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Radiation Chemical Studies of Protein Reactions: Effect of Chelating Agents on Optical Rotation

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ABSTRACT

1,2-Diamine and 1,2-dihydroxy derivatives such as ethylenediamine, ethylenediaminetetraacetic acid and propylene glycol were found to protect against changes in the internal relationships of the atoms in the protein molecule from radiation damage. The optical rotation behavior closely resembles that found with amino acid as shown by a similar dependence on the concentration.

INTRODUCTION

It is well known that some chelating agent such as mercaptoethylamine, diethyldithiocarbamate, and ethylenediaminetetraacetic acid show a protective effect against radiation damage in biological macromolecule such as protein or hyaluronic acid [1, 2]. Since 1,2-diamine and 1,2-dihydroxy derivatives such as ethylenediamine, ethylenediaminetetraacetic acid, and propylene glycol are well-known chelating agent, it was thought desirable to see 1) whether they showed such a protective property and 2) what would be the effect of their concentration on changes in the internal relationships of the atoms in the protein molecule.

The urea denaturation of protein was selected as the change mechanism in the internal relationships of the atoms in the protein molecule because it has been described in previous papers [3, 4]. The determination can be followed conveniently by measuring the optical rotation of the solution as a function of the concentration of the chelating agent.

EXPERIMENTAL

Materials

The albumin and urea used in this work were the same as those described in a previous paper [3].

The ethylenediamine, ethylenediaminetetraacetic acid, and ethylamine used were commercial materials produced by Daiichi Pure Chemical Co., Ltd.

The propylene glycol and n-propyl alcohol used were commercial materials produced by Junsei Pure Chemicals Co., 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. The 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 chelating agent or control agent. Then the optical rotation was measured [4].

RESULTS

The changes, with time, in optical rotation of albumin and of the chelating agent (1,2-diamine and 1,2-dihydroxy derivatives such as ethylenediamine, ethylenediaminetetraacetic acid, or propylene glycol) or of control agent (monosubstituted amine and alcohol such as ethylamine or n-propyl alcohol) were studied with 2% albumin in 7 M urea, 10^3 R, and 30°C.

The chelating agent used and the control agent used were selected because their compounds are well known. The results are shown in Figs. 1-5. The relationship between the final specific rotation values and the percentage of chelating agent or control agent are shown in Figs. 6-10.

In the presence of the chelating agent, the specific rotation of

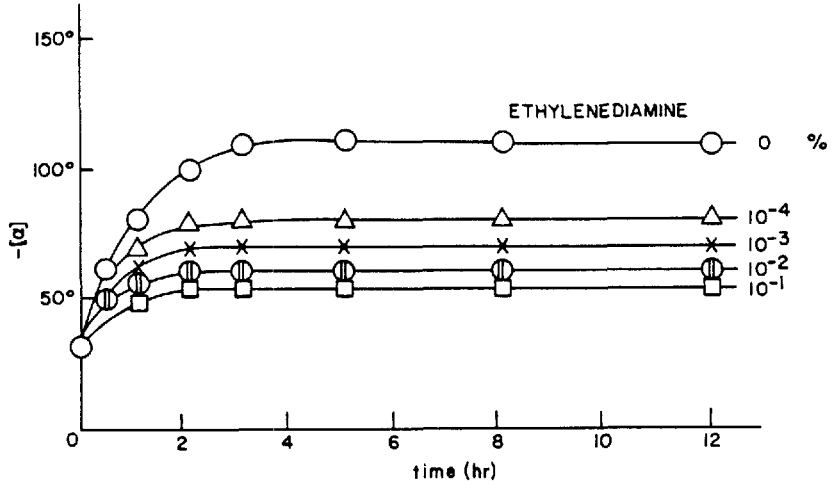

FIG. 1. Specific rotation vs. time; 2% albumin in 7 M urea, 10^3R and 30° C, in the presence and in the absence of ethylenediamine.

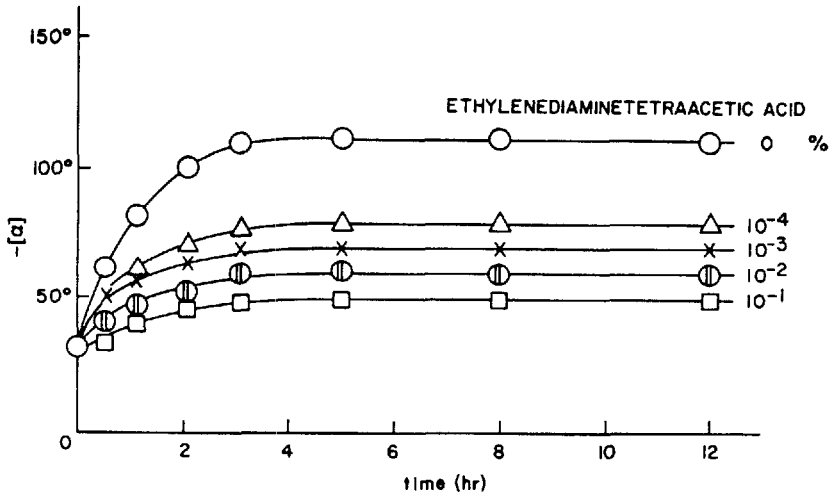

FIG. 2. Specific rotation vs. time; 2% albumin in 7 M urea, 10^3R and 30° C, in the presence and in the absence of ethylenediaminetetraacetic acid.

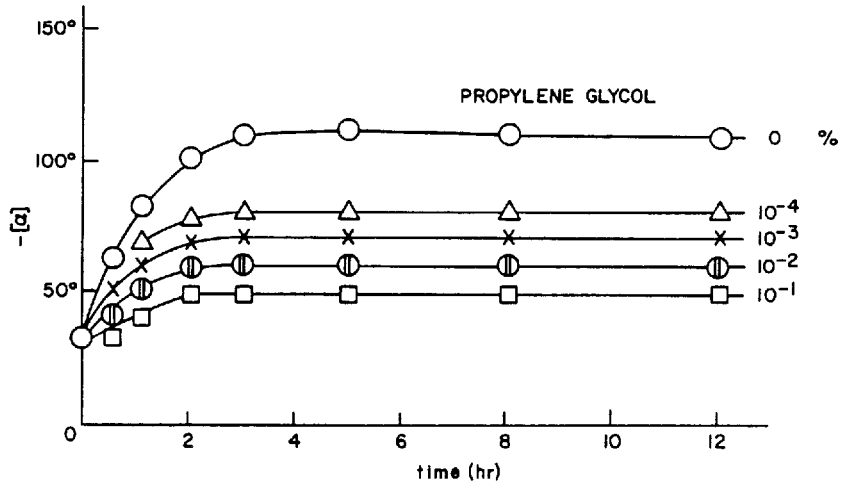

FIG. 3. Specific rotation vs. time; 2% albumin in 7 M urea, 10^3R and 30° C, in the presence and in the absence of propylene glycol.

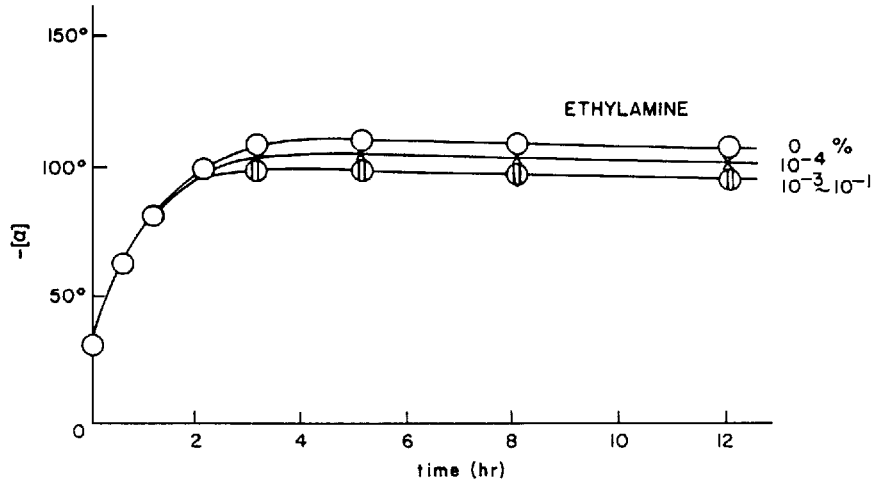

FIG. 4. Specific rotation vs. time; 2% albumin in 7 M urea, 10^3R and 30° C, in the presence and in the absence of ethylamine.

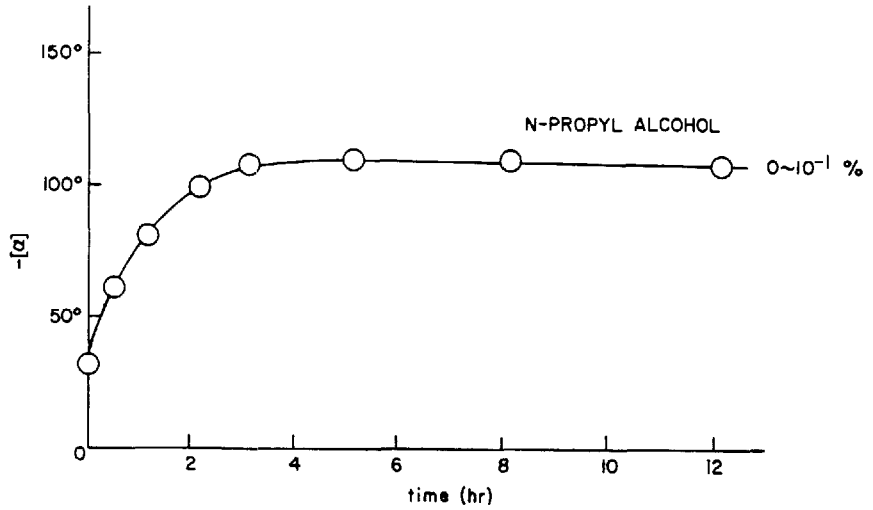

FIG. 5. Specific rotation vs. time; 2% albumin in 7 M urea, 10^3 R and 30° C, in the presence and in the absence of n-propyl alcohol.

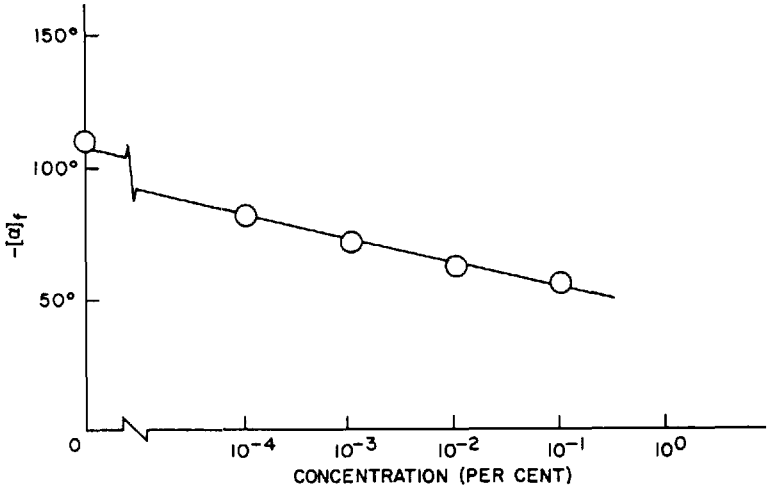

FIG. 6. Dependence of protective effect on the concentration of ethylenediamine; 2% albumin in 7 M urea, 10^3 R and 30° C.

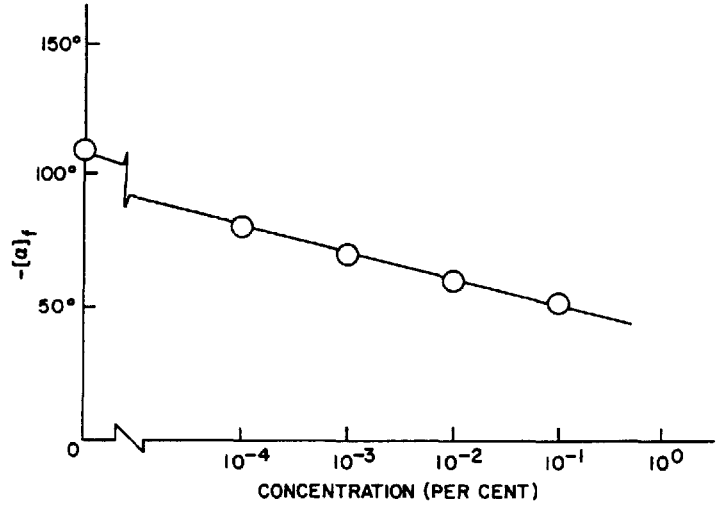

FIG. 7. Dependence of protective effect on the concentration of ethylenediaminetetraacetic acid; 2% albumin in 7 M urea, 10^3R and 30° C.

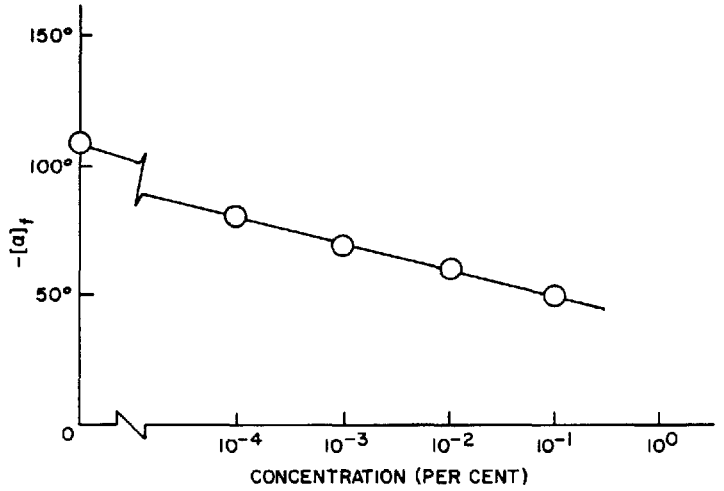

FIG. 8. Dependence of protective effect on the concentration of propylene glycol; 2% albumin in 7 M urea, 10^3R and 30° C.

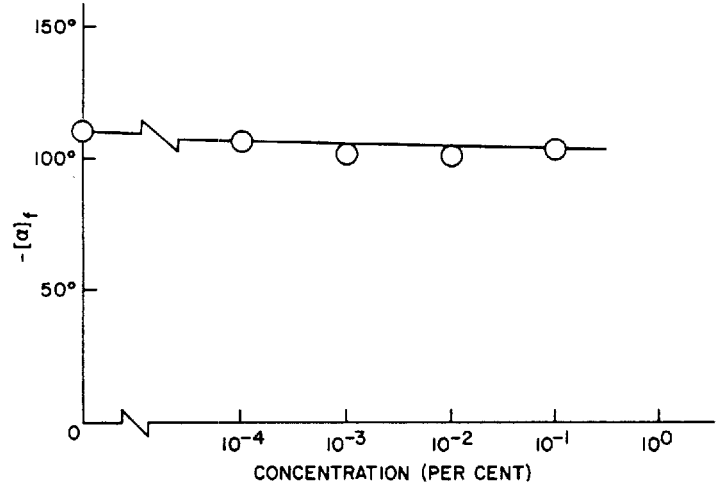

FIG. 9. Dependence of protective effect on the concentration of ethylamine; 2% albumin in 7 M urea, 10^3R and 30° C.

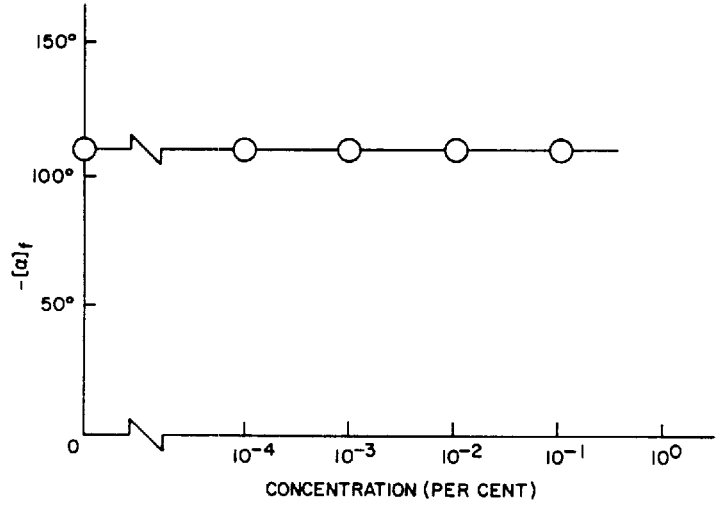

FIG. 10. Dependence of protective effect on the concentration of n-propyl alcohol; 2% albumin in 7 M urea, 10^3R and 30° C.

albumin solutions decreased in a linear fashion when the logarithmic abscissa was used for the concentration of the chelating agent in per cent, as shown in Figs. 6-8.

In the presence of the control agent, the specific rotation of albumin solutions did not decrease, as shown in Figs. 9-10.

From these graphs it is clear that the effect of the chelating agent on the optical rotation is apparently related to its protective action on the changes in the internal relationships of the atoms in the protein molecule.

DISCUSSION

As stated above, it is well known that some chelating agent such as mercaptoethylamine, diethyldithiocarbmate, and ethylenediaminetetraacetic acid show a protective effect against radiation damage in biological macromolecule such as protein or hyaluronic acid [1, 2].

The changes in the internal relationships of the atoms in protein molecule were estimated from the changes in optical rotation, as shown in Figs. 1-5. The relationship between the change in optical rotation and the concentration of the chelating agent is related to that between the change in the internal relationships of the atoms in protein molecule and its inhibition. When the concentration of protein and of urea and the radiation dose are all constant, a change in the concentration of chelating agent results in the change in optical rotation required for the internal relationships of the atoms in protein molecule; see Figs. 6-8. This behavior closely resembles that found with amino acid as shown by a similar dependence on the concentration [4]. The change in the internal relationships of the atoms in protein molecule by γ-radiation may be attributed to a change in optical rotation. The group activated by γ-radiation in the activated position in the vicinity of the asymmetric carbon atom in protein molecule may be attributed to the change in optical rotation [3]. Activated groups may be formed as a direct result of γ-radiation,

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

It may be that the groups activated by γ-radiation in the protein molecule could be moderated by a scavenging action of the chelating agent after irradiation, thereby reducing the number of activated groups formed in a protein molecule before the change in the internal relationships of the atoms in a protein molecule can occur. Since an increase in the concentration of chelating agent in per cent decreases the change in the specific rotation of protein, protection against change in the

internal relationships of the atoms in a protein molecule caused by γ-radiation must be due to the presence of the chelating agent. At the concentrations studied, this protection may be due to the scavenging reaction of the chelating agent with the activated groups formed in protein molecule by irradiation before it can attack the urea or interact with either group in a protein molecule. The following processes was assumed for the protective reaction:

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

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

where P—P is the group in the internal position in the vicinity of the asymmetric carbon atoms in the protein molecule, P* is the activated group, S is the chelating agent, and P—S—P is the chelating agent-group in protein linkage. These reactions are probably responsible for the protection against changes in the internal relationships of the atoms in protein molecule by chelating agent such as 1,2-diamine and 1,2-dihydroxy derivatives (ethylenediamine, ethylenediaminetetraacetic acid, or propylene glycol).

For the present system the observed specific rotation is expressed in linear fashion by adapting a logarithmic abscissa for the concentration of the chelating agent in per cent;

$$[a]f = b - a \log X$$

This formula agrees with the experimental data plotted in Figs. 6-8. This behavior of the chelating agent shows a concentration dependence similar to that of earlier experiments [4].

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