Studies on Irradiation of Agar–Agar in the Solid State: On the Changes of X-Ray Diffraction of Agar–Agar Film Produced by Irradiation

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Synopsis

Solid agar–agar was irradiated with ^{60}Co gamma-rays, dissolved in aqueous solutions, molded and made a thin film. Effects of radiation on the ordered conformation of agar–agar molecule were studied by measuring the X-ray diffraction intensity at different radiation doses, elapsed times after irradiation, and concentrations of added substances (sugar and starch). Empirical equations in changes of X-ray diffraction were obtained.

INTRODUCTION

It is well known that many molecules are rendered less stable when they are irradiated,[1-2] and also that some polysaccharides, such as agar–agar and carrageenans, adopt an ordered conformation in aqueous solutions, and form a rigid and thermoreversible hydrogel.[3-5] However, the corelationship of polysaccharide chains in a gel or in a film is not clear. The effects of radiation on agar–agar are also of interest to those who study the effects of radiation on organisms grown in this biomaterial. It was, therefore, considered desirable to study the effects of radiation on the ordered conformation of the agar–agar molecule.

Changes in the ordered conformation can be followed conveniently by measuring the X-ray diffraction intensity of the agar–agar film as functions of radiation dose, elapsed time after irradiation, and concentration of added substances such as sugar and starch.[3]

EXPERIMENTAL

Materials

The agar–agar used in this work was a commercial material produced by the Junsei Chemical Co., Ltd. Sugar and starch were commercial materials produced by the Koso Chemical Co., Ltd. and the Wako Chemical Industries, Ltd., respectively.

Apparatus and Procedure

The solid agar–agar was irradiated with ^{60}Co gamma-rays in air at room temperature at a dose rate of 1.3×10^5 rad/h. After irradiation, the agar–agar

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was dissolved in distilled water or added substance (sugar or starch) solution in a concentration of 3% at about 100°C, molded with 3 mm thickness in a flat plastic vessel. The gel was sun-dried to make a thin film. The X-ray diffraction was measured with a Rigaku X-ray diffractometer using Ni-filtered Cu-Kα 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 in terms of relative intensity of diffraction, which is I/I_0 of the irradiated/nonirradiated agar–agar films.

RESULTS AND DISCUSSION

The changes in relative intensity of diffraction of the agar–agar at various radiation doses were studied with a thin film. Figure 1 shows one of the X-ray diffractogram of the agar–agar film. The results are shown in Figure 2. The changes in relative intensity of diffraction of the agar–agar film at different times after gamma-irradiation (3×10^6 rad) are shown in Figure 3.

The changes in the ordered conformation of the agar–agar molecule are estimated from the changes of X-ray diffraction of the agar–agar film, since the X-ray diffraction in this system is considered to be diffraction of networks. In the case of the agar–agar the networks must be formed by crosslinks between polysaccharide chains, containing double helices of polysaccharide chains and large aggregates of the double helices. These networks must be involved in the crosslinking processes in the polysaccharide system.[3,4]

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

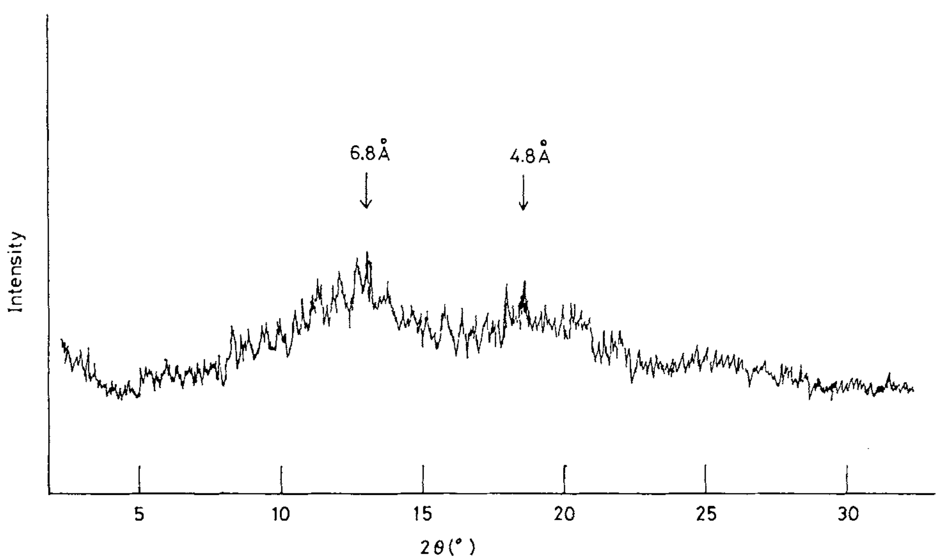

Fig. 1. X-ray diffractogram of agar–agar film.

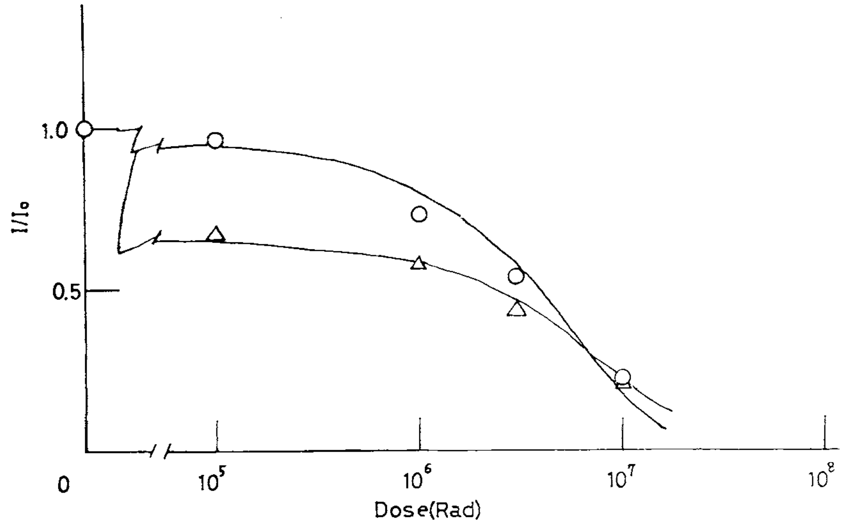

Fig. 2. Effect of radiation dose on the relative intensity of diffraction (I/I_0) of agar–agar film: (○) 6.8 Å and (△) 4.8 Å. The solid curves have been plotted according to eq. (4) with $k = 1.73 \times 10^{-7}$ for 6.8 Å and 1.22×10^{-7} for 4.8 Å.

tion of the agar–agar molecule are assumed to be

$$A \rightsquigarrow A^* \tag{1}$$

$$A\text{-}A^* \rightarrow ((A\text{-}A^*)) \tag{2}$$

$$((A\text{-}A^*)) \rightarrow ((A\text{-}A^*))_n \tag{3}$$

where A is the crosslinking loci of the agar–agar molecule, A^* is the broken crosslinking loci of an irradiated agar–agar molecule, $((A\text{-}A^*))$ is the crosslinking loci of irradiated agar–agar molecule in the double helix system, and $((A\text{-}A^*))_n$ is the crosslinking loci of irradiated agar–agar molecule in the junction zone system, then the breaking step may be reaction (1), which leads to the observed diffraction intensity changes with the radiation dose. Therefore, the response of the network formation to the radiation dose can be determined by measuring the diffraction intensity. The relative intensity of diffraction of the agar–agar film was found to follow

$$I/I_0 = \exp(-kr) \tag{4}$$

where r is the absorbed dose (rad) and k is an adjustable constant. On the other hand, with increasing radiation dose the relative diffraction intensity at 4.8 Å is lower than that at 6.8 Å. It is understood that radioresistance of crosslinking loci of the agar–agar molecule in the junction zone system (at 4.8 Å) is lower than that in the double helix system (at 6.8 Å). In the system studied here the crosslinking loci of the agar–agar 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 the irradiated agar–agar molecule

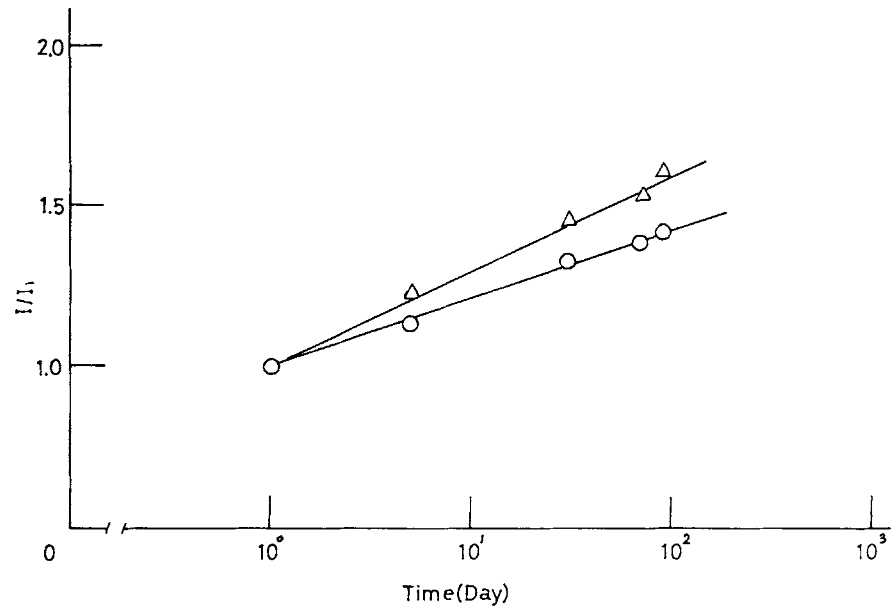

Fig. 3. Effect of post-irradiation on the relative intensity of diffraction (I/I_1) of irradiated (3×10^6 rad) agar–agar film: (○) 6.8 Å and (△) 4.8 Å. The solid lines have been plotted according to eq. (6) with $a = 0.21$ for 6.8 Å and 0.30 for 4.8 Å.

(Fig. 3). When the radiation dose is constant, the increase in the network formation of the irradiated agar–agar molecule depends on the elapsed time after irradiation. If the main actions of irradiation on the network formation of the agar–agar molecule are assumed to be

$$A \rightsquigarrow A^* \tag{1}$$

$$A^* \rightarrow A + E_a \tag{5}$$

where E_a 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. Therefore, the response of the network formation to the time after irradiation can be determined by measuring the diffraction intensity. The relative intensity of diffraction of the agar–agar film was found to follow

$$I/I_1 = 1 + a \log t \tag{6}$$

where t is the elapsed time (days) after irradiation and a is an adjustable constant. Also, with increasing elapsed time after irradiation the relative diffraction intensity at 4.8 Å is higher than that of 6.8 Å. It is understood that post-irradiation recovery of crosslinking loci of the agar–agar molecule in the junction zone system (at 4.8 Å) is higher than that in the double helix system (at 6.8 Å). In the system the recovery may occur in the broken crosslinking loci of the irradiated agar–agar molecule, and the crosslinks are restored. As a result, the X-ray diffraction is recovered until a certain value.

Third, the changes in relative intensity of diffraction of the agar–agar with added substances (sugar and starch) were studied with a thin film, and by the

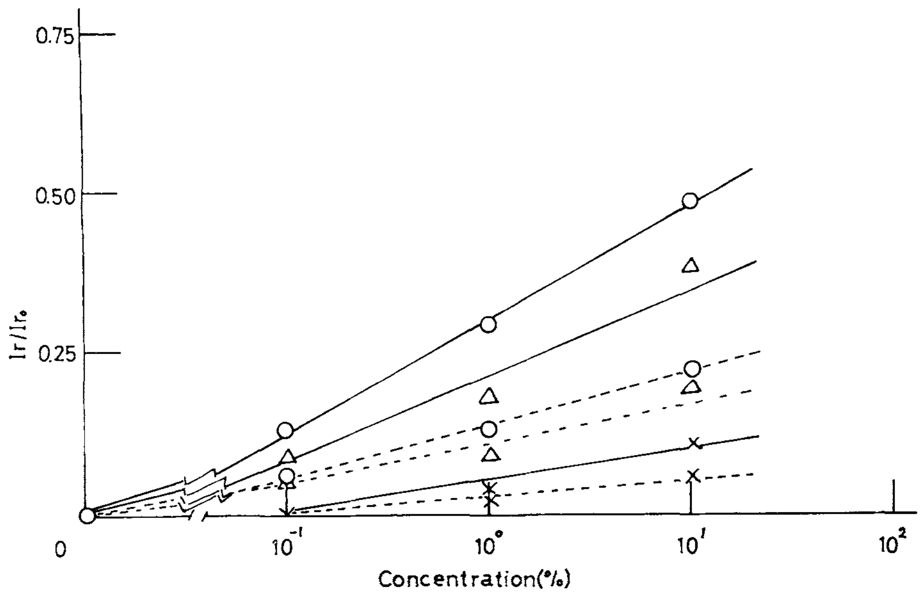

Fig. 4. Effect of sugar concentration on the relative diffraction intensity ratio (I_r/I_{r0}) of irradiated agar–agar film: (○) 10^5 rad; (△) 10^6 rad; (×) 10^7 rad; (- - -) 6.8 Å; (—) 4.8 Å. The solid and dash lines have been plotted according to eq. (10) with $b = 0.18$ for 10^5 rad, 0.13 for 10^6 rad and 0.53×10^{-1} for 10^7 rad, and 0.80×10^{-1} for 10^5 rad, 0.58×10^{-1} for 10^6 rad and 0.28×10^{-1} for 10^7 rad, respectively.

irradiation of 0, 10^5, 10^6, and 10^7 rad. The results are shown in Figures 4 and 5.

The increases in the X-ray diffraction with added substance (sugar and starch) are related to an increased density of the networks of the irradiated agar–agar molecule (Figs. 4 and 5). When the radiation dose is constant, an increase of the concentration of added substance (sugar and starch) results in a restoration of the network formation of the irradiated agar–agar molecule. If the main actions of added substance (sugar and starch) on the network formation of the irradiated agar–agar molecule are assumed to be

$$A \rightsquigarrow A^* \tag{1}$$

$$A^* + S \rightarrow A^*\text{–}S \tag{7}$$

$$A^*\text{–}S \rightarrow ((A^*\text{–}S)) \tag{8}$$

$$((A^*\text{–}S)) \rightarrow ((A^*\text{–}S))_n \tag{9}$$

where S is the added substance, $A^*\text{–}S$ is a linkage of the broken crosslinking loci of the irradiated agar–agar molecule and the added substance, $((A^*\text{–}S))$ is the restored crosslinking loci of the irradiated agar–agar molecule in the double helix system, and $((A^*\text{–}S))_n$ is the restored crosslinking loci of the irradiated agar–agar molecule in the junction zone system, then the restorative step may be reaction (7), which leads to the observed diffraction intensity changes with the concentration of added substances. Therefore, the response of the network formation to the added substances can be determined by measuring the diffraction intensity. The relative intensity of diffraction of the

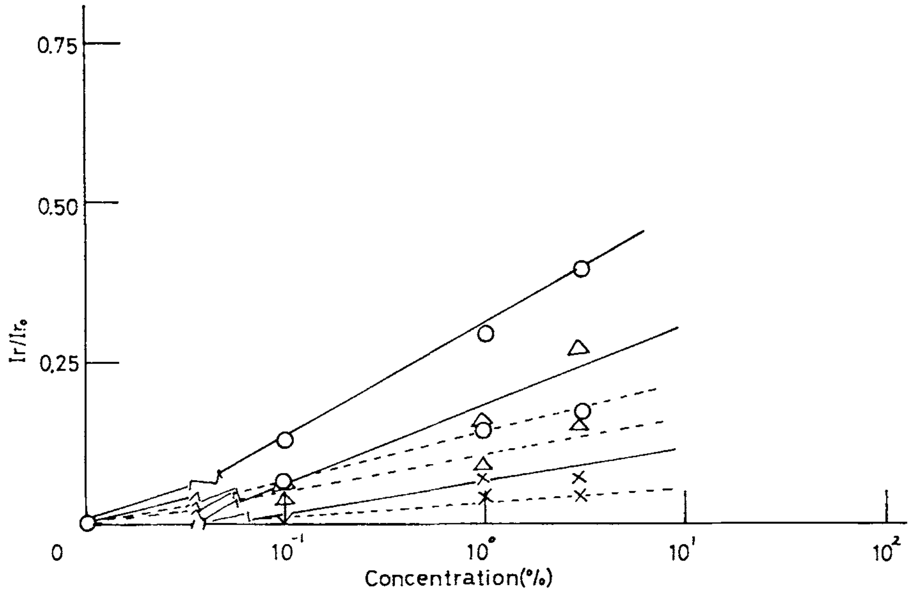

Fig. 5. Effect of starch concentration on the relative diffraction intensity ratio (I_r/I_{r0}) of irradiated agar–agar film: (○) 10^5 rad; (△) 10^6 rad; (×) 10^7 rad, (---) 6.8 Å; (—) 4.8 Å. The solid and dash lines have been plotted according to eq. (10) with $b = 0.17$ for 10^5 rad, 0.12 for 10^6 rad, and 0.54×10^{-1} for 10^7 rad, and 0.70×10^{-1} for 10^5 rad, 0.50×10^{-1} for 10^6 rad, and 0.24×10^{-1} for 10^7 rad, respectively.

agar–agar film was found to follow

$$I_r/I_{r0} = b \log S \quad (10)$$

where S is the concentration (%) and b is an adjustable constant. Furthermore, with increasing the concentration of added substances the relative diffraction intensity at 4.8 Å is higher than that at 6.8 Å. It is understood that the radiation restoration of broken crosslinking loci of the irradiated agar–agar molecule in the junction zone system (4.8 Å) is higher than that in the double helix system (at 6.8 Å). In the system the broken crosslinking loci of the irradiated agar–agar molecule may be restored by adding the added substances (sugar and starch), and the formation of the crosslinks of the irradiated agar–agar molecule is enhanced. As a result, the X-ray diffraction is restored until a certain value.

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