Studies on γ-Ray Irradiated Bovine Plasma Albumin Solution*

Masaru SOGAMI**

Department of Physiology, Yamaguchi Medical College, Ube

Yoshihiro TAMURA, Yasuo IMAI and Yoshiya SHINAGAWA***

Department of Physiology, Faculty of Medicine, Kyoto University

(*Received August 10, 1959*)

Most of the investigation on γ- or x-ray irradiated albumin solution were directed to produce the changes in viscosity, sedimentation, absorption spectrum etc. which were the most destructive process, occuring after irradiation of large dose. The authors used subfractionation technique of bovine plasma albumin (hydroxylapatite column) for the studies on γ-ray irradiated protein solutions and found that the subfractionation technique was very useful in the field of radiation chemistry of proteins. That is, the changes in chromatogram were more radiosensitive than those of viscosity, absorption spectrum and amperometric titration of -SH groups. Therefore, the effects of dose rate, protein concentration, pH and salts on γ-ray irradiation of protein solution were investigated mainly by used column chromatography. It was found that the above stated factors produced the marked effects on γ-ray irradiation of bovine plasma albumin solutions.

INTRODUCSION

The effects of radiation on enzyme or protein solutions have been studied by many investigators by using enzyme activity, absorption spectrum, viscosity, sedimentation, electrophoresis etc. Hardly, any studies using subfractionation technique have been made on effects of ionizing radiation on protein solution except by Rosen[1~3]. The authors have studied on the effects of various factors, such as protein concentration, pH, salts, temperature, oxygen tension, dose rate etc., on γ-ray irradiated bovine plasma albumin solution by using column chromatography, viscosity, absorption spectrum and amperometric titration[1,2,4~6].

EXPERIMENTAL MATERIALS AND METHODS

Armour's bovine plasma albumin was used. Paper electrophoretic analysis at pH 8.50, $\Gamma/2$ 0.045 showed that bovine plasma albumin was almost a single componet. However, chromatographic analysis (hydroxylapatite column) showed that bovine plasma albumin had three subfractions eluted by 0.07 M, 0.11 M

* This investigation was supported by a grant from the Ministry of Education of the Japanese Government in 1958.

** 曾我美 勝

*** 田村 喜弘，今井 安男，品川 嘉也

and 0.40 M of sodium phosphate buffer pH 6.8 and that ratio of three subfractions changed from sample to sample[7,8]. In the experiment described in the next section (II. Chromatographic Studies), the same sample was used.

Redistilled water was used in these experiments.

Viscosity was measured at 29.9±0.05°C in Ostwald's viscometer. Absorption spectrum was measured by using Beckman DU spectrophotometer in quartz cell of 10 mm width. Amperometric analysis of -SH groups was carried out by the method of Weissman and Benesch[9,10]. That is, 1.0 ml of 3 per cent bovine plasma albumin solution was added dropwisely to 30 ml of ammoniacal solution (60 per cent ethanol, NH_4NO_3 : 0.05 M, NH_4OH : 0.25 M) and titrated with 0.001 N silver nitrate solution.

Chromatography was performed at room temperature by using very coarse grain of hydroxylapatite (1.5 cm×12 cm column) as an adsorbent and 0.02, 0.04 M, 0.07 M, 0.11 M and 0.40 M of sodium phosphate buffer pH 6.8 as eluants[7,8]. The coarse adsorbent was prepared by authors' method and this column (1.5 cm×12 cm) could eluted 80∼120 ml per hour[8]. However, in these experiments, the elution rate was about 35 ml per hour and the effluent was fractionated into fractions of 3.5 ml each by using a fraction collector. The protein concentration was analysed by the extinction at 280 mμ, because the extinction of unirradiated protein at 280 mμ was nearly equal to that of irradiated one in the authors' experiment. The adsorbent was poisoned by γ-ray irradiated protein solution[3]. The deactivated adsorbent was washed out with distilled water from 5 chromatographic tubes and suspended in 1 liter of 0.01 M of sodium phosphate buffer pH 6.8. And then, this solution was heated with stirring untill 84°∼87°C. After standing for 1∼2 minutes, the heated solution was decanted and 1 liter of 0.01 M sodium phosphate buffer pH 6.8 was added. The same procedure was repeated several times (3∼4 times). The deactivated adsorbent was reactivated. By using this reactivation method, the same adsorbent could be used many times[8].

The irradiation of protein solutions was performed at room temperature in an open pyrex glass tube with γ-rays from Co^{60} having dose rate of 2.4×10^5 r per hour and 4×10^3 r per hour. After irradiation, pyrex glass tubes were immersed in ice-water and after standing for 24 hours, the samples were analysed.

pH values of protein solution before and after irradiation were measured by glass electrode.

EXPERIMENTAL RESULTS AND DISCUSSION

(I) Comparison of Changes of Chromatogram with those of Viscosity Absorption Spectrum and -SH-groups of Protein

0.6 per cent bovine plasma albumin solution (0.02 M sodium phosphate buffer pH 6.8) was irradiated with γ-rays having an intensity of 2.4×10^5 r/hr at 25°C. Relative viscosity was measured at 29.9±0.05°C, 24 hours, after irradiation. As reported by Barron[5], γ-ray irradiation with 9×10^4 r of albumin solution in 0.02 M sodium phosphate buffer pH 6.80 produced a small increase in viscosity as shown in the left part of Fig. 1. γ-ray irradiation of 9×10^4 r produced marked change in chromatogram. That is, 1st subfraction eluted by 0.07 M was decreased from 77 per cent to 39 per cent as shown in Fig. 1.

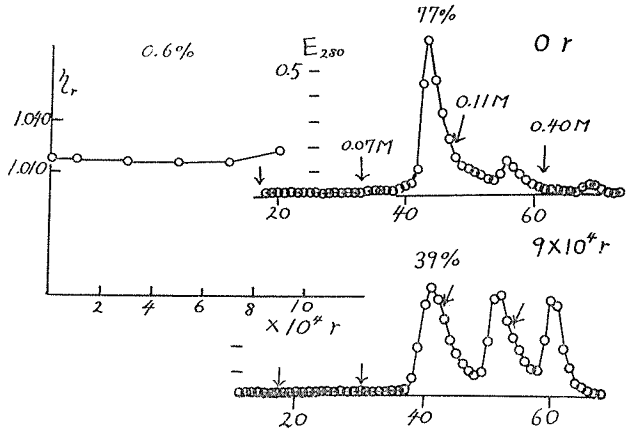

Fig. 1. The left diagram shows the relative visicosity of 0.6 per cent bovine plasma albumin solution (0.02 M sodium phosphate buffer pH 6.8) against dose of γ-rays having an intensity of 2.4×10^5 r per hour. The right upper and lower diagram show column chromatogram of unirradiated and irradiated (9×10^4r) albumin. The abscissa and ordinate show tube numbers of fraction collector and extinction at 280 mμ, respectively.

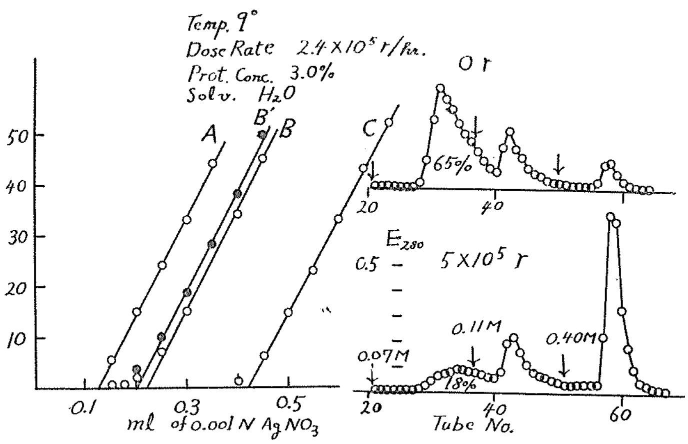

Fig. 2. The right upper and lower diagrams show column chromatograms of unirradiated and irradiated (5×10^5r) bovine plasma albumin, respectively. The ordinate and abscissa show the extinction at 280 mμ and tube numbers of fraction collector, respectively. The left diagram shows the results on amperometric analysis. The ordinate and abscissa show galvanometer reading and ml of 0.001 N silver nitrate solution, respectively. Solid and empty circles show the irradiated (5×10^5r) and unirradiated albumins. Curve A : 0.5 ml of 2.9 per cent bovine plasma albumin used for titration, curves B and B′ : 1.0 ml used for titration, curve C : 2.0 ml used for titration.

Amperometric titration of -SH-groups was performed at room temperature in ammoniacal solution (60 per cent ethanol, NH_4NO_3 0.05 M, NH_4OH 0.25 M) by titrating with 0.001 N silver nitrate solutin (9, 10). 570 mg of bovine plasma albumin was dissolved in 30 ml of redistilled water. The irradiation of γ-rays was performed at 9°C in an open pyrex tube under the following conditions : dose rate 2.4×10^5 r per hour, dose 5×10^4 r, 11.2×10^4 r, 1.5×10^5 r, 2.06×10^5 r, 2.5×10^5 r, 3×10^5 r, 5×10^5 r, γ-ray irradiation with 5×10^5 r decreased only 17 per cent of -SH groups. On the other hand, in the chromatographic analysis, 1st subfraction eluted by 0.07 *M*, was decreased from 65 per cent to 18 per cent. The results were illustlated in Fig. 2. The chromatographic changes of γ-ray irradiated protein were not mainly due to oxydation of -SH groups as in the case of SH-enzyme[11)~13)]. However, chromatographic changes were protected by the addition of cysteine.

γ-ray irradiation of 0.033 per cent bovine plasma albumin solution (solvent : redistilled water) was performed at 9°C with γ-rays having dose rate 4×10^3 r per hour. After irradiation, there was a steady rise of light absorption[5)]. Therefore, after standing for 24 hours at 0°C, the absorption spectrum was measured at 9°C. Absorption spectrum changes at 240, 251, 297 and 310 mμ of γ-ray irradiated bovine plasma albumin were illustrated in the upper part of Fig. 3. These results were consistent with those of Barron[5)]. That is, γ-ray irradiation with 4×10^3 r of 0.033 per cent albumin solutions in water produced a small increase in extinctions at 240 mμ and 251 mμ. On the chromatographic analysis, the irradiation with 4×10^3 r decreased the 1st subfraction eluted by 0.07 *M* sodium phosphate buffer pH 6.8 from 68 per cent to 28 per cent as shown in Fig. 3.

From comparison of changes of chromatogram with those of viscosity,

Temp. 9°
Dose Rate 4×10^3 r/hr.
Prot. Conc. 0.033%
Solv. H_2O

γ \ mμ	240	251	279	310	
0	0.379	0.124	0.236	0.025	*
2000	0.382	0.133	0.235	0.028	
4000	0.400	0.142	0.237	0.029	*
6000	0.406	0.145	0.243	0.030	

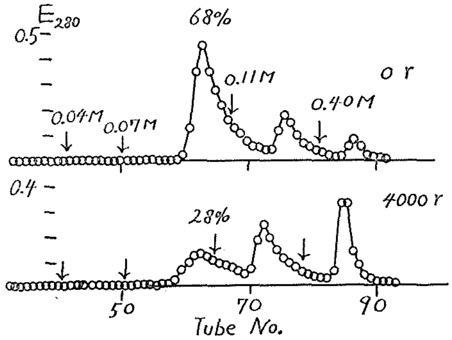

Fig. 3. The Table shows the absorption spectrum changes at 240 mμ, 251 mμ, and 310 mμ after γ-ray irradiation with 2×10^3r, 4×10^3r and 6×10^3r of 0.033 per cent bovine plasma albumin sclution. The lower diagrams shows the chromatograms of unirradiated and irradiated (4×10^3r) bovine plasma albumin solutions.

absorption specturm and -SH-groups, it was found that the chromatogram was more sensitive toward radiation than other measurements. Therefore, the authors used chromatographic analysis to study the effects of various factors such as dose rate, temperature, protein concentration, pH, salt, oxygen tension etc., on γ-ray irradiated protein solution[1~6].

(II) Chromatographic Studies

By using hyroxylapatite column, the authors studied the effect of γ-ray irradiation on protein solution under the following conditions : dose rate 4×10^3 r per hour and 2.4×10^5 r per hour, protein concentration 3 per cent~0.019 per cent, pH 6.8 and 5.25. The irradiated solutions were immersed in ice-water for 24 hours and chromatographed. As shown in Figs. 4 and 5, only log (percentages of the 1st subfraction) was linear against dose of γ-rays. These results were similar to those on Dow X-2 column[1,2]. The relation between log (percentages of 1st subfraction) and dose of γ-rays was different from that of heat denaturation of bovine plasma albumin[13]. On the heat denaturation, 1st subfraction was decreased according to reversible 1st order kinetics[13]. And also, on heat denaturation of bovine plasma albumin, percentage of 2nd subfraction was constant for about 100 minute at 60°C and then gradually decreased[13].

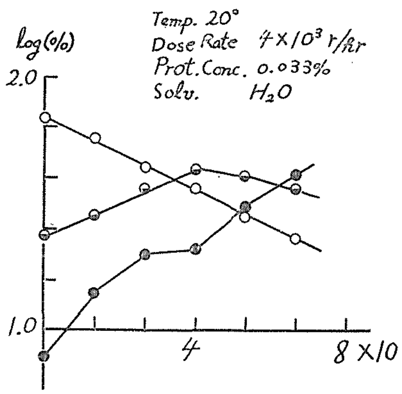

Fig. 4. Effect of γ-rays on chromato-gram of bovine plasma albumin. ○, ⊖ anc ● showed three subfractions which were eluted with 0.07 M 0.11 M and 0.40 M sodium phosphate buffer pH 6.8, respectively. The ordinate and abscissa showed log (%) of three subfractions and dose of γ-rays, respectively. γ-ray irradiation was performed under the following conditions : solvent redistilled water, protein concentration 0.033 per cent, temperature 20°C, dose rate 4×10^3r per hour.

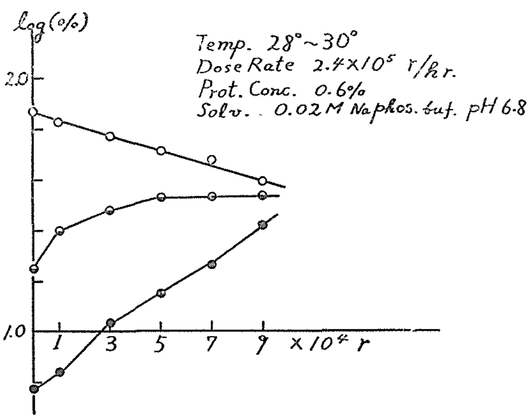

Fig. 5. γ-ray irradiation was performed under the following conditions : solvent 0.02 M sodium phosphate buffer pH 6.8, protein concentration 0.6 per cent, tempearature 28°~30°C, dose rate 2.4×10^5r per hour. See Fig. 4. for other conditions.

When the concentration was decreased, there was a proportional decrease in 37 per cent dose of the 1st subfraction (D_{37}). That is, D_{37} of 0.019 per cent, 0.038 per cent and 0.075 per cent were 3×10^3r, 5.1×10^3r, 7.5×10^3r, respectively, at

16°C, γ-rays having an intensity of 4×10^3 r per hour. The protein was dissolved in redistilled water on these experiments. Radiosensibility of albumin solution was also affected by dose rate of γ-rays as reported by Forsberg[6]. D_{37} of 0.019 per cent, 0.038 per cent and 0.075 per cent were 1.4×10^4 r, 1.6×10^4 r and 2.2×10^4 r, respectively, at 26°C, γ-rays having an intensity of 2.4×10^5 r per hour. These results were shown in Fig. 6. Therefore, the results of γ-ray irradiation on protein solution were markedly affected by protein concentration and dose rate. When the concentration of albumin and dose rate of γ-rays are decreased, D_{37} of 1st subfraction will be as low as that of SH-enzyme. Threfore, some sort of indirect action was apparently involved in the chromatographic changes[14,15].

The presence of salts (0.01 *M*, 0.02 *M*, 0.04 *M*, 0.07 *M* sodium phosphate buffer pH 6.8) in the 0.3 per cent bovine plasma albumin solutions had a marked effect on the changes of chromatogram, as shown on irradiation with 7×10^4 r at 13°C, γ-rays having an intensity 2.4×10^5 r per hour. As increasing the concentration of sodium phosphate buffer, 1st subfraction eluted by 0.07 *M* was decreased and 3rd subsfraction eluted by 0.40 *M* was increased. However, 2nd subfraction was constant as shown in Fig. 7. On the other hand, it was reported that presence of salts had a marked protecting effect on the changes of absorption spectum and precipitation of protein on irradiation of x-rays[5]. The mechanism by which the difference appears between changes in chromatogram and absorption sepctrum will be not clear until more data are available.

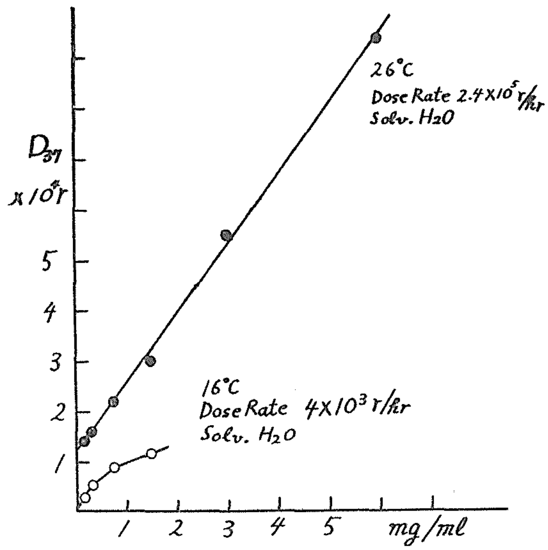

Fig. 6. 37 per cent dose of 1-st subfraction against protein concentration. γ-ray irradiation was performed at 16°C (○) and 26°C (●), γ-rays having intensities of 4×10^3r per hour (○) and 2.4×10^5r per hour (●).

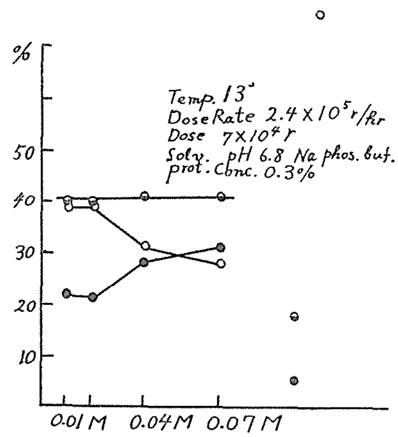

Fig. 7. The effect of salts on chromatographic changes of γ-ray irradiated bovine plasma albumin. ○, ⊖ and ● show the subfractions eluted by 0.07 *M*, 0.11 *M*, and 0.40 *M* sodium phosphate buffer pH 6.8, respectively. The ordinate and abscissa show percentages of three subfractions and moles of sodium phosphate buffer pH 6.8. The lightest circles show three subfraction of unirradiated albumin.

To study the effect of pH changes, bovine plasma albumin was dissolved either in water (pH 5.25), in 0.02 M acetate buffer (pH 4.10, 4.70, 5.78) or in 0.02 M sodium phosphate buffer (pH 6.68, 7.55). γ-ray irradiation with 7×10^4 r of 0.3 per cent albumin solution was performed at 30°C, γ-rays having an intensity 2.4×10^5 r per hour. When bovine plasma albumin solution dissolved in distilled water was irradiated with 7×10^4 r at 30°C, small amount of precipitation occured. This solution was centrifuged at 2,5000 rpm for 15 minutes. The supernatant solution was chromatographed. The greatest changes in light absorption were observed on x-ray iradiation of alkaline solution[5]. However, in chromatographic analysis, the greatest changes was observed on γ-ray irradiation of acid solution as in the case of SH-enzyme[11]. This may be due to high concentration of H_2O_2 formed at acid solution[11]. On the chromatographic studies of the effect of pH on heat denaturation, the greatest change was observed in alkaline solutions except at the isoelectric region of albumin as shown in Fig. 9.

The changes in chromatogram were more sensitive toward γ-ray irradiation than those of viscosity, absroption spectrum etc which were most destructive process. However, the mechanism by which changes in chromatogram occur will be not clear, untill more informations are available on the molecular structure of albumin subfractions. Therefore, the authors made a little discussion on the results.

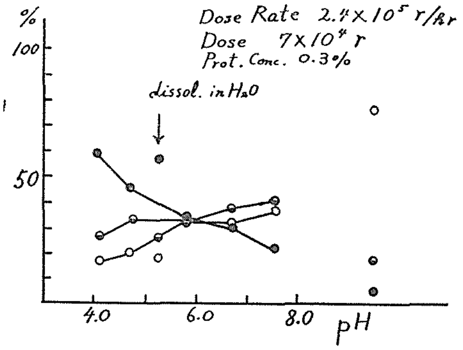

Fig. 8. Effect of pH on chromatographic changes of γ-ray irradiated albumin. γ-ray irradiation with 7×10^4 r was performed at 30°C, γ-rays having an intensity of 2.4×10^5 r per hour. ○, ◒ and ● show three subfractioas which were eluted by 0.07 M, 0.11 M and 0.40 M sodium phospphate buffer pH 6.8, respectively. The lightest three circls show percentages of three subfractions of unirradiated albumin solution (pH 6.8). The ordinate and abscissa show percentage and pH, respectively.

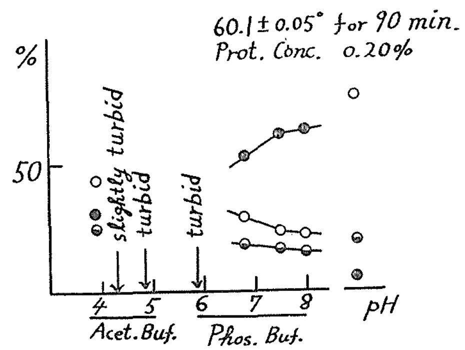

Fig. 9. Effect of pH on chromatographic changes of heat denatured albumin (0.20 per cent, 60°C for 90 minutes). See Fig. 8 for other conditions.

SUMMARY

The authors studied on the γ-ray irradiated bovine plasma albumin solutions

by using hydroxylapatite column, viscosity, absorption spectrum and amperometric titration of -SH-groupt. The results obtained were as follows:

(1) It was found that the changes in chromatograms were more radiosensitive than those of other measurments.

(2) Log (percentage of 1st subfraction) was linear against dose of γ-rays.

(3) Dose rate of γ-rays had a marked effect on the changes of chromatogram. When the dose rate of γ-rays was decreased, 37 per cent dose of 1st subfraction was decreased.

(4) When pH and protein concentration were decreased and salt concentration was increased, there was a proportional increase in the changes of chromatogram.

(5) When protein concentration and an dose rate of γ-rays are decreased, 37 per cent dose of 1st subfraction will be as low as that of SH enzyme.

The authors wish to thank Professor Akira Inouye (Department of Physiology, Faculty of Medicine, Kyoto University), Professor Goro Kawabata (Department of Physiology, Yamaguchi Medical College), Professor Seiji Matumura, and Dr. Sohei Kondo (Department of Induced Mutation, National Institute for Genetics) for their advice and discussion. The authors also express their gratitude to Mr. Yasuyuki Nakayama (Institute for Chemical Research, Kyoto University) for his cooperation in the Co^{60} γ-ray irradiation and to the workers of Institute for Tuberculosis of Kyoto University for their permission to use the Beckmann DU spectrophotometer.

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