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Radiation Chemical Studies of Protein Reactions: Radiation Dose and Viscosity Change

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Synopsis

When protein was irradiated by gamma rays from a cobalt 60 source, an activated state was caused. An empirical equation for the viscosity change was obtained, and the phenomena were explained on the basis of the molecular mechanism. The general equation for the viscosity change is given by $\eta_{red} = (b + a \log R)(1 - e^{-kt})$, where η_{red} is the reduced viscosity of the solution, R is the gamma radiation dose, and t is time.

INTRODUCTION

Experiments have suggested that many molecules are rendered less stable with respect to the stable form if they are irradiated.[1] Since the reaction mechanism of protein denaturation is a problem of general interest, it was decided to investigate the effect of radiation on the urea denaturation of protein. Egg albumin was selected as the biological macromolecule, since it has previously been used in studies of the kinetics of protein denaturation.[2] The detection can be followed conveniently by measuring the viscosity change of the solution as a function of radiation dose.

EXPERIMENTAL

Materials

Albumin. The albumin used in this work was a commercial material produced by the Wakō Pure Chemical Industries, Ltd. In the preparation of the solutions the water content of the powder was first determined by drying a small, weighed sample for approximately 1 hr. at 105°C. The solution was then made up to the desired weight of protein per unit volume of solution by mixing the calculated weights of water and protein. The reduced viscosities are expressed as the reciprocal of grams per hundred milliliters of solution.

Urea. The urea was a commercial material produced by the Junsei Pure Chemical & Co., Ltd.

Apparatus and Procedure

An irradiation source containing about 300 curies of ^{60}Co was used. The dose rates in this experiment were 1.0×10^2–1.0×10^4 r./hr. The solid albumin was irradiated at room temperature.

In the viscosity measurements Ostwald-type viscometers were used. The temperature was maintained by thermostat at 30°C. Water times of 34–51 sec. were obtained by these viscometers. The viscometers were cleaned with fresh, filtered chromic acid solution and thoroughly rinsed and dried before each run. When not in use, they were kept filled with chromic acid solution or distilled water.

The results of the viscosity experiments are expressed in terms of the reduced viscosity:

$$\eta_{red} = \eta_{sp}/c = 1/c(\eta/\eta_0 - 1) \quad (1)$$

where c is the concentration of protein in g./100 ml. of solution, η_{sp} is the specific viscosity, η is the viscosity of the protein–urea mixture, η_0 is the viscosity of the "solvent," (a solution of the same composition as the protein–urea mixture, except that distilled water was added in place of the protein solution). Throughout this paper reduced viscosities will be expressed in units of the reciprocal of g./100 ml. of solution. The ratio η/η_0 was assumed to be equal to the ratio of the outflow time of the protein solution to that of the solvent. This ignores the kinetic energy effect and the small effect of protein on the density of the solution. The protein solutions and the urea solutions were prepared for each experiment. Urea concentrations are expressed in moles per liter of solution at 30°C. The protein solutions and the urea solutions were warmed to 30°C. before being mixed. A 7 ml. sample was then pipetted into the viscometer. The viscosity reading could usually be taken within 2 or 3 min. of mixing.

RESULTS

The changes in reduced viscosity of the albumin with time at various radiation doses were studied with 3% albumin in 10*M* urea. The results are shown in Figure 1.

From this it is clear that the reduced viscosity does not go to infinity but approaches a limiting value, and the increase in the reduced viscosity with increasing radiation dose on the logarithmic scale indicates an accelerating effect of the denaturation reaction.

DISCUSSION

It is known that in protein denaturation the rupture of many weak linkages, as a condition of the initial unfolding, and the rupture of relatively few strong, intramolecular crosslinks, as a cause of the subsequent loop-opening reaction, are required for the first stage and the second stage, respectively.

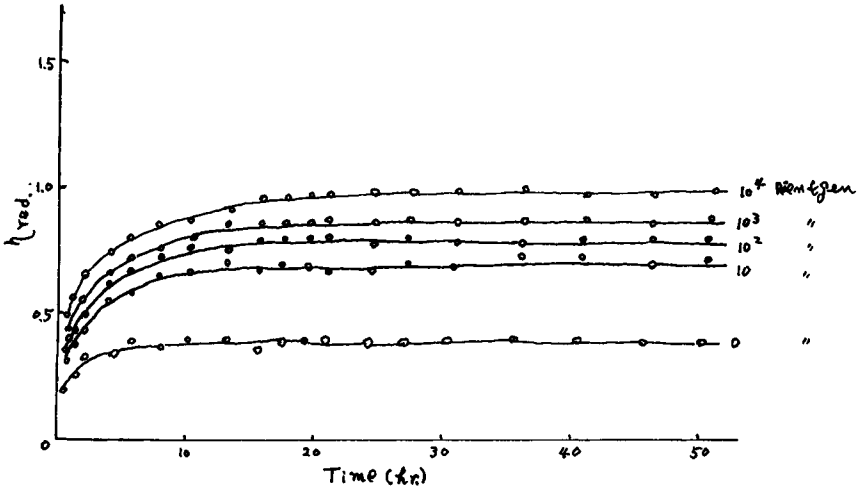

Fig. 1. Reduced viscosity versus time for various radiation doses (3% albumin in 10*M* urea).

The relation between radiation dose and viscosity change will now be discussed. The viscosity change required for the reaction of protein denaturation is estimated from Figure 1. Thus, the relation between the radiation dose and the viscosity change is parallel to that between the activation energy and the protein denaturation. When the concentrations of albumin and urea are constant, the increase in the radiation dose results in an increase in the activation energy required for the denaturation reaction. The reaction rate must, therefore, depend on the activation energy. If the main reactions for the protein denaturation are assumed to be

$$\text{P-P} \xrightarrow{h\nu} \text{P}^* + \text{P}^* \tag{2}$$

$$\text{P}^* + \text{U-U} \rightarrow \text{P-U} + \text{U}^* \tag{3}$$

$$\text{U}^* + \text{U}^* \rightarrow \text{U-U} \tag{4}$$

where P-P is the protein molecule and U-U is the urea molecule, the rate-determining step may be reaction (2), which means that the observed reaction rate is proportional to the activation energy. Therefore, the response of the albumin molecule to the radiation dose can be determined by measuring the reduced viscosity.

The phenomena, then, will be treated in terms of a molecular mechanism. In albumin molecules K^* is the number of activated linkages produced in 1 g. of irradiated albumin, N is the number of albumin molecules in 1 g. of irradiated albumin, M is the number of weak linkages in the irradiated albumin molecule, R is the radiation dose, and a is an adjustable constant. Then K^* is given by

$$K^* = NM = a \log R \tag{5}$$

Let $(\text{P-P})_{\text{intra}}$ be the number of intramolecular noncoils produced in 1 g. of irradiated albumin; the reaction rate of intramolecular noncoils $d(\text{P-P})_{\text{intra}}/dt$ will be proportional to the number of weak linkages M. If the probability of producing one intramolecular noncoil per weak linkage at unit time is K, one obtains

$$d(\text{P-P})_{\text{intra}}/dt = KT \tag{6}$$

As the increase in $(\text{P-P})_{intra}$ approaches the decrease in M,

$$-dM/dt = KM \tag{7}$$

Therefore,

$$(\text{P-P})_{\text{intra}} = M(1 - e^{-kt}) \tag{8}$$

Now, if the reaction rate of intramolecular noncoils $(\text{P-P})_{\text{intra}}/dt$ is proportional to the rate of viscosity change η_{red}/dt, one obtains

$$\eta_{\text{red}} = (b + a \log R)(1 - e^{-kt}) \tag{9}$$

This formula agrees with the experimental data that describe the curves in Figure 1.

The following mechanism was considered for the protein denaturation;

In this mechanism urea–protein linkages may be formed by a kind of linkage between the polypeptide chains and the urea molecules.

The ^{60}Co source was made available by Dr. T. Urai and his group of the First Research and Development Center, Technical Research and Development Institute, Defense Agency, Japan.

References

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