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Radiation Chemical Studies of Protein Reactions: Effect of Amino Acids, Proteins, Vitamins, Chelating Agents, S-Containing Compounds, and Temperature on Viscosity

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SUMMARY

Amino acids, proteins, vitamins, chelating agents, and S-containing compounds were found to protect the shape of the external envelope of the protein molecule from radiation damage. The behavior of the viscosity change closely resembles that found with sodium glutamate and sodium benzoate, as shown by a similar dependence on the concentration. Protein irradiated by γ-rays showed the effect of temperature on changes in the shape of the external envelope of the protein molecule. The behavior of the viscosity change was studied.

INTRODUCTION

Irradiation experiments have suggested that changes in the shape of the external envelope of the protein molecule are accelerated by γ radiation [1]. On the other hand, some amino acid such as sodium glutamate and some benzene-series hydrocarbons such as sodium benzoate protect the shape of the external envelope of the protein molecule from the activation effect of

γ radiation [2]. It was though desirable to see if such well-known materials as amino acids (such as l-arginine, l-aspartic acid, and calcium d-panthothanate), proteins (such as bovine albumin, egg albumin, and gelatin), vitamins (such as riboflavin, thiamine, and l-ascorbic acid), chelating agents (such as propylene glycol, ethylenediamine, and ethylenediaminetertra-acetic acid), and S-containing compounds (such as thiourea, thioglycol, and β, β′-thioglycolic acid) 1) showed such a protective property, 2) what would be the effect of concentration on changes in the shape of the external envelope of the protein molecule.

The irradiated biological materials are rendered more stable with respect to radiation damage if the irradiation temperature is lowered [3, 4]. Since the effect of temperature is a problem of general interest, it was thought desirable to study the effect of temperature on the changes in the shape of the external envelope of the protein molecule.

The urea denaturation of protein was selected for the study since it has been described in the previous papers [1, 2]. The determination can be conveniently followed by measuring the reduced viscosity of the solution as a function of the concentration of added substances and the temperature.

EXPERIMENTAL

Materials

The albumin (egg), albumin (bovine), gelatin, and urea used in this work were commercial materials produced by the Kanto Chemical Co., Inc. The L-arginine, calcium d-panthothanate, riboflavin, l-ascorbic acid, ethylenediamine, ethylenediaminetetraacetic acid, and thioglycolic acid used were commercial materials produced by the Daiichi Pure Chemical Co., Ltd. The L-aspartic acid, propylene glycol, and thiourea used were commercial materials produced by the Junsei Pure Chemicals Co., Ltd. The thiamine used was a commercial material produced by the Takeda Chemical Industries, Ltd.

Apparatus and Procedure

An irradiation source containing about 300 C of ^{60}Co was used. The dose rate in this experiment was 1.7×10^3 R/hr. The solid albumin was irradiated in air at room temperature. The irradiated solid albumin was dissolved with distilled water and mixed with urea solution containing the added substances or with urea solution. Then the viscosity was measured [1, 2].

RESULTS

Effect of Added Substances

The changes in reduced viscosity of albumin and added substances (amino acid, protein, vitamin, chelating agent, and S-containing compound) were studied with 3% albumin in 10 M urea, 10^3 R, and 30°C. The results are shown in Figs. 1-5. From the relation between the final reduced viscisoty and the percentage of added substance shown in Figs. 1-5 it is clear that the effect of the added substance on the viscosity is apparently related to its inhibition of changes in the shape of the external envelope of the protein molecule.

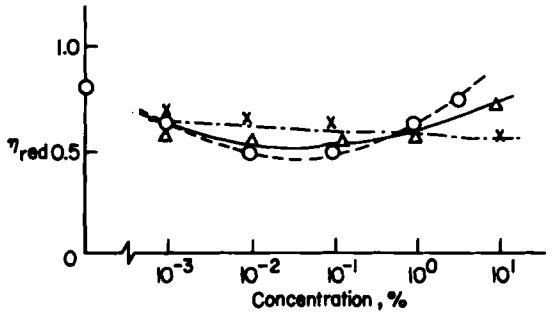

Fig. 1. Dependence of protective effect on concentration of amino acids. (○) l-arginine, (△) calcium d-panthothanate, (X) l-aspartic acid. Conditions: 3% albumin in 10 M urea, 10^3 R, 30°C.

Effect of Temperature

The changes in reduced viscosity of albumin at various temperatures were studied with 3% albumin in 6 M urea and 10^3 R. The results are shown in Fig. 6. From the relation between the final reduced viscosity and the temperature shown in Fig. 6 it is clear that the effect of temperature on the viscosity is apparently related to its temperature effect on changes in the shape of the external envelope of the protein molecule.

DISCUSSION

As stated above, it is known that changes in the shape of the external envelope of the protein molecule are accelerated by γ radiation [1]. On

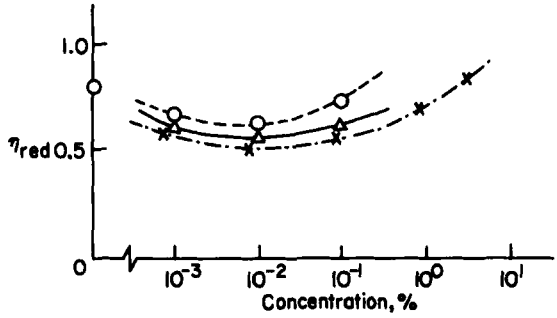

Fig. 2. Dependence of protective effect on concentration of proteins: (○) albumin (bovine), (△) albumin (egg), (X) gelatin. Conditions: 3% albumin in 10 M urea, 10^3 R, 30°C.

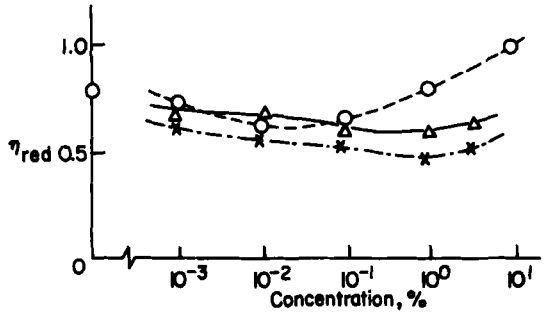

Fig. 3. Dependence of protective effect on concentration of vitamins: (○) riboflavin, (△) thiamine, (X) l-ascorbic acid. Conditions: 3% albumin in 10 M urea, 10^3 R, 30°C.

the other hand, some amino acids such as sodium glutamate and some benzene-series hydrocarbons such as sodium benzoate protect the shape of the external envelope of the protein molecule from an activation effect of γ radiation [2]. The irradiated biological materials are rendered more stable with respect to the radiation damage if the irradiation temperature is lowered [3, 4]. The change in the shape of the external envelope of the protein molecule was estimated from the viscosity change and this change is given in Figs. 1-6.

The decrease in the reduced viscosity of the irradiated albumin molecule in urea solution containing the added substance may be attributed to a

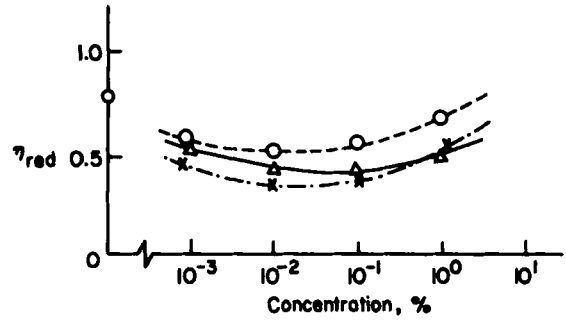

Fig. 4. Dependence of protective effect on concentration of chelating agents: (○) propylene glycol, (△) ethylenediaminetetraacetic acid, (X) ethylenediamine. Conditions: 3% albumin in 10 M urea, 10^3 R, 30°C.

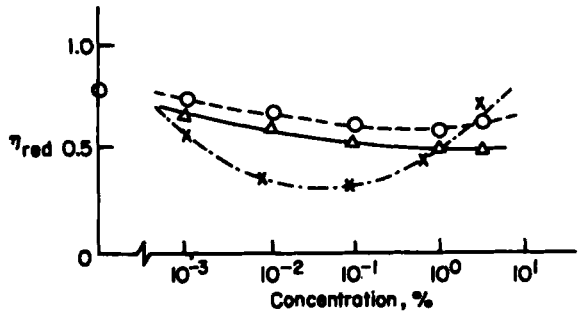

Fig. 5. Dependence of protective effect on concentration of S-containing compounds: (○) β,β'-thioglycolic acid, (△) thioglycol, (X) thiourea. Conditions: 3% albumin in 10 M urea, 10^3 R, 30°C.

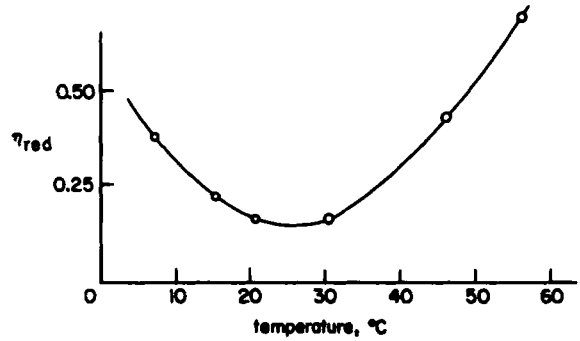

Fig. 6. Reduced viscosity as a function of temperature. Conditions: 3% albumin in 6 M urea, 10^3 R.

protection in the change in the shape of the external envelope of albumin molecule [2]. If the decrease in the reduced viscosity of albumin results from protection of the change in shape of the external envelope due to changes in the shape of the external envelope of albumin molecule, increased concentration of the added substances should result in protection against the changes in the shape of the external envelope of albumin molecule, and the reduced viscosity should continue to change under the concentration employed. This behavior indicates that the added substance protects the changes in the shape of the external envelope of albumin molecule from the activation effect of γ radiation, and that the protection effect closely parallels that found with sodium glutamate and sodium benzoate [2].

Activation in protein molecule by γ radiation may be attributed to the reaction of the activated protein molecules P* with other molecules, such as urea in this system [1]. The activated protein molecules may be formed as a direct result of γ radiation

$$P\text{–}P \longrightarrow P^* + P^*$$

Since the increase of the added substance concentration in percentage on the logarithmic scale changes the reduced viscosity of albumin, the protection of the changes in the shape of the external envelope of the protein molecule from the activation effect of γ irradiation must be due to the presence of the added substances. At the concentration studied the protection of the changes in the shape of the external envelope of the protein molecule may be due to the reaction of the added substances with the activated protein molecules formed by γ irradiation before they can attack the urea or interact with other protein molecules. The following processes were considered for the protective reaction

$$P^* + S \longrightarrow P\text{–}P + S^*$$

$$S^* \longrightarrow S + E_a$$

or

$$S^* \longrightarrow S'$$

where P–P is the protein molecule, P* is the activated protein molecule, S is the added substance, S* is the activated added substance, S′ is the reacted added substance, and E_a is the activation energy of γ rays.

For the present system the observed viscosity is expressed as a parabolic curve, a logarithmic abscissa for the added substance concentration in percentage

$$\eta_{red} = a(\log X)^2 + b(\log X) + c$$

This formula agrees with the experimental data that describe the curves in Figs. 1-5.

With an increase in the temperature, there is first a decrease, then a minimizing, and finally an increase in the reduced viscosity of the irradiated albumin molecule in urea solution that is inferred to be related to the temperature dependence on the changes in the shape of the external envelope of the albumin molecule.

The irradiated albumin molecule are rendered more stable with respect to the temperature effect on minimizing the reduced viscosity.

If T is the reaction temperature expressed in °C; T_0 is the temperature of $\eta_{red} = c$ (a minimum); and a, b, and c are adjustable constants, then

$$\eta_{red} = a(T - T_0)^2 + b(T - T_0) + c$$

This formula agrees with the experimental data which describe the curve in Fig. 6.

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