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Radiation Chemical Studies of Protein Reactions: Effect of Alkaline-Earth Metals on Viscosity

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ABSTRACT

Magnesium chloride, calcium chloride, strontium chloride, and barium chloride 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 concentration.

INTRODUCTION

It is well known that some alkaline-earth metals, such as calcium, interact with some biological macromolecule, such as protein [1], and also protect the changes of permeability of some biological macromolecular membranes, such as cell membranes, as induced by radiation [2, 3].

Since magnesium chloride, calcium chloride, strontium chloride, and barium chloride are well-known alkaline-earth metals, it was thought desirable to see 1) whether they showed such a protective property, and 2) what the effect of their concentration on changes in the shape of the external envelope of the protein molecule would be.

The urea denaturation of protein was selected for the study since it has been described in previous papers [4, 5]. The determination can be conveniently followed by measuring the reduced viscosity of the solution as a function of the concentration of the alkaline-earth metal.

EXPERIMENTAL

Materials

The albumin and the urea used in this work were the same as those described in a previous paper [4].

The magnesium chloride used was a commercial material produced by the Kanto Chemical Co., Inc.

The calcium chloride and the strontium chloride used were commerical materials produced by the Junsei Pure Chemical & Co., Ltd.

The barium chloride used was a commercial material produced by the Wako Pure Chemical Industries, Ltd.

Apparatus and Procedure

An irradiation source containing about 1500 Ci of ^{60}Co was used. The dose rate in this work was 1.2×10^4 R/hr. Solid albumin was irradiated in air at room temperature. The irradiated solid albumin was dissolved with distilled water and mixed with the urea solution containing the salt of the alkaline-earth metal. Then the viscosity was measured [5].

RESULTS

The changes in reduced viscosity of the albumin and of the salts of alkaline-earth metals were studied with 3% albumin in 10 M urea at 10^3 R and 30°C.

The salts of the alkaline-earth metals used, i.e., magnesium chloride, calcium chloride, strontium chloride, and barium chloride, were selected because their compounds are well known.

The results are shown in Fig. 1. It is clear that the effect of the alkaline-earth metal on the viscosity is apparently related to its inhibition of changes in the shape of the external envelope of the protein molecule.

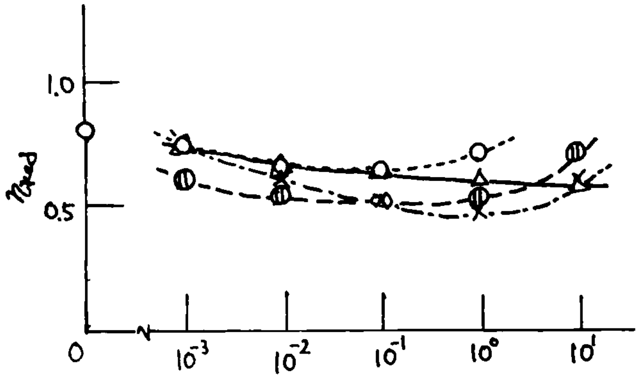

FIG. 1. Dependence of the protective effect on the concentration of the salt of alkaline-earth metals: (○) magnesium chloride, (△) strontium chloride, (×) barium chloride, and (⦶) calcium chloride. Conditions: 3% albumin in 10 M urea, 10^3 R, 30°C.

DISCUSSION

The change in the shape of the external envelope of the protein molecule was estimated from the viscosity change as shown in Fig. 1. Thus the relationship between the viscosity change and the concentration of the alkaline-earth metal is related to that between the change in the shape of the external envelope of the protein molecule and its inhibition. When the concentrations of protein and urea and the radiation dose are all constant, a change in the concentration of the salt of an alkaline-earth metal results in a change in the reduced viscosity for the shape of the external envelope of the protein molecule (see Fig. 1). This behavior closely resembles that found with sodium glutamate and sodium benzoate as shown by a similar dependence on concentration [5]. A change in the shape of the external envelope of the protein molecule by γ-radiation may lead to a change in reduced viscosity [4]. The activated protein molecules may be formed as a direct result of γ-radiation:

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

Since an increase in the concentration of the salts of alkaline-earth metals changes the reduced viscosity of proteins, protection from a change in shape of the external envelope of the protein molecule by radiation must be due to the presence of the salt of an alkaline-earth metal. At the concentration studied, the protection from a change in shape of the external envelope of the protein molecule by the salt of an alkaline-earth metal may be due to the interaction of the alkaline-earth metal with activated protein molecule formed by irradiation before it can attack the urea or interact with either protein molecule. The following process was assumed for the protective reaction:

$$P^* + S \longrightarrow P{-}S{-}P$$

$$P{-}S{-}P \longrightarrow P{-}P + S$$

where P—P is the protein molecule, P* is the activated protein molecule, S is the alkaline earth metal, and P—S—P is the linkage of the alkaline-earth metal ion to the protein molecule. The role played by the alkaline-earth metal may be understood if one assumes that it decreases the repulsion between the electrical charges on the protein molecules and permits them to approach more closely. If the activated protein molecules were important in causing the change of shape of the external envelope of the protein molecule, one would expect that the alkaline-earth metal ions would inhibit the change in shape of the external envelope of the protein molecule because they should moderate the activated protein molecules either by direct combination with them or by surrounding them with an atmosphere of ions.

In the mechanism the alkaline-earth metal ion may be bound at the peptide O,N of the protein molecule and protect the external envelope of the protein molecule from a change of shape due to radiation.

The observed reduced viscosity is expressed as a parabolic curve with a logarithmic abscissa for the concentration of the salt of alkaline-earth metal in percent;

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

This formula agrees with the experimental data that describe the curves in Fig. 1. This behavior of the alkaline-earth metal shows a dependence on the concentration similar to earlier experiments [5].

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