# Radiation-Induced Sol-Gel Transition of Protein: Effects of Radiation and Metal Ions on Melting Behavior 

Ionizing radiation strongly influences the chemical and conformational properties of biological macromolecules. ${ }^{1,2}$ Some biological macromolecules, such as proteins, form a thermoreversible hydrogel ${ }^{3-5}$ and interact with metal ions, such as $\mathrm{Cu}^{2+}$ ions. ${ }^{6}$ However, the thermal property changes that accompany a change of polypeptide chains in a gel are not clear. It was, therefore, considered desirable to study the effects of radiation and metal ions $\left(\mathrm{Cu}^{2+}\right.$ and $\mathrm{Fe}^{2+}$ ) on the cross-links of a protein molecule. Changes in the cross-links can be followed conveniently by measuring the melting point of protein hydrogel as a function of radiation dose.

The solid gelatin (Kanto Chemical Co.) as the model protein was irradiated with ${ }^{60} \mathrm{Co}$ gamma-rays in air at room temperature at dose rates of $6.0 \times 10^{4}$ to $1.3 \times 10^{5} \mathrm{rad} /$ h . The irradiated solid gelatin was dissolved in distilled water or metal ions ( $\mathrm{Cu}^{2+}$ as $\mathrm{CuSO}_{4}$ or $\mathrm{Fe}^{2+}$ as $\mathrm{FeSO}_{4}$ ) solution at about $80^{\circ} \mathrm{C}$ and gelatinized at $2^{\circ} \mathrm{C}$ for 24 h . The gelatin hydrogel was then warmed at a rate of $0.2^{\circ} \mathrm{C} /$ min and the melting point was measured.

The changes in melting point of irradiated gelatin with and without metal ions ( $\mathrm{Cu}^{2+}$ and $\mathrm{Fe}^{2+}$ ) were studied with $3-10 \%$ gelatin, $0.5 \% \mathrm{CuSO}_{4}$, and $0.5 \% \mathrm{FeSO}_{4}$ and by irradiation of $0,10^{5}, 10^{6}$, and $10^{7}$ rad. Figure 1 shows the relation between the values of gelatin concentration and the reciprocal absolute temperature of melting for various radiation doses. The melting point decreases as the radiation dose increases. If such changes in melting point are compared to changes in melting point and molecular weight, which were reported in the experiments of Eldridge and Ferry, ${ }^{7}$ it seems that the decrease in melting point with radiation is attributable to a decrease in molecular weight by scission of the main chain or cross-links.

Also, the changes in melting point of irradiated gelatin with metal ions ( $\mathrm{Cu}^{2+}$ and $\mathrm{Fe}^{2+}$ ) are shown in Figures 2 and 3. The melting point raises as the metal ions ( $\mathrm{Cu}^{2+}$ and $\mathrm{Fe}^{2+}$ ) are added. If such changes in melting point are due to the reduction of the radiation damage by the effect of ionic or polar cross-links through the bivalent metal ions, the measurement of the melting point can be used as monitoring the cross-links of the irradiated gelatin molecule.

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Figure 1 Gelatin concentration vs. absolute temperature of melting for various radiation doses: (O) $0 \mathrm{rad} ;(\Delta)$ $10^{5} \mathrm{rad}$; (x) $10^{6} \mathrm{rad}$; ( $\square$ ) $10^{7} \mathrm{rad}$.


Figure 2 Gelatin concentration vs. absolute temperature of melting with $0.5 \% \mathrm{CuSO}_{4}$ for various radiation doses: ( $O$ ) 0 rad ; ( $\triangle$ ) $10^{5} \mathrm{rad}$; (x) $10^{6} \mathrm{rad}$; ( $\square$ ) $10^{7} \mathrm{rad}$.


Figure 3 Gelatin concentration vs. absolute temperature of melting with $0.5 \% \mathrm{FeSO}_{4}$ for various radiation doses: (O) $0 \mathrm{rad} ;(\triangle) 10^{5} \mathrm{rad} ;(\mathrm{x}) 10^{6} \mathrm{rad}$; ( $\square$ ) $10^{7} \mathrm{rad}$.

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