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PHYSICO-CHEMICAL PROPERTIES OF GLYCINE SOLUTION DURING IRRADIATION

By

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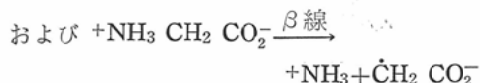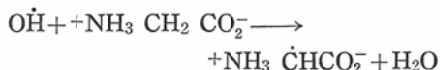Department of Radiology, Medical School, Osaka University

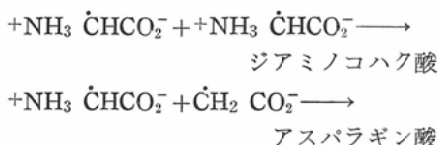### ^{3}H-β 線照射時グリシン溶液の損傷機転

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常温グリシン水溶液に ^{3}H$_2$O を加えて，β線の照射時におけるグリシン分子の解離，架橋を動力学的に追求した．ESR共鳴法により

$$\dot{O}H + ^+NH_3\,CH_2\,CO_2^- \longrightarrow$$
$$^+NH_3\,\dot{C}HCO_2^- + H_2O$$

および $^+NH_3\,CH_2\,CO_2^- \xrightarrow{\ \beta線\ }$

$$^+NH_3 + \dot{C}H_2\,CO_2^-$$

初期反応が起る．照射時の誘電率測定より上記の解離の他に双極子能率の大きい生成物が存在すると考えられた．カラムクロマトの溶離より次の反応が解明された．

$$^+NH_3\,\dot{C}HCO_2^- + ^+NH_3\,\dot{C}HCO_2^- \longrightarrow$$
$$ジアミノコハク酸$$

$$^+NH_3\,\dot{C}HCO_2^- + \dot{C}H_2\,CO_2^- \longrightarrow$$
$$アスパラギン酸$$

これらの生成物による誘電率の上昇は定性的には説明出来るが，定量的に説明には難しい．従つてこれらの生成物が更に静電的作用により，次々の反応が期待される．

Introduction

It seems generally accepted that ionising radiation alters the physico-chemical properties of the aqueous solution of amino of acids. There have been some observations made by Gordy et al (1), Week et al (2), and Henriksen (3) on the changes of physico-chemical properties of glycine solution following irradiation. However, they are referable only to a post irradiation status. Accordingly, they should have been carried out even during irradiation, since reactions caused by irradiation are very quick in their responses and their developements. In the present study it was attempted to elucidate the mechanism of radiolysis of glycine solution being exposed to ^{3}H$_2$O β-ray.

Two experimental methods were employed on a physico-chemical basis : one was carried out on the observation of free radicals formed in glycine solution, and the other on the change of dielectric properties during irradiation, in order to obtain some informations of the immediate effects of ionising radiation.

Experiment 1

On a study of radiation induced free radicals electron spin resonance (ESR) spectro-

scopy was made of glycine solution being irradiated by ^3H_2O β-ray at room temperature. Fortyfive mg and/or 7.5 mg of purified glycine were added to 1 ml of ^3H_2O (specific activity, 1 cc/0.2 ml, the weight ratio of ^3H_2O to H_2O was 1 : 500) obtained from Amersham, England, and samples of 3 M and 0.5 M glycine solutions were prepared. Thus, the samples were constantly exposed to ^3H_2O β-ray, at a dose rate of 6×10^4 rads/hr.

The ESR spectrometer used was a Varian 4500 instrument. It was operated at 9000 Mc/sec microwave with 100 Kc/sec field modulation and 30 gausses sweep. To minimize a dielectric loss in the aqueous solution, a thin sample cell with flat dimension of 50 mm $\times$ 9 mm $\times$ 0.25 mm was employed, which was located in a nodal plane of the electric field in the cavity. The spin density and line width of the irradiated samples were quantitized by comparing with that of DPPH and $(SO_3)_2NO_2^-$, respectively.

When the ESR spectroscopy of aqueous solution whith contains ^3H_2O is studied, it is very likely that free radicals from H_2O being exposed to ^3H_2O β-ray induce background spectra. At room temperature, no " back ground signal " was observed of the aqueous solution in the presence of air, which was in agreement with Henriksen (3) and Kroh et al (4).

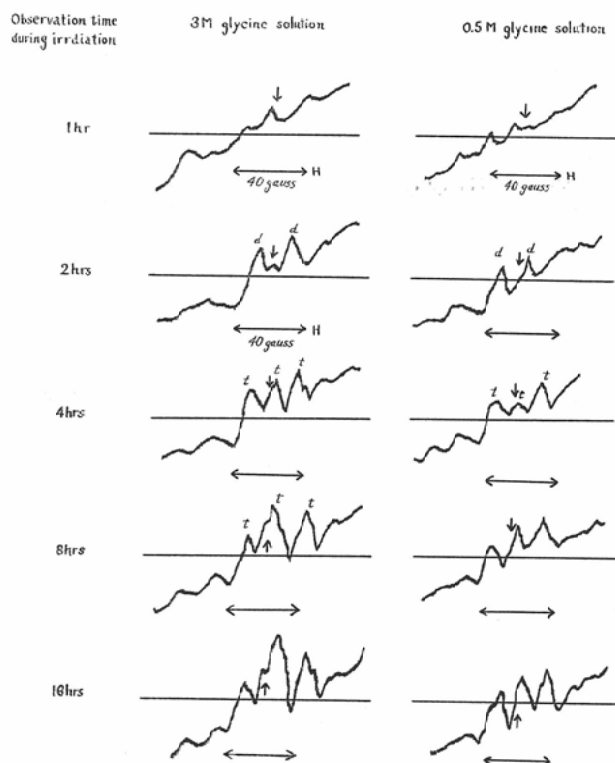Observations of the resonance spectra of 3 M and 0.5 M glycine solution were made

Fig. 1 The qualitative ESR spectrum of 3 M and 0.5 M glycine solution observed at different time during irradiation of ^3H_2O β-ray, at a dose rate of 6×10^4 rads/hr and measured at room temperature. d or t indicates a doublet or a triplet. The arrow indicates where resonance of DPPH occurs.

1 hr, 2 hrs, 4 hrs, 8 hrs and 16 hrs after preparation of the samples. As shown in Fig. 1, the spectra observed at 2 hrs exhibit a pattern of doublet with a hyperfine splitting (hfs) of 16 gausses and a peak to peak width of 8 gausses in the concentrations of 3 M and 0.5 M. The spectra at 4 hrs show a pattern of triplet with a hfs of about 12 gausses in 3 M and 0.5 M. The latter seemed to be a composite pattern, where a triplet of the relative intensity distribution of $1:1:1$ was superimposed upon a doublet of $1:1$ in 3 M, and a triplet of $1/2 \times (1:2:1)$ upon a doublet of $1:1$ in 0.5 M, since the field modulation was swept so wide as 30 gausses that the wings of a triplet were not distinguished from a doublet.

While the spin of a triplet signal was markedly increased in a concentration of 3 M solution at 8 hrs and afterwards, it was not increased in 0.5 M. With an assumption that a triplet of resonance was superimposed upon a doublet, the relations between the spin induced and the dose given to glycine solution were plotted in Fig. 2 in terms of a doublet and a triplet.
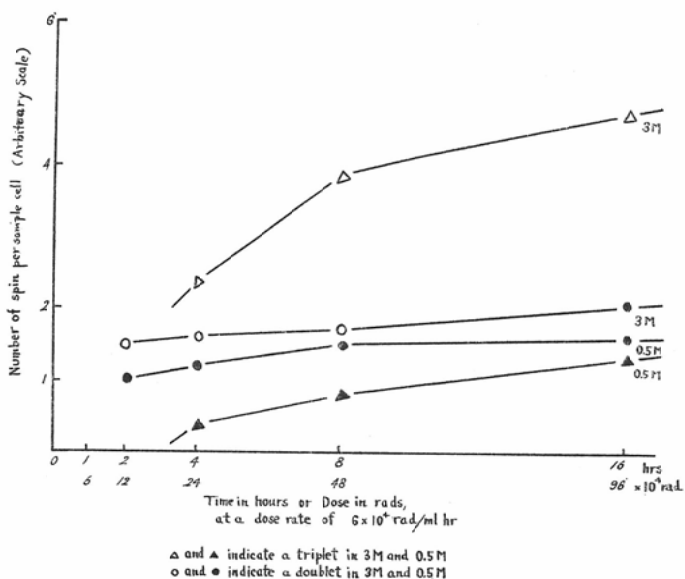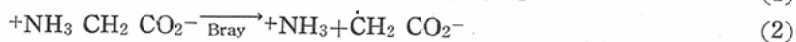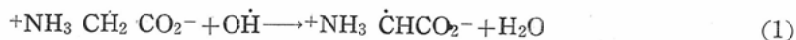

Fig. 2. The number of ESR spins being induced by ^3H_2O in 3 M and 0.5 M glycine solution as a function of the time at 0 to 16 hrs or the dose, at a dose rate of 6×10^4 rads/hr.

Observations of patterns of resonance in glycine solution led to a conclusion that the doublet observed resulted from a radical where the unpaired electron interacts with one hydrogen, and the triplet from a radical where the unpaired electron interacts with two protons. Glycine solution exists as a zwitter ion aqueous solution. The following equations are reasonably considered.
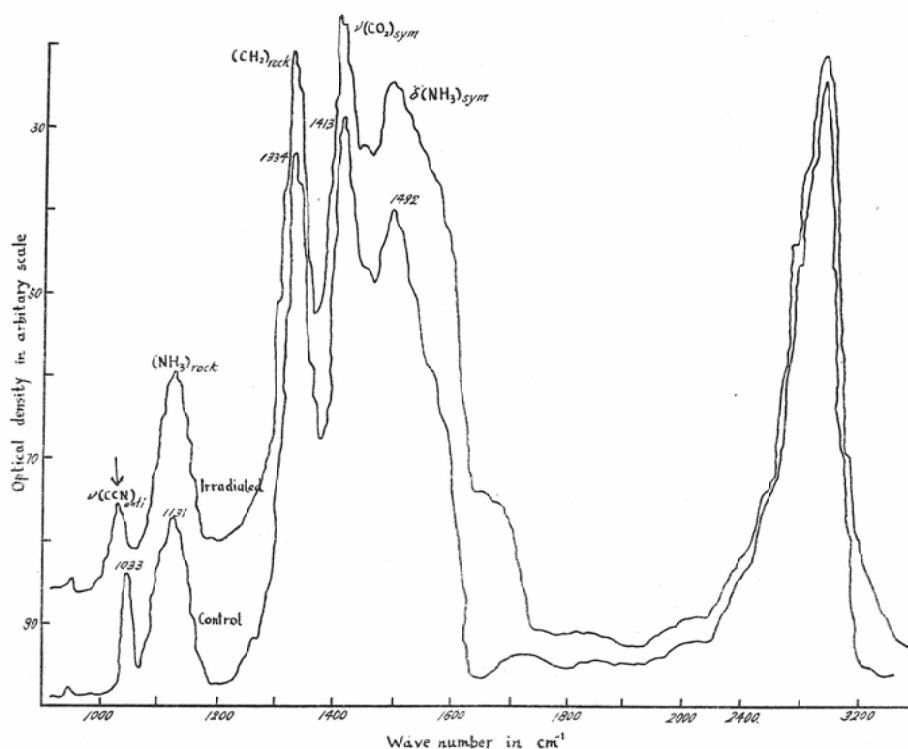
$$^+NH_3\ CH_2\ CO_2^- + O\dot{H} \longrightarrow {}^+NH_3\ \dot{C}HCO_2^- + H_2O \tag{1}$$

$$^+NH_3\ CH_2\ CO_2^- \xrightarrow{Bray} {}^+NH_3 + \dot{C}H_2\ CO_2^- \tag{2}$$

Fig. 3. Infrared spectrum of 3 M glycine solution being irradiated by ^3H_2O β-ray at 2.4×10^5 rads dose.

To make sure whether or not NH_3 was produced in the glycine solution during irradiation, infrared spectroscopy was carried out on a 3 M glycine solution being irradiated by ^3H_2O β-ray at the same dose rate mentioned above. The absorption on the spectrum at 4 hrs after preparation of the samples was studied, namely, 2.4×10^5 rads dose was given to the glycine solution.

As shown in Fig. 3 difference was hardly observed of the absorption of CH_2, NH_3, and CO_2 between the irradiated and non-irradiated samples, whereas in case of an antisymmetric stretching frequency of CCN on 1033^{-1}cm band the absorption of irradiated sample showed a marked decrease over non-irradiated sample. Such decreased absorption may be explained by the production of NH_3, resulting from the breakdown of C-C bond. And also infrared spectroscopy was carried out on a 0.5 M glycine solution being irradiated at the above mentioned dose. The absorption on 1033^{-1}cm band was hardly seen.

There is a possibility that either of or both of the direct and the indirect actions of radiation are responsible for free radicals in glycine solution being irradiated. The experimental results revealed that spins of a triplet in 3 M were increased than in 0.5 M as the dosage increased, whereas spins of a doublet in 3 M and 0.5 M solutions were kept almost equal. If the difference between a triplet and a doublet is dependent of concentration, it may be concluded that a triplet is formed as a result of the indirect action and that a doublet is formed as a result of the direct action. Further, infrared spectroscopical

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findings support this view.

Experiment 2

The dielectric properties of the glycine solution were studied during irradiation to obtain another information of the immediate effects of ionising radiation. The changes of dielectric constant during irradiation could be interpreted on the basis of degradation and/or crosslinkage caused by irradiation.

As samples, 1 ml of each 3 M and 0.5 M glycine solution which contained 500 mc of ^3H_2O (specific activity, 1 c/0.2 ml) was prepared. That is 1 ml of glycine solution was to be irradiated at a dose of 6×10^3 rads/hr.

Each sample solution was poured into a glass cell with two end walls made of 6 mm platinum black plates placed 2 mm apart. Mesurement of the dielectric constant were made with a modified Oncley's technique (5) developed by the present author, by means of alternating current bridge, at a frequency of 2 Mc/sec and/or 8 Mc/sec, with an oscilloscope used as a detector. The sensivity of the capacity reading was 0.02 $\mu\mu F$ between 400 Kc/sec and 27 Mc/sec. Dielectric constant is given by $\varepsilon = \dfrac{C_1 - C_2}{C_x} + 1$, where C_x is sample cell capacity constant, C_1 and C_2, capacity with glycine solution and without glycine solution.

Preliminary experiments were made with eight samples of glycine solution from 0.1 M

Fig. 4 Dielectric increments of 3 M and/or 0.5 M glycine solution measured at 2 Mc/sec and/or 8 Mc/sec is plotted against the doses during irradiation.

to 3 M. Dielectric constant measured in these solutions linearly increased with an increase in the concentration. Dielectric increment showed exactly 23 per Mol which agreed with Oncley's (6) (7) experiments.

The dielectric constants were measured at a frequency of 2 Mc/sec in 3 M and 0.5 M glycine solutions being irradiated at a dose rate of 6×10^3 rads/hr. Dielectric constant showed gradual increase as the dose proved to be 5×10^4 rads. Dielectric increment converted in 1 Mol against doses given is shown in Fig. 4. As is obvious from this figure, dielectric increment in a 3 M solution shows a more marked increase than in 0.5 M at more than 5×10^4 rads.

The increase of dielectric increment during irradiation may be well explained by the formation of chemicals, which have much larger dielectric constants than parent glycine, resulting from radical combination process among glycine molecules degraded by irradiation. Accordingly, it is considered that the reaction proposed in equation (1) and (2) is subjected to the following processes :
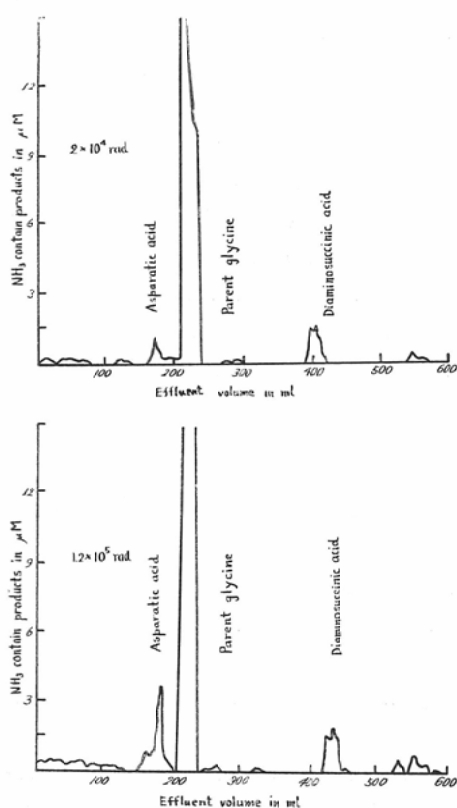

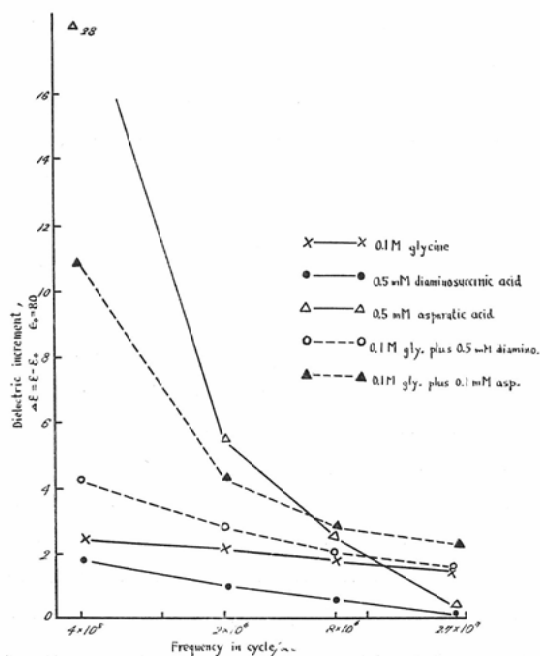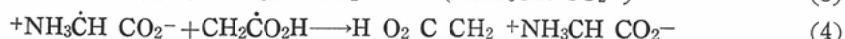Fig. 5. Elution curve of NH₃ contain products formed in the glycine solution by ^3H_2O β-ray at a 2×10^4 rads dose and 1.2×10^5 rads dose.

Fig. 6. Dieletric increment of amino acids as a function of frequency.

$$^+NH_3\dot{C}H\ CO_2^- + ^+NH_3\dot{C}H\ CO_2^- \longrightarrow 2\ (^+NH_3CH\ CO_2^-) \tag{3}$$

$$^+NH_3\dot{C}H\ CO_2^- + CH_2\dot{C}O_2H \longrightarrow H\ O_2\ C\ CH_2\ ^+NH_3CH\ CO_2^- \tag{4}$$
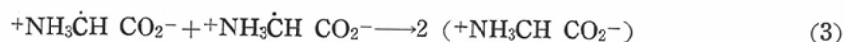
As the above products were already confirmed by Week and Garrison (2).

To examine the quantitative dependences between dielectric increments and chemical products, column chromatography was carried out following a modified method of Moore and Stein (9). Dowex 50 resin (H-form, 100 to 200 mesh, 8% crosslinked) was used. A 3 M glycine solution which contained ^3H_2O and was subjected to be exposed to ^3H_2O β-ray for 4 hrs or 20 hrs was poured onto the top of the column. Hidrocloride with progressively increased concentrations (0 to 4 N) was to be used to elute nitrogen products and parent glycine. Five ml of effluent was collected in 100 tubes serially. The position of product peaks was determined by colouring each tube for ninhidrin and quantitized by transmittance at 570 mμ. Typical elution curves of a 3 M glycine solution irradiated by ^3H_2O β-ray at 2.4×10^4 rads and 1.2×10^5 rads are represented in Fig. 5. The nitrogen products have been identified as diaminosuccinic acids and asparatic acids, which showed an exact correspondence with a preliminary elution curve in both chemicals and as well as parent glycine. Diamiosuccinic acids and asparatic acids produced by 1.2×10^5 rads dose were approximately 1.5 μM and 3.2 μM from a 1 M glycine solution (converted in 1 M from 3 M).

Measurements of the dielectric constant of 0.5 mM diaminosuccinic acids and 0.5 mM asparatic acids were performed at 400 Kc/sec, 2 Mc/sec, 8 Mc/sec and 27 Mc/sec, since it seems advisable to determine whether or not the dielectric increment was caused by the production of μM of the above chemicals. Fig. 6 shows that the dielectric increment of diaminosuccinic acids and asparatic acids was about 1 and 5 on a 0.5 mM aqueous solution at 2 Mc/sec measurement, respectively. As obvious from the above results, yield of μM products fails to explain an increase of the dielectric increment in glycine solution being irradiated, although an increase of the dielectric increments resulted from irradiation is proportional (see Conclusion) to the dielectric constants of chemicals from a parent glycine by irradiation.

Conclusion

Radiolysis of glycine solution during irradiation was studied by the spin resonance method. Free radicals formed in glycine solution during irradiation are reasonably considered to be $^+NH_3\dot{C}H\ CO_2^-$ and $\dot{C}H_2\ CO_2^-$ by the analysis of a triplet and a doublet pattern. The reaction by ionising radiation on glycine solution may be represented by equation (1) and equation (2). Experiments with infrared spectroscopy seems to provide evidence in support of the above equations. Equation (1) is very likely to be a result of indirect action, so is equation (2) of direct action.

Dielectric increment increased gradually from the beginning to 5×10^4 rads and afterwards increased markedly in a 3 M glycine solution at 2 Mc/sec. Elevation of dielectric increment was proportional to the formation of diaminosuccinic acids and asparatic acids which derived from radical recombination action as represented in equation (3) and (4), as the latter product is expected to increase markedly at more 5×10^4 rad dose, being

supported by the results of column chromatography and dielectric study(Fig.6). It must be caused by the direct process that dielectric increment in a 0.5 M dia not increase so markedly at more 5×10^4 rads as a 3 M. However, formation of diaminosuccinic acids and asparatic acids from glycine solution during irradiation cannot give an explanation quantitatively to the dielectric increment observed. On an assumption that such additional reaction as crosslinkage between products and/or parent glycine may be expected, it is supported to consider that a clue is found in interpreting the large dielectric increment of irradiated glycine. Further study is underway in order to find products by snbsequent reaction.

Acknowledgement

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