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SEPARATION AND CHARACTERIZATION OF SHORT-LIVED RADICALS IN DL-METHIONINE AQUEOUS SOLUTION BY HIGH SPEED LIQUID CHROMATOGRAPH EQUIPPED WITH ESR SPECTROMETER

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Short-lived radicals produced in a DL-methionine aqueous solution by γ-irradiation were converted into stable ones by spin-trapping, and they were separated and detected by a high speed liquid chromatograph equipped with an ESR spectrometer. The radicals separated in this way were characterized by a conventional ESR spectrometer. Consequently, the structures of four radicals were successfully identified.

It has been well known that many kinds of short-lived radicals are produced in aqueous solutions of amino acids by ionizing radiation and other methods and therefore were studied by various methods.[1),2)] Recently, a spin-trapping method was introduced to convert short-lived radicals into stable ones, and to make it possible to identify not them by an ESR spectrometer.[3),4),5)] This spin-trapping reaction is represented by the equation (1).

$$R\cdot + (\text{spin-trap}) \longrightarrow R\text{-}(\text{spin-trap})\cdot \qquad \text{----------(1)}$$

When this procedure is applied to aqueous solutions, a popular spin-trap is used, 2-methyl-2-nitrosopropane as in this study, because it is easily soluble in water under a careful treatment on its photo-sensitiveness. The reaction scheme between a short-lived radical(R·) and a reagent, 2-methyl-2-nitrosopropane, is described by the equation (2).

$$R\cdot + {}^{t}Bu\text{-}N{=}O \longrightarrow R\text{-}N(O\cdot)\text{-}{}^{t}Bu \qquad \text{----------(2)}$$

2-Methyl-2-nitrosopropane was synthesized by the Stowell's method,[6)] and was used as aqueous solution of blue color. The concentration of spin-trap was 10 mg per 10 cm^3 of water. Two mmol of DL-methionine was added to 10 cm^3 of this prepared solution. This sample solution was irradiated with ^{60}Co γ-rays at a dose

rate of 6.0×10^5 rad/hr at ice temperature to a total dose of 5×10^4 rad, and then immediately injected to a high speed liquid chromatograph (TOYO-SODA, HLC-803) equipped with an ESR spectrometer(JEOL, Model PE-3X), which was operated at 100 kHz modulation with X-band. A column, N-44 of TOYO-SODA, was attached to this chromatograph.[7),8)]

The DL-methionine aqueous solution in the presence of the spin-trap was γ-irradiated and it gave an ESR spectrum shown in Fig.1. This spectrum is too complex to be analysed, so that the chromatography mentioned above was applied. A flow diagram of the chromatograph is shown in Fig.2. An obtained chromatogram is shown in Fig.3. In the course of this chromatographic separation, the magnetic field was fixed at a position indicated by the arrow in Fig.1, and the modulation width applied was 5.0 G. And 62.5 mM borax-NaOH buffer, pH 11.5, was used as an eluent.

Four peaks were found in the observed chromatogram, as shown in Fig.3. In order to observe the ESR spectrum at each peak by a conventional ESR spectrometer, the pump was stopped to interrupt the eluting at various points. The ESR spectra obtained thus are shown in Fig. 4(a), (b) and (c), which correspond to peaks, A, B and C, respectively. However, the peak D didn't give any analysable spectrum because of low concentration of radicals though the peak in the chromatogram indicates the existence of a radical. Here, the peak intensity depends not only on the concentration of radicals but also on the line shape of the ESR spectrum and

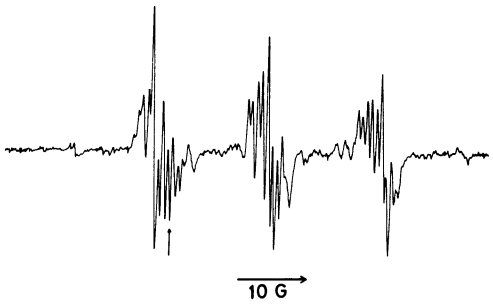

Fig.1 ESR spectrum of the γ-irradiated DL-methionine aqueous solution containing spin-trap.

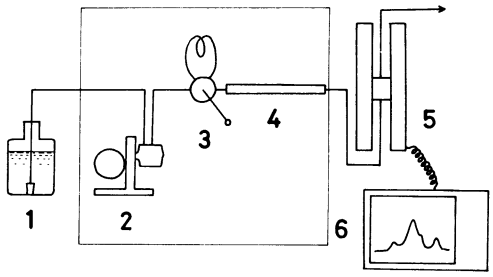

Fig.2 Flow diagram of the chromatograph.
(1) Eluent, (2) Mini-pump,
(3) Six-port sample injector,
(4) Column, (5) ESR spectrometer,
(6) Recorder.

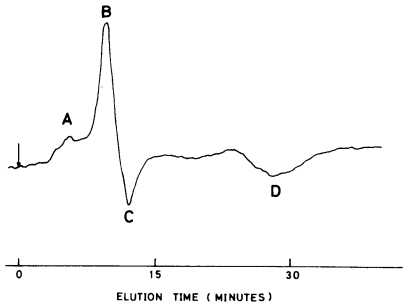

Fig.3 Chromatogram of the γ-irradiated DL-methionine aqueous solution containing spin-trap.

the magnetic field fixed.

The spectrum shown in Fig.4(a) is quite the similar to the spectrum obtained in the γ-irradiated spin-trap aqueous solution, and consequently the structure of the radical at peak A is assigned as $^{t}Bu\text{-}N(O\cdot)\text{-}^{t}Bu$.

The spectrum in Fig.4(b) consists of triple-double-triplet. The dominant splitting is a ^{14}N triplet (a_N=15.6 G), and the secondary splittings are attributed to a β-proton and two equivalent γ-protons ($a_{\beta\text{-}H}$=4.7 G, $a_{\gamma\text{-}H}$=0.8 G). From this, the structure of the trapped radical is considered to be one of these two, (A) or (B), shown below.

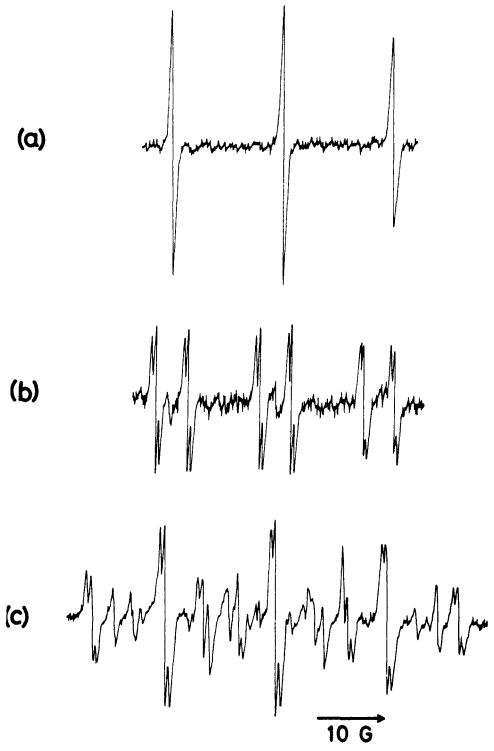

Fig.4 ESR spectra of separated radicals. (a), (b) and (c) are observed at peak A, B and C, respectively.

(A) $^{t}Bu\text{-}N(O\cdot)\text{-}CH(CH_2CH_2SCH_3)COO^{-}$

(B) $^{t}Bu\text{-}N(O\cdot)\text{-}CH(CH_2CH(NH_2)COO^{-})SCH_3$

However, as it is known that carboxyalkyl radicals are produced by reductive deamination caused by the reaction of e^{-}_{aq} with amino acids,[9] the structure (A) is assigned to the trapped radical.

In Fig.4(c), the observed complex spectrum is shown. The major component is analysed as triple-triple-triplet. The dominant splitting of this component is a ^{14}N triplet (a_N=16.8 G). And the secondary splittings are attributed to two equivalent β-protons and two equivalent γ-protons ($a_{\beta\text{-}H}$=11.2 G, $a_{\gamma\text{-}H}$=1.0 G). The structure of the corresponding trapped radical is shown by (C).

(C) $^{t}Bu\text{-}N(O\cdot)\text{-}CH_2CH_2CH(NH_2)COO^{-}$

And then the minor component is also characterized, referring the literature,[10] as trapped methyl radical, $^{t}Bu\text{-}N(O\cdot)\text{-}CH_3$. However, it can't be decided whether it was generated from DL-methionine or spin-trap, since both of them have methyl groups.

Another experiment using a longer column of the analogous type (IEX 210SC of TOYO SODA, 60cm in length) in order to get higher resolution, but it didn't give

any useful results because spin-adducts in the γ-irradiated aqueous solution of DL-methionine are not stable enough to survive for a long time in a chromatographic column. From this, it is found that these spin-adducts should be separated with relatively high speed.

According to the aforementioned discussion, it is concluded that two kinds of DL-methionine radicals can be successfully separated by the chromatography to give the each ESR spectrum of each trapped radical and that the present method is applicable to the γ-radiolysis of an aqueous solution of another amino acid.

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