Radiation Chemical Studies of Protein Reactions: Effect of Post-irradiation on Optical Rotation

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SUMMARY

When protein was irradiated by γ-rays from a ^{60}Co source, a post-irradiation effect of protein reaction was caused. An empirical equation for the optical rotation was obtained, and the phenomena were explained on the basis of the molecular mechanism. The general equation for the optical rotation is given by $[\alpha]f = a - b \log t$, where $[\alpha]f$ is the final specific rotation of the solution, t is time after γ irradiation, and a and b are adjustable constants.

INTRODUCTION

It is well known that reactivity of irradiated molecules varies with the time after irradiation [1-6]. Since the effect of radiation in protein reactions is a problem of general interest [7-9], it was decided to investigate the effect of post-irradiation on the change of internal relationships of atoms in protein molecule.

The urea denaturation of protein was selected to follow the change of internal relationships of atoms in protein molecule [8, 10]. The determination can be followed by measuring the optical rotation of the solutions as a function of the time after γ irradiation [8].

EXPERIMENTAL

Materials

The albumin and urea used in this work were commercial materials produced by the Kanto Chemical Co., Ltd.

Apparatus and Procedure

An irradiation source containing about 300 C of ^{60}Co was used. The dose rate in this work was 1.7 X 10^3 R/hr. The solid albumin was irradiated in air at room temperature. At different times after γ irradiation the irradiated solid albumin was dissolved with distilled water and mixed with urea solution. The optical rotation was measured [8].

RESULTS

The changes with time in specific rotation of albumin at different times after γ irradiation were studied with 2% albumin in 7 M urea, 10^3 R, and 30°C.

Experimental results are shown in Fig. 1. The relation between the values of the final specific rotation and the time after γ irradiation are shown in Fig. 2.

From this result it is clear that the specific rotation does not go to infinite value but approaches a limiting value, and the decrease in the specific rotation with the lapse of time after irradiation on the logarithmic scale indicates a post-irradiation effect in the protein molecule.

DISCUSSION

As stated above, it is known that a reactivity of the irradiated molecules varies with the time after irradiation [1-6]. The change of internal relationships of atoms in protein molecule is estimated from the change in optical rotation. This change is given in Figs. 1 and 2. The relation between the change in optical rotation and the time after irradiation is related to that between the change of internal relationships of atoms in protein molecule and the time after γ irradiation. When the concentration of protein and urea and the radiation dose are all constant, a change with
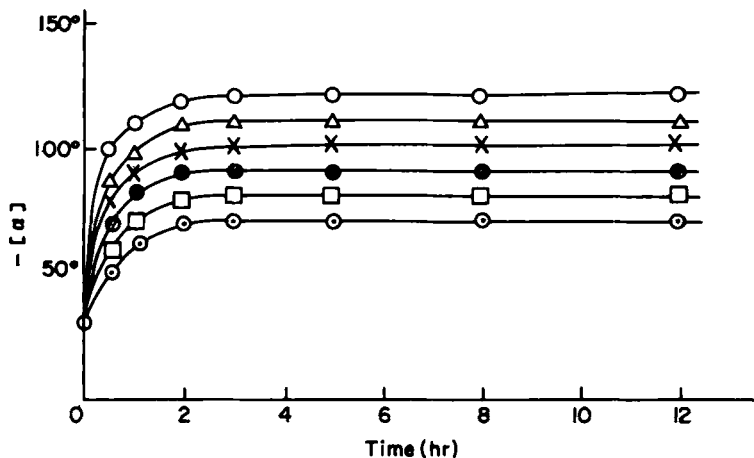
Fig. 1. Specific rotation vs. time for various times after irradiation: (○) 0.1 day, (△) 0.2 day, (✕) 1 day, (●) 3 days, (□) 10 days, (◉) 25 days. Conditions: 2% albumin in 7 M urea, 30°C.

Fig. 2. Final specific rotation vs. time after irradiation.

time after irradiation results in the change in optical rotation required for the change of internal relationships of atoms in protein molecule; see Fig. 2. The reaction mechanism must, therefore, depend on the time after

irradiation. If the main processes for the reactivity of protein are assumed to be

$$P-P \xrightarrow{h\nu} P* + P* \qquad (1)$$
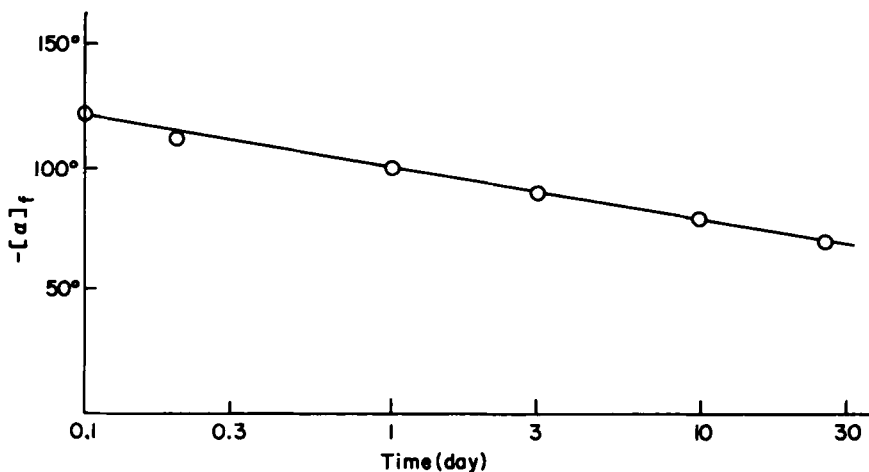
$$P* + P* \longrightarrow P-P + E_a \qquad (2)$$

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 E_a is the activation energy of the γ-rays. Then the reactivity-decay step may be process (2), which means that the observed optical rotation is related to the time after irradiation. The response of the internal relationships of atoms in protein molecule to the time after irradiation can therefore be determined by measuring the specific rotation.

The phenomena will be treated in terms of molecular mechanism. In any given unit volume of the irradiated protein there is a decay mechanism in which the relaxation process of reactivity can occur and over which the reactivity distributes itself. In this unit process of decay the effect of reactivity on the rate of radiation is twofold. First there is an activation component for which the rate of radiation in groups in positions in the vicinity of asymmetric carbon atoms in the protein molecule is related to the rate of reactivity, and second, there is a contribution to the rate of radiation of relaxation processes such as energy transfer or energy loss. It is convenient to classify the various unit processes of decay into categories which group similar processes together. Let us suppose that there are N such parallel processes of reactivity group in a cubic centimeter of unit volume, so that if the total reactivity f distributes itself on this type of unit process of decay, the average reactivity will be f/N. Suppose, too, that in the per unit distance of decay there are n such processes of decay in series. Then the total rate of radiation is given by

$$\frac{dr}{dt} = \frac{1}{R}\frac{df}{dt} + n\lambda\frac{kT}{h}\exp\left(\frac{-\Delta F*}{kT}\right) 2 \sinh\frac{f}{2NkT} \qquad (3)$$

where λ is the average distance projected in the reactivity space between equilibrium states in the relaxation process, and $\Delta F*$ is the free energy of activation for the decay process. R is the appropriate partial constant to be associated with the decay process, and the first term on the right represents the activation contribtuion. The contribution to rate of radiation due to

relaxation (energy transfer or energy loss) is given in the second term, and this term is derived by applying the theory of absolute reaction rates. The average reactivity f/N acts on the unit group and tends to favor motion from one equilibrium state to another in the reactivity space. The sum of all the partial reactivities on the decay mechanism is equal to the total reactivity, and the reactivity is distributed in such a way that the rate of radiation is the same for each.

Equation (3) can be written in a more condensed form

$$\frac{dr}{dt} = \frac{1}{R}\frac{df}{dt} + A \sinh Bf \qquad (4)$$

Integration of Eq. (4) for the condition of constant radiation, $dr/dt = 0$, gives the following for the relaxation of reactivity

$$\tanh\left(\frac{Bf}{2}\right) = \tanh\frac{Bf_0}{2}\exp(-ABRt) \qquad (5)$$

where f_0 is the initial reactivity. If $Bf/2$ is larger than unity and $f_0 > f > 1/2B$, then the approximate result is

$$f = \frac{1}{B}\ln\left(\frac{ABR}{2}\right) - \frac{1}{B}\ln t \qquad (6)$$

Equation (6) can be written in a more condensed form

$$f = a - b \log t \qquad (7)$$

Now, if the decay rate of the reactivity is proportional to the rate of final specific rotation, then

$$[\alpha]f = a - b \log t \qquad (8)$$

This formula agrees with the experimental data described in Fig. 2.

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