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Radiation Chemical Studies of Protein Reactions: Radiation Dose and Optical Rotation

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Synopsis

When protein was irradiated by gamma rays from a cobalt 60 source, an activated state on the position of the groups in the vicinity of the asymmetric carbon atoms in the protein molecule was caused. An empirical equation for the change in optical rotation was obtained, and the phenomena were explained on the basis of the molecular mechanism. The general equation for the change in optical rotation is given by $[\alpha] = (b + a \log R)(1 - e^{-kt})$, where $[\alpha]$ is the specific rotation of the solution, R is the gamma radiation dose, t is time, and a, b, and k are adjustable constants.

INTRODUCTION

Irradiation experiments have suggested that the changes in the shape of the external envelope of the protein molecule are accelerated by gamma irradiation.[1] Since the structural changes which accompany a change in the internal relationships of the atoms is a problem of general interest, it was decided to investigate the effect of radiation on the groups in the vicinity of the asymmetric carbon atoms in the protein molecule.

The urea denaturation of protein was selected as the change in the internal relationships of the atoms in protein molecule, since it has previously been studied in the kinetics of protein denaturation.[2] The determination can be followed conveniently by measuring the optical rotation of the solution as a function of radiation dose.

EXPERIMENTAL

Materials

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

Apparatus and Procedure

An irradiation source containing about 300 curies of $_{60}Co$ was used. The dose rates in these experiments were 1.0×10^2 to 1.0×10^4R/hr. The solid albumin was irradiated in air at room temperature.

In the optical rotation measurements a Polax polarimeter equipped with a sodium lamp was used. The temperature was maintained by thermostat

at 30°C. The protein solutions and urea solutions were prepared for each experiment. Protein and urea concentrations are expressed in grams per 100 ml of solution and in moles per liter of solution at 30°C, respectively. The protein and urea solutions were warmed to 30°C before being mixed. A 10-ml sample was then pipetted into the polarimeter tube having a length of 1 dm. The first reading of the optical rotation could usually be taken after 2 or 3 min of mixing.

RESULTS

The changes in optical rotation of the albumin with time at various radiation doses were studied with 2% albumin in 7*M* urea at 30°C. The results are shown in Figure 1. The relation between the values of the final rotation and the radiation dose is shown in Figure 2. From this result it is clear that the specific rotation does not go to infinity but approaches a limiting value. The increase of the specific rotation with increasing radiation dose indicates that the radiation dose does affect the groups in the vicinity of the asymmetric carbon atoms in the protein molecule.

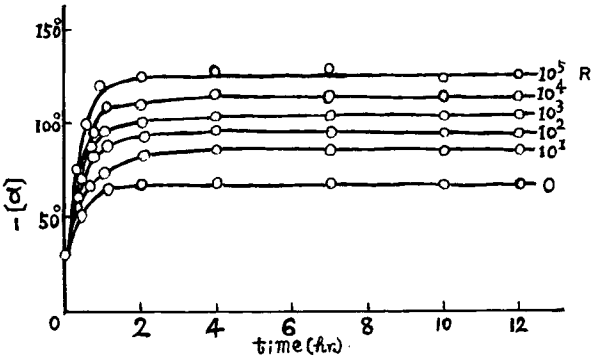


Fig. 1. Specific rotation versus time for various radiation doses: 2% albumin in 7*M* urea at 30°C.

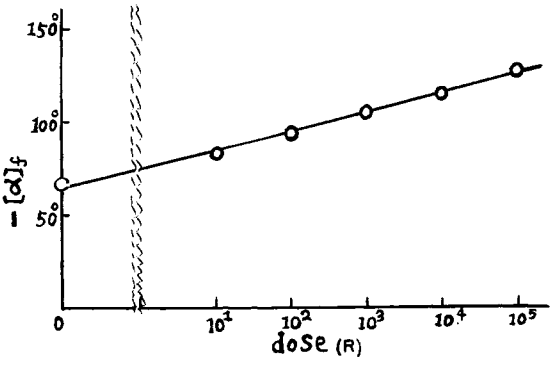


Fig. 2. Final rotation versus radiation dose: 2% albumin in 7*M* urea at 30°C.

DISCUSSION

As stated above, it is suggested that the changes in the shape of the external envelope of the protein molecule are accelerated by gamma radiation.[1] The optical rotation is sensitive to relatively subtle changes in the structure, such as the changes in the internal relationships of the atoms, and is readily measured without interfering with the course of a chemical reaction. A change in the optical rotation of a protein indicates a change in the internal relationships of the atoms. Therefore, the change in specific rotation required for the change in the internal relationships of the atoms in the protein molecule is estimated from Figure 1. Thus, the relation between the radiation dose and the optical rotation is parallel to that between the activation and the internal relationships of the atoms in protein molecule. When the concentration of albumin and urea are constant, the increase in the radiation dose results in an increase in the activation required for a change in the position of the groups in the vicinity of the asymmetric carbon atoms in the protein molecule. The reaction mechanism must, therefore, depend on the radiation dose. If the main reaction for the position change are assumed to be

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

$$\text{P}^* + \text{U—U} \rightarrow \text{P—U} + \text{U}^* \tag{2}$$

$$\text{U}^* + \text{U}^* \rightarrow \text{U—U} \tag{3}$$

where P—P is the group in the initial position in the vicinity of the asymmetric carbon atom in the albumin molecule, P* is the activated group in the activated position in the vicinity of the asymmetric carbon atom in the irradiated albumin molecule, U—U is the urea molecule, and U* is the activated urea molecule. The activated step may be reaction (1), which means that the observed rotation change is concerned with the radiation dose. Therefore, the response of the position change of the groups in the vicinity of the asymmetric carbon atoms in albumin molecule to the radiation dose can be determined by measuring the specific rotation.

The phenomena, then, can be treated by applying a molecular mechanism described in the previous paper.[1] In albumin molecules, (P—P) is the number of activated groups in the activated position in the vicinity of the asymmetric carbon atoms in the irradiated albumin molecule, N is the number of albumin molecules in 1 g of irradiated albumin, M is the number of groups in the initial position in the vicinity of the asymmetric carbon atoms in irradiated albumin molecule, R is the radiation dose, and a is an adjustable constant. Then (P—P) is given by

$$(\text{P—P}) = NM = a \log \text{R}. \tag{4}$$

Under the present experimental conditions, the reaction rate of the position change $d(\text{P—P})/dt$ will be proportional to the number of groups in the initial position in the vicinity of the asymmetric carbon atoms M. If the

probability of producing one position change per group at unit time is k, one obtains

$$d(\text{P—P})/dt = kM. \tag{5}$$

As the increase in (P—P) approaches a decrease in M,

$$-dM/dt = kM. \tag{6}$$

Therefore,

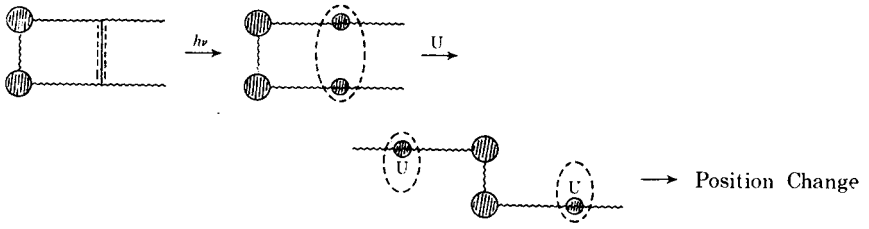
$$(\text{P—P}) = M\,(1 - e^{-kt}). \tag{7}$$

Now, if the reaction rate of position change (P—P)/dt is proportional to the rate of change in optical rotation $[\alpha]/dt$, one obtains

$$[\alpha] = (b + a \log \text{R})(1 - e^{-kt}). \tag{8}$$

This formula agrees with the experimental data that describe the curves in Figure 1. This behavior of optical rotation shows a similar dependence on radiation as shown in earlier viscosity experiment.[1]

The following mechanism was considered for the position change of the groups in the vicinity of the asymmetric carbon atoms in protein molecule:



In this mechanism, urea may inhibit the formation of secondary bonding between the NH and CO groups of the peptide chains in the protein molecule.

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References

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