

ESR Study on Radicalic Species on the Irradiation of the Aspartic Acid

Radu – Claudiu Fierascu^{*}, Mihail – Anghel Contineanu^{**}, Irina Dumitriu^{*},
Rodica – Mariana Ion^{*}

^{*} ICECHIM-București, Spl. Independenței, sect. 6, București

^{**} Universitatea București, Bd. Regina Elisabeta, 4-12, București

e-mail: radu_claudiu_fierascu@yahoo.com

Abstract

Knowing the way amino acids reacts to ionizing radiation is of great importance due to the fact that those results helps to better understanding of the effects of ionizing radiations on proteins, DNA-protein complexes, and others in which amino acids are the base structure. In the present work was study the behavior of aspartic acid (L, D and DL) at irradiation. The ESR spectrums are presented and a mechanism for the radical disappearance is proposed.

Keywords: ESR, ionizing radiation, aspartic acid

Introduction

The formation of radicals upon irradiation of simple amino acids (like α -glycine and l- α -alanine) was the subject of intense RPE studies since 1950, by the revolutionary studies of Gordy and Morton [1, 2].

Complete information on the radicalic trapped species was obtained on single crystals. Until now, ESR proved to be the most efficient technique.

In the past years the interest on amino acid irradiation in solid state was once again renewed, first due to their use in dosimetry, using RPE technique [3, 4], and second for better understanding the importance of some amino acids in the biochemical processes [5,6].

Electron paramagnetic resonance (EPR) or electron spin resonance (ESR) spectroscopy is a technique for studying chemical species that have one or more unpaired electrons, such as organic and inorganic free radicals or inorganic complexes possessing a transition metal ion. The basic physical concepts of EPR are analogous to those of nuclear magnetic resonance (NMR), but it is electron spins that are excited instead of spins of atomic nuclei. Because most stable molecules have all their electrons paired, the EPR technique is less widely used than NMR. However, this limitation to paramagnetic species also means that the EPR technique is one of great specificity, since ordinary chemical solvents and matrices do not give rise to EPR spectra.

EPR was first observed in Kazan State University by the Soviet physicist Yevgeny Zavoisky in 1944, and was developed independently at the same time by Brebis Bleaney at Oxford University.

Experimental

For the radiolysis, polycrystalline isomer acids L, D and DL Aspartic was used, commercial available (purchased from Fluka).

Samples from the amino acids were irradiated at room temperature using gamma radiation from a ^{137}Cs source with a initial activity of 1600 Ci.

The dose was $4 \cdot 10^2$ Gy/h. The RPE spectrums were recorded on an ART5 spectrograph, operating in the X band, with 100 kHz frequency modulation. The g factors were determined using a Mn^{2+} standard in CaO matrix.

Results and Discussion

By room temperature irradiation of solid state polycrystalline L and D aspartic acid identical spectrums are obtained. In figure 1, RPE spectrums of the two isomers are presented.

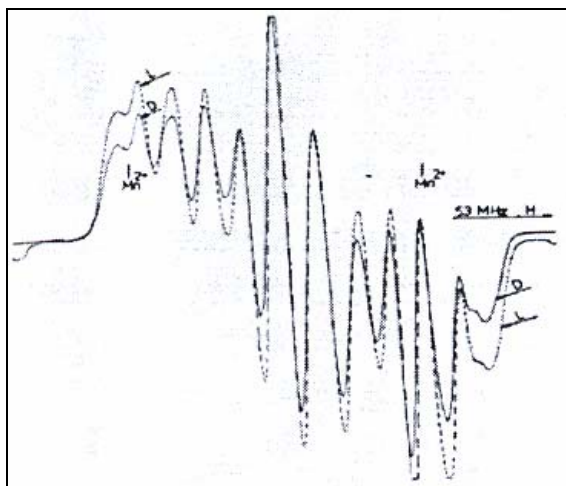


Fig. 1. RPE spectrum of polycrystalline sample irradiated with a dose of 10^4 Gy
a) line- D- aspartic and b) dotted line – L aspartic

The RPE spectrums of the DL aspartic acid are different form the one of L and D isomers. In figure 2 are shown those spectrums, recorded in the same conditions.

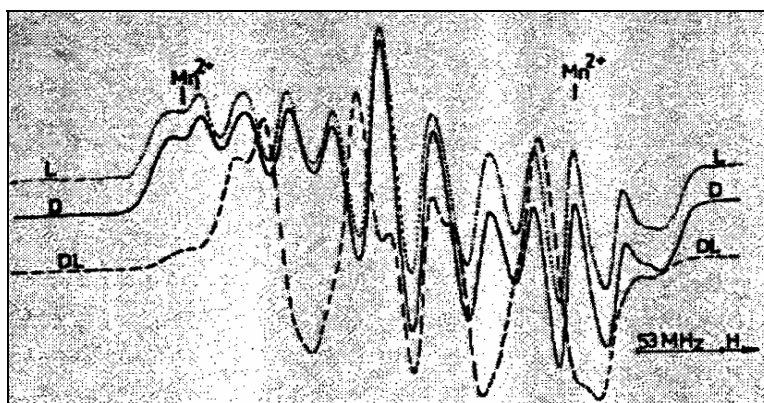


Fig. 2. RPE spectrum of aspartic acid samples irradiated with a dose of 10^4 Gy (L – aspartic acid, D- aspartic acid, DL-aspartic acid)

Radical accumulation with the irradiation dose is presented in figure 3.

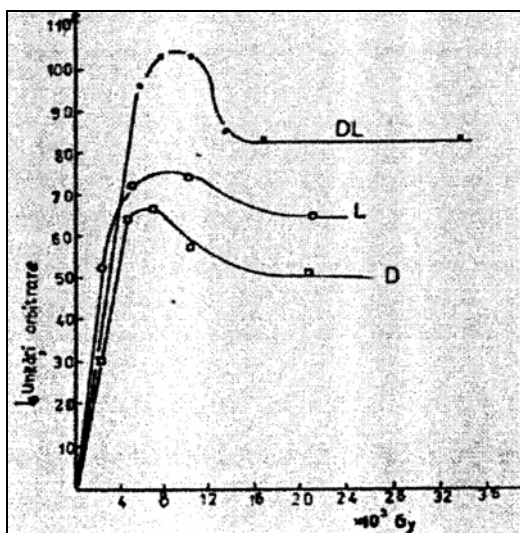


Fig. 3. RPE signal intensity variation (arbitrary units) as a function of integral dose

Those curves show that till a dose of $4 \cdot 10^3$ Gy the concentration of radicals is increasing with the irradiation dose. The curves pass through a maximum between $4 \cdot 10^3$ and $8 \cdot 10^3$ Gy, and after that begin to decrease, tending to a constant at great doses. All the isomers have similar radiolitical behaviors. The decrease after $8 \cdot 10^3$ Gy shows that the radiolitical process involves not only the formation of the radicals, but also their disappearance.

It is a known fact that the radiolitical process is non-selective, meaning that the radicalic species formed by irradiation can be transformed while the sample is irradiated, most likely in non-radicalic species.

For the kinetics of thermal recombination of the formed radicals the reaction isotherms were made. For that, the intensities of the RPE signal in time at constant temperature were recorded. The temperatures at which the kinetic study was conducted were chosen to satisfy two conditions:

1. the reaction must be completed more than 75%;
2. the speed of the reaction must be small enough to avoid experimental errors.

The following phenomenon was observed: if we compare the RPE spectrum of an irradiated amino acid sample before the heating with the one after the heating, a modification in its structure can be observed. As we know, the hyperfine structure of RPE spectrum gives information on the nature of the studied radical. The modification of the spectrum after the heating proves that the spectrum belongs to at least two radicalic species. In figure 4 it is represented the spectrum of L-aspartic acid, before (A) and after heating two minutes at 185°C (B).

At the heated sample it can be observed the disappearance of some extreme components and the center line increases about 30%. Those changes suggest that by heating the sample one of the radicalic species disappears, being less resistant at temperature.

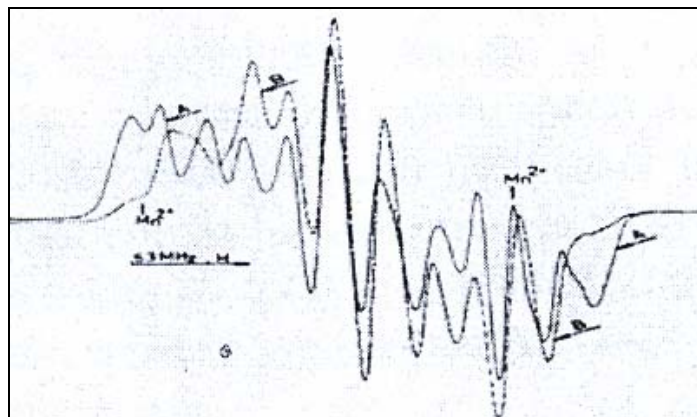


Fig. 4. RES spectrum of L-aspartic acid irradiated with a $5 \cdot 10^3$ Gy dose
a) unheated sample; b) sample heated at 185°C for 2 minutes

The increase of the center line can have two reasons:

1. a radicalic species can be transformed in another one, more stable;
2. by heating, some bonds of the radicals with neighbor molecules can be destroyed, allowing it's transformations in a more stable conformation.

The kinetics of the recombination necessitated the reaction isotherms. For that, RPE signal intensities were recorded, as a function of heating time, at constant temperature in the radical disappearance interval.

In figure 5 are presented the isotherms at 185 , 194 , 202 , 212 and 227°C .

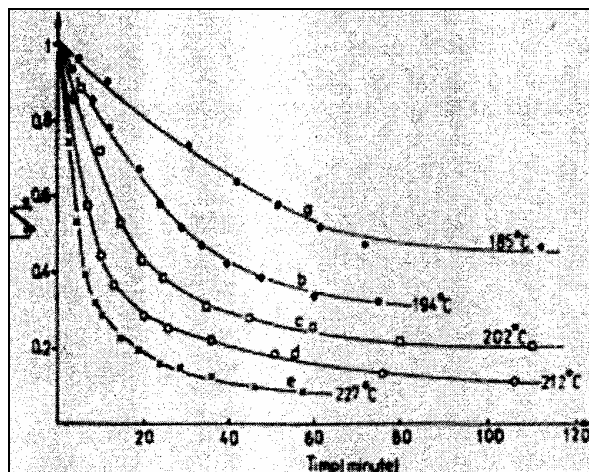


Fig. 5. Isotherm variation of RPE signal intensity at different temperature:
a) 185°C ; b) 194°C ; c) 202°C ; d) 212°C ; e) 227°C

All the spectrums were recorded at room temperature and only the central line was measured, the most intense in the spectrum.

From figure 5, we can observe that the radical concentration decreases faster at higher temperatures. Other important observation is that all tend to a constant, smaller as the temperature is higher. This suggests that at every temperature, some radicals do not disappear.

This can be explained by the fact that the paramagnetic centers are trapped different in the crystalline network, thus having different resistance to temperature.

For the kinetics of the radicals disappearance the known laws of kinetics was verified.

Experimental was observed that the variation $(I_s/I_0)^{-3/2}$ is the best fitted. This proves that the kinetics verifies a fractionary kinetics of the order 2.5. In figure 6 is represented the variation $(I_s/I_0)^{-3/2}$ at 227°C.

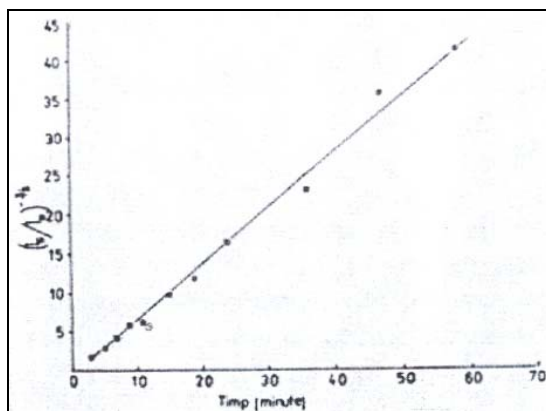


Fig. 6. $(I_s/I_0)^{-3/2}$ variation as a function of time at 227°C

The speed constants were calculated, and the values are presented in table 1.

Table 1. Speed constants values for thermal disappearing of paramagnetic centers formed at the irradiation of L-aspartic acid

Speed constants				
t°C	T K	$Y_T 10^3 K^{-1}$	$k 10^4 s^{-1}$	Ln k+y
185	458	2.183	4.8113	1.571
194	467	2.141	12.45	2.521
202	475	2.105	20.3	3.01
212	485	2.062	41.96	3.736
227	500	2.000	123.46	4.816

The activation energy was calculated from the representation $\ln k$ function of $1/T$ (figure 7) as 143.82 kJ/mol, and with a preexponential factor of $1.36 \cdot 10^{17} s^{-1}$.

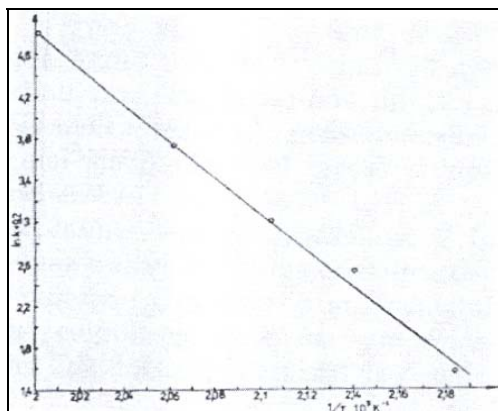


Fig. 7. Arrhenius type representation for the calculus of the activation parameters

The activation entropy was calculated from the formula:

$$\Delta S^*_0 = R[\ln(Ah / k_B T) - m] \quad (1)$$

with: $A = 1.36 \cdot 10^{17}$

$$h = 6.625 \cdot 10^{-34} \text{ J}\cdot\text{s}$$

$$k_B = 1.38 \cdot 10^{-23} \text{ J/K}$$

$$R = 8.31 \text{ J/(mol}\cdot\text{K)}$$

$$m = 2.5$$

so, $\Delta S^*_0 = 62.22 \text{ J/mol}$

The standard activation entropy (big and positive) is in good concordance with the mechanism of the process.

The proposed mechanism for the thermal disappearing of the formed radicals at the room temperature irradiation of L aspartic acid.

As we know, the radicals formed at the irradiation of the solid state samples remain trapped. At low temperatures (liquid nitrogen or liquid helium) all the radicals, even hydrogen atoms can remain trapped in the network.

As we shown when the temperature is raised above 77 K, RPE spectrums have changed their shape, proving that the less resistive radicals are transformed with the heating of the sample in more resistive species.

We can propose the existence of three radicals (R1, R2, R3), similar with those presented in the literature for l- α -alanine [7].

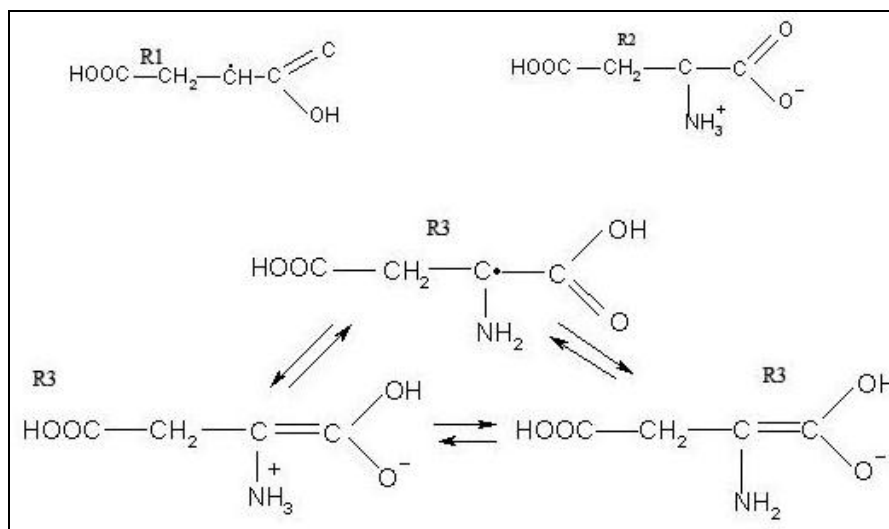
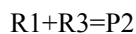


Fig. 8. The three radicals proposed for the irradiation of the aspartic acid

The kinetics of the disappearance of the radicals R1 and R3, at temperatures below the melting point of L-aspartic acid, can include more processes to explain the order 2.5.

Probably, those are:

1. The recombination of the radicals and the transformation in entities not paramagnetic.



By this mechanism, it can be observed that three radicals R1 disappears, but the first reaction is preponderant, due to the much larger concentration of R1.

2. A second possibility is the disappearance of the radical character by reaction with a neighbor molecule, with home it realize hydrogen bounds:



3. A third possibility is that, by heating, the radicalic entity decomposes by breaking weak bounds, forming stable molecules or gases like CO₂ or NH₃.

Conclusions

Knowing the way amino acids reacts to ionizing radiation is of great importance due to the fact that those results helps to better understanding of the effects of ionizing radiations on proteins, DNA-protein complexes, and others in which amino acids are the base structure.

In the present work was study the behavior of aspartic acid (L, D and DL) at irradiation.

The modification of the spectrum after the heating proves that the spectrum belongs to at least two radicalic species.

For the kinetics of the radicals disappearance the known laws of kinetics was verified. Experimental was observed that the variation $(I_s/I_0)^{-3/2}$ is the best fitted. This proves that the kinetics verifies a fractionary kinetics of the order 2.5.

The fact that all the radical concentration tend to a constant suggests that at every temperature, some radicals do not disappear. This can be explained by the fact that the paramagnetic centers are trapped different in the crystalline network, thus having different resistance to temperature.

By analogy with the literature, a mechanism for the radical disappearance is proposed. The activation entropy calculated is in good concordance with the mechanism proposed.

References

1. Morton, J. R., J. Chem. Rev. 64, 1964, p 453;
2. Gordy, W., *Theory and Application of Electron Spin Resonance*, Wiley, New York, 1980;
3. Heydary, M.Z., Malinen E., Hole E.O., Sagstuen E., J. Phys. Chem., A 106, 8971, 2002;
4. Malinen E., Hult E.A., Hole E.O., Sagstuen E., Rad. Res, 159, 149, 2003;
5. Mezzeti A., Maniero A.L., Brustolon M., Giacometti G., J. Phys. Chem., A 103, 9636, 1999;
6. Him o E., Chem. Phys. Lett., 328, 270, 2000;
7. Vanhaelewyn G.C.A.M., Mondelaers W.K.P.G., Callens F., J. Radiat. Res., 151. 590, 1999

Studiu prin RES al speciilor radicalice apărute la iradierea acidului aspartic

Rezumat

Cunoașterea modului în care aminoacizii reacționează la acțiunea radiațiilor ionizante are o mare importanță datorită faptului că aceasta ajută la mai buna înțelegere a efectelor radiațiilor ionizante asupra proteinelor, complexilor ADN-proteine și a altora în care aminoacizii reprezintă structura de bază. În lucrarea de față este studiată comportarea acidului aspartic (L, D și DL) la iradiere. Spectrele RES sunt prezentate, ca și un mecanism pentru a explica dispariția radicalilor.