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Free Radical Formation in Amino Acids Exposed to Hydrogen Atoms

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Electron spin resonance spectroscopy has been used to identify free radicals formed in powdered amino acids by the exposure to thermal hydrogen atoms at 77°K. The low temperature ESR spectra of the radicals produced at 77°K are different from the spectra at room temperature. The primary radicals are those produced by the abstraction of H atoms from the common part of amino acids (in the zwitter-ion compounds) or from some position of the amino acid residue (in neutral compounds). In most amino acids a reversible change of the radical conformation was observed. The irreversible change of the isoleucine resonance pattern is attributed to the change of the unpaired electron from the sp^3 to the p orbital. Different conformations of radicals at room temperature when they are produced at 77° and at 300°K are interpreted by the difference in the crystal lattice arrangement in the vicinity of the radical.

INTRODUCTION

The free radicals observed by electron spin resonance (ESR) spectroscopy in radiation damage study of organic molecules are often produced by secondary hydrogen atoms which have been abstracted from the molecules by the primary action of radiation. Free radicals are also formed in samples of organic molecules when these are exposed directly to thermal H atoms[1–7]. Comparison of the radicals formed in these two ways can help to explain the role of H atoms in the radiation damage of organic compounds. Such a study is of a particular interest in biological systems.

Radicals formed in amino acids when exposed to thermal hydrogen atoms have already been studied with ESR spectroscopy by Snipes and Schmidt[5]. However, these authors carried out their measurements only at room temperature and considered primarily the similarity between the ESR spectra of amino acids exposed to H atoms and those irradiated with gamma rays. The present study is aimed at gaining more information on the radicals in amino acids produced in this way. Aliphatic and aromatic amino acids react differently with H atoms. While in the former case abstraction of atoms or groups is expected, in the latter addition of H atoms takes place. Since we were not able to get much information on the aromatic amino acids, these compounds will not be discussed here.

MATERIALS AND METHODS

Samples of L and DL amino acids were obtained from various commercial sources and used without further purifications. Deuterated serine was prepared by recrystallization of serine from heavy water. Powdered specimens were placed in a chamber

described earlier[8,9], and exposed to a beam of thermal hydrogen atoms from a discharge of the hydrogen gas. The discharge was maintained by a radiofrequency generator. The pressure of hydrogen was about 0.1 Torr. The specimens were bombarded either at 77°K or at 300°K and after the exposure shaken into the sample tube emersed in liquid nitrogen and then sealed off. The radicals were studied by ESR spectroscopy with a Varian E-3 spectrometer, at temperatures from 77—300°K.

RESULTS

Glycine and Alanine

Radicals formed in glycine and alanine by gamma irradiation have been identified and analysed in detail in single crystals[10,11]. Powdered specimens exposed to H atoms at room temperature gave the same ESR spectra as the samples irradiated with gamma rays[5]. The glycine spectrum, although essentially a triplet at room temperature, was interpreted as being due to the radical $H_3N^+—\dot{C}H—COO^-$ (I). Glycine when bombarded at 77 °K shows a completely different spectrum, as shown in Fig. 1. The upper curve was recorded at 77 °K immediately after the exposure to H atoms, the middle one was recorded at room temperature and the lowest again at 77° K. The middle curve is essentially the same as that of glycine bombarded at room temperature[5]. The only difference is that in the present case the outer lines are much better expressed. The reversible change of the ESR pattern from 77° to 300 °K (after the additional line present in the original spectrum disappears) suggests that we deal with chemically identical radical species at both temperatures, *i. e.* with radicals (I). Only conformation of the radical changes with temperature. The couplings of the protons from the NH_3^+ group are the same at both temperatures (20 gauss at 77 °K and 20.5 gauss at 300 °K). The

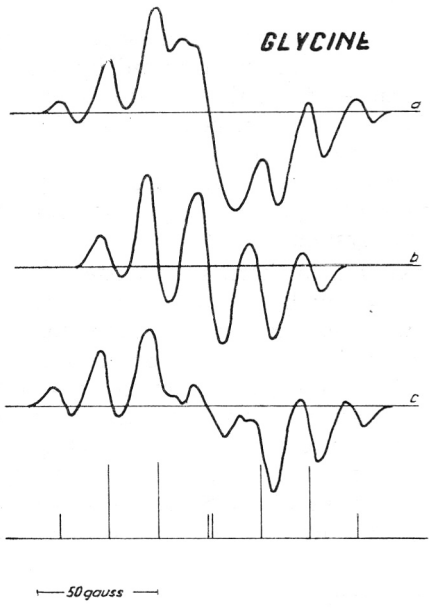

Fig. 1. ESR spectra (first derivative curves) for glycine exposed to H atoms at 77° K, recorded at 77° K (curve a), at 300° K (b) and again at 77° K (c).

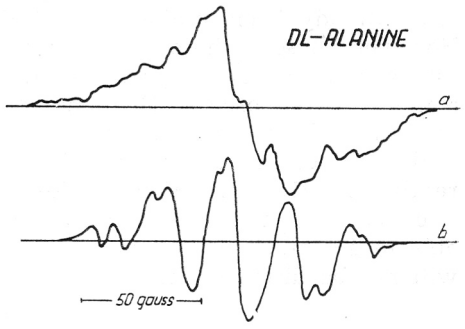

Fig. 2. ESR spectra of DL-alanine bombarded with H atoms at 77° K, recorded at 77° K (a), and at room temperature (b).

proton coupling is 20 gauss at room temperature and as large as 63 gauss at 77 °K. The latter value is too large for an α-proton in the nodal plane of the *p* orbital of the unpaired electron. If the above assignment is correct, at 77 °K the electron must be, at least partly, in the sp^3 orbital.

The ESR spectrum of alanine treated at 77 °K is completely different from the one treated at room temperature, as shown in Fig. 2. The room temperature pattern has been assigned[11,5] to the radical $CH_3—\dot{C}H—COO^-$ (II), and the conclusion was reached that the abstraction of the amino group by H atoms took place. The present experiments demonstrate that the above radicals are not the primary ones. The pattern at 77 °K, although not completely resolved, cannot be understood if the unpaired electron couples with only three methyl protons and an additional proton. We believe that the radicals at 77 °K are $H_3N^+—\dot{C}(CH_3)—COO^-$ (III). These radicals irreversibly transform into radicals (II) upon warming.

Serine and Threonine

Fig. 3 shows the spectra of serine bombarded at 77 °K and (a) recorded at the same temperature (b), at room temperature and (d) the same sample recorded again at 77 °K. These spectra can be interpreted only if we deal with the radical $H_3N^+—\dot{C}(CH_2OH)COO^-$ (IV). Namely, DL-serine is known to

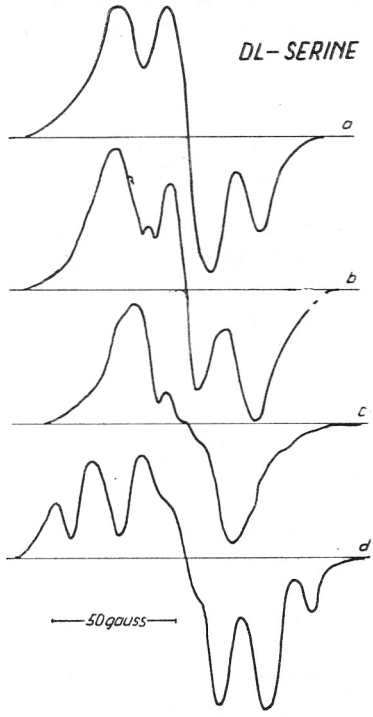

Fig. 3. Resonance curves of DL-serine. Spectra a, b and d were recorded at 77° K, 300° K and again at 77° K, respectively, after the exposure at liquid nitrogen temperature. Spectrum c is from the deuterated sample, bombarded and recorded at 300° K.

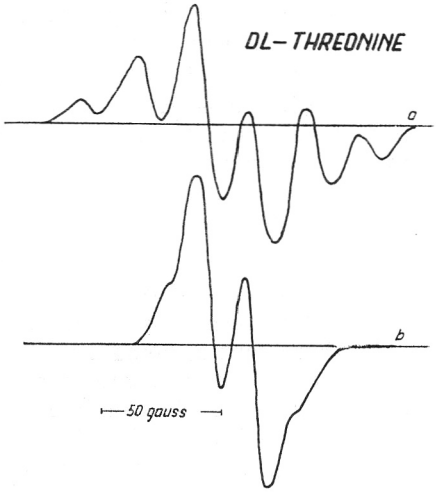

Fig. 4. Resonances of DL-threonine at 77° K (a) and at 300° K (b), after the bombardment at 77° K.

be in the zwitter-ion form[12] and for such a radical one expects the unpaired electron to couple with three protons from the rotating NH_3^+ group and with one or two additional β-protons. The observed triplet spectrum with splitting of 25 gauss can be understood, similarly as in glycine[14,5], if there are four protons with approximately equal coupling; the two weaker side lines are not observed because of the weak but significant anisotropic interaction of ^{14}N nucleus. Freezing the specimen again to 77 °K the conformation of the radical changes so that both β-protons couple with the unpaired electron and the 6-line pattern with the intensity ratio of approximately 1:6:10:10:6:1 is observed (spectrum d). If the former explanation is correct, serine with the protons from the amino group replaced by deuterium should exhibit a doublet with the same splitting of 25 gauss. This was actually observed. (Spectrum c in Fig. 3).

The low temperature spectrum of threonine, represented by the upper curve in Fig. 4, consists of 6 lines, indicating approximately equal coupling of 5 protons. It is interesting to note that such a spectrum can occur if the hydroxyl group is abstracted from the threonine molecule, yielding the radicals $H_2N—CH(\dot{C}HCH_3)—COOH$ (V). The room temperature spectrum is, in contrary to the earlier observations[5], essentially a doublet. The doublet structure is probably caused by the radical $NH_2—\dot{C}[CH(CH_3)OH]—COOH$ (VI). Since no coupling of the amino group protons is observed, the present experiment indicates that DL-threonine crystallizes in the neutral form. Transformation from radical (V) to radical (VI) is irreversible.

Valine, Leucine and Isoleucine

ESR spectra of valine exposed to H atoms at 77 °K are shown in Fig. 5. The top curve, recorded at 77 °K, shows the hyperfine interaction of 8 protons, 7 of them with equal coupling of 25 gauss and the remaining one of 12 gauss. At room temperature the same sample exhibits a little different pattern (spectrum b): predominantly the 8-line spectrum, originating from 7 equally coupled protons. Both resonances are attributed to the same radical species: $H_2N—CH[\dot{C}(CH_3)_2]—COOH$ (VII), as also found in the irradiated crystals of D,L-valine[14]. The central part of the room temperature spectrum is obscured by additional resonances from other radicals.

In leucine an 8-line spectrum was observed (not shown here). An additional doublet splitting at 300 °K and the change of the radical conformation was the same as in the gamma-irradiated leucine. The radicals have been earlier identified as $H_2N—CH[CH_2—\dot{C}(CH_3)_2]—COOH$ (VIII).

The ESR pattern of isoleucine at 77 °K consists essentially of 7 equidistant lines, the three central being 2 or 3 times as large as the remaining four. Such a spectrum cannot be interpreted by the hyperfine coupling with protons only. We believe that the ^{14}N coupling and two proton couplings are involved, as indicated by the lines under the top curve in Fig. 6. Such a pattern can be due to the radical $H_2N—CH[\dot{C}(CH_3)CH_2CH_3]—COOH$ (IX) but only if the configuration of the radical is such that the unpaired electron remains in the sp^3 orbital and does not couple with the methyl protons but does couple with the ^{14}N nucleus from the amino group and with additional two β-protons. At the temperature slightly above 100 °K the spectrum irreversibly changes into the well known 7-line pattern, which is also observed at 300 °K. However, in

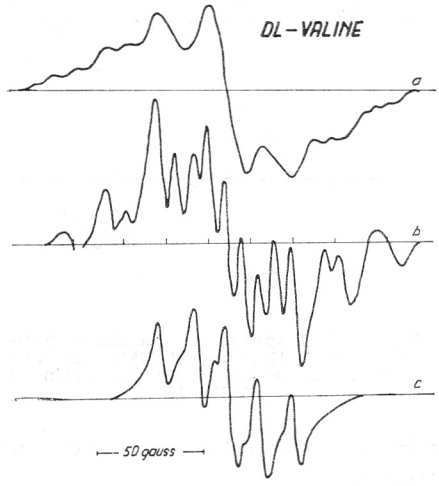

Fig. 5. ESR spectra of H-bombarded DL--valine, recorded at 77° K (a), 300° K (b) and at 300° K after a short heating at 350° K (c).

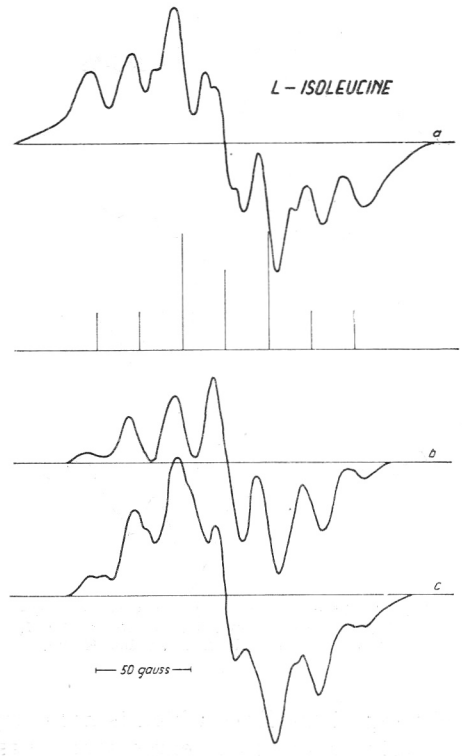

Fig. 6. Resonance curves of L-isoleucine exposed to H atoms at 77° K, recorded at 77° K (a) and at 300° K (b) and of the specimen bombarded and recorded at room temperature (c).

the specimen bombarded at room temperature an additional proton coupling was observed.

The interpretation of the unstable radical species in isoleucine may not be convincing. However, at present we do not see any other rational explanation.

Lysine and Arginine

These two amino acids are similar in structure and similar radicals in these compounds will probably be represented by similar ESR spectra. Fig. 7 shows the spectra of lysine and Fig. 8 those of arginine. The same radicals are present in both amino acids. The intensity ratio of the lines 1:2:2:2:1 suggests the coupling of 3 protons: one of them is 20 gauss and the remaining two about 40 gauss. The radical corresponding to such a pattern is $H_2N—CH(\dot{C}HCH_2R)—COO^-$ (X). One of the β-protons of the methylene group does not couple significantly. In lysine bombarded at room temperature (curve c in Fig. 7) the conformation of the radical is different; 4 protons couple with the electron, with splittings of about 30 gauss. There is also an additional

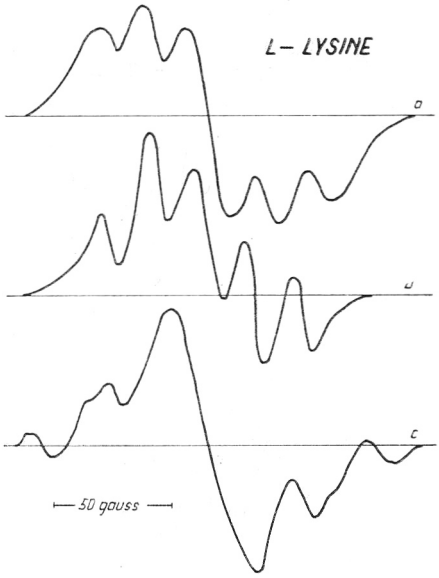

Fig. 7. ESR spectra of L-lysine H-bombarded at 77° K (curve a recorded at 77° K, curve b at 300° K) and at 300° K (c).

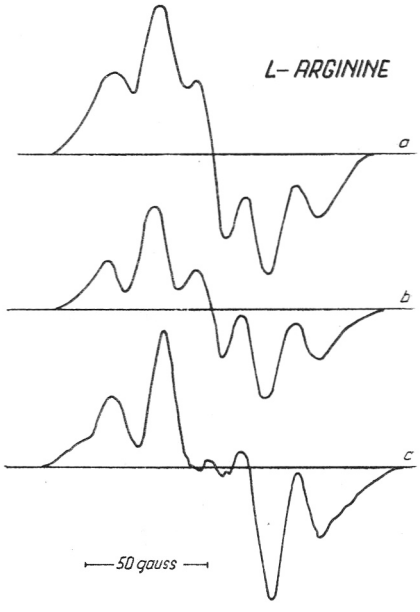

Fig. 8. ESR spectra of L-arginine bombarded with H atoms at 77° K, recorded at 77° K (a), at 300° K (b) and again at 77° K (c).

splitting of the lines which is not completely understood. No similar behaviour of arginine at room temperature was observed.

Proline

In the ESR spectra of proline bombarded at 77 °K, as shown in Fig. 9, one recognizes the hyperfine interaction of three protons of different coupling: 22, 42 and 55 gauss, respectively. One of the protons is the α-proton and the

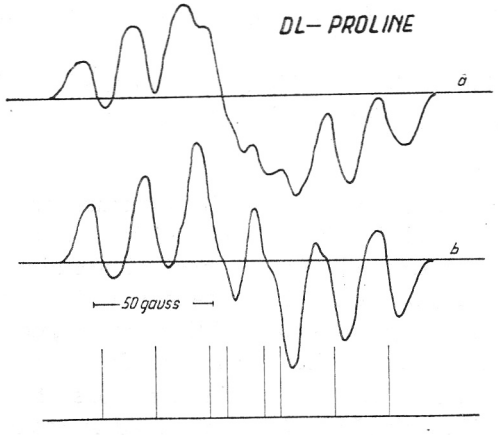

Fig. 9. Resonance of DL-proline at 77° K (a) and at 300° K (b). The sample was bombarded with H atoms at 77° K.

remaining two are the β-protons. There are many possibilities for such a structure in proline and one cannot be sure which hydrogen atom has been abstracted by the thermal atoms.

Glutamic Acid and Aspartic Acid

These two compounds have the NH_3^+ group and if a proton from the amino acid residue is abstracted (like in alanine or serine) the coupling of the NH_3^+protons should be observed. The spectra of glutamic acid show (See Fig. 10) that at 77 °K four protons couple with the electron (The coupling constants

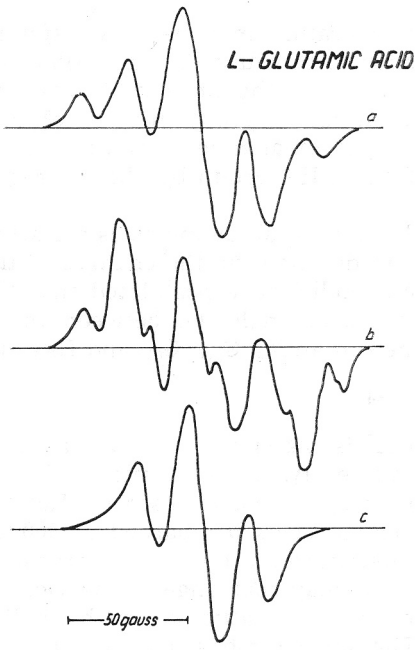

Fig. 10. ESR spectra of L-glutamic acid bombarded at 77° K (curves a and b) and at 300° K (c). The top curve was recorded at 77° K, the remaining two at 300° K.

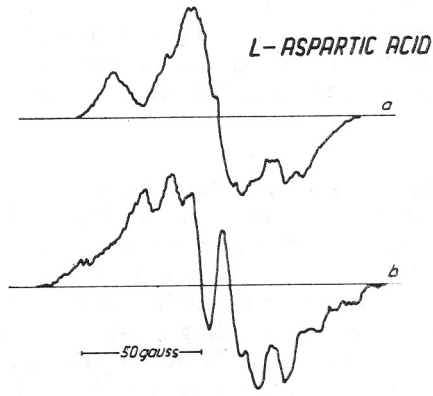

Fig. 11. Resonance spectra of L-aspartic acid exposed to H atoms at 77° K, recorded at 77° K (a) and at 300° K (b).

are 25 gauss). At 300 °K the splitting caused by the fifth proton proves that we deal with the radical H_3N^+—$\dot{C}(CH_2R)$—COOH (XI) in two different configurations. The spectrum recorded at 300 °K after the exposure at the same temperature may be attributed to the same radical if the two outer lines are not seen (similarly as in serine and glycine).

The aspartic acid spectra (Fig. 11) are not resolved enough to be interpreted. They indicate, however, that here radicals (XI) may as well be present.

DISCUSSION

In general, the ESR spectra of amino acids exposed to H atoms are rather complex. The complexity of the spectra is partly due to the large number of nuclei taking part in the hyperfine interaction and partly due to the anisotropy of the couplings. The latter phenomenon makes sometimes the interpretation of the spectra of powdered specimens difficult. In the investi-

gated radicals both α and β protons are involved in the hyperfine couplings. Although these protons should be distinguished by their couplings, in some cases such a distinction was not possible. In spite of this, some definite and general conclusions could be reached.

From the above results one can conclude that the primary effect of the thermal H atoms is abstraction of hydrogen atoms from amino acids. It is interesting to note that the abstracted atoms are those from the carbon atom of the common part of the amino acids if the molecule is in the zwitter-ion configuration, while in the neutral molecule abstraction occurs from some other part.

Secondary radicals observed at room temperature may be of a different structure. From these radicals it is not possible to conclude on the mechanism of the H atom reaction. Thus, as demonstrated by the present results, the mechanism proposed for the abstraction for the amino group in alanine[5] does not seem to be correct. Furthermore, both L- and DL-alanine crystallize in the zwitter-ion form[15,16] and the abstraction of the NH_3^+ group by the hydrogen atom is improbable.

The reversible transformations observed in almost all amino acids are attributed to the conformational changes. They are detected via the changes of the methylene proton couplings. Heller and McConnell[15] have postulated that the β-proton coupling, A^β, depends upon the dihedral angle, Θ, between the p orbital of the unpaired electron and the plane containing $C_{(\alpha)}$, $C_{(\beta)}$ and $H_{(\beta)}$, as:

$$A^\beta = Q^\beta \cdot \cos \Theta$$

where Q^β is a constant. Hence a change in A^β is associated with a change of conformation. In some amino acids (isoleucine, lysine, glutamic acid) the spectra recorded at room temperature from the specimens exposed to H atoms at 77 °K are different from the spectra of amino acids bombarded at 300 °K. That fact might be explained if at room temperature more energy is transferred to the surrounding crystalline matrix which may rearrange in the vicinity of the radical. At 77 °K the transfer of energy to the lattice is much smaller and its rearrangement may be different. The same reasons may account for some differences in the radical conformation of the gamma-irradiated and the hydrogen atom-bombarded amino acids.

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IZVOD

Slobodni radikali u amino kiselinama, nastali reakcijom s termalnim vodikovim atomima

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Slobodni radikali, nastali reakcijom termalnih vodikovih atoma s amino kiselinama, proučavani su elektronskom spinskom rezonancijom. Primarni radikali nastaju otcjepljenjem vodikova atoma, i to onoga iz zajedničkog dijela amino kiselina ako je spoj u zwitter-ion obliku, a vodika iz ostatka amino kiseline ako spoj kristalizira u neutralnom obliku. U većini amino kiselina kemijska struktura radikala koji su stvoreni na 77°K ne mijenja se zagrijavanjem. Mijenja se samo njihova konformacija. U nekim amino kiselinama radikali, nastali na 77°K, imaju na sobnoj temperaturi različitu konformaciju od radikala nastalih na toj temperaturi. To se tumači različitom preraspodjelom okoline radikala pri različitim uvjetima.

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