A DSC Study on Gamma Irradiated Isomers of the Aspartic Acid

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The behaviour on heating of irradiated with γ rays and non-irradiated L-, D- and DL- isomers of the aspartic acid (Asp. acid) were investigated by means of differential scanning calorimetry (DSC). All isomers have two decomposition steps which partially overlap. The measurements have shown that after the irradiation of the three isomers the decomposition temperatures and enthalpies decrease. The isomers of Asp. acid can be arranged in the following ascending order of radiation stability DL < D < L.

Keywords: aspartic acid, isomers, DSC, gamma radiation

Radiation effects on biological materials are of multi-disciplinary interest. The study of the effect of ionizing radiations upon amino acids is important for several reasons (theoretical and practical): (i) the investigation of the kinetics and mechanism of the radiolysis of a class of organic compounds of exceptional biological importance; (ii) the use of the results of the above mentioned study as a starting point in the evaluation of the effect of ionizing radiations upon proteins and protein-DNA complexes (the cleavage of the peptide chain is the significant way of macromolecular structure destruction of DNA and leads to biological activity loss); (iii) the use of the results of the above mentioned study as a starting point for extrapolating the effects in irradiated foodstuffs.

The radiation effect on biological materials has practical application in many fields such as food preservation, radioprotection [1] surface modification of materials [2].

Biological macromolecules such as proteins or DNA are known to be very sensitive to ionizing radiation. This problem has a fundamental importance in biology and medicine (e.g. mechanism of mutagenesis and methods of radiation protection), on one hand, but on the other hand, it hinders the investigation of biologically important systems by physical methods using intense beams of photons or charged particles.

The irradiation of proteins (enzymes included) in solid state produces the deamination and decarboxylation of final groups and peptidic bonds breaking. Investigation of radiation effects on small biomolecules like amino acids would be helpful to understand the radiation effects on more complex biological system, such as proteins or even tissues and organisms [3]. Study on aspartic acid may also be a value in aspects of evolution of life, as it is a kind of optical active molecule that was present in the early solar system before life began [4].

The understanding of chemical transformations caused by radiations in aspartic acid isomers is important because of their role in biological systems. Aspartic acid is a specific cleavage site for caspases and thus is a key residue in apoptosis processes [5].

It is the primary amino acid, synthesized by bacteria which are able to assimilate molecular nitrogen (living on the roots of some plants). Aspartic acid is used to treat chronic fatigue because of its role in cellular energy production in the mitochondria, participating in the generation of adenosine triphosphate, required fuel for cell activity. Aspartic acid participates indirectly in the synthesis of antibodies and the proper functioning of RNA and DNA and helps to remove ammonia and some toxins from blood (being necessary for the brain and nervous system functioning). The thermal analysis of irradiated amino acids is a useful tool in the investigation of the effects of irradiation on living mater. It is important also because some of them can be identified on the basis of their decomposition temperature because these values are distinct and do not overlap with those of adjacent α amino acids [6, 7].

Experimental part

The Asp acid isomers (L-, D- and DL-) were Merck reagents of purity ≥ 99.5%. Amino acid samples were irradiated with gamma rays from a 137Cs source (Gammator type). The dose rate, determined by means of the Fricke dosimeter was 1.05·10² Gy/h. The DSC thermograms of irradiated and non-irradiated isomers of aspartic acid were obtained by means of a Perkin Elmer 1B calorimeter, in static air. The samples, weighing 3 to 7 mg, were placed in standard aluminium crucibles, not tightly closed. The measurements were performed between 42 and 400°C, at 4 K/min heating rate and with a sensitivity of 4 mcal/s. The calibration of the calorimeter was done using indium as a standard. The peak area was evaluated using tangential sigmoidal baseline correction.

Results and discussion

Although the chemical properties of the isomers L-, D-, DL-aspartic acid are known to be the same, the DSC thermograms have shown different thermal behavior of both non-irradiated and irradiated isomers.

Figures 1 and 2 present the DSC thermograms recorded for non-irradiated and irradiated isomers of aspartic acid respectively. Analyzing these thermograms it can be observed that the three optical isomers have both similarities and differences regarding their thermal behavior. A similarity consists of the endothermic thermal effect for all three non-irradiated and irradiated isomers extending on a wide temperature range between 200-280°C.

The data obtained by us from the DSC curves for the non-irradiated L-aspartic acid are shown in figure 1a. Two
peaks are registered with maxima at about 240 and 260°C. These data are similar with those obtained by other authors [8, 9]. The thermal analysis of dicarboxylic amino acids shows that they start to decompose at about 200°C, that is just before melting. In the first step a weight loss of almost 30% was noticed. According to literature [8], aspartic acid decomposes before melting and the wide range of temperature on which this process takes place proves that it occurs at a relatively low rate.

The thermal fragmentation of various amino acids is a very complex process, which involves many pathways such as decarboxylation, deamination, dehydration and condensation reactions. In a recent study, the researchers concluded that the most favored process in the thermal decomposition of aspartic acid is decarboxylation [10]. Some information about the fragmentation scheme of amino acids is obtained from gas chromatography–mass spectroscopy studies of their esters. Such a study of the diethyl ester of the aspartic acid indicates a complicate process with several final products, such as: propiolactame, ethyl amine, β-amino propion-aldehyde [11].

Some discrepancies between the thermogravimetric data of different authors [8, 9, 12] can be observed. According to DSC thermograms the decomposition -melting process of L-aspartic acid is proceeding in two steps, partially overlapping. These data suggest that the first peak, of lower intensity, indicates probably the start of the thermal decomposition, while the second one belongs mostly to melting (also accompanied by decomposition, but the weight loss is slower above 260°C).

The size of the second peak showing a pronounced endothermic effect cannot be attributed only to melting as its maximum temperature is lower than the melting temperature (270°C) reported for this amino acid [13]. The melting point commonly used to characterize an organic compound is unlikely to be applied to α-amino acids because of their decomposition. Neutron diffraction studies established that aliphatic amino acids are found in solid state as zwitterions [14], which interact electrostatically. The melting points of α-amino acids are high because of electrostatic interactions and hydrogen bonding between the COO⁻ and NH₃⁺ groups linked to the same α-carbon atom. This dipolar ion structure is the main factor influencing their thermal behavior [15].

The two endothermic peaks from figures 1 and 2 are close to one another. This feature proves that several endothermic processes occur in close temperature ranges and therefore they overlap. It means that after the sample heating to the temperature of the first peak, the decomposition process continues at higher temperatures.

The explanation is as follows: we have worked with closed but not sealed crucibles and therefore the decomposition products have accumulated in the space inside the crucible and their evacuation took place gradually with increasing temperature. The accumulation of gases inside the crucible produced the decrease of the decomposition rate.

This finding is fully consistent with the thermal analysis performed on L-aspartic acid [9]. Working at a higher heating rate (10°C/min) the overlap of the two processes was more evident. This is because the development of the decomposition process depends on both temperature and heating rate.

A different behaviour is met in the case of the D-isomer and in that of the racemic (fig. 1b and 1c, respectively). A “shoulder” is observed instead of the first peak. The EPR study of aspartic acid enantiomers performed in a previous paper [16] showed similar spectra, that is the radiolysis mechanism is the same. However the thermal behavior of
the three isomers is different including that of the radicals formed during the irradiation, although only the racemic presents an EPR spectrum which differs from those of enantiomers.

While the different behaviour of the L-isomer and of the racemic (which was also observed in the EPR spectra) [16] is easily explained by their different molecular conformations in crystalline state, it is more difficult to find a reason for the fact that the enantiomers give almost the same EPR spectrum (irrespective of the irradiation dose), but unlike thermograms and that the effect of irradiation on the thermal effect is different too. As the peaks of the thermograms are due at least partially to chemical processes, their shape is greatly influenced by the reaction kinetics and consequently by the morphology of the solid reactant. While our samples of L-aspartic acid were formed from white small crystals, those of D-enantiomer and of racemic were under the form of powders, which decompose more easily.

The morphology of samples explains probably some discrepancies observed between the thermogravimetric data of different authors [8, 9, 12], as well.

The temperatures of the onset ($t_i$), completion ($t_f$) and peaks maxima ($t_{m1}$, $t_{m2}$) were determined, as well as the temperature range of the endothermic processes ($\Delta t$), for each isomer.

The variation with the irradiation time of ($\Delta t = t_f - t_i$) for the three isomers is shown in figure 3a, while the decrease of the temperatures corresponding to the maximum of the first ($t_{m1}$) and second ($t_{m2}$) peaks with the irradiation time is given in figures 3b and 3c, respectively.

The effect of $\gamma$ radiation upon the aspartic acid isomers is sustained by:

- the decrease of $\Delta t$ range (fig. 3a) and the shift of the maxima of the decomposition and melting peaks, $t_{m1}$ and $t_{m2}$ (figs. 3b and 3c) towards lower temperatures with the increase of the irradiation time.

- From figures 3a, 3b and 3c it can be remarked that the effect of radiations on the decrease of $\Delta t$, $t_{m1}$, $t_{m2}$ is significant for all three isomers, at low doses; a tendency towards a plateau with increasing dose is observed at higher doses. The same comment was formulated in [17] following the plot of the alanine decomposition temperature versus the irradiation dose. This behaviour is due to the formation of radiolysis products of amino acids. The presence of radiolytic products in the irradiated samples of amino acids even in small concentration has the same effect as the presence of impurities: they modify the crystalline structure of the substance and produce the decrease of the above-mentioned parameters.

The process is eased by irradiation, because deamination was already started under the action of gamma rays [16]. All temperature values $\Delta t$, $t_i$, $t_f$, $t_{m1}$, $t_{m2}$ and the enthalpies $\Delta H$ on the DSC thermograms decrease with the irradiation dose. We consider that the weight of this reaction in the overall decomposition process increases with the irradiation dose.

Reaching of a plateau is due to the accumulation of radiolysis products with increasing irradiation dose. As a consequence, the irradiation products have a protective effect on the amino acid parent molecule.

The average values of the temperatures $t_i$, $t_f$, $t_{m1}$, $t_{m2}$, calculated for a wide range of irradiation doses from $10^3$ to $6 \times 10^4$ Gy (where there are no significant changes in the DSC thermograms) are listed in table 1.

A decrease of the four temperatures $t_i$, $t_f$, $t_{m1}$, $t_{m2}$ is noticed in the order L-Asp > D-Asp > DL-Asp isomers for both non-irradiated and irradiated.

### Table 1

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Dose, Gy</th>
<th>$t_i$ ($^\circ$C)</th>
<th>$t_f$ ($^\circ$C)</th>
<th>$t_{m1}$ ($^\circ$C)</th>
<th>$t_{m2}$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L Asp.</td>
<td>0</td>
<td>223.0</td>
<td>273.6</td>
<td>241.9</td>
<td>264.5</td>
</tr>
<tr>
<td></td>
<td>$(0.1-6) \times 10^4$</td>
<td>213.2 ±0.9</td>
<td>259.4±1.4</td>
<td>227.2±2.1</td>
<td>251.4±2.0</td>
</tr>
<tr>
<td>D Asp.</td>
<td>0</td>
<td>215.9</td>
<td>261.4</td>
<td>227.0</td>
<td>253.2</td>
</tr>
<tr>
<td></td>
<td>$(0.1-6) \times 10^4$</td>
<td>212.7±0.9</td>
<td>258.5±1.7</td>
<td>224.6±0.8</td>
<td>249.9±2.0</td>
</tr>
<tr>
<td>DL Asp.</td>
<td>0</td>
<td>203.0</td>
<td>245.9</td>
<td>223.7</td>
<td>243.1</td>
</tr>
<tr>
<td></td>
<td>$(0.1-6) \times 10^4$</td>
<td>200.2±1.5</td>
<td>245.2±2.3</td>
<td>218.1±3.5</td>
<td>235.2±1.8</td>
</tr>
</tbody>
</table>
The decomposition enthalpy values, corresponding to both endothermic peaks for the three irradiated and non-irradiated isomers were calculated from DSC curves and are listed in table 2.

A linear decrease of the decomposition enthalpy with increasing irradiation time is noticed for all three isomers. The decrease in decomposition enthalpy with irradiation time is illustrated in figure 4.

A similar diminishing in the melting enthalpy caused by irradiation has been observed earlier for some antibiotics and steroids [18, 19].

It should be noted that the values from table 1 and figure 4 depend on experimental and structural factors such as: the amount of substance, the morphology of the sample and the tightness insured when sealing the crucible.

All the effects observed including a decrease of the melting point and of the values of the enthalpies of decomposition and melting of aspartic acid after irradiation are due to the break of covalent bonds produced by the high energy ionizing radiation. Weaker interactions stabilizing the crystal lattice are also perturbed in this way and as a result defects are appearing in the crystal lattice.

The thermal effect is registered on a wide temperature range (200 - 280°C) because of the increase in the partial pressure of reaction products (closed crucibles were used).

Although the general properties of the isomers L-, D- and DL-Asp are known to be the same, the present DSC study showed a different thermal behavior. These differences were found both for non-irradiated and irradiated isomers and are assigned to the difference in morphology of the enantiomers crystals.

**References**


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