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# Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Liquids Containing Aromatic Hydrocarbons and pH on Optical Rotation

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# Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Liquids Containing Aromatic Hydrocarbons and pH on Optical Rotation

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## SUMMARY

When protein in various liquids containing aromatic hydrocarbons, such as benzene, naphthalene, and phenanthrene, is irradiated by γ-rays from a $^{60}Co$ source the changes in the internal relationships of the atoms in the protein molecule vary with the irradiation liquids containing aromatic hydrocarbons. An empirical equation for the optical rotation was obtained, and the phenomena were explained on the basis of the molecular mechanism. Protein irradiated by γ-rays from a $^{60}Co$ source in air showed the effect of pH on the changes in the internal relationships of the atoms in the protein molecule. An empirical equation for the optical rotation 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 on physical mixing of aromatic hydrocarbons with the

macromolecule [1, 2], and also that on increasing the pH the changes in the shape of the external envelope of the protein molecule irradiated increase [2]. Since the effect of irradiation liquid containing aromatic hydrocarbons and the effect of pH on protein reaction are problems of general interest [2], it was therefore felt important to see 1) the effect, if any, of the irradiation liquid, and 2) the effect of pH on the internal relationships of the atoms in protein molecule.

The urea denaturation of protein was selected to study the changes in the internal relationships of the atoms in the protein molecule [3, 4]. The determination can be conveniently followed by measuring the optical rotation of the solutions [3].

## EXPERIMENTAL

### Materials

The materials used in this work were the same as those described in the previous paper [2].

### Apparatus and Procedure

An irradiation source containing about 300 C of $^{60}Co$ was used. The dose rate in this experiment was $1.7 \times 10^3$ R/hr.

In the studies of the effect of irradiation liquid, the solid albumin was put into each irradiation bottle and the bottles were filled with irradiation liquid (carbon tetrachloride) containing various amounts of benzene, naphthalene, or phenanthrene. The irradiation was carried out at room temperature. The irradiated solid albumin was cleaned with fresh carbon tetrachloride, dried at 30°C under vacuum, powdered, dissolved with distilled water, and mixed with urea solution. Then the optical rotation was measured [3].

In the pH studies the solid albumin (powder) was irradiated in air at room temperature. The irradiated albumin was dissolved with distilled water and mixed with urea–buffer mixtures. Then the optical rotation was measured [3].

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

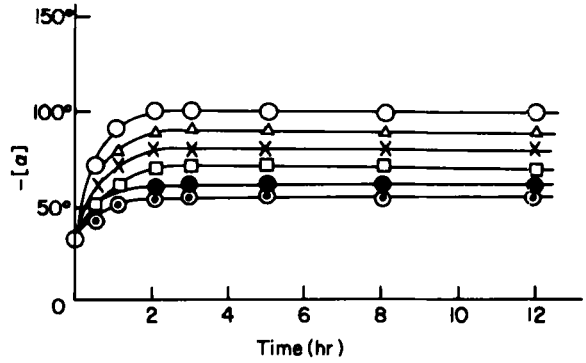


**Fig. 1.** Specific rotation vs. time in the presence of various amounts of benzene in $CCl_4$: (○) none, (X) 2 mole, (□) 4 mole, (⊙) 6 mole, (⊘) 8 mole, (△) 11 mole. Conditions: 2% albumin in 7 M urea, $10^3$ R, 30°C.



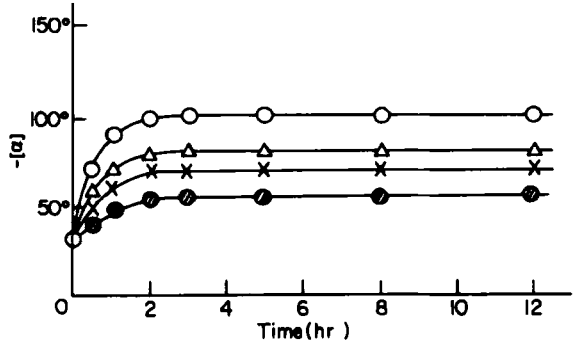


**Fig. 2.** Specific rotation vs. time in the presence of various amounts of naphthalene in $CCl_4$: (○) none and 1.2 mole, (△) 0.2 and 1 mole, (X) 0.4 and 0.8 mole, (⊘) 0.6 mole. Conditions: 2% albumin in 7 M urea, $10^3$ R, 30°C.

## RESULTS

### Effect of Irradiation Liquid

The changes with time in optical rotation of albumin irradiated by γ-rays ($10^3$ R) in liquid containing small amounts of aromatic hydrocarbons were studied with a 2% albumin in 7 M urea at 30°C.

Irradiation liquid and aromatic compounds used were carbon tetrachloride

(because it is a nondenaturant), benzene, naphthalene, and phenanthrene (because they have previously been used to study the changes in the shape of the external envelope of the protein molecule) [2].

Experimental results are shown in Figs. 1-3. From these it is clear that the specific rotation does not continue to increase but approaches a limiting value. When the values of the final specific rotation in Figs. 1-3 are plotted against the concentration of aromatic hydrocarbons, the relationships shown in Figs. 4-6 are obtained. With increasing concentration of aromatic hydrocarbons the specific rotation first decreases, reaches a minimum, and then increases. The minimum in the final specific rotation indicates the maximum effective protective effect for the changes in the internal relationships of the atoms in the protein molecule.

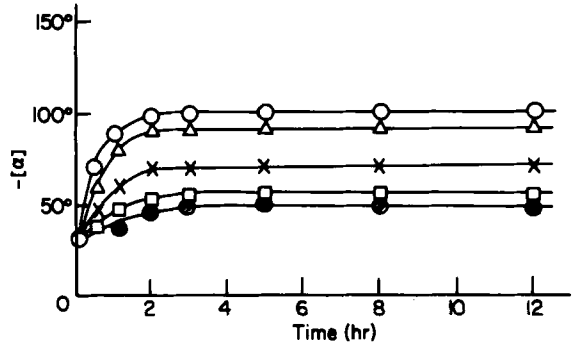


**Fig. 3.** Specific rotation vs. time in the presence of various amounts of phenanthrene in $CCl_4$: (○) none, (△) 0.1 and 0.7 mole, (×) 0.2 and 0.6 mole, (□) 0.3 and 0.5 mole, (●) 0.4 mole. Conditions: 2% albumin in 7 M urea, $10^3$ R, 30°C.

## Effect of pH

The changes with time in the specific rotation of albumin irradiated by γ-rays ($10^3$ R) in buffer solution at various pH values was studied with 2% albumin in 7 M urea at 30°C.

Experimental results are shown in Fig. 7. From these it is clear that the specific rotation does not continue to increase, but approaches a limiting value. When the values of the final specific rotation in Fig. 7 are plotted against the pH values, the relation shown in Fig. 8 is obtained. With increasing pH the final specific rotation of the irradiated albumin in urea increases. This increase shows the effect of pH on the changes in the internal relationships of the atoms in the protein molecule.

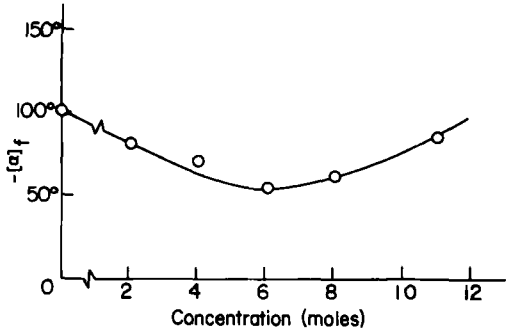


**Fig. 4.** Dependence of the protective effect on the concentration of benzene. Conditions: 2% albumin in 7 M urea, $10^3$ R, 30°C.

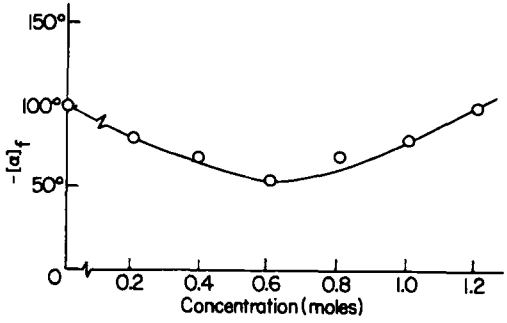


**Fig. 5.** Dependence of the protective effect on the concentration of naphthalene. Conditions: 2% albumin in 7 M urea, $10^3$ R, 30°C.

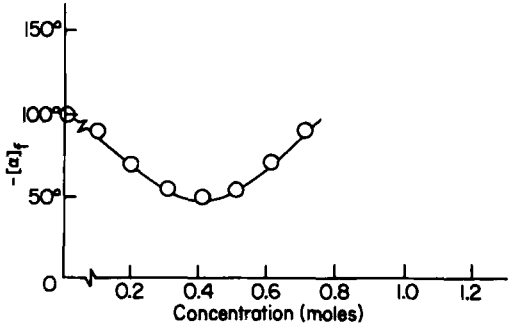


**Fig. 6.** Dependence of the protective effect on the concentration of phenanthrene. Conditions: 2% albumin in 7 M urea, $10^3$R, 30°C.

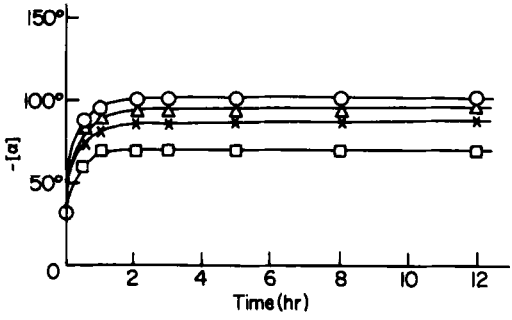


**Fig. 7.** Specific rotation vs. time at various pH values: (□) 2.1, (X) 4.1, (△) 6.2 and 8.1, (○) 10.0 and 12.5. Conditions: 2% albumin in 7 M urea, $10^3$ R, 30°C.

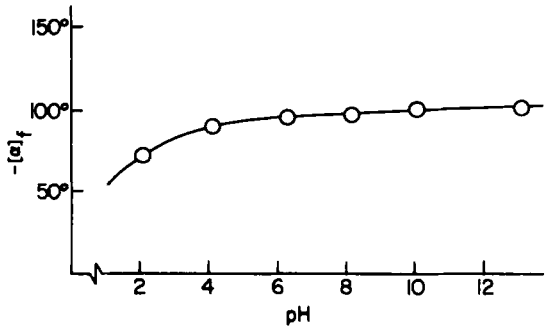


**Fig. 8.** Specific rotation as a function of pH. Conditions: 2% albumin in 7 M urea, $10^3$ R, 30°C.

## DISCUSSION

As stated above, it is known that macromolecules can be more stable to radiation by physical mixing of aromatic hydrocarbons [1, 2], and also that on increasing the pH the changes in the shape of the external envelope of the protein molecule irradiated increase [2]. The changes in the internal relationships of the atoms in protein molecule is estimated from the changes in optical rotation as shown in Figs. 1-8.

First, the relation between the change in optical rotation and the concentration of aromatic hydrocarbons is related to that between the change in

the internal relationships of the atoms in the protein molecule and its inhibition. When the concentration of protein and urea and the radiation dose are constant, a change in the concentration of aromatic hydrocarbons results in a change of activation required for the change in the internal relationships of the atoms in the protein molecule; see Figs. 1-6. The reaction mechanism must, therefore, depend on the concentration of aromatic hydrocarbons. If the main processes for the protective action are assumed to be

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

$$P^* + P^* \longrightarrow P\text{–}P + E_a \tag{2}$$

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

$$A^* \longrightarrow A + (E_a - E_r) \tag{4}$$

$$P\text{–}P + (E_a - E_r) \longrightarrow P^* + P^* \tag{5}$$

where P–P is the group in the initial position in the vicinity of the asymmetric carbon atoms in the albumin molecule, P* is the activated group in the activated position in vicinity of the asymmetric carbon atoms in the irradiated albumin molecule, $E_a$ is the activation energy of $\gamma$-rays, A is the aromatic hydrocarbons, A* is the activated aromatic hydrocarbons, and $E_r$ is the resonance energy of aromatic hydrocarbons. 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 internal relationships of atoms in protein molecule to aromatic hydrocarbons may be determined by measuring the specific rotation.

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 hydrocarbons x and also the activation rate by fluorescence 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 γ-rays by the aromatic hydrocarbons d(P)/dx is proportional to the rate of change in optical rotation $[\alpha]/dx$, then

$$d(P)/dx = d[\alpha]/dx \tag{7}$$

From Eqs. (6) and (7)

$$d[\alpha]/dx = (a1 + a2)X + b1 + b2 \tag{8}$$

Integration of Eq. (8) yields

$$[\alpha] = \frac{(a1 + a2)}{2} X^2 + (b1 + b2)X + c \tag{9}$$

Stated otherwise, Eq. (9) becomes

$$[\alpha] = aX^2 + bX + c \tag{10}$$

This formula agrees with the experimental data plotted in Figs. 4-6.

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 changes in optical rotation and the pH changes is related to the swelling of protein molecule caused by the electrostatic repulsion associated with the increased net charge of the molecule activated by γ-rays. When the concentration of protein and urea and the radiation dose are constant, the increase in pH results in an increase of the optical rotation required for the changes in the internal relationships of atoms in the protein molecule; see Fig. 7. The reaction mechanism must, therefore, depend on the pH. If the main processes for the effect of pH are assumed to be

$$P\text{–}P \xrightarrow{h\nu} P^* + P^* \tag{11}$$

$$P^* \xrightarrow{OH^-} P \tag{12}$$

where P–P is the group in the initial position in the vicinity of the asymmetric carbon atoms in the albumin molecule, P* is the activated group in the activated position in the vicinity of the asymmetric carbon atoms in the irradiated albumin molecule, and $\dot{O}H^-$ is a hydroxyl radical, the rate-determining step is Reaction (12), which means that the observed

optical rotation is related to the pH value. The response of the internal relationships of atoms in protein molecule to $OH^-$ radical may therefore be determined by measuring the specific rotation.

The phenomena, then, will be treated in terms of a molecular mechanism. In albumin molecules K* is the number of activated groups in the activated position in the vicinity of the asymmetric carbon atoms in 1 g of irradiated albumin, N is the number of albumin molecule in 1 g of irradiated albumin, M is the number of groups in the position in the vicinity of the asymmetric carbon atoms in irradiated albumin molecule, and X is the concentration of hydroxyl radical. Then K* is given by

$$K^* = NM \tag{13}$$

Let $(P)_{inter}$ be the number of changed internal relationships of the atoms in 1 g of irradiated albumin; the changing rate of internal relationships $d(P)_{inter}/dx$ will be proportional to the number of activated groups M. If the probability of changing one internal relationships of the atoms per molecule at unit $OH^-$ concentration is k, then

$$d(P)_{inter}/dx = KM \tag{14}$$

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

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

Therefore

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

Now, if the changing rate of internal relationships of the atoms $(P)_{inter}/dx$ is proportional to the rate of change in optical rotation $[\alpha]/dx$, then

$$[\alpha] = a(1 - e^{-kx}) + b \tag{17}$$

This formula agrees with the experimental data plotted in Fig. 8.

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