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# Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Atmosphere and Temperature on the Breaking of Secondary Bonding in Protein

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# Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Atmosphere and Temperature on the Breaking of Secondary Bonding in Protein

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## ABSTRACT

When protein in various atmospheres such as $N_2$, $CO_2$, $O_2$, $H_2$, and $NH_3$ is irradiated by $\gamma$ rays from a $^{60}Co$ source, the radiation effects of the breaking of secondary bonding in protein vary with the gases composing the irradiation atmosphere. An empirical equation for the viscosity change was obtained. Protein irradiated by $\gamma$ rays showed the effect of temperature on the breaking of secondary bonding in protein. An activation energy required to break secondary bonding in irradiated protein was obtained.

## INTRODUCTION

Irradiation experiments have suggested that changes in the shape of the external envelope of the protein molecule and in the internal relationships of the atoms in the protein molecule are affected by irradiation atmospheres such as $N_2$, $O_2$, and $NH_3$ [1, 2], and also that on increasing the temperature the changes in the shape of the external envelope of the irradiated protein molecule and in the internal relationships of the atoms in the irradiated protein molecule are affected [2, 3]. The effects of irradiation atmospheres, such as $N_2$ and $O_2$, and temperature in biological macromolecules are of interest since structural changes in biological macromolecules are caused by the breaking of inter- or intramolecular bonds [4-12]. It was therefore decided to investigate the effects of irradiation atmospheres, such as $N_2$, $CO_2$, $O_2$, $H_2$ and $NH_3$, and temperature on the breaking of secondary bonding in protein.

The breaking of secondary bonding in gelatin molecules caused by urea was selected for study of the breaking of secondary bonding in protein [12, 13]. The determination can be conveniently followed by measuring the reduced viscosity of the solutions [13, 14].



## EXPERIMENTAL

### Materials

The gelatin used in this work was a commercial material produced by the Kanto Chemical Co. The urea used was a commercial material produced by the Junsei Pure Chemical Co. The $N_2$, $CO_2$, $O_2$, $H_2$, and $NH_3$ used were commercial materials.

### 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. The solid gelatin was put into each irradiation bottle, and gases were displaced with $N_2$, $CO_2$, $O_2$, $H_2$, or $NH_3$, and the irradiation was carried out at room temperature. The irradiated solid gelatin was dissolved with distilled water and mixed with urea solution. Then the viscosity was measured [13, 14].

## RESULTS

### Effect of Irradiation Atmosphere

Changes in the reduced viscosity of gelatin irradiated by $\gamma$ rays ($10^5$ R) in various atmospheres were studied with a 5% gelatin in 8 M urea at 30 °C.

The gases used were $N_2$, $CO_2$, $O_2$, $H_2$, and $NH_3$ (because they have previously been used 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 [1, 2]. The values of dissociation energy ($E_d$), resonance energy ($E_r$), and the energy difference ($E_d$ - $E_r$) between dissociation energy and resonance energy of the gases used are well known [15]. These values are shown in Table 1.

Experimental results are shown in Fig. 1. From these results it is clear that the increase of the reduced viscosity with increasing energy difference ($E_d$ - $E_r$) of the gases composing the irradiation atmosphere indicates that the irradiation atmosphere does affect the breaking of secondary bonding in protein.

## Effect of Temperature

Changes in the reduced viscosity of irradiated gelatin at various temperature were studied with a 5% irradiated gelatin ($10^3$ R) in 8 M urea.

Experimental results are shown in Fig. 2. From these results it is clear that with an increase of temperature the reduced viscosity decreases. This effect of temperature on the reduced viscosity is apparently related to its temperature effect in the breaking of secondary bonding in protein molecule.



TABLE 1. Gases Used in Irradiation of Solid Protein

| Gas | Dissociation energy, $E_d$ (kcal/mole) | Resonance energy, $E_r$ (kcal/mole) | Energy difference, ($E_d$ - $E_r$) (kcal/mole) |
|---|---|---|---|
| $N_2 \longrightarrow 2\,N$ | 225.1 | 0 | 225.1 |
| $CO_2 \longrightarrow C + 2\,O$ | 191.0 | 33 | 158.0 |
| $O_2 \longrightarrow 2\,O$ | 117.2 | 0 | 117.2 |
| $H_2 \longrightarrow 2\,H$ | 103.2 | 0 | 103.2 |
| $NH_3 \longrightarrow NH_2 + H$ | 92.0 | 0 | 92.0 |

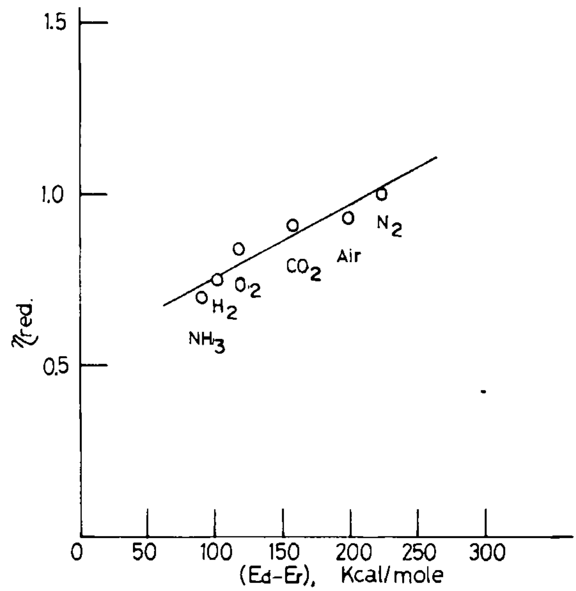


FIG. 1. Reduced viscosity as a function of energy difference ($E_d$ - $E_r$) of the gases in the irradiation atmosphere (5% gelatin in 8 M urea, $10^3$ R, 30°C).

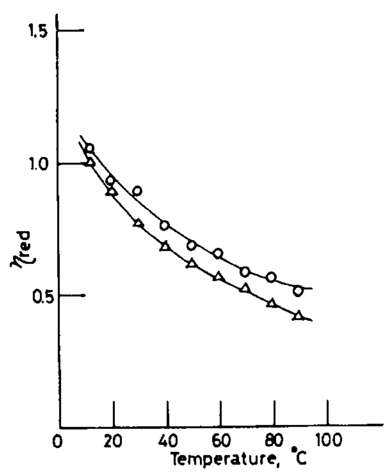


FIG. 2. Reduced viscosity as a function of temperature (5% gelatin in 8 M urea): (○) $10^3$ R and (△) no radiation.

## DISCUSSION

The viscosity change required for the breaking of hydrogen bonds was estimated from Figs. 1 and 2.

The relation between the viscosity change and the energy difference (dissociation energy and resonance energy) of the gases composing the irradiation atmosphere is related to that between the breaking of hydrogen bonds in gelatin molecule and its effect. When the concentrations of gelatin and urea and the radiation dose are all constant, a change of energy difference ($E_d$ - $E_r$) results in a change of the activation energy required for the breaking of hydrogen bonds in gelatin molecule (see Fig. 1). The reaction mechanism must, therefore, depend on the energy difference for the gases composing the irradiation atmosphere. If the main processes for the activation reaction are assumed to be

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

$$\text{X}^* + \text{X}^* \longrightarrow \text{X—X} + (E_d - E_r) \tag{2}$$

$$\text{P—P} \xrightarrow{(E_d - E_r)} \text{P}^* + \text{P}^* \tag{3}$$

where P—P is the gelatin molecule, P* is the irradiated gelatin molecule, X—X is the molecule of gases composing the irradiation atmosphere, and X* is the activated molecule of gases composing the irradiation atmosphere. The activation step caused by the irradiation atmosphere is Process (3), which means that the observed viscosity change is related to the energy difference for the gases composing the irradiation atmosphere. Therefore the response of the breaking of hydrogen bonds in gelatin molecule to the irradiation atmosphere can be determined by measuring the reduced viscosity.

If in the system the activation rate by the energy difference for the gases composing the irradiation atmosphere d(P)/dx is a, then

$$d(P)/dx = a \tag{4}$$

If the activation rate by the energy difference d(P)/dx is proportional to the rate of the reduced viscosity $d(\eta_{red})/dx$, then

$$(P)/dx = d(\eta_{red})/dx \tag{5}$$

From Eqs. (4) and (5)

$$d(\eta_{red})/dx = a \tag{6}$$

Integration of Eq. (6) yields

$$\eta_{red} = aX + b \tag{7}$$

This formula agrees with the experimental data plotted in Fig. 1.

With increasing temperature the reduced viscosity of the irradiated gelatin molecule in urea solution decreases. It is inferred to be related to the temperature dependence of the breaking of hydrogen bonds in gelatin molecule, (see Fig. 2).

If T is the reaction temperature expressed in °K, and A and B are adjustable constants, then

$$\eta_{red} = Ae^{B/T} \tag{8}$$

This formula (Andrade type) agrees with the experimental data which describe the curve in Fig. 2. Figure 2 is converted to Fig. 3 for

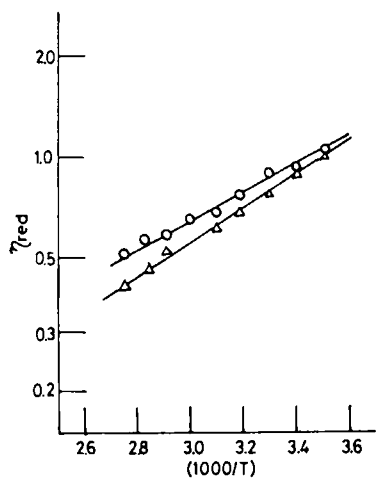


FIG. 3. Relation between reduced viscosity and absolute temperature (5% gelatin in 8 M urea): (○) $10^3$ R and (△) no radiation.

calculation of the activation energy required to break hydrogen bonds in an irradiated gelatin molecule. From the slope of the line shown in Fig. 3, the activation energy for the breaking of hydrogen bonds in an irradiated gelatin molecule ($10^3$ R) is obtained as $\Delta H^\circ = 1.97$ kcal/mole (for no radiation, $\Delta H^\circ = 2.36$ kcal/mole). From these it is clear that the decrease in the activation energy of the reduced viscosity with irradiation indicates an accelerating effect of the breaking of secondary bonding in protein.

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