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Abstract

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ON THE RADIOLYTIC PROPERTIES OF AMINO ACIDS AND PEPTIDES

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In the present work an attempt was made, by means of the EPR method, to determine the influence of various functional groups and of their relative arrangement in the molecule on the radiation-chemical properties of molecules, using certain amino acids and peptides as examples.

The samples—dry polycrystalline powders—were irradiated under vacuum at low ($\geq -196^{\circ}$) and room temperatures on the electron accelerator of the L. Ya. Karpov Institute and with ^{60}Co preparations. To record the EPR spectra, a radiospectrometer of the EPR-2IKhF type was used ([1]).

In terms of radiation stability, α-amino acids may be provisionally divided into three groups: aliphatic amino acids containing no heteroatoms (G_R of the order of unity), aromatic amino acids ($G_R \sim 0.3$ and lower), and aliphatic amino acids containing various functional groups with heteroatoms O, S, N ($1 > G_R > 0.3$). It may be noted that increasing the number of methylene units in the series of amino acids studied in groups 1 and 3 has practically no effect (or only an insignificant effect) on the values of G_R. However, replacement of hydrogen atoms bonded to secondary carbon atoms by a methyl group, as can be seen from the data of Table 1, somewhat lowers the radiation stability of the corresponding amino acids (for example, norvaline and leucine, serine and threonine). As can be seen from the data of Table 1, replacement of one of the hydrogen atoms in α-alanine by groups containing heteroatoms (O or S), on the contrary, has a protective effect (α-alanine and serine, cystine, cysteine, asparagine, aspartic acid). The low yield of radicals in amino acids of the second group is evidently associated with the presence in the molecule of aromatic rings—systems known to be the most resistant to the action of radiation. It is interesting to note that the radiation-chemical behavior of aromatic amino acids is completely determined by the aromatic ring present in them. The initial yields of radical accumulation in aromatic α-amino acids correlate with the G_R values observed in the corresponding aromatic compounds (β-phenyl-α-alanine (0.3)—benzene (0.2) ([1]), tyrosine (0.1)—phenol (0.13), tryptophan (0.03)—indole (0.03)).

The presence of aromatic rings in amino acids also determines the type of EPR

spectrum. The hyperfine structure of the spectrum indicates that a considerable fraction of the spin density falls on the aromatic ring.

In contrast to α-amino acids, irradiation of β-phenyl-β-alanine gives a higher yield of radicals. The described features of the EPR spectra of aromatic amino acids, as well as their radiation stability, can be explained, in our opinion, if one assumes that the principal radical in these amino acids is formed as a result of cleavage of the C^{β}—H bond in the case of α-amino acids and of the C—NH_2 bond in the case of β-phenyl-β-alanine.

Of the aliphatic amino acids, only sulfur-containing amino acids, upon irradiation, give a spectrum whose type is determined by the presence of the functional group (—S—H, —S—S—, —S—CH_3). A characteristic feature of the generally similar spectra of these compounds (see Table 1) is asymmetry. In methionine the asymmetry of the spectrum is less pronounced than in the case of cystine and cysteine. From the literature it is known that radicals,

Table 1

Name of amino acid	G_R^*	ESR spectral characteristics at −196° (ΔH, oersteds)	ESR spectral characteristics upon warming the sample to 25° (ΔH, oersteds)	ESR spectral characteristics upon repeated cooling to −196° (ΔH, oersteds)
Glycine	1	Doublet (27)	Triplet (37)	Triplet (42)
α-alanine	1.4 (1)	Triplet 1 : 2 : 1 (35)	Quintet 1 : 4 : 6 : 4 : 1 (92)	Quintet 1 : 2 : 2 : 2 : 1 (88)
β-alanine	1.7 (0.2)	Doublet (26)	Quartet + triplet (53)	Quartet + triplet (53)
Valine	2.4	Singlet ($\Delta H_{1/2}$ = 18)	11 lines (104)	Complex, weakly resolved multiplet spectrum (150)
Norvaline	0.9	5 lines (90)	15 lines (100)	Complex multiplet spectrum (100)
Leucine	1.2	Triplet with additional lines (45)	Quartet 1 : 3 : 3 : 1 (67)	6 lines with additional splitting (116)

Name of amino acid	G_R^*	ESR spectral characteristics at -196° (ΔH, oersteds)	ESR spectral characteristics upon warming the sample to 25° (ΔH, oersteds)	ESR spectral characteristics upon repeated cooling to -196° (ΔH, oersteds)
Norleucine	1.9	6 lines (115)	Complex spectrum (105)	Sextet 1 : 5 : 10 : 10 : 5 : 1 (105)
Serine	0.4	Doublet (20)	Doublet of triplets + triplet (56)	Doublet of triplets + triplet (60)
Threonine	3.4	Triplet, weakly resolved (60)	4 lines with additional splitting (50)	Triplet (60)
Aspartic acid hydrochloride	0.4	Singlet ($\Delta H_{1/2} = 32$)	7 lines, weakly resolved (66)	Singlet ($\Delta H_{1/2} = 22$)
Asparagine acid	0.4	7 lines, weakly resolved (60)	Triplet of doublets (55)	Complex multiplet spectrum (60)
Glutamic acid	0.4	7 lines, weakly resolved (70)	Triplet with additional splitting (40)	Triplet, weakly resolved (40)
Cysteine	0.5	Asymmetric multiplet spectrum (130)	Asymmetric spectrum (70)	Asymmetric spectrum (90)
Cystine	0.3	Asymmetric multiplet spectrum (55)	Asymmetric spectrum (80)	Asymmetric spectrum (90)
Methionine	0.6 (0.3)	Asymmetric triplet (34)	3 lines (64)	3 lines (64)
Ornithine hydrochloride	0.6	Singlet (23)	Triplet with additional splitting (60)	Triplet (50)

Name of amino acid	G_R^*	ESR spectral characteristics at -196° (ΔH, oersteds)	ESR spectral characteristics upon warming the sample to 25° (ΔH, oersteds)	ESR spectral characteristics upon repeated cooling to -196° (ΔH, oersteds)
Lysine hydrochloride	1.1	7 lines + singlet at the center (100)	Triplet with additional splitting (52)	Triplet (52)
Arginine	1.2	5 lines (80)	5 lines (75)	5 lines (70)
Proline	1 (1)	Triplet (52)	Triplet (35)	Triplet (43)
Histidine	0.26	Triplet (40)	Triplet, weakly resolved (35)	7 lines, weakly resolved (40)
β-phenyl-α-alanine	0.3	Triplet, weakly resolved (43)	Triplet (40)	Triplet, weakly resolved (35)
β-phenyl-β-alanine	12	Quartet 1 : 3 : 3 : 1 (80)	Quartet 1 : 3 : 3 : 1 (46)	Quartet 1 : 3 : 3 : 1 (80)
Tyrosine	0.1 (0.1)	Singlet ($\Delta H_{1/2} =$ 20)	Singlet ($\Delta H_{1/2} =$ 22)	Singlet ($\Delta H_{1/2} =$ 20)
Tryptophan	0.03 (0.03)	Singlet ($\Delta H_{1/2} =$ 20)	Triplet, weakly resolved (45)	Singlet ($\Delta H_{1/2} =$ 40)

* The yield of radicals at low ($-196 \div -160^\circ$) temperature is given; in parentheses, G_R at room temperature. The G_R values were calculated from the initial linear portions of the radical-accumulation curves as a function of dose (or from tangents drawn to the initial portions of the curves). The absolute G_R values are determined with an accuracy not exceeding 30-40%.

in which the spin density is localized mainly on the sulfur atom have an analogous spectral structure ([3,4]).

The hyperfine structure (hfs) of the spectra of radicals formed in the other aliphatic amino acids, in essence, reflects little of the "individuality"of the amino acids associated with the presence of functional groups in them. This is especially manifested in the case where the amino acid has a relatively long aliphatic

tail and contains no side branches. Functional groups (amino, carboxyl), located 2–4 units away from C^{α}, practically do not affect the appearance of the spectrum (for example, the ESR spectra of glutamic acid, ornithine, and lysine at room temperature). Analysis of the spectra shows that, with rare exceptions, in aliphatic amino acids the radicals recorded by ESR at low temperatures apparently form upon removal from the molecule of the group $-NH_2$. The hfs of the spectrum is determined mainly by H nuclei located near the free valence that has formed. Owing to the structural features of one radical or another, the H nuclei may evidently participate differently in the splitting of the Zeeman levels of the unpaired electron, which apparently accounts for the observed differences in the hfs of irradiated amino acids: 7–3 hfs lines (sometimes broadened).

Heating of the samples, causing the motions of individual segments of the molecule to unfreeze, leads (sometimes to identical) changes in the hfs spectra (glutamic acid, ornithine, lysine). In the radiolysis of β-alanine and valine, H atoms are mainly split off from C^{α} and C^{β}, respectively*. Mass-spectrometric measurements of the gas phase may serve as confirmation of this assumption: in irradiated valine the gas composition is: H_2 31.4%, NH_3 6.6%, CO_2 62% (no methane); in the case of β-alanine, H_2 10%, NH_3 75%, CO_2 15% (for comparison, in α-alanine H_2 7.8%, NH_3 33.4%, CO_2 59%).

Somewhat unexpected for us were the temperature changes in the hfs spectrum of irradiated glycine. In glycine, low-temperature radiolysis clearly reveals a doublet hfs ($\Delta H \sim 27$ Oe), analogous to the hfs of irradiated β-alanine. When the sample is warmed to room temperature and then frozen again, instead of the doublet a well-resolved triplet $1:2:1$ is recorded. The total concentration of radicals does not change in this process.

The doublet hfs indicates interaction of the unpaired electron with one hydrogen nucleus. Apparently, the radical is formed as a result of rupture of one of the $H—C^{\alpha}$ bonds. In this case the interaction of the unpaired electron with the NH_2 group should be considerably smaller than with the remaining H. On the other hand, the temperature changes in the spectrum (a $1:2:1$ triplet at room temperature presupposes interaction of the unpaired electron with two H atoms), as well as mass-spectrometric measurements of the gas phase (H_2 0.8%, NH_3 40%, CO_2 –59%) indicate that, in the radiolysis of the glycine molecule, the NH_2 group is more likely to be detached.

It seemed of interest to determine how the "individualities" of amino acids (both in radiation stability and in hfs spectra) are manifested in molecules of the simplest peptides and of a protein that include amino acids of different stability and contain groups whose presence is reflected in the EPR spectrum (aromatic rings, heteroatoms). For this purpose peptides and cottonseed globulin were studied (Table 2).

Table 2

Peptide name	G_R	EPR spectra characteristic: at -196° (ΔH, Oe)	EPR spectra characteristic: on heating the sample to 25° (ΔH, Oe)	EPR spectra characteristic: on repeated cooling to -196° (ΔH, Oe)
Glycyl-glycine	2.5	Weakly resolved triplet (~ 40)	Doublet (~ 18)	Doublet (~ 15)
α-alanyl-α-alanine	0.5	7 lines (~ 77)	Quartet + doublet (~ 78)	Singlet ($\Delta H_{1/2} \sim 30$); $[R]$ decreased by a factor of 3
β-alanyl-β-alanine	0.4	7 lines (~ 77)	Quartet + doublet (~ 78)	Weakly resolved triplet (~ 35)
Glycyl-glycyl-leucyl-glycine	0.8	Triplet (~ 43)	Doublet (~ 20)	Doublet with additional splitting (20)
α-alanyl-glycyl-glycine	3.2	Weakly resolved triplet (~ 37)	Doublet (~ 17)	Doublet (~ 18)
Glycyl-proline	3.3	Doublet of doublets + singlet (~ 93)	Doublet of doublets + singlet (~ 93)	–
Globulin	0.4	Singlet (~ 25)	Asymm. spectrum (~ 50)	Singlet ($\Delta H_{1/2} \sim 25$)
Glutathione	4.4	Triplet (~ 40)	Asymm. triplet (~ 45)	Asymm. spectrum
Glycyl-β-phenyl-α-alanine	0.7	Triplet (~ 45)	Singlet ($\Delta H_{1/2} \sim 34$)	Singlet ($\Delta H_{1/2} \sim 20$)
Glycyl-glycyl-leucine	1.6	4 lines (~ 68)	4 lines (~ 70)	–

As is evident from the data given in Table 2, peptides that include glycine (1, 2, 3 residues) or alanine are destroyed under the action of radiation with a yield of radicals that differs from G_R observed in the radiolysis of each of the individual amino acids, deviating in one direction or the other. The EPR spectra of radicals formed in peptides, as a rule, differ in their basic structure from the EPR spectra of radicals formed during the radiolysis of amino acids. The temperature changes in the spect-

* Analysis of the EPR spectrum of irradiated valine makes it possible to assume the existence of the radical formed in 2 conformations.

of irradiated peptides and simple amino acids are different (glycine, α-alanine, β-alanine, and the corresponding dipeptides). The concentration of radicals also changes differently upon warming the peptide and amino-acid samples. All this may indicate that, in low-temperature radiolysis of peptides, the primary radicals are formed mainly as a result of the rupture of bonds other than those in amino-acid radiolysis (for example, C—H). It is not excluded, however, that in the terminal amino acid of the peptide rupture of the C—NH_2 bond may occur.

The lower yield of radicals in the dipeptides of α-alanine and β-alanine, as compared with G_R in the corresponding amino acids (by a factor of 2–3), gives grounds to conclude that formation of the peptide bond in these peptides makes the molecule more resistant to the action of radiation. The similarity of the ESR spectral structures of irradiated α-alanyl-α-alanine and β-alanyl-β-alanine (the number of hfs lines and the width of the spectra) at low and room temperatures, and their difference from the spectra of α-alanine and β-alanine on the one hand, and, on the other hand, the difference in the thermal stability of the radicals formed and the type of ESR spectra of the amino acids under discussion and the corresponding dipeptides upon repeated freezing—all this makes it possible to suppose that the free valence is apparently formed on the carbon atom near the peptide bond on the nitrogen side*.

The ESR spectra of these two peptides, recorded upon repeated cooling, are poorly resolved (especially in the case of α-alanyl-α-alanine) absorption lines ($\Delta H_{1/2} \sim 30$ Oe). Such a structure of the spectrum apparently indicates delocalization of the unpaired electron over the molecule, which should be manifested to an even greater extent in the case of a protein molecule ([4]). Indeed, upon irradiation of cottonseed globulin, the presence in the molecule of each of the amino acids (more precisely, of the functional groups of these amino acids) does not affect the hfs of the spectrum. The ESR spectrum is a comparatively narrow, poorly resolved line (Table 2). The protective action of the sulfhydryl group and of the aromatic ring, studied by the examples of glutathione and glycyl-β-phenyl-α-alanine, respectively, at low temperature evidently is not manifested. The yield of radical formation in glutathione, on the contrary, is even higher than the yield of radicals in the irradiated amino acids that make up this peptide. Upon warming the sample to room temperature the radical concentration falls (in the case of glutathione, by approximately an order of magnitude in 5–10 min), and the structure of the spectrum assumes the form characteris-

tic of amino acids containing sulfur atoms (glutathione) and an aromatic ring (β-phenyl-α-alanine) (see Table 2).

In the case of aliphatic peptides (except glycylproline), significant changes in the ESR spectra are also observed upon warming (see Table 2). It may be assumed that such changes in the hfs are associated with rearrangement of the radical structure or with its chemical transformations (it is indicative that the radical concentration changes only slightly in this case). Unfortunately, the limited amount of data gives no basis for preferring either of these two possibilities.

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* It is not excluded, however, that in low-temperature radiolysis, in addition to these radicals, radicals are formed upon removal of an H atom from the C^{α} nearest to the carbonyl group (in the "alanyl" unit).

Note: Figure translations are in progress. See original paper for figures.