Radiation-Induced Sol–Gel Transition of Protein: Effects of Metal Ions on Flow and Melting Properties

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SYNOPSIS

Solid protein was irradiated with ^{60}Co gamma rays, and the effects of metal ions (Cu^{2+} and Fe^{2+}) on irradiated protein molecules were studied by measuring the viscosity of the protein hydrosol and the melting point of the protein hydrogel at different radiation doses and different concentrations of the metal ions. The effects of metal ions on the flow and thermal properties of irradiated protein molecules were discussed.

INTRODUCTION

Ionizing radiation strongly influences the chemical and conformational properties of biological macromolecules.[1,2] Some biological macromolecules, such as proteins, form a thermoreversible gel[3–5] and interact with metal ions, such as Cu^{2+} ions.[6] However, the interrelationships between metal ions and polypeptide chains in the radiation-induced sol–gel transition are not clear. It was, therefore, important to study the effects of metal ions (Cu^{2+} and Fe^{2+}) on the flow and melting properties of the irradiated protein molecule. The protein used in this work was gelatin, since it was described in a previous article.[7]

Changes in the flow and melting properties can be followed conveniently by measuring the viscosity and the melting point of irradiated protein as a function of concentration of metal ions (Cu^{2+} and Fe^{2+}).

EXPERIMENTAL

Materials

Gelatin as the model protein, cupric sulfate, and ferrous sulfate used were commercial materials produced by the Kanto Chemical Co., Ltd., The Junsei Chemical Co., Ltd., and the Wako Chemical Industries, Ltd., respectively.

Apparatus and Procedure

In irradiation, the solid gelatin was irradiated with ^{60}Co gamma rays in air at room temperature at dose rates of 6.0×10^4 to 1.3×10^5 rad/h.

In viscometry, the irradiated solid gelatin was dissolved in distilled water or a metal ion (Cu^{2+} as $CuSO_4$ or Fe^{2+} as $FeSO_4$) solution at about 80°C, and was cooled at 25°C for 24 h. The viscosity of the solution was then measured.[7]

In thermometry, the irradiated solid gelatin was dissolved in distilled water or a metal ion (Cu^{2+} as $CuSO_4$ or Fe^{2+} as $FeSO_4$) solution at about 80°C, and was gelatinized at 2°C for 24 h. The gelatin hydrogel was then warmed at a rate of 0.2°C/min, and the melting point of the hydrogel was measured.

In the calculation of heat of reaction for crosslinking process, the heat energy required to dissociate crosslinks of the gelatin hydrogel was calculated using the melting point given by the equation of Eldridge and Ferry:

$$\log_{10} C = \Delta H / 2.303\, RT + \text{const} \qquad (1)$$

where C is the gelatin concentration (g/L), ΔH is the heat of reaction for the crosslinking process of the gelatin hydrogel (Kcal/mol of crosslinks), R is the gas constant, and T is the melting point of the gelatin hydrogel (K). Equation (1) is converted to eq. (2)

$$\Delta H = (k \log_{10} C_1 / C_2)(1/T_1 - 1/T_2)$$
$$k = 2.303 \times R \qquad (2)$$

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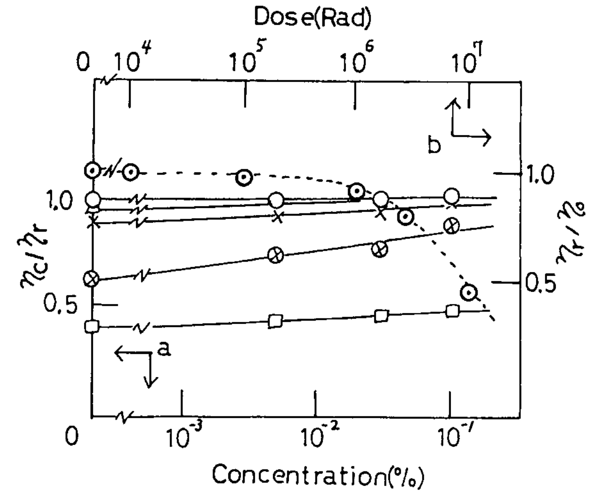

Figure 1 (a) Viscosity vs. concentration of $CuSO_4$ for various radiation doses: (○) 0 rad, (△) 10^5 rad, (×) 10^6 rad; (⊗) 5×10^6 rad, and (□) 10^7 rad. Conditions: 10% gelatin hydrosol at 25°C. (b) Viscosity vs. radiation dose without $CuSO_4$. Conditions: 10% gelatin hydrosol at 25°C.[7]

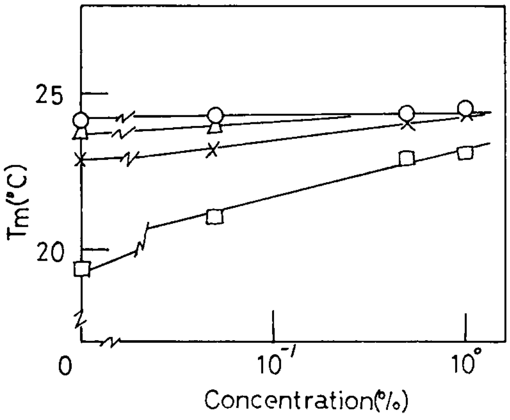

Figure 3 Melting point vs. concentration of $CuSO_4$ for various radiation doses: (○) 0 rad, (△) 10^5 rad, (×) 10^6 rad, and (□) 10^7 rad. Conditions: 8% gelatin hydrogel.

RESULTS AND DISCUSSION

The changes in viscosity of irradiated gelatin with and without metal ions (Cu^{2+} and Fe^{2+}) were studied with 10% gelatin, and by the irradiation of 0, 10^5, 10^6, 5×10^6 and 10^7 rad, and at 25°C. Figures 1 and 2 show the relations between the values of viscosity and the concentration of metal ions (Cu^{2+} and Fe^{2+}) for various radiation doses. From these results, it is clear that the viscosity of irradiated protein is increased, depending upon the addition of the metal ions under the concentration employed. It seems that the increases of viscosity values are related to a restoration of the sol form of the irradiated gelatin molecule by adding the metal ions. As a result, the viscosity value is recovered to a certain extent.

The changes in melting point of irradiated gelatin, with and without metal ions (Cu^{2+} and Fe^{2+}), were studied with 3–10% gelatin, and by the irradiation of 0, 10^5, 10^6, and 10^7 rad. Figures 3 and 4 show some of the relations between the values of melting point and the concentration of metal ions (Cu^{2+} and Fe^{2+}) for various radiation doses. The concentration

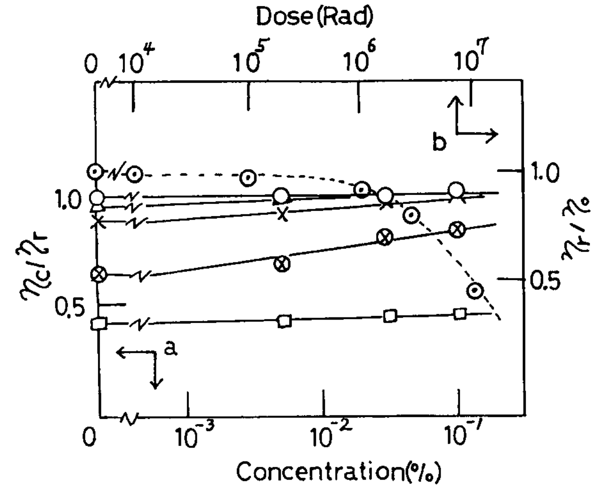

Figure 2 (a) Viscosity vs. concentration of $FeSO_4$ for various radiation doses: (○) 0 rad, (△) 10^5 rad, (×) 10^6 rad, (⊗) 5×10^6 rad, and (□) 10^7 rad. Conditions: 10% gelatin hydrosol at 25°C. (b) Viscosity vs. radiation dose without $FeSO_4$. Conditions: 10% gelatin hydrosol at 25°C.[7]

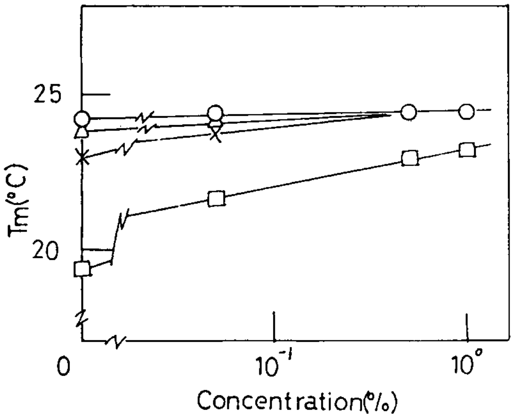

Figure 4 Melting point vs. concentration of $FeSO_4$ for various radiation doses: (○) 0 rad, (△) 10^5 rad, (×) 10^6 rad, and (□) 10^7 rad. Conditions: 8% gelatin hydrogel.

of metal ions (Cu^{2+} and Fe^{2+}) in the melting changes of irradiated gelatin hydrogel can be used more excessively than that in the viscosity changes of the irradiated gelatin hydrosol. From these results, it can be seen that the restoration of the T_m value is related to an increase of the crosslinks of the irradiated gelatin molecule by adding the metal ions. As a result, the T_m value is recovered to certain extent.

Also, the changes in heat energy required to dissociate crosslinks of irradiated gelatin hydrogel, with and without metal ions (Cu^{2+} and Fe^{2+}), were estimated by the equation of Eldridge and Ferry. Figures 5 and 6 show the relations between the values of the heat of reaction and the radiation dose, with and without metal ions (Cu^{2+} and Fe^{2+}). From the results, it is clear that the heat of reaction is increased, depending upon the addition of metal ions (Cu^{2+} and Fe^{2+}) under the concentration employed. If such changes in melting point and heat of reaction are due to the restoration of the crosslinks of the irradiated gelatin molecule, increased concentration of the metal ions should result in further restoration, and the melting point and the heat of the reaction should continue to recover up to certain values.

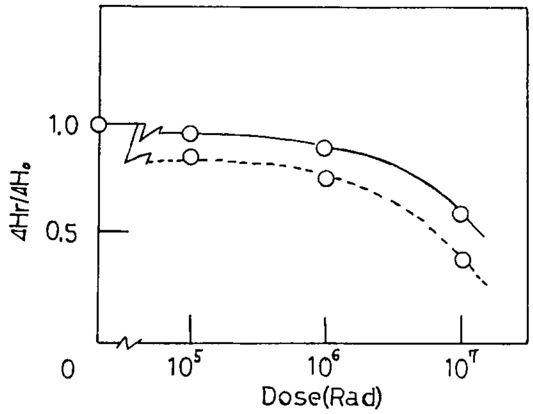

Figure 5 Heat of reaction (based on the equation of Eldridge and Ferry) vs. radiation dose with and without $CuSO_4$: (——) 0.5% $CuSO_4$ and (- - - -) no-$CuSO_4$.

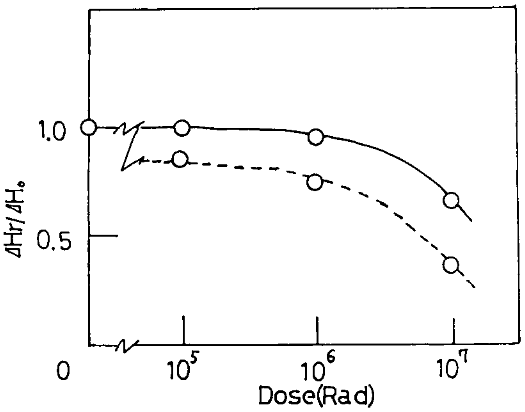

Figure 6 Heat of reaction (based on the equation of Eldridge and Ferry) vs. radiation dose with and without $FeSO_4$: (——) 0.5% $FeSO_4$ and (- - - -) no-$FeSO_4$.

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