# Radiation-Induced Sol–Gel Transition of Protein: Effects of Radiation on Diffraction Property

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## SYNOPSIS

Solid gelatin was irradiated with $^{60}$Co gamma rays, and the effects of radiation on the diffraction property of the protein molecule were studied by measuring the X-ray diffraction intensity at different radiation doses and elapsed times after irradiation. The effects of radiation on the ordered conformation of the protein molecule were discussed. © 1993 John Wiley & Sons, Inc.

## INTRODUCTION

It is well known that ionizing radiation strongly influences the chemical and conformational properties of biological macromolecules,[1–2] and also that some biological macromolecules, such as proteins, form a rigid and thermoreversible hydrogel.[3–5] However, the corelationship of polypeptide chains in a gel or in a film is not clear. The effects of radiation on protein, such as radiation-induced crosslinking and graft polymerization, are also of interest to those who study radiation processing. It was, therefore, decided to investigate the effects of radiation on the diffraction property of protein. Gelatin was selected as the protein molecule, since it had been well known to adopt an ordered conformation in aqueous solutions and to form a rigid and thermoreversible hydrogel.[3,6]

The changes in the diffraction property can be followed conveniently by measuring the X-ray diffraction intensity of the protein as functions of radiation dose and elapsed time after irradiation.

## EXPERIMENTAL

### Material

Gelatin used in this work was the same as that described in a previous article.[6]

### Apparatus and Procedure

In irradiation, the solid gelatin was irradiated with $^{60}$Co gamma rays in air at room temperature at a dose rate of $1.3 \times 10^5$ rad/h.

In X-ray diffractometry, the irradiated solid gelatin was dissolved in distilled water in a concentration of 10% at 80°C and was molded to a thickness of 3 mm in a flat plastic vessel. The gel was dried to make a thin film at about 5°C. The X-ray diffraction was measured with a Rigaku X-ray diffractometer using Ni-filtered Cu–K radiation. The diffraction data were corrected for background and diffraction curve by base line and smooth line on the curve where intensity was measured. The results were expressed by relative intensity of diffraction, which is $I/I_0$ of the irradiated/nonirradiated gelatin films.

## RESULTS AND DISCUSSION

The changes in relative intensity of diffraction of gelatin at various radiation doses were studied with a thin film. Figure 1 shows some of the X-ray diffractograms of gelatin films. The results are shown in Figure 2. The changes in relative intensity of diffraction of gelatin film at different times after gamma-irradiation ($3 \times 10^6$ rad) are shown in Figure 3.

From these results, the diffraction intensity decreased, depending upon the irradiation, and when

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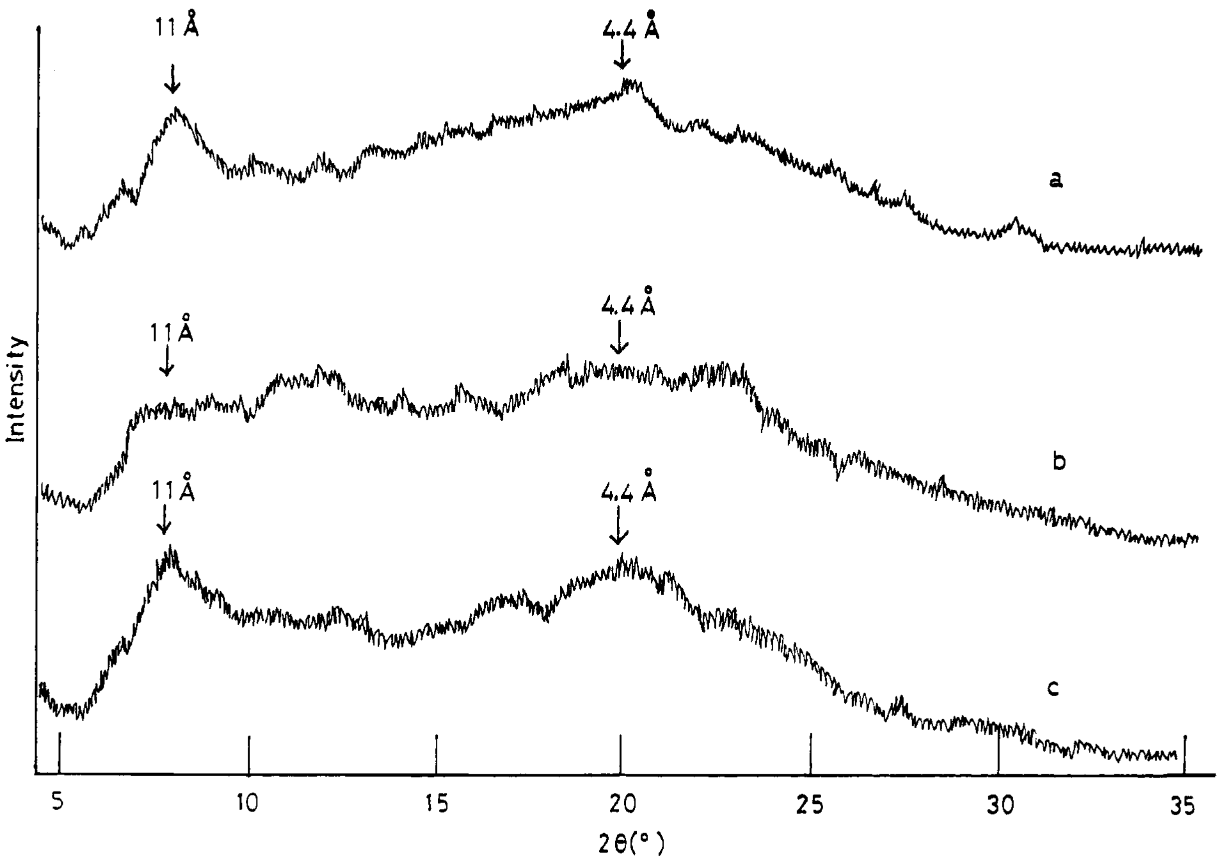

**Figure 1** X-ray diffractograms of gelatin films: (a) nonirradiation, (b) 1 day after irradiation (3 × $10^6$ rad) and (c) 70 days after irradiation (3 × $10^6$ rad).

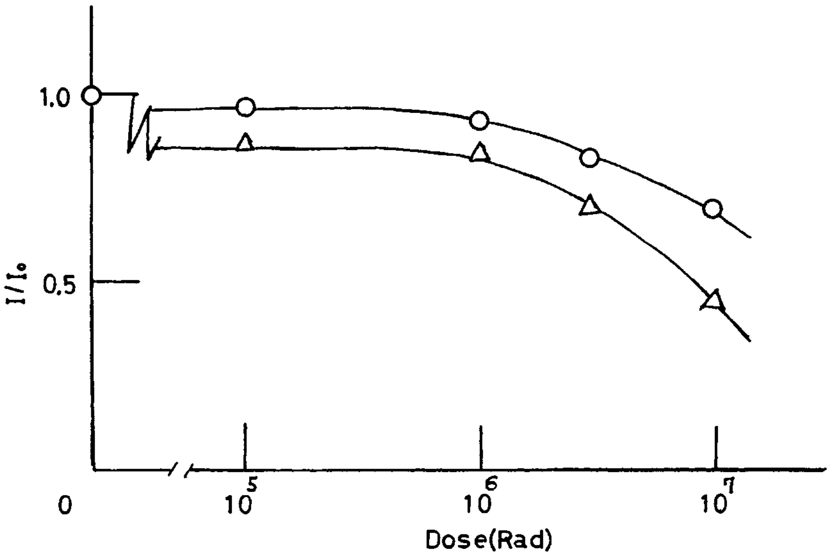

**Figure 2** Relative intensity of diffraction ($I/I_0$) vs. radiation dose: (○) 4.4 Å and (△) 11.0 Å.

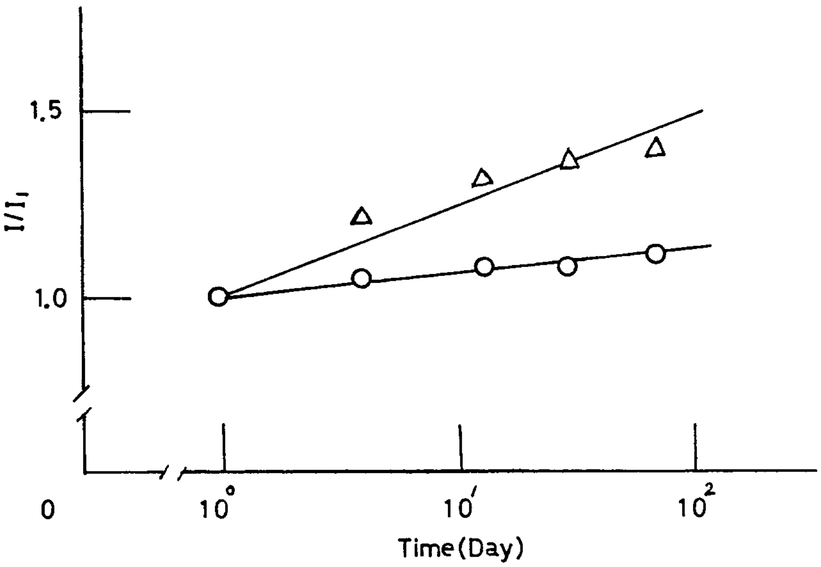

**Figure 3** Relative intensity of diffraction ($I/I_1$) vs. time after irradiation ($3 \times 10^6$ rad): (○) 4.4 Å and (△) 11.0 Å.

recovered, depending upon the elapsed time after irradiation until certain values. If such changes in diffraction intensity are compared to changes in heat of reaction, which were reported in a previous article,[6] it seems that the changes in the diffraction intensity with radiation are involved in the crosslinking processes of gelatin molecule. Therefore, the changes in the ordered conformation of gelatin are obtained from the changes in X-ray diffraction of gelatin film, since the X-ray diffraction in this system is considered to be a diffraction of networks. In the case of the gelatin, the networks must be formed by crosslinks between polypeptide chains, containing helices of polypeptide chains and large aggregates of the helices. These networks must be related to the crosslinking processes in the polypeptide system.

First, the decreases in the X-ray diffraction are related to a decreased density of the networks of gelatin molecule (Fig. 2). An increase in the absorbed dose results in a decrease in the density of the networks of gelatin molecule. If the main actions of irradiation on the network formation of gelatin molecule are assumed to be

$$P \rightsquigarrow P^* \qquad (1)$$

$$P\text{-}P^* \rightarrow (P\text{-}P^*)_h \qquad (2)$$

$$(P\text{-}P^*)_h \rightarrow (P\text{-}P^*)_0 \qquad (3)$$

where $P$ is the crosslinking loci of gelatin molecule, $P^*$ is the broken crosslinking loci of an irradiated gelatin molecule, $(P\text{-}P^*)_h$ is the crosslinking loci of irradiated gelatin molecule in the helix system, and $(P\text{-}P^*)_0$ is the crosslinking loci of irradiated gelatin molecule in the orientation system, then the breaking step may be reaction (1), which leads to the observed diffraction intensity changes with radiation dose. On the other hand, with increasing radiation dose, the diffraction intensity at 11.0 Å is lower than that at 4.4 Å. It is understood that radiation resistance of the crosslinking loci of the gelatin molecule in the orientation system (at 11.0 Å) is lower than that in the helix system (at 4.4 Å). In the system studied here, the crosslinking loci of gelatin molecule seem to be broken by ionizing radiation, and the formation of the crosslinks is depressed. As a result, the X-ray diffraction is decreased.

Second, the increases in the X-ray diffraction after irradiation are related to an increased density of the networks of gelatin molecule (Fig. 3). When the radiation dose is constant, then the increase in the network formation of the irradiated gelatin molecule depends on the elapsed time after irradiation. If main actions of irradiation on the network formation of gelatin molecule are assumed to be

$$P \rightsquigarrow P^* \qquad (1)$$

$$P^* \rightarrow P + Ea \qquad (5)$$

where *Ea* is the ionizing energy of gamma rays, then the scission decay step may be reaction (5), which leads to the observed diffraction intensity changes with the elapsed time after irradiation. Also, with increasing elapsed time after irradiation, the diffraction intensity at 11.0 Å is higher than that at 4.4 Å. It is understood that the radiation recovery of crosslinking loci of gelatin molecule in the orientation system (at 11.0 Å) is higher than that in the helix system (at 4.4 Å). In the system, the recovery may occur in the broken crosslinking loci of irradiated gelatin molecule, and the crosslinks are restored. As a result, the X-ray diffraction is recovered to a certain extent.

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