The Effect of Gamma-Irradiation on Aqueous Solutions of Triglycine

2. Electrophoretic and Paper Chromatographic Study of Irradiated Triglycine[1]

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Triglycine 水溶液에 미치는 감마線의 영향

2. Electrophoresis와 Paper Chromatography에 依한 硏究

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摘 要

Triglycine 水溶液에 미치는 gamma 線의 影響을 low voltage electrophoresis, paper chromatography 및 carbonyl 化合物定量의 세 方法으로 硏究하여 다음 結果를 얻었다.

1. 照射된 triglycine 分解産物中 한 成分이 銅 ion과 錯化合物을 이루었으며 triglycine에 比하여 높은 陰電性을 나타냈다.

2. 照射된 triglycine 分解産物中 다섯 成分이 paper chromatography에 依해 分離되었으며 이中 둘이 同定되었다.

3. 照射된 triglycine으로부터 生成되는 carbonyl 化合物의 量은 5×10^{20} ev/ml 까지는 增加하였으며 이로부터 1.9×10^{21} ev/ml 까지의 高線量에서는 오히려 減少를 나타내어 complex system 內에 있어서 二次反應에 關與함을 알 수 있었다.

4. 照射된 triglycine에서 glycine을 分離함으로써 peptide bond의 加水分解의 可能性을 보여주었다.

INTRODUCTION

In the first paper of this series, spectrophotometric study on irradiated triglycine revealed the decreasing rate of peptide bond rupture with increasing dose. This may be attributed to the reduction in the rate of formation of products as the result of the successful competition of the products.

The present experiments were designed to determine whether triglycine contributes to produce several compounds which can compete with triglycine in reactions with the free radicals.

For this purpose the effect of gamma-irradiation on aqueous solution of triglycine was studied using low voltage electrophoresis, paper chromatography and determination of carbonyl compound.

MATERIALS AND METHODS

Sample preparation and irradiation to gamma-ray were performed as described previously (Leone and Kang, 1963).

Electrophoresis of Copper Complex. Copper complexes were prepared for normal and irradiated triglycine using

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biuret reagent as described for spectrophotometric study. In addition, glycine, diglycine, and tetraglycine copper complexes were prepared in like manner and were tested for comparison.

The apparatus used for electrophoretic study was a Spinco Model R electrophoresis cell, operating in the potential range of 0-500 milliamperes. Borate-NaOH buffer, pH 10, ionic strength 0.05 (Szwarf and Kandaki, 1960) was employed in this experiment. The paper strips (Scheidher and Schull 2043-A mgl) were 30.6 cm long and 3 cm wide. The most satisfactory results were obtained at a potential gradient of 2.5 v/cm of paper strip length, 0.3 mA/cm of paper strip width and electrophoresis for 2 hours. A twenty lambda volume of sample was applied to each paper strips which had been previously saturated with buffer. After electrophoresis, the paper strips were taken from the cell and dried horizontally in an oven at 105°C for 15 minutes. The dried paper strips were dipped into a 10 % solution of acetic acid in acetone, dried at room temperature for approximately 15 minutes, and the color was developed by dipping the strips into a 0.1% solution of dithiooxamide in methanol (Smith, 1950). The positive reaction for copper complex appeared almost immediately.

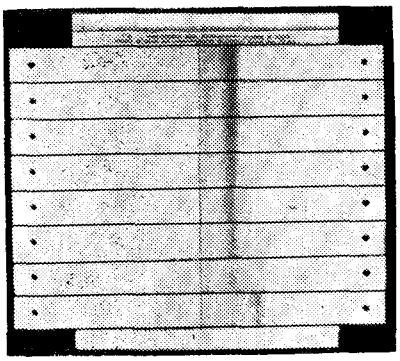

Fig. 1. Representative paper electrophoretic patterns of copper complex of normal and irradiated triglycine. 2 hour run.

Strip scanning and quantitation were performed using a Spinco Analytrol. The electrophoretic mobilities were determined in millimeters as they appeared on the scanning chart and recorded as (+) or (−) with respect to localization of detected compound in the anodic or cathodic zone, respectively, of the paper strip. The relative amounts of compounds separated were recorded as square millimeters of peak areas on the scanning chart.

Paper Chromatography. An effort was made to separate the irradiation products of triglycine by the use of simple chromatographic techniques. For the separation of irradiated triglycine, seven solvent mixtures were tested. The most satisfactory results were obtained with *n*-butanol-acetic acid-water (4 : 1 : 5, v/v) for one dimensional separation.

The volume of each irradiated triglycine solution (7.5 m*M*) applied to the paper was 20 lambda. except for the 5 m*M* solutions where a 30 lambda volume was applied in six portions, with drying between each applica-

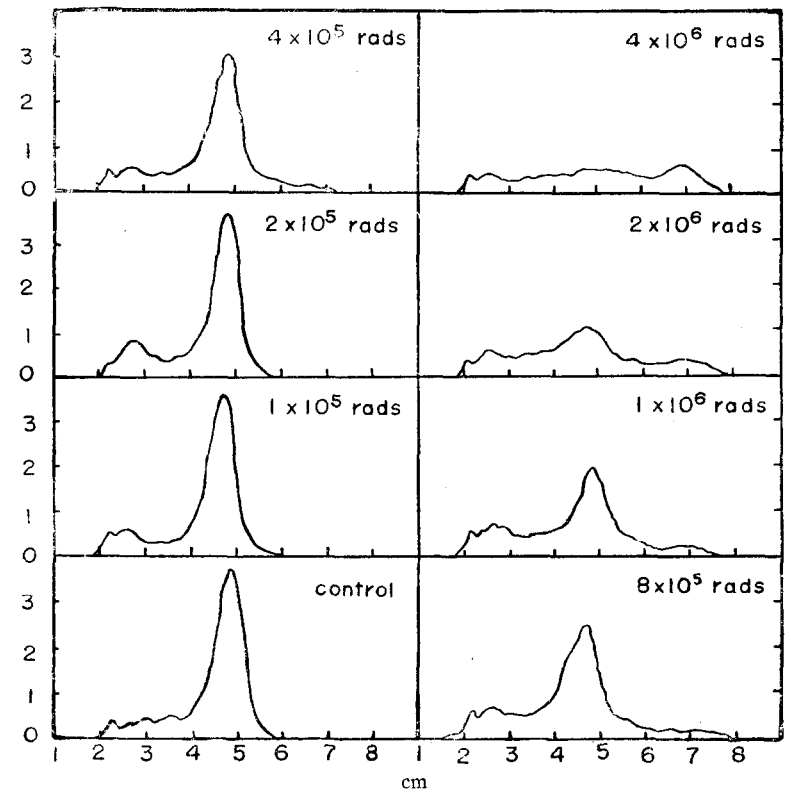

Fig. 2. Analytrol scanning patterns for paper strip electrophoresis of normal and irradiated triglycine, 2 hour run. First small peak indicates starting point where sample was applied. Borate-NaOH buffer, pH 10, ionic strength 0.05.

tion. The solutions were spaced at 1.5 inch intervals along a line one inch from the bottom of the paper. Glycine, diglycine and tetraglycine were run on each sheet as references. For two dimensional chromatography, three times as much sample as that used in a one dimensional run was applied. Ascending chromatograms were run for 20 hours in a Reco chromatographic chamber at 23°±1°C. The sheets were dried for 20 to 30 minutes in a hood, dipped in a 0.25% ninhydrin solution in acetone, and again dried in a hood. Chromatograms were then heated at 65°C for 30 minutes.

Table I. Electrophoretic mobility and amount of components (in mm^2) separated by electrophoresis from irradiated triglycine copper complex.

tube	dose (rad)	peak 1 distance migrated	peak 1 amount (mm^2)	peak 2 distance migrated	peak 2 amount (mm^2)
1	control	26.6	380	—	—
2	1×10^5	25.7	319	—	—
3	2×10^5	22.5	312	—	—
4	4×10^5	26.3	292	44.3	19.7
5	1×10^6	27.0	241	47.5	29.2
6	2×10^6	26.3	190	47.0	48.8
7	4×10^6	24.6	146	47.1	82.2

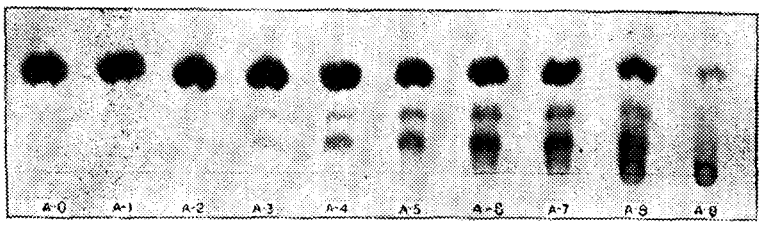

Fig. 3. Paper chromatographic separation of normal and irradiated triglycine in butanol-acetic acid mixture. Whatman No. 1, 23°±1°C, 24 hour run, color developed with 0.25% ninhydrin.

A—0	7.5 mM	control	A—5	7.5 mM	4×10^5 rads
A—1	〃	1×10^4 rads	A—6	〃	8×10^5 rads
A—2	〃	5×10^4 rads	A—7	〃	1×10^6 rads
A—3	〃	1×10^5 rads	A—8	〃	2×10^6 rads
A—4	〃	2×10^5 rads	A—9	〃	4×10^6 rads

Spots were eluted from chromatograms of irradiated triglycine, for quantitative study, using a modification of Giri's (1953) method. The chromatograms treated as described in the previous paragraph were placed in the dark for 24 hours for full color development. Spots were cut from the chromatograms and were placed in test tubes containing 4 ml of a 0.005% solution of $CuSO_4\cdot5H_2O$ in 75% ethanol. Tubes were then stored away from light for 60 minutes and were occasionally shaken. Colors were then read in the Model B spectrophotometer at 510 mμ, which was found to be the Emax of these eluates. Standard curves for glycine and triglycine were prepared for the determination of the amounts of compounds separated chromatographically.

Determination of Carbonyl Compound. The determination of carbonyl compound formed in irradiated triglycine was performed using the colorimetric method of Lippin and Clark (1951). To 0.5 ml of normal and irradiated solutions of triglycine were added 0.5 ml of the 2,4-dinitrophenylhydrazine solution and one drop of concentrated hydrochloric acid. A blank determination was made simultaneously using 0.5 ml of distilled water. The tubes were loosely stoppered with aluminum foil and heated in a water bath at 50°C for 30 minutes. After cooling, 2.5 ml of a 10% potassium hydroxide solution in 80% methanol was added. A standard curve was obtained with methylethylketone. Colors were read in a Model B spectrophotometer at 480 mμ.

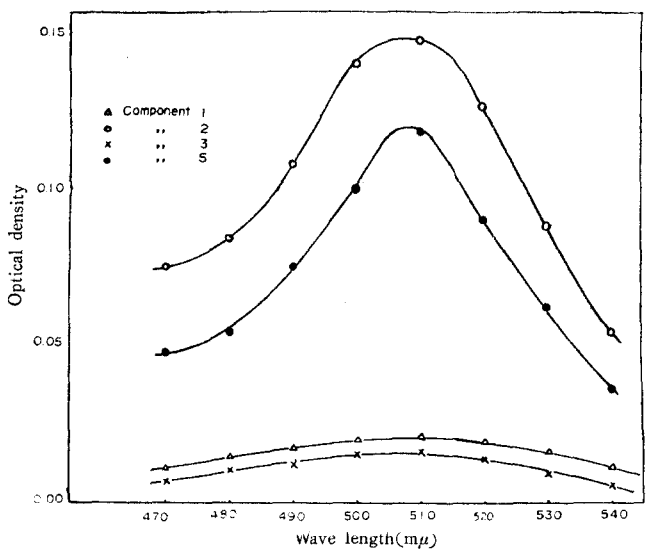

Fig. 4. Absorption spectra for eluates separated chromatographically from irradiated triglycine.

RESULTS AND DISCUSSION

Low Voltage Electrophoresis. The results obtained with normal and irradiated triglycine are shown in figs. 1 and 2. It is clear from the data that there are at least two components which complex with copper ions, and one of these two components moves faster than the other (triglycine-copper complex) toward the anode in the electric field. This indicates that the compound produced by irradiation which might be a carbonyl compound, has higher electronegativity in its molecule than triglycine has. It was also observed that the increase in copper complexing of this component was proportional to the dose up to 2.5×10^{20} ev/ml. The mobility of this complex and relative amounts of these two copper complexes separated in the electric field are shown in table 1.

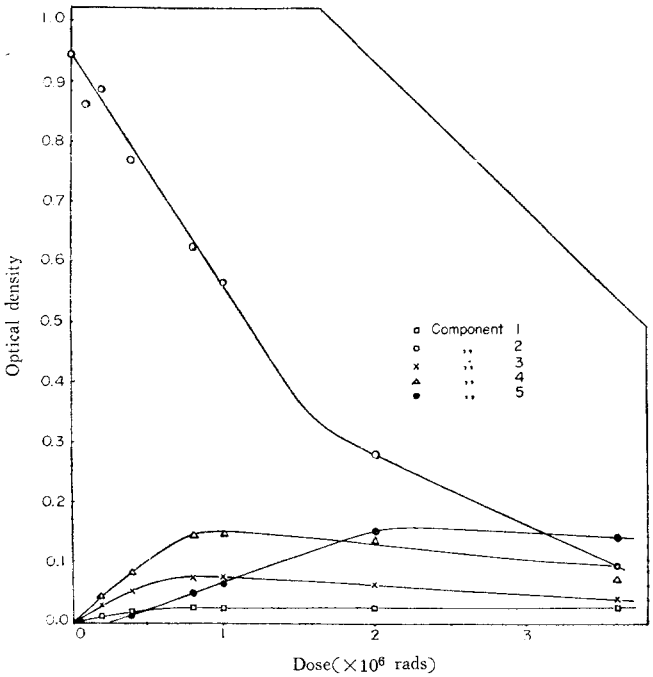

Fig. 5. Dose-yield curve for the five eluates from irradiated triglycine chromatograms. The components of spots were eluted from the chromatogram with 75% ethanol containing 0.003% copper sulfate, 24 hours after the detection of the spots with 0.25% ninhydrin.

Paper Chromatography. Diglycine, tetraglycine and triglycine were used as references. Five components were found to be present in irradiated triglycine; four moving and one nonmoving. After treatment with ninhydrin, the components first appeared as dark yellow spots which gradually turned to blue purple color following heating at 65°C for 30 minutes. On the other hand, the glycine reference and the first moving component of irradiated triglycine in butanol acetic acid mixture gave a pink color with ninhydrin treatment. This first moving component has the same Rf value as glycine. In the alpha amino acids, the amino and carbonyl groups are sufficiently close together to allow the formation of chelates with metal ions; whereas non-alpha-amino acids, amines and peptides do not form such compounds. By applying copper carbonate technique to the first moving component, it was found that the first moving component showed an orange-red color and high enough Rf value, whereas other four components gave the typical purple color and Rf values remained constant. Therefore, the first component was identified as glycine. The second moving component was identified as triglycine, and the other three remained unidentified.

Quantitative measurement of the five components separated chromatographically from irradiated triglycine solution was performed using elution technique. Glycine and triglycine standard curves were prepared and determination was made using these standard curves. Absorption spectra for the eluates from irradiated triglycine chromatograms are shown in fig. 4 and Emax was determined at 510 mμ for all components.

Dose-yield curves for irradiated triglycine determined by elution are shown in fig. 6. The dose-yield curve for peptide bond breakage in triglycine shows proportionality up to

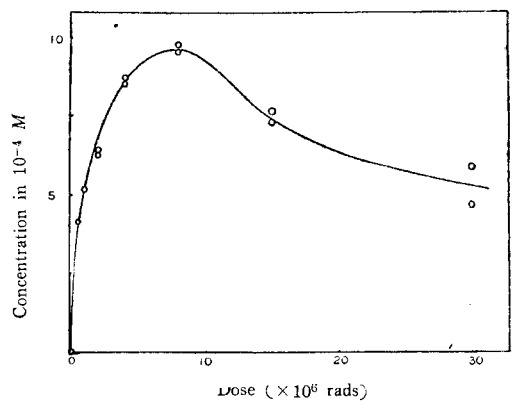

Fig. 6. Dose-yield curve for carbonyl compound from irradiated triglycine

9.3×10^{19} ev/ml, and these curves show much higher G values than those determined colorimetrically. As was shown previously, irradiated triglycine forms at least two different complexes with copper ions so that something other than triglycine copper complex contributes to the absorbance at 510 mμ. The G value for peptide breakage determined from the elution study is 3.0.

The dose-yield data for the four components other than triglycine show a decreasing rate of formation with increasing dose, indicating that they participate in secondary reactions. The concentration of the stationary component is highest at an exposure of approximately 1.2×10^{20} ev / ml, the concentration of component 4 is highest about 5×10^{19} ev/ml and gradually decreases with increasing dose. This indicates that complex reactions occur in the system.

Determination of Carbonyl Compound. Formation of carbonyl compound in irradiated triglycine solutions increases in direct proportion to dose up to 5×10^{20} ev/ml; a decreasing rate of formation with increasing dose beyond 5×10^{20} ev/ml indicates that a secondary reaction occurs.

A standard curve for the determination of carbonyl compound on a molar basis was obtained using methylethylketone. The amounts of carbonyl compound produced in irradiated triglycine solutions calculated, using this curve, are shown in fig. 7. G (c=o) is determined to be 0.79.

SUMMARY

Gamma-irradiated aqueous solutions of triglycine were studied using low voltage electrophoresis, paper chromatography, and determination of carbonyl compound.

Four generalizations can be made as follows:

1. One component from irradiated triglycine formed a complex with copper ions and had higher electronegativity than did triglycine.
2. Five components from irradiated triglycine were separated by paper chromatography; of these, two were identified.
3. Carbonyl compound formation in irradiated triglycine increased up to 5×10^{20} ev/ml and decreased at higher doses up to 1.9×10^{21} ev/ml indicating its participation in secondary reaction in the complex system.
4. The possibility of hydrolytic breakage of peptide bond was indicated by the separation of glycine from irradiated triglycine.

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