated and observed at room temperature. The EPR spectra of NCLGA exhibit a doublet with an intensity distribution of 1:1 with 5.84 mT spacing. These experimental data indicate that the unpaired electron spin interacts with a β-proton and a nitrogen nucleus ($I = 1$). The spectrum in Fig. 4a is a doublet partly due to some broadness of the lines, and due to some smallness of the nitrogen coupling. The hyperfine coupling constants for the ^{14}N nucleus are obtained using the EPR simulation program of McKelvey [11] in Table 2. Therefore we attribute the species to the $H_2C\dot{C}NH_2$ radical. The anisotropy of this doublet was observed to be very small when the crystal was turned around the three orthogonal axes, and its slightly anisotropic

Table 1. The EPR parameters of $\dot{C}H$ and $\dot{N}H_2$ radicals. The error for all the calculated g values is estimated as ± 0.0005.

Radical	Principal values of A (mT) and g		Direction Cosines		
$\dot{C}H$	A_{CH}	$A_{xx} = 9.81 \pm 0.1$	−0.9974	−0.0006	−0.0709
		$A_{yy} = 8.13 \pm 0.1$	0.0015	0.9996	−0.0297
		$A_{zz} = 7.85 \pm 0.1$	−0.0709	0.0297	0.9970
		$a = 8.60 \pm 0.1$			
		$g_a = 2.0049$	0.9989	0.0364	−0.0263
		$g_b = 2.0033$	−0.0443	0.8962	0.4415
		$g_c = 2.0028$	−0.0075	0.4422	0.8969
		$g_{av} = 2.0037$			
$\dot{N}H_2$	A_{H_2}	$A_{xx} = 0.92 \pm 0.1$	0.8427	−0.5201	0.1392
		$A_{yy} = 0.89 \pm 0.1$	0.5224	0.8524	0.0224
		$A_{zz} = 0.65 \pm 0.1$	0.1303	−0.0538	−0.9901
		$a = 0.82 \pm 0.1$			
	A_N	$a_{iso} = 2.04$			
		$g_{av} = 2.0037$			

hyperfine coupling was obtained to be $a_\beta = 2.76$ mT. The measured g value is nearly isotropic and $g = 2.0036 \pm 0.0005$. The principal values of the hyperfine coupling tensor of the methylene proton and ^{14}N nucleus with unpaired electron are given in Table 2. These values of the hyperfine coupling constants agree

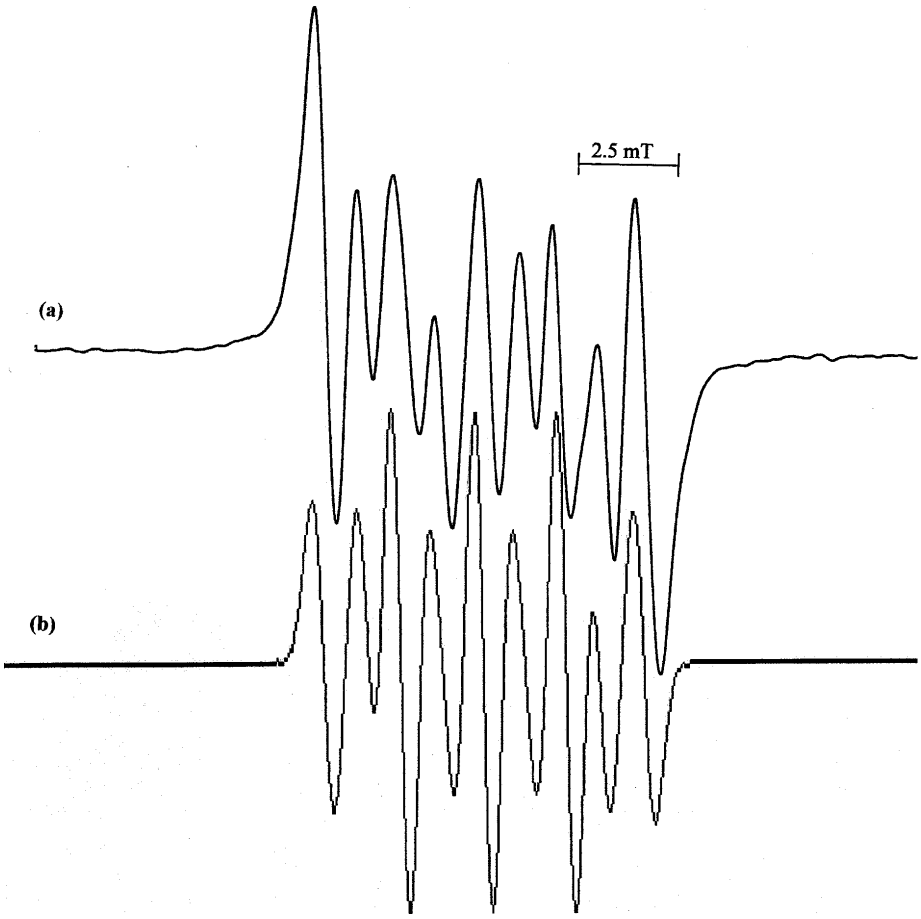

Fig. 3. (a) The EPR spectrum of γ-irradiated L-glutamine hydrochloride obtained along the $B_0//a$-axis, (b) simulated form of the spectrum using $\dot{C}H$, $a_{CH} = 7.90$ mT; $\dot{N}H_2$, $a_N = 2.05$ mT, $a_H = 0.82$ mT and linewidth 0.30 mT.

with other literature data [3, 5, 13]. A simulation of the spectrum is shown in Fig. 4b, using the hyperfine coupling constants $a_\beta = 2.76$ mT, $a_N = 0.41$ mT. The coupling to the β-proton in this radical occurs primarily through hyperconjugation, and the magnitude of these splittings depends on the dihedral angle θ between the C,H bond and the P orbital which contains the unpaired electron. The β-proton coupling constant is given by [14]

$$a_\beta = B_0 + B_1 \cos^2 \theta, \tag{1}$$

where B_0 is a constant and includes the contribution of spin density which arises from conformation independent mechanisms, in particular spin polarisation, and B_1 includes the hyperconjugative contributions. In the case of rapid free rotation about C_α-C_β the average value of a_β becomes

$$a_\beta = B_0 + \frac{1}{2} B_1 \tag{2}$$

Table 2. The principal values of the hyperfine coupling tensors of the unpaired electron with one of the protons bonded to the adjacent methylene group for $H_2C\dot{C}(NH_2)COOH$ radical in γ-irridiated *N*-carbamoyl-L-glutamic acid.

Principal values (mT)	H	^{14}N
A_{xx}	2.94	0.43
A_{yy}	2.71	0.41
A_{zz}	2.62	0.38
a	2.76	0.41
$g_{av} = 2.0036 \pm 0.0005$		

with $B_0 = 0-0.35$ mT and $B_1 = 5$ mT, which was earlier measured [14, 15]. If these values are replaced in (1), $a_\beta = 2.5-2.85$ mT is obtained. The value $a_\beta = 2.76$ mT obtained in this study is in this range. Hence we conclude that the β-proton in the $H_2C\dot{C}(NH_2)COOH$ radical rotates about $C_\alpha - C_\beta$. We can state that this rotation of the β-proton exists at room temperature.

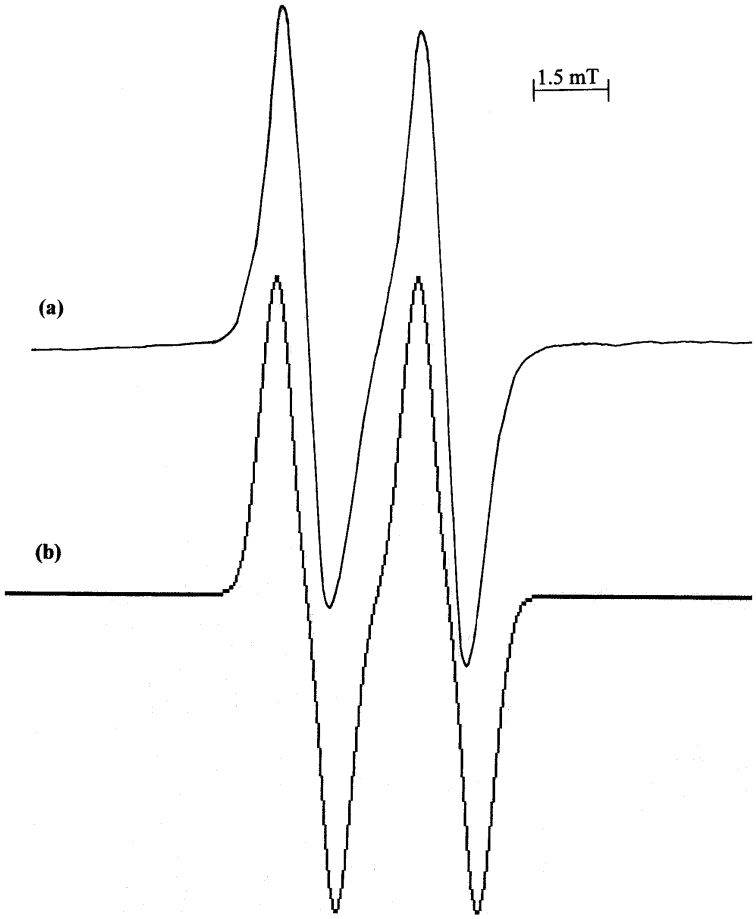

Fig. 4. (a) The EPR spectrum of γ-irradiated *N*-carbamoyl-L-glutamic acid obtained along the $B_0//c$-axis, (b) simulated form of the spectrum using $a_\beta = 2.76$ mT, $a_N = 0.41$ mT and linewidth 0.38 mT.

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