

Study on the Radical Species formed by Gamma Radiolysis of Alpha Aminobutyric Acid (α AMB)

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An EPR study of the paramagnetic centers formed by irradiation of polycrystalline α aminobutyric acid was made. In the EPR spectra of the α AMB gamma irradiated powder samples at room temperature, the presence of two types of radicals was observed. A radiolysis mechanism is proposed.

Keywords: thermal annealing, hyperfine splitting, paramagnetic centers

Ionising radiation has been used for a long time in order to sterilize medical devices, to improve hygienic quality of food [1] or for sterilization of drugs [2] and cosmetics.

Biological molecules such as proteins and DNA are very sensitive to the ionizing radiation and this problem has a fundamental importance in biology and medicine (e.g. mechanism of mutagenesis and radioprotection [3]).

Amino acids, which are the building blocks of proteins are among the simplest organic molecules of biological relevance and thus serve as convenient model systems in studies of radiation damage.

Understanding the radiation chemistry of the amino acids is of substantial importance with respect to realizing how radiation interacts with proteins of which the amino acids are the elementary building blocks and also with protein-DNA complexes like chromatin [4].

The study of amino acids irradiation in solid state has a double importance: first because of the role they play in biological processes [4] and secondly due to the use of some amino acids as dosimetry systems [5].

EPR was proposed as a method to measure radiation doses and alanine dosimetry has now already been used for a long time as a standard method by National Metrology Institutes [6].

It is well known that if the crystals of organic acids are irradiated by ionizing radiation at low temperature, an ejected electron is captured by neighboring molecule to form carboxyl anions. Since amino acids are known to form a zwitterion in the crystal, there is no acidic proton in the carboxyl group [7].

Alpha-amino-butyric acid (α AMB) is a non-protein amino acid that can protect certain plants from pathogens through activation of natural defense mechanisms of the plant.

Alpha-amino-butyric acid (α AMB) like all the amino acids performs a zwitterion structure as a result of mutual neutralization of the two groups: acidic and alkaline. From multiple studies of solid state radiolysis of glycine [8] and alanine [9] resulted that amino acids have a complex chemical behaviour. The EPR spectra of the α AMB irradiated samples are complex because of simultaneous trapping of some radiolytically formed radicals. The paramagnetic entities having different thermal stabilities could be identified through increasing the temperature from 77 K to 423 K.

Experimental part

Reagents

Polycrystalline DL- α -amino-butyric acid was Fluka reagent of purity $\geq 99\%$.

The samples were irradiated at room temperature with γ radiation using a ^{137}Cs source at a dose rate of $0.9 \cdot 10^2$ Gy/h. The EPR spectra of irradiated samples were recorded by means of an ART 6 instrument (Institute of Nuclear Physics-Bucharest), which operates in the X band, with a high frequency modulation of 100 kHz. The g factors were determined using the Mn^{2+} ion in CaO matrix, as a standard.

Results and discussions

In the present study polycrystalline samples of α AMB were gamma irradiated at room temperature and the EPR spectra were recorded.

In figure 1 the EPR spectrum of α AMB irradiated sample with $5 \cdot 10^3$ Gy dose is presented.

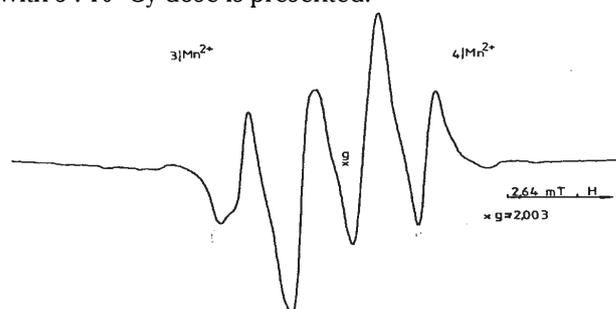


Fig.1. The EPR spectrum of α AMB polycrystalline sample irradiated at room temperature

Figure 1 spectrum is attributed to $\text{CH}_3\dot{\text{C}}\text{H}(\text{NH}_3^+)\text{CO}_2^-$ (R_4) radical which is stable up to 60°C .

According to literature data [10] the structure of this radical spectrum should be a double quintet but, as it is seen in figure 1 the spectrum is not properly resolved. This is a normal behaviour because α AMB acid was irradiated in polycrystalline powder. In these conditions the magnetic anisotropy of the irradiated sample produces lines broadening and deformation so that the spectral components (or the spectral lines) partially overlap. The spectrum from figure 1 is characterized by five components structure, the two left overlap resulting four well resolved lines, the separation of the peaks being ranged between 2.3-2.7 mT. Similar to literature information [10] the R_4

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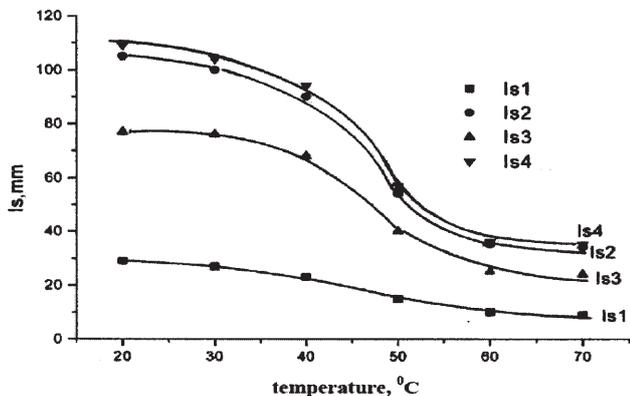


Fig.4. Variation of the spectral lines intensities versus temperature

occurs through the presented mechanism, but also the disappearance one by recombination.

The $R_4 \rightarrow R_7$ conversion is also sustained by the fact that with increasing temperature, the decrease of the four spectral lines intensity does not occur in the same proportion. Each spectral component presents a particular variation because it belongs simultaneously to two (R_4, R_7) radicals entities having different chemical structures. Due to this transformation process is ascertained that through heating, the modification both of the shape and each spectral lines structure occurs. Comparing the irradiated sample spectrum recorded after 5 min heating at 120°C with the same sample spectrum after 5 min heating at 150°C, it was ascertained the decrease of the lines intensities without their structure modification, which proves that the R_7 radical disappears without turning in to other radicals (fig. 5).

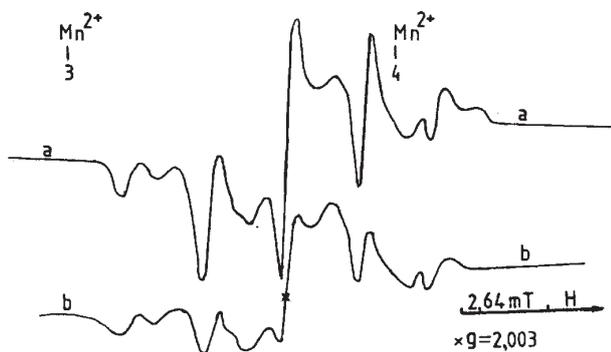


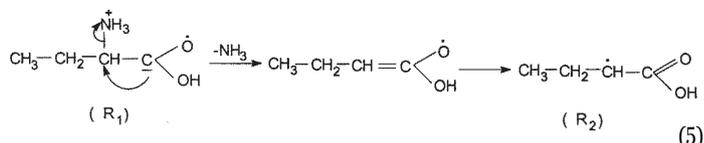
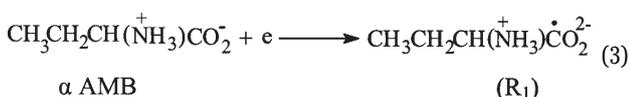
Fig.5. The EPR spectra of α AMB irradiated sample heated 5 min at: 120°C (a) and 150°C (b)

This behavior is a proof that R_7 radical can disappear both through a recombination process but also through conversion into other non-paramagnetic species having unknown structure.

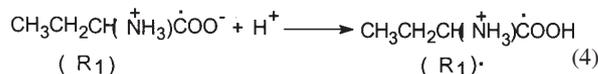
Radiolysis mechanism

The reduction route

The primary radiolytic process is the oxidation one and consist from expulsion of an electron from the carboxyl group of the α AMB acid. The expelled electron is captured after thermalization by the α AMB carbon atom of the carboxyl group, forming the reduced radicalic species having R_1 structure.



In all irradiated amino acids at 77 K [12] it was experimentally found that the EPR spectrum comes from a protonated structure of R_1 radical-anion. Electrostatic interaction between amino group positive charge and the carboxyl group negative one performs hydrogen bonds. The amino group protons perform three hydrogen bonds with the carboxyl groups of the surrounding molecules. Carboxyl group protonation occurs to compensate the excess of this group negative charge and takes place after the breaking of the shortest bond performed by the carboxyl anion with the amino group proton. The protonated radical-anion was identified on alanine irradiation [13].



In figure 6 is shown the EPR spectrum of the γ irradiated at 77 K α AMB with $5 \cdot 10^3$ Gy dose rate.

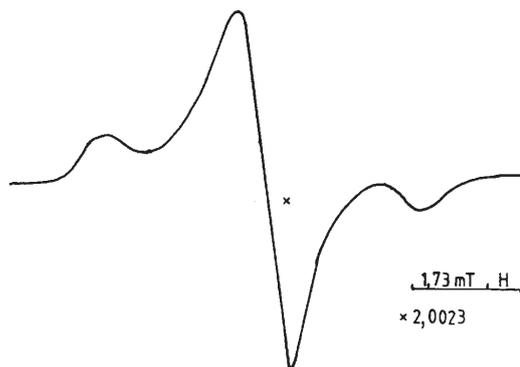


Fig.6. The EPR spectrum of the irradiated α AMB at 77 K and recorded at the same temperature

The spectrum from figure 6 consists of an intense singlet, the line width being about 1.7 mT, accompanied by one shoulder on each side. The two satellites are separated by about 6.8 mT.

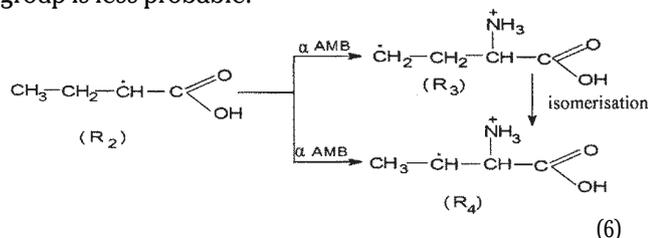
α AMB acid was deuterated by recrystallization after dissolving in D_2O (98.6%). The polycrystalline samples were irradiated at 77 K and their EPR spectra were recorded at the same temperature. The recorded spectra were similar with those of the non-deuterated ones which prove that there has been no exchange of hydrogen with deuterium. The central singlet spectrum of the irradiated polycrystalline sample at 77 K (fig. 1) is attributed to the protonated R_1 radical-anion having the odd electron located on the $2p\pi$ orbital of the carboxyl carbon atom. The weak splitting in two satellite components is due to the electron interaction with β proton of the adjacent C-H group. It was fully established that the anionic species formed by ionizing radiations in other amino acids: glycine [14], alanine [15], α -aminoisobutyric acid [16] and valine [17] spontaneously deaminate and generates the R_2 intermediate radical which at low temperature can undertake other reactions. The R_1 radical-anion deamination is a characteristic process of all amino acids.

The $R_1 \rightarrow R_2$ deamination occurs between 100-115 K and is similar to the found on glycine [18], α -alanine [19], α -aminoisobutyric acid [20] irradiation.

The amino group is extracted from R_1 radical by a non-paramagnetic path through the following intra molecular process:

In the 103-115 K temperature range, R_2 radical is converted into R_3 and R_4 radicals, reactions having very low activation energy and without the modification of the total concentration [11]. The R_3 and R_4 radicals formation is produced by the extraction of an H atom from the parent molecule neighbouring to R_2 radical, because the temperature being low, the radical diffusion in to the lattice can not take place.

It should be noted that the C-H bond breaking from a carbon atom is a known process from long chain n-alkanes radiolysis, irradiated at 77 K [21]. But, the formation of a radical through the H atom extraction from the CH_3 terminal group is less probable.



In the temperature range of 125-145 K, R_3 radical disappears and R_4 shows an increase [10]. This behaviour proves the transformation of R_3 radical into R_4 through an isomerisation process [13]. The chemical structure of R_3 and R_4 radicals are correlated with the corresponding structures from literature of the EPR spectra recorded on α -AMB (BDH) irradiation [10].

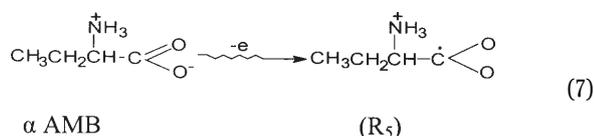
R_3 radical spectrum consists of four triplets (quartet of triplets). The splitting of the spectrum into three components varies from 1.95-2.58 mT, indicating the odd electron interaction with two α equivalent protons. The splitting into four of each of the three components suggests the interaction with two β non-equivalent protons.

The stable radical R_4 spectrum consists of two quintets having the intensities ratio 1:4:6:4:1. The five lines spectrum recorded by Ayscough and Olsen [10] is almost isotropic but the peak separation varies between 2.4-2.5 mT, proving that in the R_4 radical, the odd electron interacts with an α proton and four β protons. Indeed, the quintet duplication agrees with the R_4 radical structure, being attributed to the electron interaction with one α proton and four equivalent β protons. This radical is stable at room temperature for several days.

According the information given by Mach [11], the equivalence of β protons of the methyl group with the other β proton from R_4 radical is due to the rapid rotation of the CH_3 group to the carbon atom having an odd electron, a process that takes place even at 100 K. The primary radiolytic process characteristic to all amino acids is also the oxidation.

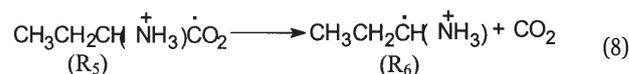
The oxidation route

α AMB oxidation consists in the removal of one electron from an oxygen atom of the carboxyl group [22]. The lack of the electron results in creating a positive vacancy on the carboxyl group. The primary oxidation species is the R_5 radical cation.



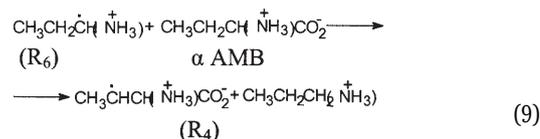
On α AMB solid state irradiation, the electron deficient species (R_5) was not detected experimentally even at 26 K

and therefore is considered to decompose spontaneously because the excited state is formed. The disappearance of the (R_5) radical-cation involves only decarboxylation followed by R_6 radical formation.



This process is sustained by highlighting CO_2 formation through gas chromatography by chemical analyzing of α AMB irradiated samples [23]. It is important to note that R_6 radical was identified neither in this study nor in the previous ones.

In conclusion, on amino acids irradiation it was found that the deamination product results from the protonated anion ($R_1 \rightarrow R_2$) and the radical-cation decomposes by decarboxylation [24]. Decarboxylation is supported by a pulse radiolysis combined with an EPR study [25], when it was found that the decarboxylation process of the glycine radical cation presents the same activation energy of alanine. A particular behaviour fully established was that in the case of all irradiated aliphatic amino acids the decarboxylation product is very unstable [26]. A possible disappearance path of the R_6 decarboxylation product is the removing of a hydrogen atom from a neighboring molecule to form the R_4 radical.



The R_6 radical seems to extract only the methylene group proton from the neighboring parent molecule, at low temperature. There is no indication of $\text{CH}_3\text{CH}_2\dot{\text{C}}(\text{NH}_3^+)\text{CO}_2$ radical formation.

At 175 K all unstable radicals were converted into R_4 stable radical, which was formed from R_2 through an hydrogen atom extraction from an α AMB adjacent molecule but also from other less stable radicals (R_3 and R_6).

Conclusions

The reactions occurring in the α AMB radiolysis process are more numerous and more complex than the known ones from the other studied amino acids irradiation.

The sequence of reactions that include: electron capture, deamination and a hydrogen atom removing from the parent molecule is a complicated process because takes place on limited temperature ranges. It is also considered the hydrogen atoms multiplicity that can be extracted and also the intermediates radicals isomerization reactions. Of the seven radicals involved in the radiolytic mechanism, only two are stable at room temperature (R_4 , R_7).

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