

EPR Analysis on Radiation Dosimetry Material Spectra

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Abstract

Radiation dosimetry work on L- α -alanine based on EPR analysis is carried out to identify radicals induced by irradiation in the system. A Varian E-12 EPR spectrometer with a rectangular parallelepiped microwave cavity to produce TE_{102} mode was utilized to measure first derivative absorption lines of X-band CW spectra of γ -irradiated L- α -alanine single crystals at room temperature. Six different resonance spectra measured from a number of defined crystal orientations to the magnetic field were obtained. The dominating feature of the spectrum along the $\langle c \rangle$ is a quintet with the intensity ratio close to 1:4:6:4:1. Along the $\langle a \rangle$ and $\langle b \rangle$, the quintet is split into a quartet of line pairs with the intensity ratio 1:1:3:3:3:3:1:1, accompanied by weaker resonance features. When the magnetic field is fixed on crystal planes, however, the spectrum is of superposition of two different spectra. Crystallographic and computed spectral simulation studies on the system clarified these analyses.

Keywords: EPR, alanine, radiation dosimetry.

Abstrak

Analisa EPR terhadap spektrum bahan dosimetri radiasi L- α -alanine telah dilakukan guna mengidentifikasi kandungan radikal yang terbentuk pada sistem kristal tunggal alanine akibat proses radiasi sinar- γ . Untuk itu, spektrometer EPR Varian E-12 yang beroperasi pada band-X dan yang dilengkapi dengan pemandu gelombang mikro untuk menghasilkan mode gelombang TE_{102} dipergunakan untuk mencatat spektrum absorpsi CW alanine pada suhu ruang. Enam macam spektrum yang diperoleh dari berbagai orientasi kristal terhadap medan magnet dianalisa. Spektrum pada sumbu $\langle c \rangle$ didominasi oleh lima garis resonansi dengan rasio intensitas 1:4:6:4:1, sedangkan spektrum pada sumbu $\langle a \rangle$ maupun $\langle b \rangle$, masing-masing terdiri atas empat pasang garis resonansi dengan rasio intensitas 1:1:3:3:3:3:1:1. Spektrum yang diperoleh dari ketiga bidang kristal merupakan hasil superposisi dari dua macam resonansi. Analisa kristalografi dan simulasi komputasi terhadap sistem memperjelas pemahaman terhadap hasil-hasil di atas.

Kata kunci: EPR, alanine, dosimetri radiasi

1. Introduction

It has commonly been assumed that the polycrystalline EPR from L- α -alanine (Fig. 1a) resulted from one single radiation induced free radical, the so-called SAR, the stable alanine radical. This species has been shown to exhibit structure-1 (Fig. 1b), formed by deamination from a protonated alanine radical anion¹⁻⁶. The EPR of the SAR is mainly due to interactions of the unpaired electron with the proton bonded to C2 and with three equally coupled (rotationally averaged) methyl protons.

However, due to peculiarities in both the radiation dose dependency and the microwave power dependency of the powder-EPR from alanine pellets, it has been speculated if several resonances possibly could be present simultaneously^{2,5,7,8}.

This paper reported the results of EPR spectral analysis on L- α -alanine single crystals to identify the existence of radicals induced by irradiation.

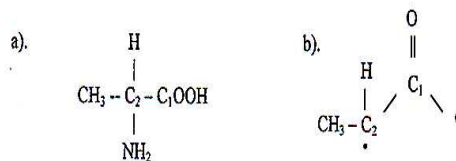


Figure 1. a) L- α -alanine structure, and b) stable alanine radical (SAR) structure¹⁰.

2. Experimental Procedure

Single crystals L- α -alanine were grown by slow evaporation of aqueous solutions of the commercial compound (BDH Chemical Ltd. Pole England). Isomedix Gammacell 1000 ¹³⁷Cs source capable to deliver a dose of 0.24 kGy per hour was occupied to irradiate the samples to the dose 28

kGy. A Varian E-12 EPR spectrometer equipped with a rectangular parallelepiped microwave cavity to produce TE₁₀₂ mode was utilized to measure X-band CW-single crystal spectra at room temperature. Experimental parameters were set to record an approximately first-derivative signal.

A goniometer with two degrees of freedom, design due to Morton *et al.*¹¹⁾ was used to fix the crystal in the cavity such that the desired orientation of the crystal with respect to the dc magnetic field can be obtained. Measurements were carried out for the B field fixed along <a>, , and <c> crystal axes, as well as on ab, bc and ca crystal planes. The results are shown in Figures 2 and 4.

3. Results and Discussions

Figures 2a-c show the experimental spectra with the magnetic field fixed to the three crystal axes, <c>, <a>, and . The dominating feature of the <c>'s spectrum in Fig. 2a is a quintet with the relative intensity close to 1:4:6:4:1. To the first order of approximation, this is due to 4 equivalent proton couplings of 26 gauss, the α -proton and the 3 protons of the freely rotating methyl group. This equivalency is explained by a different rate of reorientation of the methyl group of the radical at different temperature. At low temperature, for example 77K, it was found that the reorientation is completely quenched, that is, the rate of reorientation is much smaller than any of the hyperfine couplings constant belong to the system (A). The opposite behavior takes place at room temperature.

Fig. 2a B // <c>:

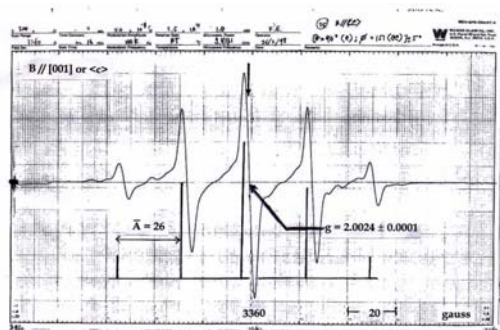


Fig. 2. The calculated and the experimental spectra of γ -irradiated L- α -alanine measured at room temperature when the B field applied: parallel to **a)** <c>, **b)** <a>, **c)** axes, respectively. The g-factors when the spectrum is centered are labeled. The arrow pointing downward indicate the position of the g-

factor of the standard sample: DPPH powder (2,0036). Experimental parameters are 200 gauss, 1 mW, 0.4 gauss 4.0×10^4 , 9.45 GHz, 4 sec and 16 min.

The hyperfine term of the Hamiltonian belongs to the three hydrogens of the methyl group, when quenching of the reorientation occurs, is approximated by the diagonal terms,

$$H_{\text{HF}} = (A^1 \mathbf{I}_{1z} + A^2 \mathbf{I}_{2z} + A^3 \mathbf{I}_{3z}) \cdot \mathbf{S}_z \quad (1)$$

Where A^1 , A^2 and A^3 are the hyperfine constants of the hydrogens. The \mathbf{S}_z and \mathbf{I}_z 's are, respectively, the components of the electronic spin of the paramagnetic electron and the nuclear spins of the protons, each of them is parallel to the external magnetic field. Energy of the Hamiltonian is, to the first order,

$$W = (A^1 \mathbf{I}_{1z} + A^2 \mathbf{I}_{2z} + A^3 \mathbf{I}_{3z}) \cdot M_z \quad (2)$$

This gives rise to eight nuclear spin states for each of the electronic spin states. These states are categorized into the following four, namely: [i] (+ + +); [ii] (+ + -), (+ - +), (- + +); [iii] (+ - -), (- + -), (- - +); and [iv] (- - -), where for example (+ - -) is the state for $\mathbf{I}_{1z} = +\frac{1}{2}$, $\mathbf{I}_{2z} = -\frac{1}{2}$ and $\mathbf{I}_{3z} = -\frac{1}{2}$.

When the rate of the reorientation becomes very much larger than the magnitude of A, the states which belong to [ii] and [iii] start to mix with each other. The mixing, however, should not occur to the inter-molecular interactions, because the states of [i] and [iv] are independent of reorientation and therefore of temperature. As a result, at room temperature, the Hamiltonian in Eq. (1) reduces to, (where it is assumed A^1 , A^2 and A^3 are all equivalent)

$$H_{\text{HF}} = A (\mathbf{I}_{1z} + \mathbf{I}_{2z} + \mathbf{I}_{3z}) \cdot \mathbf{S}_z \quad (3)$$

The analysis given above resulted in the quintet pattern as shown in Figure 2a. If the A^4 is the hyperfine coupling constant belongs to the H_α group, hence, the quintet pattern of the spectrum implies the equivalency of the methyl proton's splitting due to A^4 and the previously discussed splitting due to three methyl protons, 26 gauss each.

When the magnetic field is fixed along the <a> and axes, the quintet lines are then split into a quartet of line pairs with the intensity ratio close to 1:1:3:3:3:3:1:1 because at these orientations the α -proton coupling is smaller than that of the methyl proton. The experimental values are 18.7 gauss against 24.5 gauss for the spectrum along the <a> axis (Fig. 2b); and 19.7 gauss against 25.4 gauss for that along the axis (Fig. 2c). Weaker resonance features are also shown in addition to that forming the quartet of line pairs spectrum. The additional feature occurring between the main EPR transitions of the main radical (SAR)

is believed due to "second order effects"^{1,2)} which arose from the anisotropic dipole-dipole coupling of the CH group. This effect gives rise to off-diagonal elements that might be comparable in magnitude with the nuclear Zeeman term, ($g_N \beta_N B I_z$) in the spin Hamiltonian of the system.

Fig. 2b B // <a>:

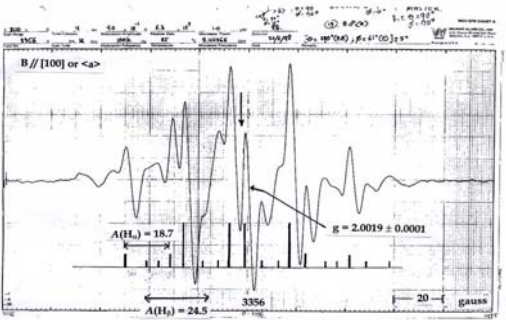
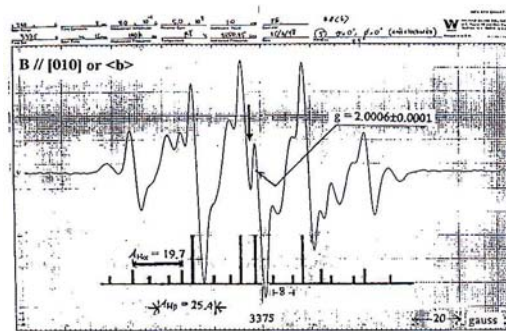


Fig. 2c B // :



The expectation values of the H for the electronic spin states are therefore,

$$H(\pm) = \pm \frac{1}{2} \{ g\beta B + (A_{zz} \pm 2 g_N \beta_N B) I_z + A_{zx} I_x + A_{zy} I_y \}$$

Here the (+) and (-) signs correspond, respectively, to the upper state with $M_s = + \frac{1}{2}$ and to the lower state with $M_s = - \frac{1}{2}$. The non-vanishing matrix elements of the $H(+)$ with $I = \frac{1}{2}$ and $M_I = + \frac{1}{2}$ for the hydrogen are,

$$\begin{aligned} \langle \frac{1}{2} | H(+)| \frac{1}{2} \rangle &= \frac{1}{2} (g\beta B) + \frac{1}{4} A_{zz} - \frac{1}{2} (g_N \beta_N B) \\ \langle -\frac{1}{2} | H(+)| -\frac{1}{2} \rangle &= \frac{1}{2} (g\beta B) - \frac{1}{4} A_{zz} + \frac{1}{2} (g_N \beta_N B) \\ \langle \frac{1}{2} | H(+)| -\frac{1}{2} \rangle &= \frac{1}{4} (A_{zx} - I A_{zy}) \\ \langle -\frac{1}{2} | H(+)| \frac{1}{2} \rangle &= \frac{1}{4} (A_{zx} + I A_{zy}) \end{aligned}$$

The secular equation that gives the energy eigen values of $H(+)$ for the upper state, $M_s = + \frac{1}{2}$, is

M_I	$\frac{1}{2}$	$-\frac{1}{2}$
$\frac{1}{2}$	$\frac{1}{2} (g\beta B) + \frac{1}{4} A_{zz} - \frac{1}{2} (g_N \beta_N B) - W$	$\frac{1}{4} (A_{zx} - I A_{zy})$
$-\frac{1}{2}$	$\frac{1}{4} (A_{zx} + I A_{zy})$	$\frac{1}{2} (g\beta B) - \frac{1}{4} A_{zz} + \frac{1}{2} (g_N \beta_N B) - W$

... (6)

The energy levels for the upper and lower states ($M_s = \pm \frac{1}{2}$), respectively

$$W_{\frac{1}{2}}(\pm) = \frac{1}{2} (g\beta B) \pm \frac{1}{4} A_{\pm}$$

$$W_{-\frac{1}{2}}(\pm) = \frac{1}{2} (g\beta B) \pm \frac{1}{4} A_{\pm}$$

(7)

Here $A_{\pm} = \{(A_{zz} \pm 2g_N \beta_N B)^2 + O^2\}^{1/2}$ and $O^2 = (A_{zx}^2 + A_{zy}^2)$. The possible transitions in those levels are shown in Figure 3.

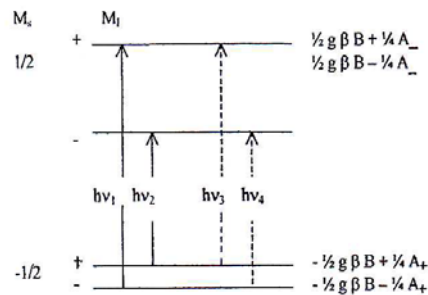
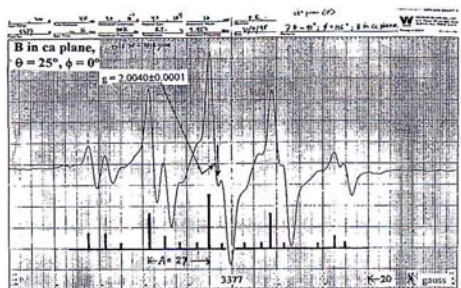
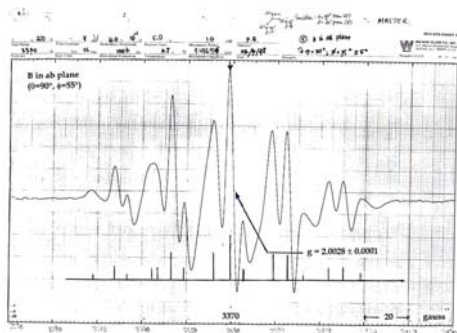


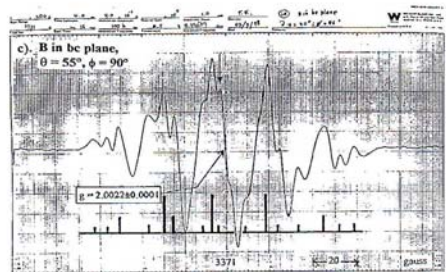
Figure 3. Energy diagram for the system of $S = \frac{1}{2}$ and $I = \frac{1}{2}$ for the Hamiltonian that include nondiagonal, the hyperfine as well as the nuclear, Zeeman terms. The levels are not to scale. The possible transitions, the doublets, are indicated by vertical lines: (—) = with the spacing d_+ ; (---) = with the spacing d_- . Frequencies that lead to the transitions are $h\nu_{1,2} = \{g\beta B \pm \frac{1}{4} (A_- + A_+)\}$; $h\nu_{3,4} = \{g\beta B \pm \frac{1}{4} (A_- - A_+)\}$. The spacing of $d_{\pm} = (A_- \pm A_+) / 2g\beta$ (in gauss).

Further, the change of the resonance (4) spectrum from the quintet for the <c> orientation with respect to the magnetic field to the nine-doublet lines for the <a> orientation is shown in Figures 4a-c.

In Fig. 4a, the B field of the spectrum is imposed on the ca plane: 25° from the <c> and perpendicular to the direction. The resonance spectra at this, and any other intermediate direction (see Figures 4b-c) are superposition of two different spectra. Referred to the data of the nuclear coupling constant for the CH group given by Miyagawa, *et al.*³⁾, it is found that one of the patterns is (5) practically a quintet at a direction as far as 60° from the <c> axis.

Fig. 4a B on ca plane:**Fig. 4b B on ab plane:**

Figures 4a-c: Using the same parameters as in Fig. 2 but the B field were applied on: a) ca plane, b) ab plane and c) bc plane. As above, the g-factors of the central lines and that of the DPPH powder are labeled.

Fig. 4c B on bc plane:

Superposition of the spectra on the crystal planes is expected because in those directions the four molecules in a unit cell are reduced into two pairs, each of which is magnetically equivalent. Explain to this answer is given in the results of the crystallographic and computed spectral simulation works that was

done by the author of the paper^{13,14}. This results are discussed briefly in the following sections.

For all the spectra depicted in Fig. 2 and 4 the magnetic field increases from the left to right, the g-factor at which the spectrum sweep is centered, and that of the DPPH powder are also indicated.

Symmetry related paramagnetic sites in the L- α -alanine crystal system.

The structure of L- α -alanine crystal is orthorhombic with the space group of $P2_12_12_1$. It contains four molecules (and therefore contains four paramagnetic sites) in the unit cell^[12]. Position of the four sites can be generated by applying a two-fold screw axis symmetry, that is, rotation of π about the crystal $\langle c \rangle$, $\langle b \rangle$ and $\langle a \rangle$ axes, respectively. Hence, coordinates of the site-1 = $\{x, y, z\}$; site-2 = $\{\bar{x}, y, z\}$, site-3 = $\{x, \bar{y}, z\}$ and site-4 = $\{x, y, \bar{z}\}$ (see Fig. 5).

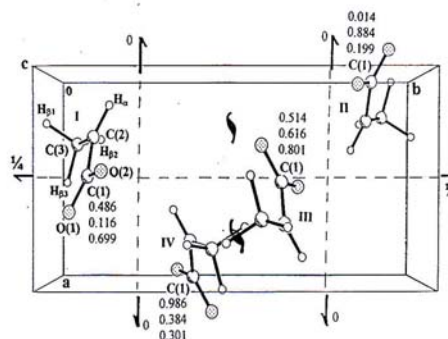


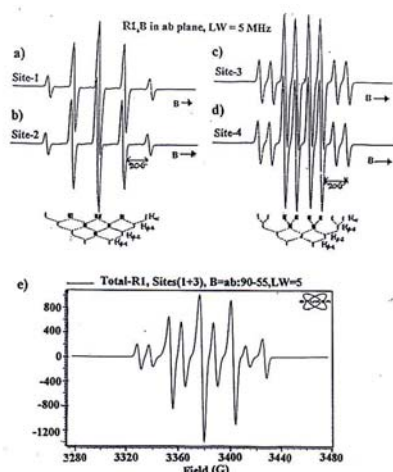
Figure 5: Four sites of L- α -alanine in unit cell (I-IV), viewed along the $\langle c \rangle$. The positions were generated from the SAR coordinates given by Kuroda, *et al.*⁵⁾. The fraction of atomic positions of C(1) to the origin 0 for the 4 sites are labeled. The --- and () symbols show the 2-fold screw axis symmetry applied in this system to generate 4 paramagnetic sites in the system¹³⁾.

For given experimental parameters, the electronic spin resonance pattern of L- α -alanine crystal is determined by orientation of the applied magnetic field B to the crystal frame. Given the coordinates in Fig. 5, when the B field fixed parallel to crystal axes, it is found that the four sites are equivalent to each other. From the point a view of symmetry operation, this is due to $\{x, y, z\} = \{\bar{x}, y, z\} = \{x, \bar{y}, z\} = \{x, y, \bar{z}\}$. It is said that all four sites are now magnetically equivalent. Application of the B field on crystal planes, however, results in equivalency of only two sites.

Pairings of this spectra are: [1] site-1 \equiv site-2, and site-3 \equiv site-4, when the B field on *ab* plane, because $\{x, y, z\} \equiv \{\bar{x}, \bar{y}, \bar{z}\}$, and $\{\bar{x}, \bar{y}, \bar{z}\} \equiv \{x, y, z\}$. [2] Site-1 \equiv site-4, and site-2 \equiv site-3, when the B field fixed on *bc* plane, because $\{x, y, z\} \equiv \{x, \bar{y}, \bar{z}\}$, and $\{\bar{x}, \bar{y}, \bar{z}\} \equiv \{\bar{x}, y, z\}$. [3] Site-1 \equiv site-3, and site-2 \equiv site-4, when the field applied on *ca* plane, because $\{x, y, z\} \equiv \{\bar{x}, y, \bar{z}\}$, and $\{\bar{x}, \bar{y}, \bar{z}\} \equiv \{x, y, z\}$. It is said that the two sites are magnetically equivalent.

Spectral simulation results on alanine system if the B field is applied on a crystal plane.

Computed spectral simulation (using the SOPHE and XEPR softwares Version 3.0 of Bruker) due to SAR in four sites for the B field on *ab* plane is shown in Figures 6a-e. As expected from the crystallographic results above, (see Fig. 6a-d) the spectra are equivalent in pairs, that is, Site-1's spectrum = Site-2's, and Site-3's = Site-4's. The thick diagrams under the spectra indicate the hyperfine structures expected from the H_β and H_α proton couplings. The linewidth was set, as trials, to be 5 MHz. Figure 6e shows the resultant spectrum due to addition between the normalized spectra in Site-1 and Site-3 in Fig. 6a and c.



Figures 6a-e. Computed simulation results on L- α -alanine system when the B field imposed on *ab* plane ($\theta = 90^\circ$, $\phi = 55^\circ$). The LW data, that depends on the θ and ϕ values of the B field as well as the M_I values, is estimated^[14].

As shown in the Fig. 6, one of the patterns is practically a quintet at a direction as far as 60° from the $\langle c \rangle$ axis, and this confirmed the results provided by Miyagawa²⁾.

Conclusion

Determination of free radicals induced by irradiation in the system of L- α -alanine based on EPR analysis is challenging, this might be due to the complexness of the paramagnetic sites existence in the system. However, as alanine has been proposed as one of the radiation dosimetry materials for number of years, thus elucidation of the radicals is very important.

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