The Effect of Gamma-irradiation on Aqueous Solutions of Triglycine

1. Spectrophotometric Study of Irradiated Triglycine.(1)

LEONE, Charles A.* and KANG, Man Sik**

(* Department of Zoology, University of Kansas, Kansas, U.S.A.
**Department of Zoology, Seoul National University)

Triglycine 水溶液에 미치는 감마線의 영향

1. 分光光度計에 依한 研究

Leone, Charles A.* · 姜萬植**

(* Kansas 大學 · 動物學科
**서울大 · 文理大 · 動物學科)

(Received Nov. 25, 1963)

摘　要

Gamma線을 照射한 triglycine溶液을 spectrophotometric technique와 ultraviolet absorption을 써서 研究하여 다음 結果를 얻었다.

1. 照射된 triglycine-biuret complex의 dose-yield curve는 放射線의 間接作用을 보여주는 典型的인 例를 보여주었다.

2. G(CO-NH)는 1.75였다.

3. Dose-yield data에서 보면 peptide bond rupture는 線量을 增加함에 따라서 減少하는 增加率을 나타내어 分解產物들이 free radical과 compete함을 알 수 있었다.

4. 照射된 triglycine의 molecular extinction coefficient는 5×10^{20} ev/ml까지는 比例的增加를 나타내었고 이보다 높은 線量에서는 1.9×10^{21} ev/ml에 이르기까지 直線的 減少를 보여 carbonyl compound의 G値는 0.80이였다.

INTRODUCTION

Much attention has been paid in recent years to the physicochemical changes in biochemically important substances produced by ionizing radiations on aqueous systems (Dale, 1940; Friedman and Zeltmann, 1958; Barr and Allen, 1959). One of the major reasons for this great interest has been the fact that energy absorbed in the water molecules present in cells leads to biologically important chemical reactions. An understanding of the mechanisms of action of ionizing radiations on living cells requires a knowledge of the changes brought about in the physicochemical as well as the biological properties of proteins. Some of the amino acids and proteins have been studied thoroughly using various ionizing radiations (Garrison *et al.*, 1959; Barron *et al.*, 1955; Fricke, 1952).

The radiolysis of aqueous solutions on some simple amino acids, such as glycine and alanine, has been investigated (Maxwell *et al.*, 1954, 1955). However, much less has been reported on the effect and mechanisms of ionizing radiations on peptides. Since proteins are made up of amino acids by peptide linkages, it is hoped that an investigation of a series of peptides will lead to generalizations which will enable one to extrapolate to more complex systems not amenable to

(1) This work was supported, in part, by the United States Atomic Energy Commission, contract AT(11—1)—1073 and by the Division of Radiological Health, Bureau of State Service, Public Health Service, U.S.A., grant RH—63 (14).

direct thorough investigation.

This paper deals with the effect of gamma-irradiation on aqueous solutions of triglycine by means of spectrophotometric study as the first step of a series of experiments on peptides.

MATERIALS AND METHODS

Chromatographically pure triglycine was obtained from the Mann Research Laboratories. Because of the importance of free radicals produced by irradiation of water in aqueous solution, special care was taken to use only triple glass-distilled water in these experiments. Irradiation was done at the High Gamma Facility at the Argonne National Laboratory, Argonne, Illinois. The dose given ranged from 6.24×10^{18} ev/ml to 1.87×10^{20} ev/ml and were measured using the Fricke ferrous sulfate dosimeter. The triglycine concentrations used ranged from 1.25 m*M* to 50 m*M*.

Spectrophotometry of Biuret Complex. Modified Weichselbaum's reagent (Dittebranst, 1948) was employed throughout the experiment. Two ml of normal or irradiated triglycine and 2 ml of biuret reagent were mixed in test tubes and the mixtures were incubated at room temperature for 30 minutes for full color development. At the end of this time, colors were read in the Beckman Model B spectrophotometer at 560 mμ.

Ultraviolet Spectrophotometry. The absorption peak for triglycine has been reported to be about 186 mμ. This is attributed to peptide linkages in the molecules (Saidal *et al.*, 1951). It was also reported that the peptide bond has some absorption from 270 mμ to 280 mμ, even though no absorption peak was apparent (Goldfard *et al.*, 1951). In our irradiated triglycine it was expected that damage in peptide bonds, the formation of the carbonyl group and the formation of imino group as intermediates, would occur (Jayko and Garrison, 1956, 1958). The imino group (N=H) has an absorption maximum about 190 mμ; the carbonyl group (C=O) about 280 mμ (Oster, 1956). For this reason it was desired to read at 186 mμ, 190 mμ and 280 mμ for irradiated triglycine. Unfortunately, it was not possible to read below 200 mμ with the Beckman Model DU spectrophotometer, so absorption coefficients for irradiated triglycine were obtained for 205 mμ, 210 mμ and 275 mμ.

The objection might be raised that results obtained from the shoulders of an absorption curve, rather than from the peak, have no valid interpretation (Jayko and Garrison, 1956). The practical analysis of information obtained from a absorption maximum is based on the consideration that this specific wavelength is representative of the most probable structure or of the more probably transition typical of the structure. This makes the use of Emax desirable but not necessary, since the shape of the curve is also typical of a structure. Therefore, any point on a curve related to a given structure is useful in describing that structure so long as little or no interferences from other chromatophores occurs in that region.

The absorption spectra of normal and irradiated triglycine in the region of 200 mμ and 300 mμ were scanned with a Spectronic 505 recording spectrophotometer for qualitative study. Optical densities at 205 mμ, 210 mμ and 275 mμ were read in a Model DU spectrophotometer for quantitative study.

RESULTS AND DISCUSSION

Spectrophotometry of Biuret-Complex. Absorption spectra for diglycine, triglycine and tetraglycine were determined. Emax for diglycine, triglycine, and tetraglycine were 625 mμ, 560 mμ and 515 mμ, respectively. For the quantitative determination of triglycine remaining following irradiation the standard curve for triglycine-biuret complex was prepared in terms of optical density-concentration. This curve follows Beer's Law up to 2.0 mg/ml. Mixtures of diglycine-triglycine and triglycine-tetraglycine were made in varying ratios and were read in the Spectronic 505 and Model B spectrophotometers. From these curves it was found that the shift in Emax as well as the changes in optical density were due to the kinds and amounts of peptides in the solution.

When solutions of triglycine were irradiated with doses up to 2.5×10^{20} ev/ml, decreases in optical density were observed with increases in dose. However, when the dose was higher than 2.5×10^{20} ev/ml, it was observed that the Emax shifted to longer wavelength in direct proportion to the dose given (figs. 1 and 2). In figure 1 no shift of Emax

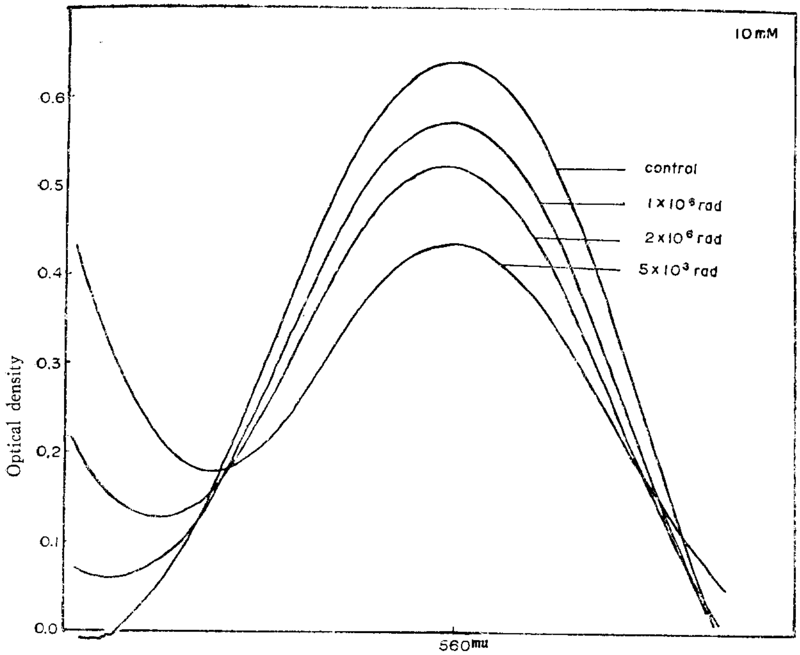

Fig. 1. Absorption spectra for copper-biuret complex of irradiated triglycine. Low dosages, no significant changes in absorption maxima.

is shown, whereas in figure 2 both a shift of Emax and a decrease in optical density are shown. For calculation of the yield of peptide bond rupture, G (CO-NH), optical densities were read in the spectrophotometer using 1.25 m*M*, 4 m*M*, 7.5 m*M*, 25 m*M* and 50 m*M* solutions of triglycine. The summary of these results is shown in fig. 3 in terms of dose-yield. G values for peptide bond rupture in various concentrations of triglycine were obtained from dose-yield curves and are plotted in fig. 4. The G value increases with the increase in concentration up to 10 m*M* solution of triglycine and keeps constant value of 1.75 up to 50 m*M*. This is a typical dose-yield curve for indirect action of free radicals produced by irradiation.

The dose-yield curves for triglycine breakdown, obtained by a study of the biuret complex, show a decrease in slope as the dose increases. This may be ascribed to either or both of the followings: 1) the reduction of the amount of a product present as the result of its decomposition by secondary reactions; or 2) the reduction in the rate of formation of product as the result of the successful competition of the products for the free radicals, with a corresponding reduction in the number of triglycine molecules reacting. As will be shown in chromatographic study, triglycine contributes to produce several compounds which can compete with triglycine in reactions with the free radicals.

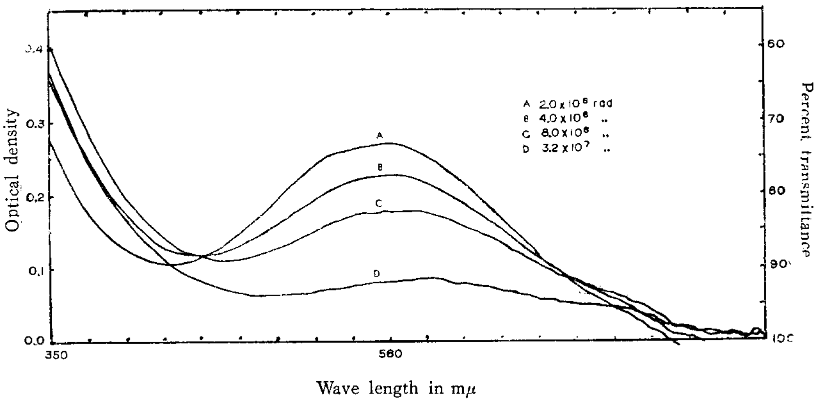

Fig. 2. Absorption spectra for copper-biuret complex of irradiated triglycine. High dosages, absorption maximum shifts to longer wavelengths.

Ultraviolet Spectrophotometry. The absorption spectra of normal and irradiated triglycine in the region of 200 mμ to 300 mμ were scanned in the Spectronic 505. It is evident from these spectra that solutions of triglycine given a dose of 9.36×10^{20} ev/ml and 1.87×10^{21} ev/ml behave differently from those given lower doses. The absorption spectra of normal and irradiated triglycine in low doses up to 2.5×10^{20} ev/ml were read with constant slit width in the Model DU spectrophotometer in the region of 205 mμ and 220 mμ. Molecular extinction coefficient changes in irradiated triglycine were measured at 205 mμ with a slit width of 1.6 mm. The result is shown in table

Table 1. Spectrophotometric determination of normal and irradiated triglycine copper biuret complex (5 mM concentration).

tube	dose	optical density at 560mμ	% peptide remaining	μg triglycine per ml
1	control	.230	100	945
2	1×10^5 rad	.221	96	900
3	5×10^5 rad	.198	86	805
4	1×10^6 rad	.180	78	730
5	2×10^6 rad	.155	67	630
6	4×10^6 rad	.130	57	530
7	8×10^6 rad	.105	46	425
8	1.5×10^7 rad	.079	34	325
9	3×10^7 rad	.060	26	246

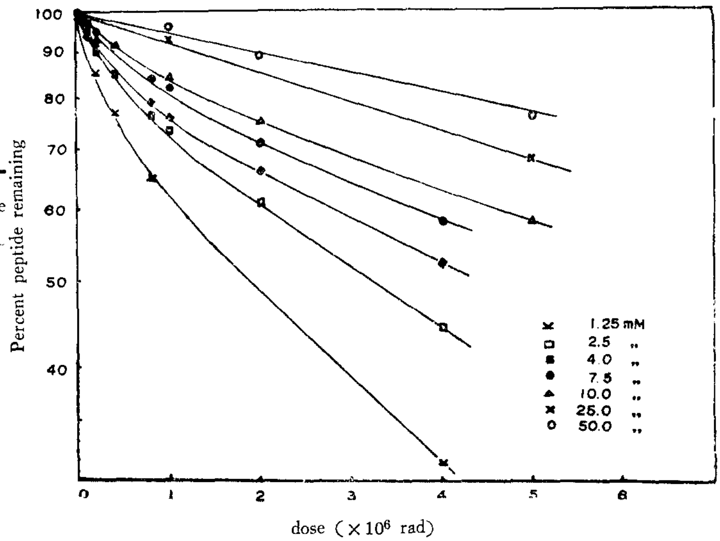

Fig. 3. Per cent peptide remaining following irradiation.

Table 2. Ultraviolet absorption spectrophotometry of irradiated triglycine.

tube	dose	optical density at 205 mμ	em 205	No. of peptide bonds*	peptide absorption coefficient	other than peptide absorption
1	control	.508	5080	2.00	2540	0
2	1×10^5 rad	.518	5180	1.92	2697	157
3	5×10^5 rad	.530	5300	1.72	3081	541
4	1×10^6 rad	.548	5480	1.56	3512	972
5	2×10^6 rad	.570	5700	1.34	4253	1713
6	4×10^6 rad	.637	6370	1.14	5587	3047
7	8×10^6 rad	.642	6420	0.92	6978	4438
8	1.5×10^7 rad	.610	6100	0.68	8970	6430
9	3×10^7 rad	.558	5580	0.52	10730	8190

* Calculated from biuret-complex study.

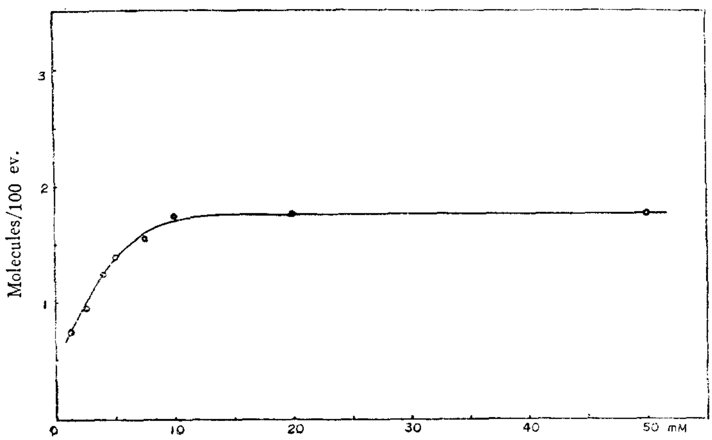

Fig. 4. Yield-concentration curve for peptide bond breakage following irradiation.

2 and fig. 5. In the wavelegnth tested, the molecular extinction coefficent increases linearly with an increase of dose up to 2.5×10^{20} ev/ml. The peak was at 5.0×10^{20} ev/ml (fig. 5). At points higher than this, molecular extinction coefficient decreases linearly with an increase in dose. From this curve G value is calculated to be 0.80.

The peptide absorption coefficients in normal and irradiated triglycine were calculated, assuming that the number of peptide bonds in irradiated triglycine was proportional to biuret color readings. As the peptide bonds in triglycine molecules are ruptured by irradiation, the peptide

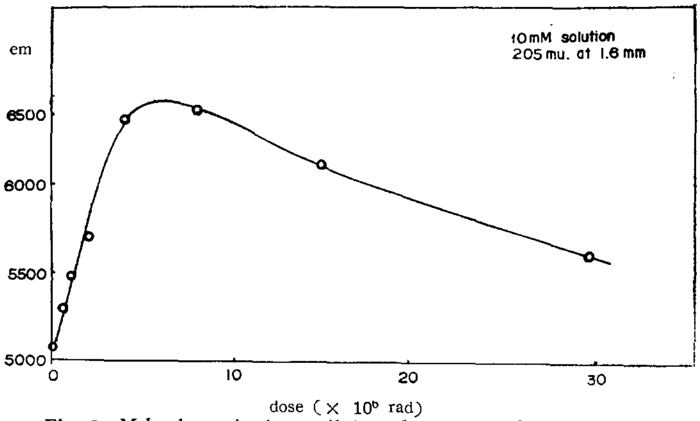

Fig. 5. Molecular extinction coefficient change in irradiated triglycine.

absorption coefficient of irradiated solutions should remain constant. However, experimental data show that the peptide absorption coefficient increases, indicating the formation of new compounds. This is assumed to be due to either carbonyl compounds or imino compounds which are produced as intermediates by the mechanism discussed by Garrison *et al.* (1959). The increase in optical density of irradiated triglycine at 205 mμ indicates that carbonyl compound is formed in irradiated samples. This observation suggested that the quantitative determination of carbonyl compound in irradiated triglycine would be of value.

SUMMARY

Gamma-irradiated aqueous solutions of triglycine were studied using spectrophotometric techniques and ultraviolet absorption studies.

1. The dose-yield data for irradiated triglycine-biuret complex showed a typical dose-yield curve for indirect action of radiation.
2. G value of peptide bond rupture in irradiated aqueous solution of triglycine was found to be 1.75.
3. The dose-yield data for irradiated triglycine-biuret complex indicated a decreasing rate of peptide bond rupture with increasing dose showing the successful competition of the products for the free radicals.
4. Molecular extinction coefficient of irradiated triglycine increased proportionally with the dose up to 5×10^{20} ev/ml and at higher doses the molecular extinction coefficient decreased linearly with increasing dose up to 1.9×10^{21} ev/ml. From these data G value for carbonyl compound was found to be 0.80.

REFERENCES

Barr, N.F. and A.O. Allen, 1959. *J. Phys. Chem.* 63, 928.

Barron, E.S.G., J. Ambrose and P. Johnson, 1955. Studies on the mechanism of action of ionizing radiations. XIII. The effect of X-irradiation on some physicochemical properties of amino acids and proteins. *Rad. Res.* 2, 145.

Dale, W.M., 1940. Formation of C=O bonds in the radiation induced oxidation of protein in aqueous solutions. *Biochem. J.* 34, 1367.

Dittebranst, M., 1948. Application of the Weichselbaum buiret reagent to the determination of spinal fluid protein. Am. *J. Clin. Pathol.* 18, 439.

Fricke, H., 1952. *J. Phys. Chem.* 56, 769.

Friedman, H.L. and A.H. Zeltmann, 1958. *J. Chem. Phys.* 28, 878.

Garrison, W.F., H.R. Haymond, W. Bennett and S. Cole, 1959. *Rad. Res.* 10, 273.

Goldfard, A.R., L.J. Saidal and E. Mosovitch, 1951. The ultraviolet absorption spectra of proteins. *J. Biol. Chem.* 193, 397.

Jayko, M.E. and W.M. Garrison, 1956. *J. Chem. Phys.* 25, 1084.

Jayko, M.E. and W.M. Garrison, 1958. *Nature* 181, 413.

Maxwell, C.R., D.C. Peterson and N.E. Sharpless, 1954. The effect of ionizing radiation on amino acids. I. The effect of X-rays on aqueous solution of glycine. *Rad. Res.* 1, 530.

Maxwell, C.R., D.C. Peterson and N.E. Sharpless, 1955. The effect of ionizing radiation on amino acids. II. The effects of X-rays on aqueous solutions of alanine. *Rad. Res.* 2, 133,

Oster, G. and A.W. Pollister, 1956. Physical Techniques in Biological Research.

Saidal, L.J., A.R. Goldfard and W.B. Kalt, 1951. False absorption bands in the region of 200—230 mμ caused by stray radiation in the Beckman spectrophotometer. *Science* 113, 683.