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Radiation Chemical Studies of Protein Reactions: Effect of Amino Acids and Vitamins on the Breaking of Secondary Bonding in Protein

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ABSTRACT

The radiation protective effect of the breaking of secondary bonding in protein was examined with such amino acids as disodium inosine-5'-monophosphate and disodium guanosine-5'-monophosphate, and vitamins such as thiamine and ℓ-ascorbic acid. The behavior of the viscosity change closely resembles that found with the sodium l-glutamate as shown by a similar dependence on concentration.

INTRODUCTION

Irradiation experiments have suggested that the breaking of secondary bonding in protein is accelerated by γ-radiation [1]. On the other hand, some amino acid such as ℓ-glutamate protect the breaking of secondary bonding in protein from the activation effect of γ-radiation [2].

It was thought desirable to see if such well-known materials as amino acids (such as disodium inosine-5'-monophosphate and disodium guanosine-5'-monophosphate) and vitamins (such as thiamine and ℓ-ascorbic acid) (1) show such a protective property, and (2) have an effect through concentration on the breaking of secondary bonding in protein.

The breaking of hydrogen bonds caused by urea in a gelatin molecule was selected for this study [1, 2]. The determination can be made by measuring the viscosity of the solution as a function of the concentration of amino acids or vitamins.

EXPERIMENTAL

Materials

The gelatin and urea used in this work were commercial materials produced by the Kanto Chemical Co. The thiamine used was a commercial material produced by the Takeda Chemical Industries. The ℓ-ascorbic acid used was a commercial material produced by the Junsei Pure Chemical Co. The disodium inosine-5'-monophosphate and the disodium guanosine-5'-monophosphate used were a gift from Dr. Y. Komata of the Ajinomoto Central Research Laboratory.

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. The solid gelatin was irradiated in air at room temperature. The irradiated solid gelatin was dissolved with urea solution containing an amino acid or a vitamin. Then the viscosity was measured [1, 2].

RESULTS

The effect of amino acids and vitamins on the breaking of secondary bonding in protein irradiated by γ-rays (10^3 R) was studied with a 5% gelatin in 8 M urea at 30°C.

The amino acids used, i.e., disodium inosine-5'-monophosphate and disodium guanosine-5'-monophosphate, and the vitamins, i.e., thiamin and ℓ-ascorbic acid, were selected because their compounds are well known.

In the presence of the amino acids and the vitamins, the reduced viscosity of the gelatin solutions decreased in a linear fashion on a logarithmic scale with the amino acids and the vitamins concentration

in percent, as shown in Figs. 1 and 2. The slope of the lines in Figs. 1 and 2 changed depending upon the structure of the amino acid and the vitamin.

From these results it is clear that the effect of amino acids and vitamins on the viscosity is apparently related to its protection from the breaking of secondary bonding in protein. These effects can be presented in the following order of concentration dependency: disodium inosine-5'-monophosphate > disodium guanosine-5'-monophosphate, and thiamine > ℓ-ascorbic acid.

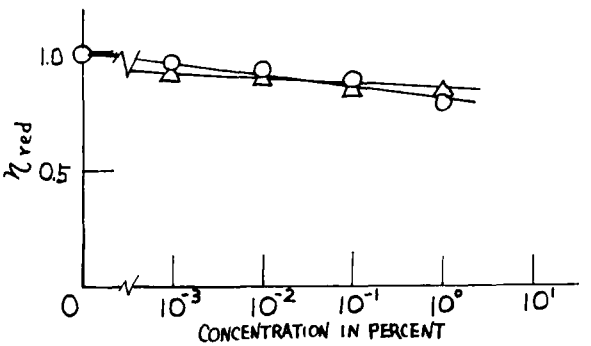

FIG. 1. Dependence of the protective effect on the concentration of amino acids (○, disodium inosine-5'-monophosphate; △, disodium guanosine-5'-monophosphate); 5% gelatin in 8 M urea, 10^3R, and 30°C.

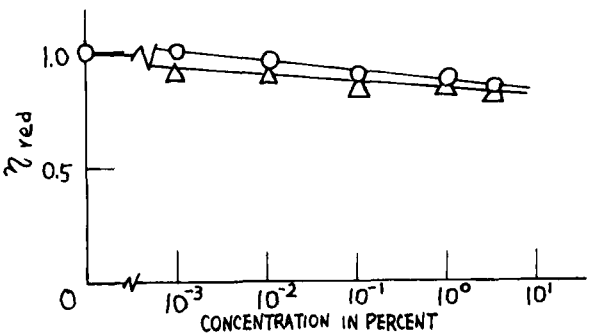

FIG. 2. Dependence of the protective effect on the concentration of vitamins (○, thiamine; △, ℓ-ascorbic acid); 5% gelatin in 8 M urea, 10^3R, and 30°C.

DISCUSSION

The effect of the amino acids and the vitamins against radiation was estimated from the viscosity, as shown in Figs. 1 and 2. The decrease in the reduced viscosity of the irradiated gelatin in urea solution containing the amino acids or vitamins may be attributed to a protection against the breaking of secondary bonding in the gelatin molecule [2]. If the decrease in the reduced viscosity of gelatin results from a protection of the structural change due to the scission of hydrogen bonds, increased concentration of the amino acids or the vitamins should result in protection against scission of hydrogen bonds, and the reduced viscosity should continue to decrease under the concentration employed. This behavior indicates that amino acids and vitamins protect against the breaking of secondary bonding in gelatin molecule from the activation effect of γ-radiation, and the protection effect closely parallels that found with sodium ℓ-glutamate [2].

Activation in protein molecules by γ-radiation may be attributed to the reaction of the activated protein molecule 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.

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

Since the increase of the concentration of amino acids and vitamins in percent on the logarithmic scale decreases the reduced viscosity by γ-radiation must be due to the presence of the amino acids and the vitamins. At the concentration studied, protection from the scission of hydrogen bonds by the amino acids and vitamins may be due to the reaction of the amino acids or vitamins with the activated gelatin molecules formed by irradiation before they can attack the urea or interact with other gelatin molecules. The following process was assumed for the protective reaction

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

where P—P is the gelatin molecule, P* is the activated gelatin molecule, and S and S' are the amino acid or vitamin.

For the present system the observed viscosity is expressed in a linear fashion on a logarithmic scale with the concentration of the amino acid and the vitamin in percent by

$$\eta_{red} = b - a \log X$$

This formula agrees with the experimental data described in Figs. 1 and 2. This reduced viscosity behavior shows a dependence on the concentration similar to that of earlier experiment [2].

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