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Radiation Chemical Studies of Protein Reactions: Effect of Protectors on the Breaking of Secondary Bonding in Protein

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Synopsis

Radiation protective effect of the breaking of secondary bonding in protein was examined with sodium benzoate, monosodium 1-glutamate, 1-arginine, and thiourea, and the behavior of viscosity against the protector was obtained. An experimental equation for the viscosity change is given by $\eta red = b - a \log X$, where ηred is the reduced viscosity of the solution, X is the protector concentration, and a and b are adjustable constants.

INTRODUCTION

The scission of primary bonds such as chemical bonds or of secondary bonds such as hydrogen bonds in protein occur by radiation or by urea.[1–3] The scission of secondary bonds by urea as a well-known protein-denaturing agent is a very important act in the structural changes in protein.[3] While urea denaturation of protein can be accelerated by radiation and protected against an activation effect of denaturation from radiation by the protectors.[4,5] Since the breaking of secondary bonding in protein is a problem of general interest, it was decided to investigate the effect of protectors against radiation on the breaking of secondary bonding. The breaking of hydrogen bonds in gelatin molecules caused by urea was selected as the breaking of secondary bonding in protein. The determination can be followed conveniently by measuring the reduced viscosity of the solution as a function of the protector concentration.

EXPERIMENTAL

Materials

Gelatin, urea, monosodium 1-glutamate, and thiourea used in this work were commercial materials produced by the Kanto Chemical Co., Inc.

Sodium benzoate and 1-arginine were commercial materials produced by the Koso Chemical Co., Ltd.

Apparatus and Procedure

An irradiation source containing about 300 curies of ^{60}Co was used. The dose rate in this experiment was 1.67×10^3 R/hr. The solid gelatin was irradiated in an air atmosphere at room temperature. The irradiated solid gelatin was dissolved with the urea solution containing protector. Then, the viscosity was measured at 30°C.[5] Each of these measurements was repeated three times.

RESULTS

The changes in reduced viscosity of the gelatin with protectors were studied with 5% gelatin in 8 *M* urea, 10^3 R, and 30°C. The results are shown in Figures 1–4.

From these it is clear that the decrease in the reduced viscosity with increasing protector concentration in percent on the logarithmic scale indicates a protection of the breaking of hydrogen bonds in protein.

DISCUSSION

The scission of primary bonding or of secondary bonding in protein occur by radiation or by urea.[1–3] While urea denaturation of protein can be accelerated by radiation and protected against an activation effect of denaturation from radiation by the protectors.[4,5]

The effect of protectors against radiation and the effect of protector concentration on the breaking of hydrogen bonds in gelatin molecules caused by urea will be now discussed. The viscosity change required for the breaking of hydrogen bonds is estimated from Figures 1–4. Thus the relation between the viscosity change and the protector concentration is parallel to that between the breaking of hydrogen bonds and protective reaction. When the concentration of gelatin and urea, and radiation dose are constant, the increase in the protector concentration results in a decrease in the

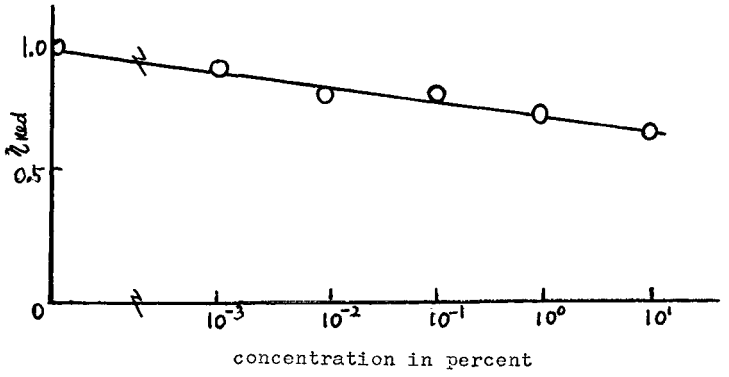

Fig. 1. Dependence of the protective effect on the concentration of sodium benzoate (5% gelatin in 8 *M* urea, 10^3 R, and 30°C).

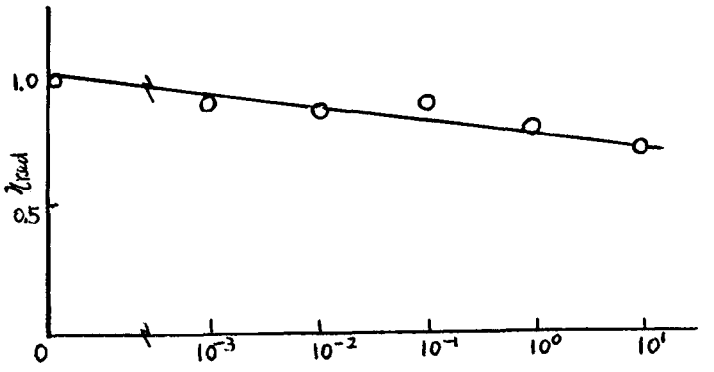

Fig. 2. Dependence of the protective effect on the concentration of monosodium l-glutamate (.5% gelatin in 8 *M* urea, 10^3 R, and 30°C).

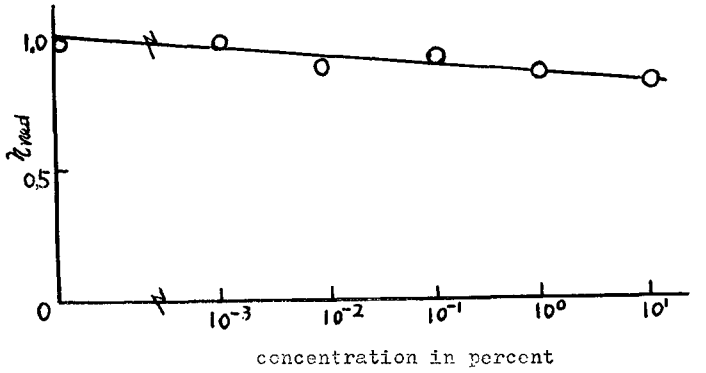

Fig. 3. Dependence of the protective effect on the concentration of l-arginine (5% gelatin in 8 *M* urea, 10^3 R, and 30°C).

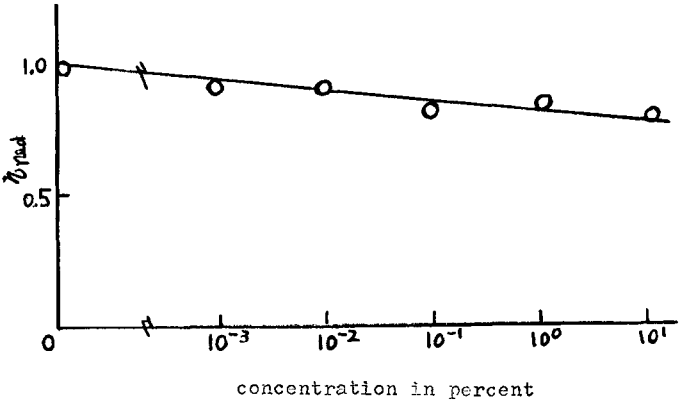

Fig. 4. Dependence of the protective effect on the concentration of thiourea (5% gelatin in 8 *M* urea, 10^3 R, and 30°C).

activation required for the breaking of hydrogen bonds obtained from Figures 1–4. The reaction mechanism, therefore, depends on the protector concentration. The following reactions were considered for the protective reaction,

$$P\text{—}P \xrightarrow{h\nu} P^* + P^*$$
$$P^* + S \rightarrow P\text{—}P + S^*$$
$$S^* \rightarrow S + Ea, \text{ or } S^* \rightarrow S'$$

where P—P is the gelatin molecule, S is the protector, and Ea is activation energy of γ-rays. These reactions are probably responsible for the protection on the breaking of secondary bonding in protein by protectors.

For the present system, the observed viscosity change is expressed as linear line, a logarithmic abscissa for the protector concentration in percentage;

$$\eta\text{red} = b - a \log X$$

This formula agrees with the experimental data plotted in Figures 1–4.

In the mechanism the protectors may be protected by the breaking of secondary bonding in protein by energy transfer, or energy absorbing of γ-rays.

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