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Mizuho Nisizawa[a]
[a] Department of Chemistry, Defense Academy, Yokosuka, Japan

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

MIZUHO NISIZAWA

Department of Chemistry
Defense Academy
Yokosuka, Japan

SUMMARY

The radiation protective effect of the breaking of secondary bonding in proteins was examined with 1,2 diamine and 1,2 dihydroxy derivatives such as ethylenediamine, ethylenediaminetetraacetic acid, and propylene glycol, and monosubstituted amines and alcohols such as ethylamine and n-propyl alcohol. An empirical equation for the viscosity change was obtained.

INTRODUCTION

It is well known that chelating agent such as mercaptoethylamine, diethyldithiocarbamate, and ethylenediaminetetraacetic acid show a protective effect against radiation damage on biological macromolecules such as protein or hyaluronic acid [1, 2].

The effect of a radical-scavenger such as a chelating agent against radiation of biological macromolecules is of interest since structural changes in biological macromolecules were caused by breaking of inter-or intramolecular bonds [3]. It was therefore decided to investigate the effect of chelating agents such as ethylenediamine, ethylenediaminetetraacetic acid, and propylene glycol against radiation in the breaking of secondary bonds in protein.

The breaking of hydrogen bonds caused by urea in a gelatin molecule was selected for this study [4, 5, 7]. The determination can be made by measuring the viscosity of the solution as a function of the chelating agent concentration.

EXPERIMENTAL

Materials

The gelatin and urea used in this work were commercial materials produced by the Kanto Chemical Co., Inc. The ethylenediamine, ethylenediaminetetraacetic acid, and ethylamine used were commercial materials produced by the Daiichi Pure Chemical Co., Ltd. The propylene glycol and n-propyl alcohol used were commercial materials produced by the Junsei Pure Chemicals Co., 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. Solid gelation was irradiated in air at room temperature. The irradiated solid gelatin was dissolved with urea solution containing chelating agent. Then the viscosity was measured [5].

RESULTS

The effect of chelating agents, such as 1,2-diamine and 1,2-dihydroxy derivatives, and control agents, such as monosubstituted amine and alcohol, on the breaking of secondary bonding in proteins irradiated by γ-rays (10^3 R) was studied with a 5% gelatin in 8 M urea at 30°C.

The chelating agents used, i.e., ethylenediamine, ethylenediaminetetraacetic acid and propylene glycol, and the control agents used, i.e., ethylamine and n-propyl alcohol, were selected because their compounds are well known.

In the presence of a chelating agent the reduced viscosity of the gelatin solutions decreased in a linear fashion on the logarithmic scale with the chelating agent concentration in per cent, as shown in Figs. 1-3. In the presence of a control agent the reduced viscosity of gelatin solutions did not decrease as shown in Figs. 4-5.

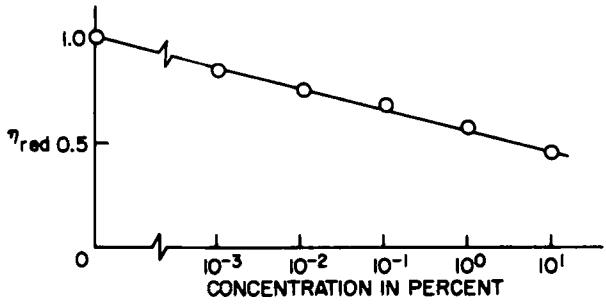

Fig. 1. Dependence of the protective effect on the concentration of ethylenediamine. Conditions: 5% gelatin in 8 M urea, 10^3 R, 30°C.

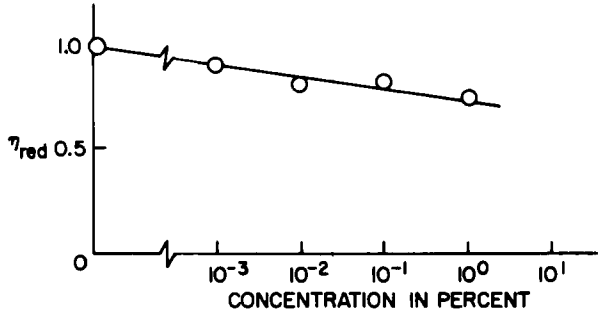

Fig. 2. Dependence of the protective effect on the concentration of ethylenediaminetetraacetic acid. Conditions: 5% gelatin in 8 M urea, 10^3 R, 30°C.

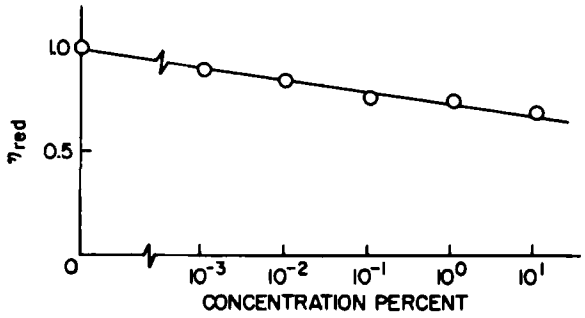

Fig. 3. Dependence of the protective effect on the concentration of propylene glycol. Conditions: 5% gelatin in 8 M urea, 10^3 R, 30°C.

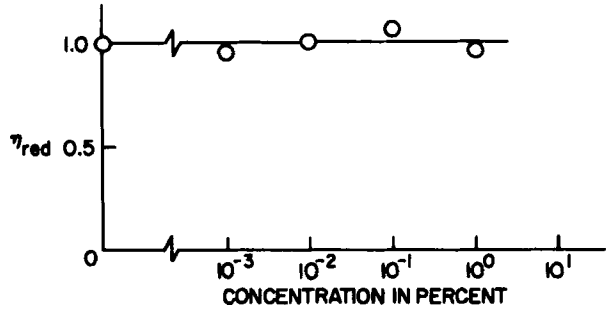

Fig. 4. Dependence of the protective effect on the concentration of ethylamine. Conditions: 5% gelatin in 8 M urea, 10^3 R, 30°C.

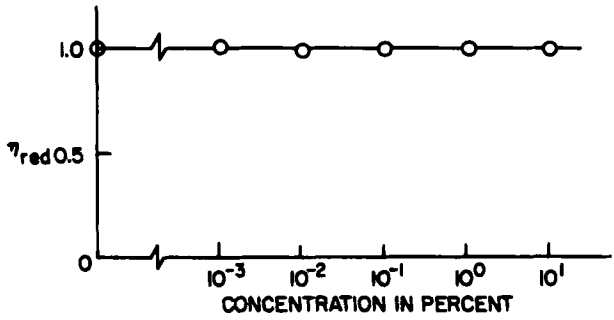

Fig. 5. Dependence of the protective effect on the concentration of n-propyl alcohol. Conditions: 5% gelatin in 8 M urea, 10^3 R, 30°C.

From these results it is clear that the effect of a chelating agent on the viscosity is apparently related to its protection from the breaking of secondary bonds in protein.

DISCUSSION

It is well known that chelating agent such as mercaptoethylamine, diethyldithiocarbamate, and ethylenediaminetetraacetic acid show a protective effect against radiation damage in biological macromolecules such as protein and hyaluronic acid [1, 2].

The effect of the chelating agent against radiation was estimated from the viscosity, as shown in Figs. 1-5. The decrease in the reduced viscosity of the irradiated gelatin in urea solution containing the chelating agent

may be attributed to protection from structural change. If the decrease in the reduced viscosity of gelatin results from protection of structural changes due to scission of hydrogen bonds, increased concentration of the chelating agent should result in protection against scission of hydrogen bonds, and the reduced viscosity should continue to decrease under the condition employed. Structural change in protein as a result of scission of secondary bonding caused by γ-irradiation has been described in previous papers [6, 7].

It was observed that addition of a chelating agent to a gelation solution after γ-irradiation reduces the values in the reduced viscosity of gelation. This behavior indicates that the chelating agent protects gelatin molecule from structural change as a result of the scission of hydrogen bonds caused by γ-radiation.

Scission of secondary bonds in protein by γ-radiation may be attributed to interaction of the activated protein molecule P* with molecules such as urea in this system. The activated protein molecules may be formed as a direct result of γ-radiation

$$\text{P–P} \xrightarrow{h\nu} \text{P*} + \text{P*}$$

It may be that the scission of secondary bonds in protein by γ-radiation could be moderated by the scavenging action of the chelating agent after irradiation, thereby reducing the number of activated protein molecules formed before structural change can occur. Since the increase of the chelating agent concentration in per cent on a logarithmic scale decreases the reduced viscosity of gelatin, the protection from the scission of hydrogen bonds caused by γ-radiation must be due to the presence of the chelating agent. At the concentration studied, protection from the scission of hydrogen bonds by the chelating agent may be due to the reaction of the chelating agent with the activated protein molecules formed by irradiation before they can attack the urea or interact with the protein molecules. The following process was assumed for the protective reaction

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

where P–P is the protein molecule, P* is the activated protein molecule, and S and S′ are the chelating agent.

For the present system, the observed reduced viscosity is expressed in linear fashion on a logarithmic scale with the chelating agent concentration in per cent by

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

This formula agrees with the experimental data that describe the curves in Figs. 1-3.

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