

The Effect of Gamma-Irradiation on Aqueous Solutions of Triglycine

3. Mechanism for Gamma-ray Induced Degradation Products¹

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Triglycine 水溶液에 미치는 감마線の 영향

3. 감마線에 의한 分解產物에 關하여

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摘 要

酸素 溶存下의 triglycine 水溶液을 γ -線으로 照射하여 carbonyl 化合物, glycine, ammonia, CO₂ 및 水素를 檢出하였다. 이 水溶液系에서 일어나는 放射化學反應을 初過程과 分解產物을 가지고 論議하였다.

Triglycine 分子는 먼저 中間生成物을 이루게 되고 이것이 carbonyl 化合物과 acid amide 로 分解하게 된다. 이들은 一次의 또는 二次의 作用에 의하여 acetamide, glycine, glyoxylic acid, formic acid, oxalic acid, ammonia, 및 CO₂ 로 分解하게 된다.

또한 radical-radical 結合에 의하여 生成物이 생길 未確認反應의 可能性도 있으며 妥當한 物質 平衡은 앞으로의 研究에 期待한다.

INTRODUCTION

A number of early studies on irradiated glycine solutions showed that ammonia was an important reaction product. Maxwell and co-workers (1954) obtained a complete analysis of all the products and found a good material balance in the production of irradiation of 1M glycine with X-rays. The main products turned out were ammonia, glyoxylic acid, acetic acid, and hydrogen with smaller quantities of formaldehyde, methylamine, formic acid and carbon dioxide.

Barron (1955) suggested the imino acid, NH=CHCOOH, as an important intermediate in the reaction. Weeks and Garrison (1956) studied the irradiated glycine solutions and suggested the reaction sequence to explain their results and those of Maxwell *et al.* (1954).

Alanine was also studied by Sharpless and co-workers (1955) and the similarity of results obtained from 1 M solutions of glycine and alanine was quite remarkable. The only essential difference was that the products arising from the alkyl end of the alanine molecule contained an additional methyl group. The alanine solutions gave ethylamine, propionic acid, pyruvic acid and acetaldehyde in essentially the same yields. The reaction mechanism is evidently the same for both as postulated.

Weeks and Garrison (1958) have studied the chemical nature of the compounds formed on radiolytic oxidation of proteins. The mechanism proposed by Garrison for oxidation of amino acid could well be expected to produce in a similar fashion, with the protein splitting at the peptide bond to yield a carbonyl compound and an amide.

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The previous papers of this series (Leone and Kang, 1963, 1964) have discussed the action of gamma-irradiation on aqueous solutions of triglycine. The studies on products from gamma-irradiated triglycine include glycine, carbonyl compound, and acid amide expected and secondary reactions taking place in the irradiated aqueous systems. The studies also showed that the competitive action of degradation products with triglycine to free radicals produced.

This paper continues the possible mechanism for the degradation products arising from irradiated aqueous solutions of triglycine in the presence of oxygen.

MATERIALS AND METHODS

The materials and methods described previously (Leone and Kang, 1963, 1964) for irradiation and experimental procedures were adopted unchanged in this study otherwise noted.

In order to know the significance of absorption maximum shift in irradiated triglycine-biuret complex (dose higher than 2.5×10^{20} ev/ml), biuret complex of mixtures of diglycine-triglycine and triglycine-tetraglycine were made in varying ratios and were read in spectronic 505 and in Model B spectrophotometer.

Annalytrol scanning of paper chromatograms of separated compounds from irradiated triglycine was followed for the analysis of reaction scheme in the aqueous systems. The dose ranged from 5×10^{19} ev/ml to 2.5×10^{20} ev/ml. Whatman No. 1 chromatography paper was used in this experiment using butanol-acetic acid-water as solvent mixture.

As was shown previously the first moving compound in this solvent in ascending chromatography was glycine. The confirmation of this compound was done using copper carbonate technique (Crumpler, 1949). The procedure employed was as follows; some solid copper carbonate was first applied to the origin of chromatography paper (Whatman No. 4) by means of a small cotton pad and, after gently rubbing into the paper, the excess was blown off. Samples were then applied to the paper and, when dry, chromatography was carried out as usual with butanol-pyridine-water (1 : 1 : 1, v/v, neutral pH) for 3 hours. After air drying chromatograms were dipped in 0.25% ninhydrin.

Qualitative determination of ammonia was performed directly by nesslerization after steam distillation from the sample. Hydrogen and carbon dioxide were identified on a mass spectrometer but quantitative determination of these compounds was not performed.

Specific identification and isolation of glyoxylic acid by precipitation of the 2,4-dinitrophenylhydrazine at 0°C were tried since carbonyl compounds were identified and determined quantitatively using the colorimetric method of Lappin and Clark (1951) previously.

RESULTS AND DISCUSSION

Discussion on the mechanism for gamma-ray induced degradation of triglycine is made with the result of the present study and those of previous ones (Leone and Kang, 1963, 1964).

Absorption spectra for copper-biuret complex of diglycine-triglycine and of triglycine-tetraglycine mixtures in varying ratios are shown in figs. 1 and 2. As is evident

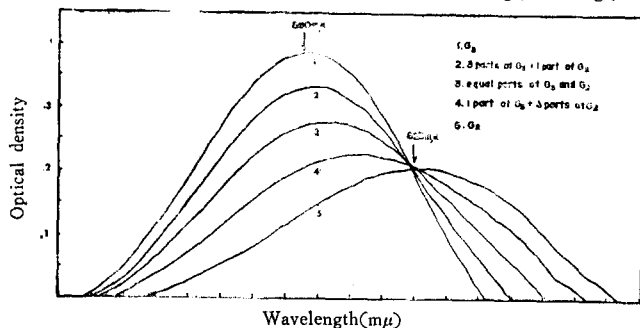


Fig. 1. Absorption spectra for copper-biuret complex of diglycine-triglycine mixtures in varying ratios, showing the change in absorption maxima. G_3 , triglycine and G_2 , diglycine.

from these figures maximum peak for triglycine at $560 \text{ m}\mu$ moves to longer wavelength as the increased ratios of diglycine and that for triglycine to shorter wavelength with increase of tetraglycine mixed. The absorption maxima for diglycine and tetraglycine are $625 \text{ m}\mu$ and $515 \text{ m}\mu$, respectively. By analyzing the significance of absorption maxima shift in irradiated triglycine-biuret complex to longer wavelength and the shift of absorption maximum of biuret-copper complex of triglycine-diglycine mixtures to longer wavelength, diglycine

in the system might be assumed or other degradation products which form copper-biuret complex leading to absorption maximum shift to longer wavelength. The possibility of this occurrence is not available from the present study but a possibility arises if only the C-terminal peptide bond is attacked by free radicals producing $\text{NH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{NH}_2$ and this, in turn, converted to $\text{NH}_2\text{CH}_2\text{CONHCH}_2\text{COOH}$ and NH_3 by a secondary reaction. Maxwell and co-workers (1959) observed $\text{HOOCCH}_2\text{CHNH}_2\text{COOH}$ and $\text{HOOCCHNH}_2\text{CHNH}_2\text{COOH}$ in their evacuated glycine solutions but not in the presence of oxygen. This fact strongly suggests that the formation of products of higher molecular weight by the combination of carbon radicals is prevented by oxygen and oxidized products are found instead in our irradiated

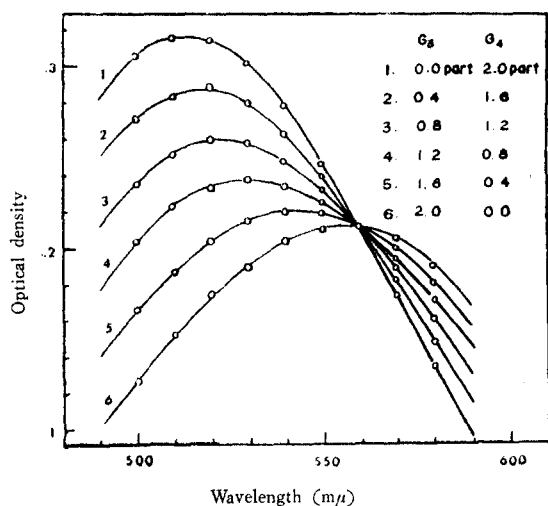


Fig. 2. Absorption spectra for copper-biuret complex of triglycine-tetraglycine mixtures in varying ratios, showing the changes in absorption maxima. G_3 , triglycine and G_4 , tetraglycine.

nism proposed by Weeks and Garrison (1958). If direct hydrolytic splitting of peptide bond by free radical gives rise to glycine, the possibility of diglycine production seems to be quite adequate but it seems to be impossible from the standpoint of free radical attack on $-\text{CO}-\text{NH}-$ group though it was conjectured in the previous paper. The latter assumption seems to be quite reasonable since acid amide is one of the degradation products. Glycine is probably produced by the oxidation of amino acetamide, $\text{NH}_2\text{CH}_2\text{CONH}_2 \rightarrow \text{NH}_2\text{CH}_2\text{COOH} + \text{NH}_3$, primarily or secondarily from the irradiated triglycine.

Ammonia was identified qualitatively. Ammonia yield probably is arisen by deamination from triglycine and degradation products available. Ammonia was found to be the most abundant product when amino acids, such as glycine and alanine, are irradiated with X-rays (Maxwell *et al.*, 1954 and Sharpless *et al.*, 1955). G-value was found to be about 4 for one molar concentrations of glycine and alanine. The mechanism extended by Maxwell *et al.* (1954) and Sharpless *et al.* (1955) is also attributable to the production of ammonia in irradiated triglycine. Possibly the N-terminal glycine residues of triglycine and degradation products yield much ammonia. Free radical attack on amino group in one of the degradation products, $\text{NH}_2\text{CH}_2\text{CONH}_2$, gives rise to CH_3CONH_2 and NH_3 in return.

Hydrogen and carbon dioxide were identified as degradation products from irradiated triglycine. Hydrogen seems to be produced by a similar reaction, suggested by Barron (1955), in the formation of an important intermediate in amino acid degradation and by radical-radical interactions. Carbon dioxide could be produced by decarboxylation mainly in its degradation products such as carboxylic acid. Carbon dioxide is produced by primary reaction as well as by secondary reaction in which HCOCOOH gives rise to $\text{HCOOH} + \text{CO}_2$ and $(\text{COOH})_2 + \text{CO}_2$.

Specific identification of glyoxylic acid was tried in vain. Isolation of hydrazone of this compound from hydrazone mixtures in the system was not possible since the precipitate formed by the addition of 2,4-dinitrophenylhydrazine at

triglycine solutions.

Analytical scanning patterns for paper chromatograms of irradiated triglycine is shown in fig. 3. It is clear from the scanning patterns that the compounds separated chromatographically undergo secondary reactions competing with triglycine or with each other to free radicals available in the system.

Chromatograms obtained using copper carbonate technique is shown in fig. 4 with glycine, diglycine, and tetraglycine for comparison. From this chromatograms it is evident that an α -amino acid has a high enough R_f value compared with those of other compounds which are detectable with ninhydrin. The occurrence of glycine in irradiated triglycine suggests two possibilities for its production. One is by direct hydrolytic splitting of peptide bond in triglycine molecule and the other by the oxidation of an acid amide produced either as primary or secondary product by the mechanism

0°C was not adequate to isolate and purify for further treatment.

Qualitative and quantitative determinations of this compound have to be done with samples at high concentrations.

Jayko and Garrison (1956, 1958) have studied the chemical nature of the compounds formed on radiolytic oxidation of proteins. The =CH-NH₂ is first oxidized to the unstable imino compound =C=NH which then hydrolyzed to ammonia and a carbonyl compound =C=O + NH₃. Attack on peptide could well be expected to proceed in a similar fashion, with the peptide splitting at the peptide bond to yield a carbonyl compound and an amide. It was, in fact, found (Leone and Kang, 1963, 1964) that on irradiation of triglycine solutions with gamma-rays, carbonyl compounds occurred in the system, as shown by the reaction of products with 2,4-dinitrophenylhydrazine to give a characteristic hydrazone color. The initial G-value for carbonyl formation was 0.80 by ultraviolet spectrophotometric study and 0.79 by colorimetric quantitation. The corresponding amide groups were not identified. These occurred in the presence of oxygen.

It is possible to postulate the following reactions as occurring in the irradiated aqueous systems of triglycine; aqueous solutions of triglycine undergo a reaction for the formation of an intermediate by free radicals produced in irradiated water.

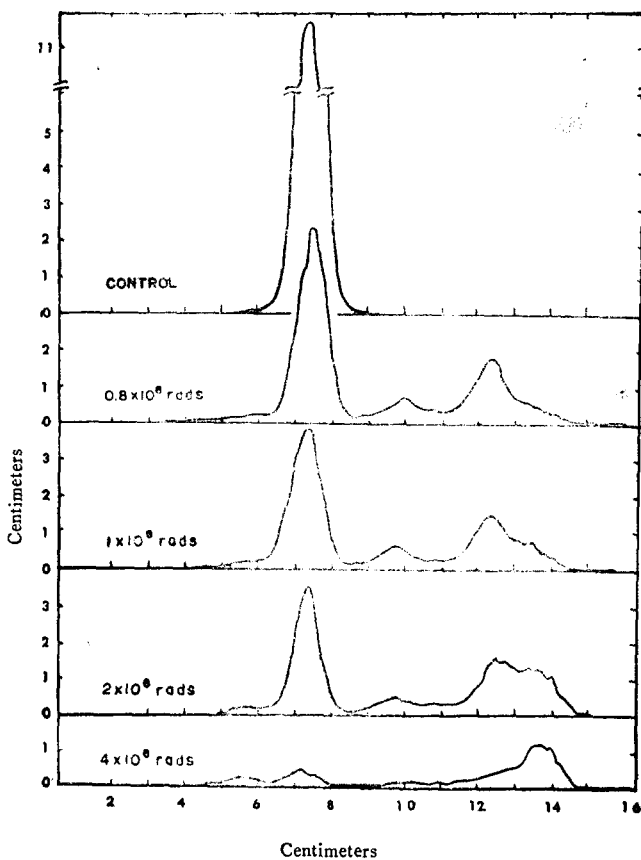
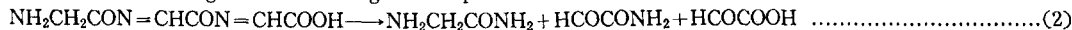


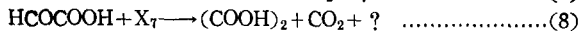
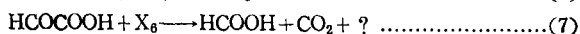
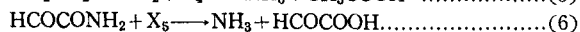
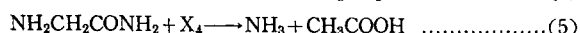
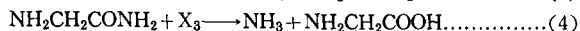
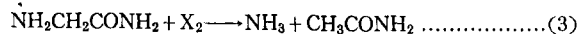
Fig. 3. Annalytrol scanning patterns of irradiated triglycine separated by paper chromatography.



The intermediate then gives rise to a degradation process:



At the same time these compounds are degraded further giving rise to primary products and secondary products which are assumed to be produced by:



It is also possible that the unrecognized reactions may give rise to products formed by radical-radical combinations. The nature of the free radicals, X₁, X₂, X₃, X₄, X₅, X₆, and X₇ could be either one of the irradiation products from the water in the presence of oxygen.

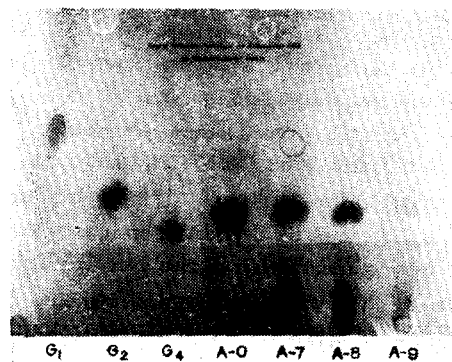
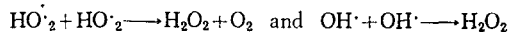


Fig. 4. Chromatographic separation of irradiated triglycine using copper carbonate technique for specific identification of glycine. G₁, glycine; G₂, diglycine and G₄, tetraglycine.

The indirect action of radiation on solutes is generally ascribed to the formation of chemically reactive free radicals, $H\cdot$ and $OH\cdot$ by decomposition of water; $H_2O \longrightarrow H\cdot + OH\cdot$

In the presence of oxygen following reaction is believed to occur with great ease: $H\cdot + O_2 \longrightarrow HO_2\cdot$

The principal reactions leading to the formation of hydrogen peroxide are believed to be:



Competing with these reactions will be the radical removal reaction: $HO_2\cdot + OH\cdot \longrightarrow H_2O + O_2$

Since it is beyond the scope of this discussion to enter into the mechanism by which these various radicals caused the transformation of triglycine molecules, reactions (1 to 8) are postulated only as representing the initial process and the final products. Satisfactory material balance for all the products found have to wait the study now in progress.

SUMMARY

Carbonyl compounds, glycine, ammonia, carbon dioxide and hydrogen have been identified in gamma-irradiated oxygenated aqueous solutions of triglycine. The reactions occurring in this system are postulated only by representing the initial process and the final products.

The triglycine molecule undergoes a reaction to form an intermediate giving rise to carbonyl compounds and acid amides. These compounds are decomposed to acetamide, glycine, glyoxylic acid, formic acid, oxalic acid, ammonia, and carbon dioxide by free radical attack in the primary and/or secondary reactions.

It is also possible that the unrecognized reactions may give rise to products formed by radical-radical combinations. Satisfactory material balance for all the products have to wait until the completion of a study now in progress.

REFERENCES

- Barron, E.S.G., J. Ambrose and P. Johnson, 1955. *Rad. Res.* 2, 145.
- Crumpler, H.R. and C.E. Dent, 1949. Distinctive test for amino acids in paper chromatography. *Nature* 164, 441.
- Garrison, W.M. and B.M. Weeks, 1956. Mechanism in the radiolysis of aqueous glycine solution. I. *J. Chem. Phys.* 24, 616.
- _____, 1956. Mechanism in the radiolysis of aqueous glycine solution. II. *J. Chem. Phys.* 25, 585.
- Jayko, M.E. and W.M. Garrison, 1956. Indirect action of radiation on the $-NH-CH_2-$ linkage in diethylamine (A mechanism for radiation induced decomposition of the peptide chain). *J. Chem. Phys.* 25, 1084.
- _____, 1958. Formation of C=O bonds in the radiation-induced oxidation of proteins in aqueous systems. *Nature* 181, 413.
- Lappin, G.R. and L.C. Clark, 1951. Colorimetric method for determination of traces of carbonyl compounds. *Anal. Chem.* 23, 541.
- Leone, C.A. and M.S. Kang, 1963. The effect of gamma-irradiation on aqueous solutions of triglycine. I. Spectrophotometric study of irradiated triglycine. *Kor. Jour. Zool.* 6 : 2, 48—52.
- _____, 1964. The effect of gamma-irradiation on aqueous solutions of triglycine. II. Electrophoretic and paper chromatographic study of irradiated triglycine. *Kor. Jour. Zool.* 7 : 1, 1—5.
- Maxwell, C.R., D.C. Peterson and N.E. Sharpless, 1954. The effect of ionizing radiation on amino acid. 1. The effects of X-rays on aqueous solutions of glycine. *Rad. Res.* 1, 530.
- Maxwell, C.R. and D.C. Peterson, 1959. The effect of γ -radiation on aqueous glycine. *J. Phys. Chem.* 63, 935.
- Sharpless, N.E., A.E. Blair, and C.R. Maxwell, 1955. The effect of ionizing radiation on amino acid. II. The effects of X-rays on aqueous solutions of alanine. *Rad. Res.* 2, 135.
- Weeks, B.M. and W.M. Garrison, 1958. *Rad. Res.* 9, 291.