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# Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Liquids Containing Aromatic Hydrocarbons and pH on the Breaking of Secondary Bonding in Protein

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# Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Liquids Containing Aromatic Hydrocarbons and pH on the Breaking of Secondary Bonding in Protein

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## ABSTRACT

When protein in various liquids containing aromatic hydrocarbons, such as benzene, naphthalene, and phenanthrene, is irradiated by $\gamma$-rays from a $^{60}$Co source, the breaking of secondary bonding in the protein molecule varies with the irradiation liquids containing aromatic hydrocarbons. Protein irradiated by $\gamma$-rays from a $^{60}$Co source in air showed the effect of pH on the breaking of secondary bonding in the protein molecule. In both cases an empirical equation for the viscosity change was obtained, and the phenomena were explained on the basis of the molecular mechanism.

## INTRODUCTION

Irradiation experiments have suggested that macromolecules are more stable to radiation after physical mixing of aromatic hydrocarbons with the macromolecule [1-3], also that after increasing the pH the changes in the shape of the external envelope of the protein molecule irradiated and in the internal relationships of the atoms in the protein molecule irradiated increase [2, 3]. Since the effect of the irradiation of liquids containing aromatic hydrocarbons and the effect of pH on protein reactions are problems of general interest [2, 3], it was felt important to see (1) the effect, if any, of the irradiation liquid, and (2) the èffect of pH on the breaking of secondary bonding in protein molecule.

The breaking of hydrogen bonds in gelatin molecules caused by urea was selected to study the breaking of secondary bonding in protein [4-7]. The determination can be conveniently followed by measuring the reduced viscosity of the solutions [2, 8].

## EXPERIMENTAL

### Materials

The gelatin, urea, sodium hydroxide, boric acid, and phenanthrene used in this work were commercial materials produced by the Junsei Pure Chemical Co. The carbon tetrachloride and benzene used were commercial materials produced by the Kanto Chemical Co., respectively. The naphthalene and phosphric acid used were commercial materials produced by the Wako Chemical Industries. The acetic acid used was a commercial material produced by the Ieda Pure Chemical Ltd.

### Apparatus and Procedure

An irradiation source containing about 1500 Ci of $^{60}Co$ was used. The dose rate in this work was $1.2 \times 10^4$ R/hr.

In the studies of the effect of the irradiation of liquids the solid gelatin was put into irradiation bottles and the bottles were filled with the irradiation liquid (carbon tetrachloride) containing various amounts of benzene, naphthalene, or phenanthrene. The irradiation was carried out at room temperature. The irradiated solid gelatin was cleaned with fresh carbon tetrachloride, dried at 30°C under vacuum, and dissolved with urea solution. Then the viscosity was measured at 30°C [2, 8].

In the pH studies, the solid gelatin was irradiated in air at room temperature. The irradiated gelatin was dissolved with urea-buffer mixtures, and then the viscosity was measured at 30°C [2, 8].

The pH values were measured with a glass electrode pH meter.

## RESULTS

### Effect of Irradiation Liquid

Changes in the reduced viscosity of gelatin irradiated by $\gamma$-rays ($10^3$ R) in liquid containing small amounts of aromatic hydrocarbons were studied with a 5% gelatin in 8 M urea at 30°C.

Irradiation liquid and aromatic hydrocarbons used were carbon tetrachloride (because it is a nondenaturant), benzene, naphthalene, and phenanthrene (because they have been used previously to study changes in the shape of the external envelope of the protein molecule and in the internal relationships of the atoms in the protein molecule [2, 3].

Experimental results are shown in Figs. 1-3. From these it is clear that with increasing concentration of aromatic hydrocarbons, the reduced viscosity first decreases, reaches a minimum, and then increases. The minimum in the reduced viscosity indicates the maximum effective protective effect for the breaking of secondary bonding in the protein molecule.

### Effect of pH

The changes in the reduced viscosity of gelatin irradiated by $\gamma$-rays ($10^3$ R) in buffer solutions of various pH values were studied with a 5% gelatin in 8 M urea at 30°C.

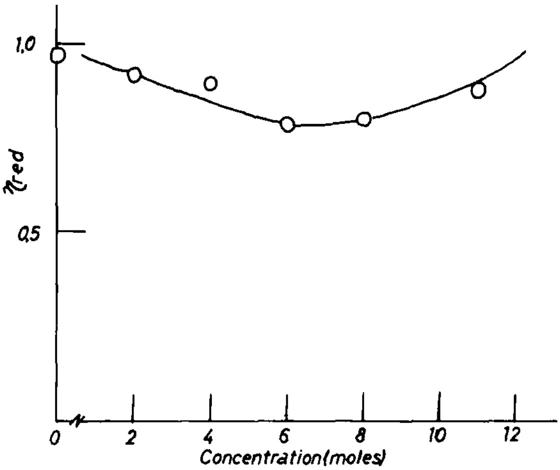


FIG. 1. Dependence of the protective effect on the concentration of benzene (in $CCl_4$ as irradiation liquids). Conditions: 5% gelatin in 8 M urea, $10^3$ R, 30°C.

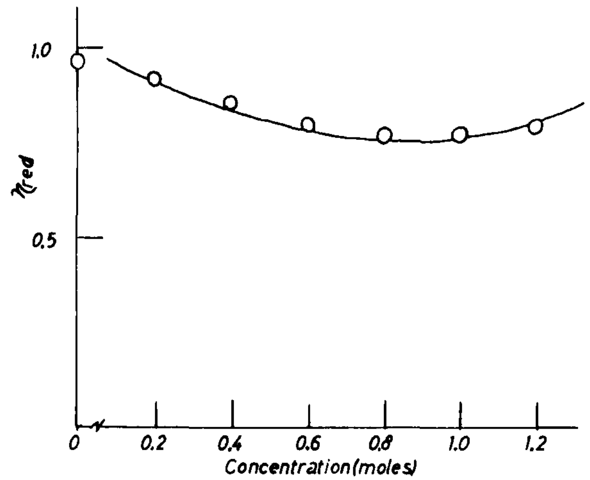


FIG. 2. Dependence of the protective effect on the concentration of naphthalene (in $CCl_4$ as irradiation liquids). Conditions: 5% gelatin in 8 M urea, $10^3$ R, 30°C.

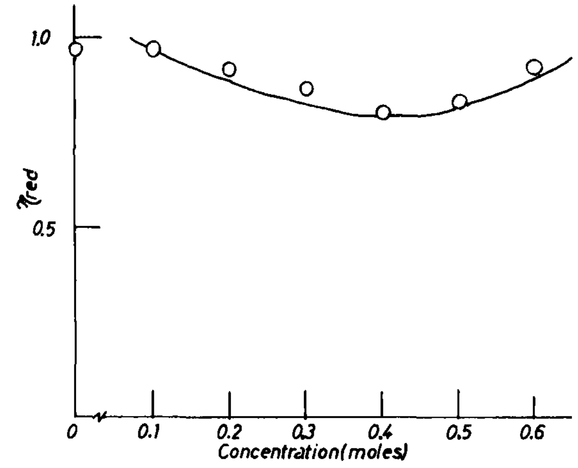


FIG. 3. Dependence of the protective effect on the concentration of phenanthrene (in $CCl_4$ as irradiation liquids). Conditions: 5% gelatin in 8 M urea, $10^3$ R, 30°C.

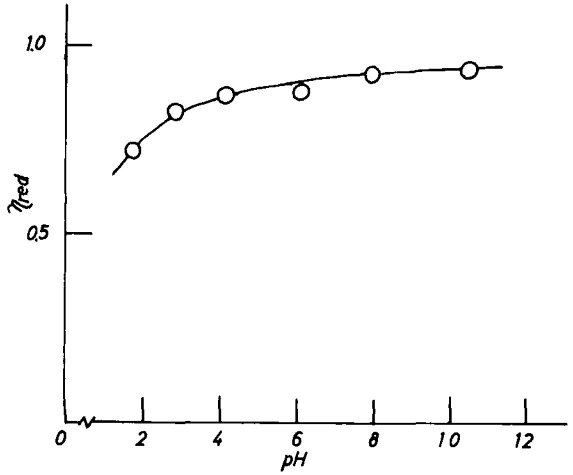


FIG. 4. Reduced viscosity as a function of pH. Conditions: 5% gelatin in 8 M urea, $10^3$ R, 30°C.

Experimental results are shown in Fig. 4. From these it is clear that with increasing pH the reduced viscosity of the irradiated gelatin in urea increases. This increase shows the effect of pH on the breaking of secondary bonding in the protein molecule.

## DISCUSSION

The relation between the change in reduced viscosity and the concentration of aromatic hydrocarbons is related to that between the breaking of hydrogen bonds in the gelatin molecule and its inhibition. When the concentration of gelatin and urea and the radiation dose are all constant, a change in the concentration of aromatic hydrocarbons results in a change of the activation required for the breaking of hydrogen bonds in gelatin molecule; see Figs. 1-3. The reaction mechanism must, therefore, depend on the concentration of the aromatic hydrocarbons. If the main processes for the protective action are assumed to be

$$P{-}P \xrightarrow{h\nu} P^* + P^* \tag{1}$$

$$P^* + P^* \longrightarrow P{-}P + Ea \tag{2}$$

$$A + Ea \longrightarrow A^* \tag{3}$$

$$A^* \longrightarrow A + (Ea - Er) \tag{4}$$

$$P{-}P + (Ea - Er) \longrightarrow P^* + P^* \tag{5}$$

where P—P is the gelatin molecule, P* is the irradiated gelatin molecule, Ea is the activation energy of $\gamma$-rays, A is the aromatic hydrocarbons, A* is the activated aromatic hydrocarbons, and Er is the resonance energy of aromatic hydrocarbons, then the protective step is Reaction (3), which means that the observed protective effect follows a parabolic curve vs the concentration of aromatic hydrocarbons. Therefore the response of the breaking of hydrogen bonds in gelatin molecule to aromatic hydrocarbons may be determined by measuring the reduced viscosity.

The phenomena, then, will be treated in terms of a molecular mechanism. If in the system the loss rate of activation energy of $\gamma$-rays by the aromatic hydrocarbons $d(P^*)_{loss}/dx$ is proportional to the concentration of aromatic hydrocarbonx X, and also the activation rate by fluorescene of aromatic hydrocarbons $d(P^*)_{act}/dx$ is proportional to the concentration of aromatic hydrocarbons X, then the total activation rate of $\gamma$-rays by the aromatic hydrocarbons $d(P)/dx$ is given by

$$d(P)/dx = d(P^*)_{loss}/dx + d(P^*)_{act}/dx = a1X + a2X + b1 + b2 \tag{6}$$

If the total activation rate of $\gamma$-rays by the aromatic hydrocarbons $d(P)/dx$ is proportional to the rate of reduced viscosity $d(\eta_{red})/dx$, then

$$d(P)/dx = d(\eta_{red})/dx \tag{7}$$

From Eqs. (6) and (7)

$$d(\eta_{red})/dx = (a1 + a2)X + b1 + b2 \tag{8}$$

Integration of Eq. (8) yields

$$\eta_{red} = (1/2)(a1 + a2)X^2 + (b1 + b2)X + c \tag{9}$$

Stated otherwise, Eq. (9) becomes

$$\eta_{red} = aX^2 + bX + c \tag{10}$$

This formula agrees with the experimental data plotted in Figs. 1-3.

In this mechanism these aromatic hydrocarbons may be involved in energy loss by fluorescence from the electron system of the aromatic rings.

Second, the relation between the change in reduced viscosity and the pH change is related to the swelling of the gelatin molecule caused by the electrostatic repulsion associated with the increased net charge of the molecule activated by $\gamma$-rays. When the concentration of gelatin and urea and the radiation dose are constant, the increase in pH results in an increase of the reduced viscosity required for the breaking of hydrogen bonds in the gelatin molecule; see Fig. 4. The reaction mechanism must, therefore, depend on the pH. If the main processes for the effect of pH are assumed to be

$$P{-}P \longrightarrow P^* + P^* \qquad (11)$$

$$P^* \xrightarrow{OH^-} P \qquad (12)$$

where P—P is the gelatin molecule, P* is the irradiated gelatin molecule, and $OH^-$ is a hydroxyl radical, the rate-determining step is Reaction (12), which means that the observed viscosity change is related to the pH value. Therefore the response of the breaking of hydrogen bonds in the gelatin molecule to the $OH^-$ radical may be determined by measuring the reduced viscosity.

The phenomena, then, will be treated in terms of a molecular mechanism. In gelatin molecules K* is the number of activated hydrogen bonds produced in 1 g of irradiated gelatin, N is the number of gelatin molecules in 1 g of irradiated gelatin, M is the number of hydrogen bonds in irradiated gelatin molecule, and X is the concentration of hydroxyl radical. Then K* is given by

$$K^* = NM \qquad (13)$$

Let $(P{-}P)_{sec}$ be the number of broken hydrogen bonds in 1 g of irradiated gelatin; the breaking rate of hydrogen bonds $d(P{-}P)_{sec}/dx$ will be proportional to the number of hydrogen bonds M. If the probability of the breaking one hydrogen bond per molecule at unit $OH^-$ concentration is K, then

$$d(P{-}P)_{sec}/dx\ KM \qquad (14)$$

As the increase in $(P{-}P)_{sec}$ approaches the decrease in M

$$-dM/dx = KM \tag{15}$$

Therefore

$$(P{-}P)_{sec} = M_0(1 - e^{-kx}) \tag{16}$$

Now, if the breaking rate of hydrogen bonds $d(P{-}P)_{sec}/dx$ is proportional to the rate of viscosity change $d(\eta_{red})/dx$, then

$$\eta_{red} = a(1 - e^{-kx}) + b \tag{17}$$

This formula agrees with the experimental data plotted in Fig. 4.

This reduced viscosity behavior shows a dependence on the concentration of aromatic hydrocarbons and pH similar to that of earlier experiments [2, 3].

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