NOTE

Radiation-Induced Sol-Gel Transition of Protein: Effects of Radiation and Metal Ions on Conformational Property

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 thermoreversible hydrogel,[3–5] and interact with metal ions, such as Cu^{2+} ions.[6,7] It was, therefore, considered desirable to study the effects of radiation and metal ions (Cu^{2+} and Fe^{2+}) on the conformational property of protein hydrosol. 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.[3,7] The changes in the conformational property can be followed conveniently by measuring the optical rotations of the protein hydrosol as functions of radiation doses and concentrations of metal ions (Cu^{2+} and Fe^{2+}).

EXPERIMENTAL

The solid gelatin (Kanto Chemical Co.) as the model protein was irradiated with ^{60}Co gamma rays in air at room temperature at a dose rate of 1.0×10^5 rad/h. The irradiated solid gelatin was dissolved in 0.2*M* KCl solution or a metal ion (Cu^{2+} as $CuSO_4$ or Fe^{2+} as $FeSO_4$) solution at room temperature, and held at 45°C for 2 h. Then the optical rotation of the solution was measured at various temperatures. In the calculation of the activation energy required for the conformational change of the gelatin molecule, the general linearity of plots of $\log(\alpha)$ versus $1/T$ indicates a constant activation energy for conformational change of gelatin hydrosol and the valid application of the Andrade equation over a range of the temperatures was thus employed:

$$(\alpha) = Ae^{-E/RT}$$

where E is the activation energy (Kcal/mol) of conformational change, T is the absolute temperature (K), R is the gas constant (1.987 cal/mol·K), and A is an adjustable constant.

RESULTS AND DISCUSSION

The changes in specific rotation of irradiated gelatin at various radiation doses were studied with 0.6% gelatin in 0.2*M* KCl and by the irradiation of 0, 10^4, 10^5, 10^6, and 7×10^6 rad. Figure 1 shows some of the relationships between the values of the specific rotation and the reciprocal absolute temperature. The specific rotation initially increased very slowly until a transit point, and then it increased very rapidly, depending upon the reciprocal temperature. From these results it is clear that the changes in the optical rotation of gelatin hydrosol with the temperature are due to the transition of the random coil structure of gelatin molecule (Sol_I) to the helical structure (Sol_{II}). Also, the changes in activation energy required to induce conformational change of gelatin molecule with irradiation were estimated from a plot of $\log(\alpha)$ versus $1/T$. Figure 2 shows the relationships between the values of the activation energy for conformational change and the radiation dose for various hydrosol states. The activation energy decreased, depending upon the irradiation, and the activation energy of Sol_I is lower than that of Sol_{II}. If such changes in activation energy with irradiation are assumed to be changes in the relation of optical rotation and conformational order, it seems that the decrease in activation energy with irradiation is due to destruction of the conformational order of the gelatin molecule, and the radiation resistance of the gelatin molecule in the random coil state is lower than that in the helical state.

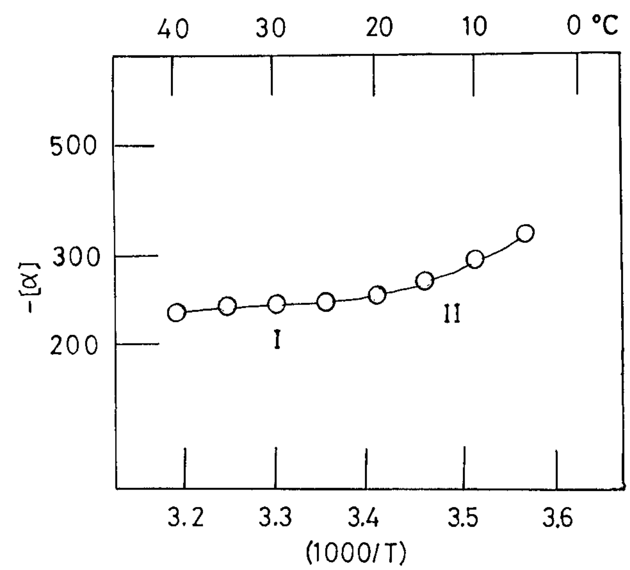

Figure 1 Specific rotation versus absolute temperature. Conditions: 0.6% gelatin in 0.2*M* KCl.

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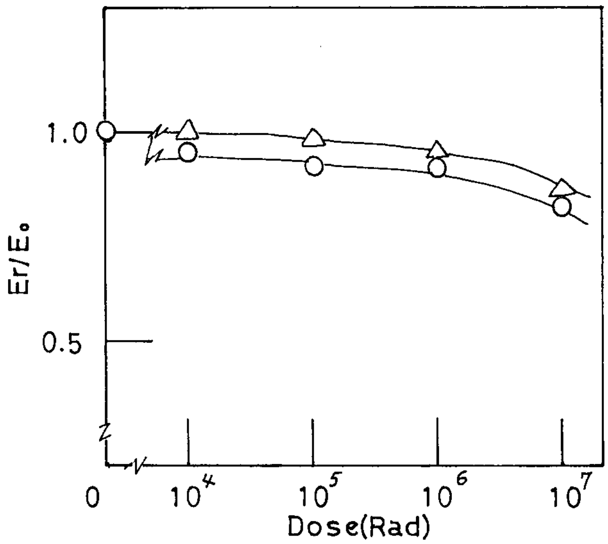

Figure 2 Activation energy versus radiation dose for various sol states: (○) Sol_I and (△) Sol_{II}.

The changes in specific rotation of irradiated gelatin with and without metal ions (Cu^{2+} and Fe^{2+}) were studied with 0.6% gelatin in 0.2*M* KCl and by the irradiation of 7×10^6 rad. Figures 3 and 4 show the relationships between the values of the activation energy for conformational change and the concentration of metal ions (Cu^{2+} and Fe^{2+}), for various gelatin hydrosol states. The activation energy increased, depending upon the concentration of metal ions (Cu^{2+} and Fe^{2+}), and the increase rate of activation energy of Sol_I is higher than that of Sol_{II}. If such changes in activation energy are due to the reduction of the radiation damage

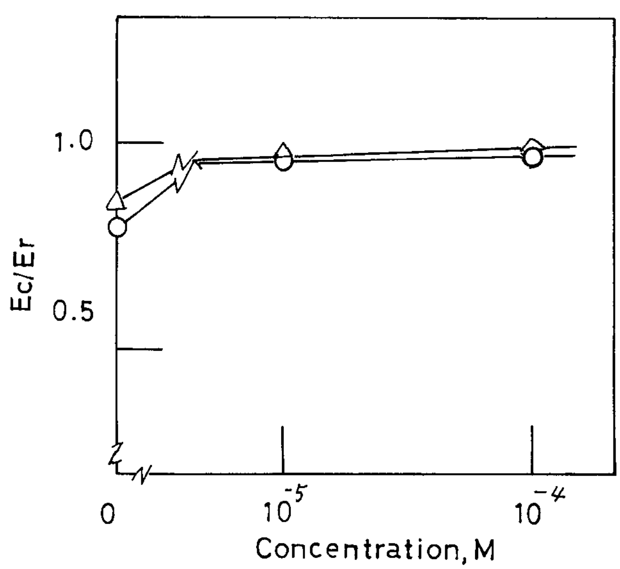

Figure 3 Activation energy versus concentration of $CuSO_4$ for irradiation (7×10^6 rad): (○) Sol_I and (△) Sol_{II}.

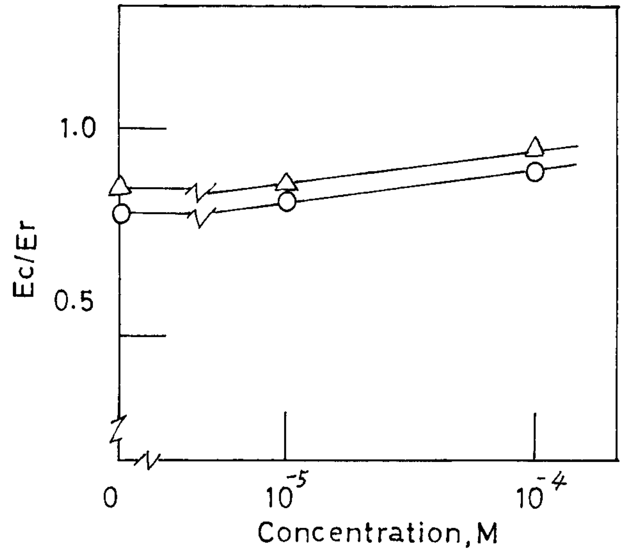

Figure 4 Activation energy versus concentration of $FeSO_4$ for irradiation (7×10^6 rad): (○) Sol_I and (△) Sol_{II}.

by the effect of ionic or polar crosslinks through the bivalent metal ions, it can be seen that the restorative effect of the metal ions (Cu^{2+} and Fe^{2+}) on the gelatin molecule in the random coil state is higher than that in the helical state. Thus, the measurement of the optical rotation can be used as monitoring the changes in activation energy required to induce the conformational changes of the irradiated gelatin molecule.

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