NOTE

Radiation-Induced Sol–Gel Transition of Protein: Effects of Radiation on Amino Acid Composition and Viscosity

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

The changes in the chemical structure and the flow property can be followed conveniently by measuring the infrared spectra, amino acid composition, and viscosity of the irradiated protein.

EXPERIMENTAL

Materials

Gelatin used in this work was a commercial material produced by the Kanto Chemical Co., Ltd. All other reagents were of analytical grade.

Apparatus and Procedure

In irradiation, the solid gelatin was irradiated by ^{60}Co gamma rays in air at room temperature at a dose rate of 1.0×10^5 rad/h.

In infrared spectrometry, the irradiated solid gelatin was molded into 1.5% pellets with KBr, and then the infrared spectra were measured with a Hitachi dispersive-type infrared spectrophotometer.

In amino acidimetry, the irradiated solid gelatin was dissolved with 6*N* HCl, hydrolyzed at 110°C for 24 h in sealed nitrogen flushed tube. Then the amino acid composition of the solution was measured with a JEOL 5AH amino acid analyzer.

In viscometry, the irradiated solid gelatin was dissolved in 0.2*M* KCl solution at room temperature, and held at the desired temperature for 2 h. Then the viscosity of the solution was measured with a Cannon-Fenske type viscometer.

In the calculation of the activation energy required for the sol flow of the gelatin molecule, the general linearity of plots of log η_{red} vs. $1/T$ indicates a constant flow activation energy for gelatin hydrosol and the valid application of the Andrade equation over a range of the temperatures. The following relation for the shift in viscosity with temperature was thus employed:

$$\eta_{red} = Ae^{-E/RT}$$

where E is the activation energy (kcal/mol) of the sol flow, T, absolute temperature, R, the gas constant, 1.987 cal/mol and A, an adjustable constant.

RESULTS AND DISCUSSION

Chemical Structure Change

The changes in infrared spectra of gelatin at various radiation doses were studied with 1.5% gelatin in KBr pellet at 15°C. Figure 1 shows the relation between the values of the infrared absorbance ratio and the radiation dose.

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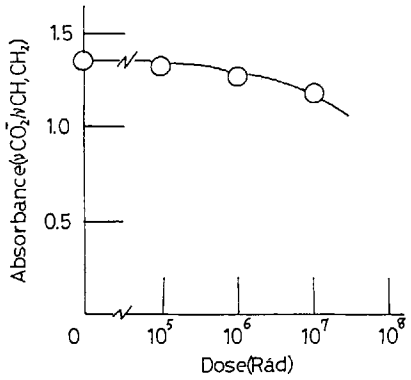

Fig. 1. IR absorbance ratio ($\nu_{CO_2^-}/\nu_{CH,CH_2}$) vs. radiation dose. Conditions: 1.5% gelatin in KBr pellet at 15°C.

TABLE I
Amino Acid Composition Ratio of Irradiated (10^7 rad) and Nonirradiated Gelatin[a]

Major amino acid	10^7 rad irrad./nonirrad.
Hyp	1.0
Asp	0.8
Glu	1.0
Pro	1.0
Gly	1.1
Ala	0.8

[a] Conditions: 24 h hydrolysis in 6*N* HCl at 110°C.

Also, the changes in amino acid composition of gelatin with the irradiation were studied with irradiated (10^7 rad) and nonirradiated gelatin in 6*N* HCl, 24 h hydrolysis at 110°C. Table I shows the amino acid composition of irradiated and nonirradiated gelatin. From these results it is clear that the infrared absorbance and the amino acid composition are decreased, depending upon the irradiation. If such changes in infrared absorbance and amino acid composition are due to destruction of some amino acid residues in the bonds of the gelatin molecule, then the destruction may occur in the residues such as Asp and Ala of the gelatin molecule and lead to the observed

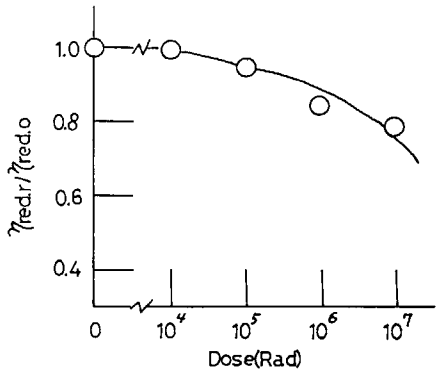

Fig. 2. Reduced viscosity ratio ($\eta_{red.\ r}/\eta_{red.\ 0}$) vs. radiation dose. Conditions: 0.6% gelatin in 0.2*M* KCl at 20°C.

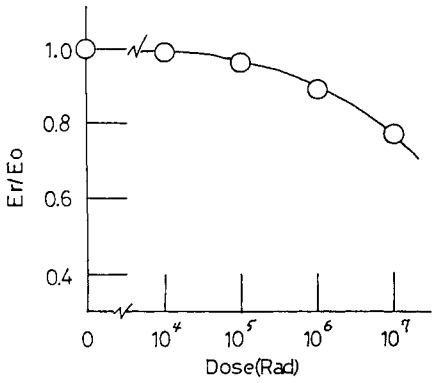

Fig. 3. Activation energy ratio (E_r/E_0) vs. radiation dose.

infrared absorbance and amino acid composition changes with the irradiation (see Fig. 1 and Table I). Therefore, the infrared absorbance and amino acid composition of the irradiated gelatin are decreased depending upon the irradiation. It seems that the changes in the residues (Asp and Ala) with irradiation are involved in transitions from Asp and Ala to Gly in the gelatin molecule.

Flow Property Change

The changes in reduced viscosity of gelatin with the irradiation were studied with 0.6% gelatin in 0.2*M* KCl. Figure 2 shows the relation between the values of the reduced viscosity and the radiation dose. Also, the changes in activation energy required to sol flow of gelatin with irradiation were estimated from a plot of log η_{red} vs. $1/T$. Figure 3 shows the relation between the values of the flow activation energy and the radiation dose. From these results it is clear that the reduced viscosity and the activation energy are decreased, depending upon the irradiation. If such changes in viscosity and activation energy with the irradiation are due to destruction of sol form of gelatin molecule, increased radiation dose should result in further destruction of sol form and the viscosity and activation energy should continue to decrease with increasing absorbed dose. This corresponds to the observed viscosity and activation energy changes (see Figs. 2 and 3).

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MIZUHO NISIZAWA

Department of Chemistry
Defense Academy
Yokosuka, Japan

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