

Scheme.

and β protons, respectively $a(2H)=1.8$ mT and $a(2H)=0.55$ mT. The Cl substituent remaining in the IA molecule probably stabilises the paramagnetic centre in the crystalline matrix. The spectrum of the other component (radical B) reveals a broad anisotropic signal reflecting a strong hyperfine interaction, $a(1P)=5.3$ mT, characteristic of a phosphorus atom, together with a narrow splitting of 0.7 mT due to unidentified protons. If the major, or even a significant spin-density, was localised on the 5-valent phosphorus atom, then one should expect very high hyperfine coupling constants [1,2] as A_{iso} for the ^{31}P nuclei equals to 363.2 mT. Since radical B does not reveal such strong interactions and, moreover, the influence of other atoms is distinct, we believe that the paramagnetic centre is not situated at the phosphorus atom. The much lower stability of radical B (its characteristic spectrum decays after 1 day, Fig.2) indicates, that, for example, a fast hydrogen atom transfer can be taking place.

Only 30% of the initial radicals remains after 6 days, which might result from the low melting point of IA (47°C) and, therefore, from the lower rigidity of the microcrystalline matrix as compared to that exhibited by other drugs at the storage temperature. This property of IA influences the level of radicals produced with different sources of ionising radiation. The gradual character of irradiation using a γ -source, at a dose rate of only 5 kGy/h means the sample is only slightly warmed due to effective heat exchange between the irradiated object and its

environment. On the other hand, if irradiation is performed with an electron beam, then the temperature increases significantly [3] due to the adiabatic character of energy absorption, which results in

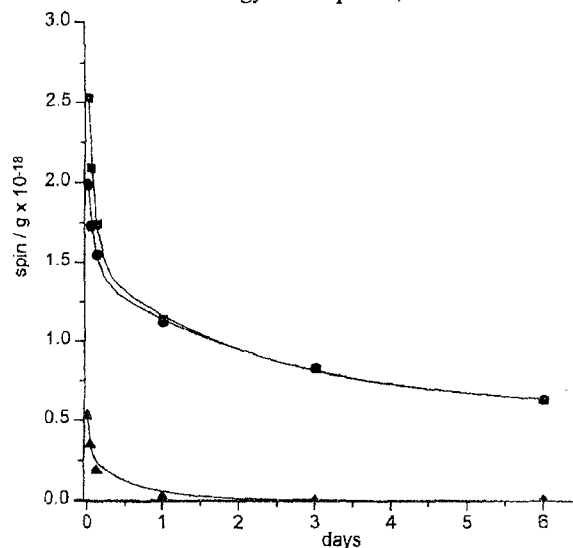


Fig.2. Concentrations of IA radicals: (■) all radicals, (●) radical A and (▲) radical B, vs. time.

a pronounced thermal effect and a subsequent reduction of spin population in radicals derived from IA. For a system of such a low melting point even variations in ambient temperature can modify considerably the radical concentration. These thermal effects are the reasons why, 5 months after electron beam irradiation, no radical species were detected, whereas in previous studies [4], using gamma-irradiation, the radicals seem to be highly populated and more stable.

References

- [1]. Begum A., Lyons A.R., Symons M.C.R.: *J. Chem. Soc. (A)*, 2388-2392 (1971).
- [2]. Begum A., Symons M.C.R.: *J. Chem. Soc., Faraday Trans., 2*, 62, 43-48 (1973).
- [3]. Zagórski, Z.P.: Thermal and electrostatic aspects of radiation processing of polymers. In: *Radiation processing of polymers*. Eds. A. Singh, J. Silverman. Hanser Publishers, Munich 1992, pp. 272-287.
- [4]. Ambroź H.B., Kornacka E.M., Marciniak B., Ogródowczyk M., Przybytniak G.: *Radiat. Phys. Chem.*, 58, 357-366 (2000).

OH RADICAL INDUCED OXIDATION OF α -METHYLALANINE TIME-RESOLVED AND SPIN-TRAPPING ESR STUDY

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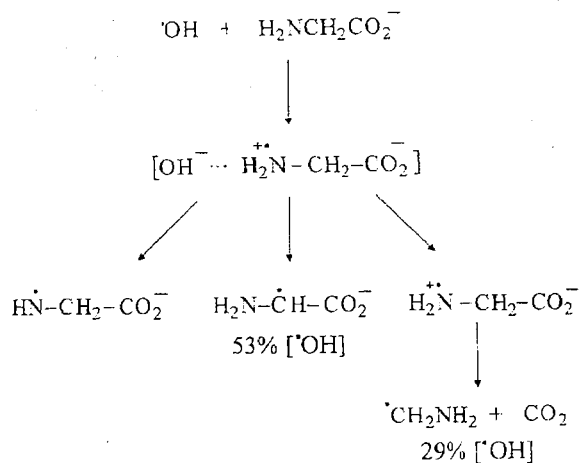
Introduction

Precise chemical mechanisms of amino acid oxidation are of interest to understanding the fate of proteins under oxidative stress in biological systems. Recently, the oxidation mechanism of glycine anions was proposed that attempts to explain systematically all the processes from subnanosecond to steady-state time scales. Several radicals were postulated [1] as being involved in the beginning steps of glycine anions oxidation with two main primary radicals,

$H_2N^+ \cdot -CH_2-CO_2^-$ and $HN \cdot -CH_2-CO_2^-$ (Scheme). Initially, the aminyl radicals $HN \cdot -CH_2-CO_2^-$ were only inferred from scavenging reactions with hydroquinones [1], but they were subsequently identified in steady-state radiolysis experiments with the aci-anion ($CH_2=NO_2^-$) of nitromethane as a spin-trap [2].

Aim

The aminyl radicals appeared to play a more significant role when glycine was substituted with methyl groups [3]. β -Scission of the aminyl radicals



Scheme.

with the formation of $\cdot\text{CO}_2^-$ radicals, was thought to be a significant decay channel for these radicals, especially for $\text{HN}^{\bullet} - \text{C}(\text{CH}_3)_2 - \text{CO}_2^-$ from α -methylalanine (2-aminoisobutyric acid). The purpose of the current work was to look closely at TRESR (Time-Resolved ESR) and also spin-trapping experiments involving α -methylalanine in order to see if this aminyl radical does exist and to see if β -scission of any resulting aminyl radical does occur. Even though, α -methylalanine has been studied previously under steady-state in situ radiolysis with ESR detection [4], no radicals left over from a decarboxylation were observed, however, carbon dioxide has been determined in steady-state γ -radiolysis [5]. Thus, the radical corresponding to the decarboxylated fragment of α -methylalanine should be present.

Experimental

Radiolysis was carried out with electrons from a Van de Graaff accelerator. The chemical samples were contained in a quartz cell of 0.4 mm thickness, and the cell was irradiated edge-on. The solution to be irradiated was passed through a heat-exchange unit before entering the ESR cell in order to cool the solution, so that the formation of N_2O bubbles could be avoided. The measurements were made at a temperature of about 288 K. A bubble trap was also used in most of the experiments.

Time-resolved ESR experiments

The TRESR was operated in both the kinetic-profile and the BOXCAR mode. Both of these modes are based on obtaining kinetic profiles with no frequency modulation [6]. The signal-averaged data are accumulated by summing pairs of matched kinetic traces. One trace in each pair is acquired at the nominal external magnetic field. The matching trace is acquired at an external magnetic field that is shifted to a spectral position where no transition is located (ideally, at least in the kinetic-profile mode). This shifted field is produced by a current through external coils on the cavity. These on- and off-resonance traces are subtracted, and the procedure is repeated with each intermediate result being folded into the average. In the BOXCAR-scanning mode of operation of the ESR spectrometer, the external field is slowly scanned, at the

same time that the kinetic traces are being accumulated. The data consists of the amplitude difference between traces over a selected time interval. The external magnetic field is slowly scanned through a range to display a spectrum. Since the field steps are generally much smaller than in the kinetic-profile mode, the spectra have a first derivative form.

Spin-trapping ESR experiments

The spin-trapping experiments were done in the steady-irradiation mode. The external magnetic fields were scanned, but now there was a frequency-modulation signal employed as part of the detection unit. The spin-trap was the aci-anion of nitromethane, $\text{CH}_2 = \text{NO}_2^-$. Radicals are trapped by radical addition to the carbon across the double bond, forming an N-centered radical on the nitrogen of the nitro group.

Results

Time-resolved ESR in α -methylalanine

The reducing radical $\text{H}_2\text{N}^{\bullet} - \text{C}(\text{CH}_3)_2$ was found to be present immediately following the radiolytic pulse, consistent with the spin-relaxation dynamics of the radicals in the joint magnetic field of the

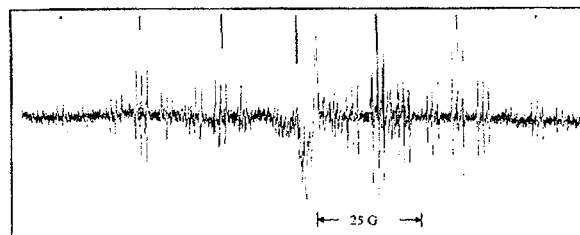


Fig.1. Time-resolved ESR spectrum (BOXCAR method) of the $\text{H}_2\text{N}^{\bullet} - \text{C}(\text{CH}_3)_2$ radical.

external magnet and the microwave radiation. From TRESR ESR (Figs.1 and 2), the pattern can be seen to be made up of 7 groups of lines with a splitting between the centers of adjacent groups of 18.86 G. The pattern is indicative of 6 equivalent protons. Furthermore, the intensities of these groups are not inconsistent, if account is taken for the spin polarization, with the 1:6:15:20:15:6:1 expected for such a set of equivalent protons. Each of the 7 major groups of lines is further split into a 1:2:1 triplet by the two equivalent protons from the NH_2 group. The splitting corresponds to a hyper-

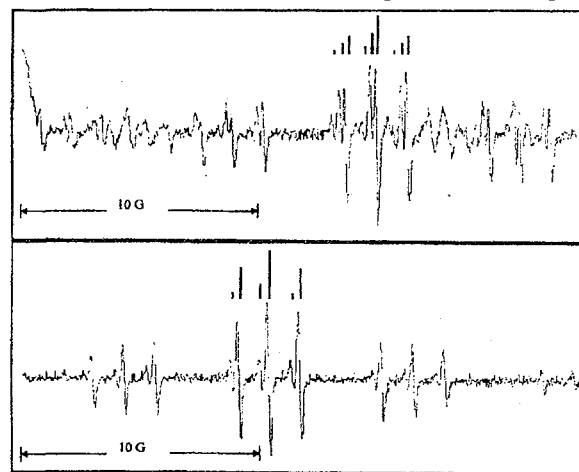


Fig.2. Second order splittings of the $\text{H}_2\text{N}^{\bullet} - \text{C}(\text{CH}_3)_2$ radical.

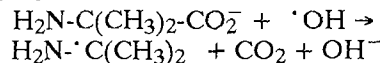
fine coupling of 6.06 G. Finally, each of these smaller groups of lines are split into an even triplet with a hyperfine coupling of 1.3 G for the contribution from the nitrogen nuclei. The g-factor associated with this spectrum is 2.0027. This spectrum is assigned to the decarboxylated radical, $\text{H}_2\text{N}\cdot\text{C}(\text{CH}_3)_2$. This radical has not been reported earlier, even in steady-state radiolysis.

The central major group of lines (corresponding to relative intensity 20 for 6 equivalent protons) is severely distorted by the electron signal from the irradiated quartz. However, the next two major line groupings (corresponding to relative intensities 15 and 6 for 6 equivalent protons) show typical second-order splittings. The lines making up the high field "intensity 15" group of lines should all be split into three lines of relative intensity 9:5:1. The lines, making up the high field "intensity 6" group, should, for the same reasons, each be split into two lines with a relative intensity 5:1. The existence of these second-order splitting patterns, associated with six equivalent protons, further supports the assignment of all these lines to the $\text{H}_2\text{N}\cdot\text{C}(\text{CH}_3)_2$ radical. Relatively broad lines from another radical can be seen in the middle of the sharper lines from the $\text{H}_2\text{N}\cdot\text{C}(\text{CH}_3)_2$ radical. There are indications that other lines of this radical are present at lower magnetic fields, and additional experiments are scheduled for more detailed scanning of these spectral regions. However, it is significant to note that these lines do not match the lines assigned to the $\text{NH}_2\text{-C}(\text{CH}_3)(\cdot\text{CH}_2)\text{CO}_2^-$ radical seen in steady-state in situ radiolysis of α -methylalanine [4]. These lines also do not correspond to the $\cdot\text{N}=\text{C}(\text{CH}_3)_2$ seen in steady-state radiolysis [4] and later assigned [7]. Lines corresponding to neither of these radicals were seen in the time window of the scan. This is not all that surprising for the $\cdot\text{N}=\text{C}(\text{CH}_3)_2$ radical since it is likely derived from secondary processes.

Spin-trapping in α -methylalanine

Aminyl radical $\text{NH}\cdot\text{-C}(\text{CH}_3)_2\text{-CO}_2^-$. As an alternative, the spin-trapping ESR method was used to trap the aminyl radical with the nitromethane aci-anion $\text{CH}_2=\text{NO}_2^-$, following in situ steady-state radiolysis. The spin-adduct of the $\cdot\text{CO}_2^-$, $^-\text{O}_2\text{C-CH}_2\cdot\text{NO}_2^-$ radical was detected. The $\cdot\text{CO}_2^-$ radical was previously proposed to be formed by b-scissions of aminyl radicals.

Conclusions



A new carbon-centered radical $\text{H}_2\text{N}\cdot\text{C}(\text{CH}_3)_2$ has been discovered in the radiolytic oxidation of α -methylalanine. A little-used TRESR method of collecting data was employed that permitted the observation of a short-lived radical that had previously escaped detection when steady-state ESR was used. The existence of $\cdot\text{CO}_2^-$ in the spin-trapping experiments is consistent with the aminyl radical being present and the β -scission process, taking place as predicted.

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References

- [1]. Bonifacic M., Stefanic I., Hug G.L., Armstrong D.A., Asmus K.-D.: J. Am. Chem. Soc., **120**, 9930-9940 (1998).
- [2]. Hug G.L., Fessenden R.W.: J. Phys. Chem. B, submitted.
- [3]. Bonifacic M., Armstrong D.A., Carmichael I., Asmus K.-D.: J. Phys. Chem. B, **104**, 643-649 (2000).
- [4]. Neta P., Fessenden R.W.: J. Phys. Chem., **75**, 738-748 (1971).
- [5]. Asmus K.-D., Stefanic I., Bonifacic M., Armstrong D.A.: private communication.
- [6]. Fessenden R.W.: J. Chem. Phys., **58**, 2489-2500 (1973).
- [7]. Fessenden R.W.: Chem. Phys. Lett., **29**, 364-367 (1974).

GENERATION OF AROMATIC THIOETHER RADICAL CATIONS CONTAINING PHENYL, VINYL, ALLYL, AND METHYL SUBSTITUENTS IN ORGANIC SOLVENTS

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Radical cations derived from organic sulfides, containing aromatic and unsaturated substituents, play an important role in a variety of chemical processes extending from those of industrial importance (organic synthesis) [1] to the enzymatic oxidations in biological systems [2,3]. A certain amount of information on the nature and kinetic parameters of intermediates formed from aryl-substituted sulfides is available [4-6], however, no similar studies have been performed for the sulfides with unsaturated substituents. Quantitative information on these sys-

tems would be of chemical and biochemical interest since the nature of the species is expected to be influenced by the degree of spin delocalization in the aromatic ring and the double bond. The last feature should affect the propensity of the radical cations to form dimers.

The basic spectral and kinetic behaviour of radical cations and radicals observed in pulse radiolysis of the corresponding organic sulfides with the aromatic and unsaturated substituents are provided in n-butyl chloride and cyclohexane solutions. In



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