Radiolysis of Solid Glycine

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The mechanism of radiation-induced reactions in a glycine aqueous solution has been extensively studied in many laboratories.[1,2] The radiolysis of solid glycine, however, has not been investigated thoroughly; although some of the radicals produced have been examined from the ESR spectra of irradiated solid glycine,[3,4] there has been insufficient interpretation of the spectra.

In the present work, products are measured after dissolving irradiated solid glycine in water, and mechanisms for the primary reaction of solid glycine radiolysis and for the secondary reaction, giving final products, are proposed with reference to the results of ESR studies.

Experimental

Materials. — Glycine (Yuki Gosei Kogyo Co., Special grade) was purified by recrystallization from water at least four times. Sodium chromotropate (Merck, Reagent) and *p*-dimethylaminobenzaldehyde (Daiichi Kagaku Yakuhin Co., Special grade) were used without further purification as reagents for the analysis of the products.

Irradiation.—Glycine powder (100∼200 mesh) in glass vessels was evacuated for 8 hr. at 110°C in vacuo ($<10^{-4}$ mmHg) and then sealed. A 3000-curie cobalt source was used for γ-ray irradiation. The dose range employed was 0.5×10^{20} eV. g^{-1} to 2.7×10^{21} eV. g^{-1} at dose rates of $9.5\times10^{18}\sim4.1\times10^{19}$ eV. g^{-1} hr^{-1}. Dosage was determined by the Fricke-dosimeter technique with a $G(\mathrm{Fe}^{3+})$ value of 15.6.

Analysis. — Hydrogen collected by conventional techniques directly from the irradiated solid samples was analyzed with a gas chromatograph (Shimadzu, GC-1A) and a mass spectrometer (Hitachi, RMU-5B).

All the other products, volatile bases, organic acids, formaldehyde etc. were analyzed from the solutions after dissolving the irradiated samples in water in and out of the presence of oxygen.

Nitrogen-containing Products.—The total amount of the volatile bases (NH_3, CH_3NH_2) was measured by two different methods, the Kjeldahl method and separation with an anion-exchange resin. In the latter case, Dowex 1 resin (OH form, 100∼200 mesh, 8% crosslinked) was used. Aqueous solutions of the irradiated glycine were poured onto the top of the column (40 cm. high by 2 cm. in diameter), and the effluents were collected in the excess of sulfuric acid and back-titrated with sodium hydroxide, using phenolphthalein as an indicator. The results of the two methods agreed well. In some runs, a radiofrequency titration method was also applied.

Methylamine was identified and measured by an infrared absorption spectrum of methylamine hydrochloride obtained by evaporating the effluents after neutralization with hydrochloric acid. Methylamine was measured also from extinction at 540 mμ induced by the reaction with lactose.[5] The amount of ammonia was obtained by substracting the amount of methylamine from that of the total volatile base.

The amount of hydrazine tested colorimetrically with *p*-dimethylbenzaldehyde[6] was negligibly small. Amino acids were not detected by the use of an amino acid analyzer or by paper chromatography.

Acids and Formaldehyde.—Organic acids were separated from glycine and other products with cation exchange resin Dowex 50 (H form, 50∼100 mesh, 8% crosslinked). Aqueous solutions of the irradiated glycine were poured onto the column (40 cm. high, 2 cm. in diameter), and the effluents were titrated with sodium hydroxide, using cresol red as an indicator, under an atmosphere of nitrogen. In some runs, the effluents were degassed by conventional techniques to remove the carbon dioxide and then titrated in order to measure the real amount of organic acids. The amount of carbon dioxide was obtained from the decrease in the total of acid produced by the degassing. The components of the organic acid were identified as glyoxilic acid and acetic acid from the infrared absorption spectra of the sodium salts obtained by freeze-drying after titration.

Glyoxilic acid was measured by techniques modified from the method used for the analysis of formaldehyde by MacFadyen.[7] The irradiated glycine (0.01∼0.05 g.) was dissolved in 0.5 ml. of water and 5 ml. of 2 N hydrochloric acid; 0.05 ml. of sodium chromotropate (0.1 g. ml^{-1}) and 5 ml. of concentrated sulfuric acid were then added, and

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[1] C. R. Maxwell, D. C. Peterson and N. E. Sharpless, *Radiation Research*, **1**, 530 (1954); C. R. Maxwell, D. C. Peterson and W. C. White, ibid., **2**, 431 (1955).

[2] B. M. Weeks and W. M. Garrison, ibid., **9**, 291 (1958).

[3] D. K. Ghosh and D. H. Whiffen, *Mol. Phys.*, **2**, 285 (1959); D. K. Ghosh and D. H. Whiffen, *J. Chem. Soc.*, **1960**, 1869.

[4] R. F. Weiner and W. S. Koski, *J. Am. Chem. Soc.*, **85**, 873 (1963).

[5] A. A. Ormsby and S. Johnson, *J. Biol. Chem.*, **187**, 711 (1950).

[6] "Bunseki Kagaku Binran," Maruzen, Tokyo (1961), pp. 463–464.

[7] D. A. MacFadyen, *J. Biol. Chem.*, **158**, 107 (1945).

the mixture was boiled for 30 min., cooled, and diluted to 10 ml. with water. From the absorption spectra of the solution, glyoxilic acid was measured by the extinction at 440 mμ.

The amount of acetic acid was obtained by subtracting the amount of glyoxilic acid from the total of organic acid. A small amount of formaldehyde was measured by the same method as glyoxilic acid from the extinction at 568 mμ.

Results and Discussion

Yields.—Figures 1 and 2 show the relations between the amounts of the products and the irradiation doses. All products except H_2 increase linearly with the dose. Hydrogen shows a plateau from a relatively low dose. Figure 1 shows that oxygen dissolved in water, which is used to dissolve the irradiated samples, does not remarkably affect the yields of the total base. The yields of the products obtained from the slopes of Figs. 1 and 2 are listed in Table I. The initial yield of H_2 was estimated to be approximately 0.2 from the initial slope of the curve.

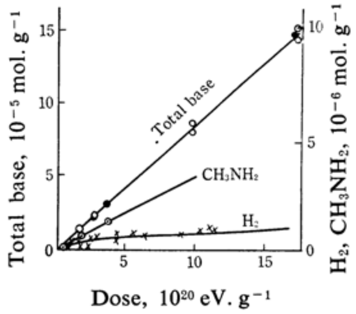

Fig. 1. The yields of basic products and H_2 from glycine irradiated by ^{60}Co γ-rays. Total base was obtained by dissolving the sample in oxygen containing water (○) or degassed water (●).

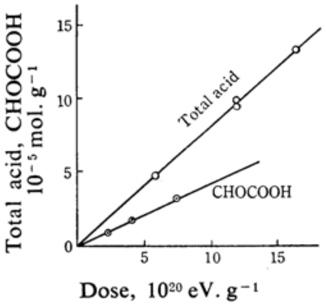

Fig. 2. The yields of acidic products from glycine irradiated by ^{60}Co γ-rays.

TABLE I. YIELDS OF PRODUCTS

Product	*G*
H_2	~0.2
NH_3	4.8
CH_3NH_2	0.2
$CHOCO_2H$	2.5
CH_3CO_2H	2.3
CO_2	~0.2
HCHO	trace (~0.0₃)

Mechanism. — From the studies of ESR spectra of the irradiated solid glycine, some evidence concerning radicals in irradiated solid glycine was obtained.[8] The results were interpreted by assuming that the free radicals primarily produced are NH_2 and $CH_2CO_2^-$, with a trace of $\overset{+}{N}H_3CHCO_2^-$, and that NH_2 and $CH_2CO_2^-$ slowly decrease to produce $\overset{+}{N}H_3CHCO_2^-$ and an unknown radical (presumably $\overset{+}{N}H_2CH_2CO_2^-$).

Irradiated glycine was dissolved in water, immediately after irradiation or after having been kept at room temperature for a long time. In the latter case, the radicals originally produced should have completely changed. Nevertheless, the chemical analysis of the final products of the two cases gave the same results.

The CH_3NH_2 obtained ($G=0.2$) appears to be due to a direct molecular dissociation of the glycine molecule, as was proposed by Garrison[2] in the radiolysis of aqueous solutions.

From these results, the following mechanism of the radiolysis of solid glycine may be proposed:

$$\overset{+}{N}H_3CH_2CO_2^- \rightsquigarrow H^+ + NH_2 + CH_2CO_2^- \qquad (1)$$

$$\overset{+}{N}H_3CH_2CO_2^- \rightsquigarrow CH_3NH_2 + CO_2 \qquad (2)$$

$$\overset{+}{N}H_3CH_2CO_2^- \rightsquigarrow \overset{+}{N}H_3CHCO_2^- + H \qquad (3)$$

$$H + \overset{+}{N}H_3CH_2CO_2^- \longrightarrow H_2 + \overset{+}{N}H_3CHCO_2^- \qquad (4)$$

With an increasing dose, radicals are accumulated (the estimated value is, for instance, 10^{-5} mol. g^{-1} at a dose of 1×10^{20} eV. g^{-1}) and scavenge H atom to give a plateau of the H_2 yield.

$$H + R \longrightarrow RH \qquad (5)$$

The primary process 1 seems to be not so simple; it may contain dissociative electron-capture mechanism to give the same resultant radicals. The results of the investigation of this point will be published elsewhere.

When the irradiated glycine is dissolved in

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[8] G. Meshitsuka, Y. Shinozaki and K. Shindo, *Annual Report, Tokyo Metropolitan Isotope Center*, 2, 181 (1963).

water, particular radicals, NH_2 and $CH_2CO_2^-$, abstract H from the glycine molecule to produce ammonia, acetic acid and $\overset{+}{N}H_3CHCO_2^-$:

$$NH_2 + \overset{+}{N}H_3CH_2CO_2^- \longrightarrow NH_3 + \overset{+}{N}H_3CHCO_2^- \qquad (6)$$

$$CH_2CO_2^- + \overset{+}{N}H_3CH_2CO_2^- \longrightarrow CH_3CO_2^- + \overset{+}{N}H_3CHCO_2^- \qquad (7)$$

Hydrogen abstraction may occur at the amino group to form $\overset{+}{N}H_2CH_2CO_2^-$, but it is probable that the radical changes to a more stable radical, $\overset{+}{N}H_3CHCO_2^-$, by the H atom migration:

$$\overset{+}{N}H_2CH_2CO_2^- \longrightarrow \overset{+}{N}H_3CHCO_2^- \qquad (8)$$

Glycine radicals give NH_3, $CHOCO_2H$, HCHO and CO_2 by the following reactions, as proposed in the radiolysis of aqueous solutions:²⁾

$$2\overset{+}{N}H_3CHCO_2^- \longrightarrow \overset{+}{N}H_2CHCO_2^- + \overset{+}{N}H_3CH_2CO_2^- \qquad (9)$$

$$\overset{+}{N}H_2CHCO_2^- + H_2O$$

$$\longrightarrow NH_3 + CHOCO_2H \qquad (10)$$

$$\longrightarrow NH_3 + HCHO + CO_2 \qquad (11)$$

Reaction 9′ has been proposed by Garrison²⁾ in the radiolysis of oxygen-containing aqueous solutions:

$$\overset{+}{N}H_3CHCO_2^- + O_2 \longrightarrow \overset{+}{N}H_2CHCO_2^- + HO_2 \qquad (9')$$

However, oxygen in water does not have any remarkable effect on the yields of the total base. It seems, therefore, that $\overset{+}{N}H_3CHCO_2^-$ reacts mainly in the disproportionation reaction 9 instead of in reaction 9′, even in the presence of dissolved oxygen. If the scavenging reaction 5 holds, $G(CH_3CO_2H)$ should be a little higher than $G(CHOCO_2H)$. On the contrary, however, the results show $G(CHO\cdot CO_2H)$ to be a little higher than $G(CH_3CO_2H)$, indicating that reaction 9′ also occurs to a small extent.

According to the mechanism, the following relationships may be expected:

$$G(\text{total acid}) = G(\text{total base})$$

$$G(NH_3) = G(CHOCO_2H) + G(CH_3CO_2H)$$

$$G(CO_2) = G(CH_3NH_2) + G(HCHO)$$

As Table I shows, the experimental results satisfy these relationships well. The yields of the primary processes are estimated as follows:

$$G(1) = G(CH_3CO_2H) = 2.3$$

$$G(2) = G(CH_3NH_2) = 0.2$$

$$\text{and} \quad G(3) = G(H_2) \sim 0.2$$

Summary

Solid glycine has been radiolyzed by ^{60}Co γ-rays in vacuo at room temperature, and all the products except H_2 have been measured after dissolving the irradiated samples in water. The hydrogen has been collected and measured directly from the irradiated solid samples. All products except H_2 increase linearly with the dose, while H_2 shows a plateau from a relatively low dose. The yields of products are as follows: $G(H_2) \sim 0.2$, $G(NH_3) = 4.8$, $G(CH_3NH_2) = 0.2$, $G(CHOCO_2H) = 2.5$, $G(CH_3CO_2H) = 2.3$, $G(CO_2) \sim 0.2$, and $G(HCHO) \sim 0.03$. The mechanisms of the primary process and the reactions to give final products have been proposed with reference to the results of the ESR studies.

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