Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Atmosphere and Temperature on Optical Rotation

Mizuho Nisizawa[a]
[a] Department of Chemistry, Defense Academy, Yokosuka, Japan

To cite this Article Nisizawa, Mizuho(1974) 'Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Atmosphere and Temperature on Optical Rotation', Journal of Macromolecular Science, Part A, 8: 6, 1051 — 1058
To link to this Article: DOI: 10.1080/00222337408066419
URL: http://dx.doi.org/10.1080/00222337408066419

J. MACROMOL. SCI.–CHEM., A8(6), pp. 1051-1058 (1974)

Radiation Chemical Studies of Protein Reactions: Effect of Irradiation Atmosphere and Temperature on Optical Rotation

MIZUHO NISIZAWA

Department of Chemistry
Defense Academy
Yokosuka, Japan

ABSTRACT

When protein in various atmospheres such as N_2, O_2, H_2, CO_2 and NH_3 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 gases composing the irradiation atmosphere. An empirical equation for the optical rotation was obtained. Protein irradiated by γ rays showed the effect of temperature by changes in the internal relationships of the atoms in the protein molecule. An empirical equation for the optical rotation was obtained. The behavior of optical rotation shows a similar dependence on irradiation atmosphere and temperature as shown in earlier viscosity experiments.

INTRODUCTION

Irradiation experiments have suggested that changes in the shape of the external envelope of the protein molecule are affected by irradiation atmospheres such as N_2, O_2, and H_2 [1], and also that on increasing the temperature the changes in the shape of the external envelope of the irradiated protein molecule are affected [2]. Since the effect of irradiation atmospheres such as N_2, O_2, H_2, CO_2, and NH_3 and the effect of temperature on protein reactions are problems of general interest, it was felt important to see 1) the effect, if any, of the irradiation atmosphere, and 2) the effect of temperature by changes in the internal relationships of the atoms in the 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]. The determination can be conveniently followed by measuring the optical rotation of the solutions [3].

EXPERIMENTAL

Materials

The albumin used in this work was a commercial material produced by the Kishida Chemicals & Co. The urea used was a commercial material produced by the Junsei Pure Chemicals Co. The N_2, O_2, H_2, CO_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 experiment was 1.2×10^4/hr. The solid albumin was put into each irradiation bottle, and gases were displaced with nitrogen, oxygen, hydrogen, carbon dioxide, or ammonia gas, and the irradiation was carried out at room temperature. The irradiated solid albumin was dissolved with distilled water and mixed with urea solution. Then the optical rotation was measured [3].

RESULTS

Effect of Irradiation Atmosphere

The changes with time in optical rotation of albumin irradiated by γ rays (10^3 R) in various atmospheres were studied with a 2% albumin in 7 M urea at 30°C.

The gases used were N_2, O_2, H_2, CO_2, and NH_3 (because they had previously been used to study changes in the shape of the external envelope of the protein molecule) [1]. Their dissociation energies (E_d), resonance energies (E_r), and the differences between dissociation and resonance energies ($E_d - E_r$) are well known. These values are shown in Table 1.

Experimental results are shown in Fig. 1. The relation between the values of the final specific rotation and the energy difference of the gases composing the irradiation atmosphere is shown in Fig. 2. From this result it is clear that the specific rotation does not continue to increase but approaches a limiting value. The increase of the final specific rotation with the increasing energy difference of the gases composing the irradiation atmosphere indicates that the irradiation atmosphere does affect the changes in the internal relationships of the atoms in the protein molecule.

Effect of Temperature

The changes with time in optical rotation of albumin at various temperatures were studied with a 2% albumin in 7 M urea and 10^3 R.

Experimental results are shown in Fig. 3. The relation between the final specific rotation and the temperature are shown in Fig. 4. From these results it is clear that the specific rotation does not continue to increase but approaches a limiting value. With increasing temperature the final specific rotation first decreases, reaches a minimum, and then increases. This effect of temperature on the optical rotation is apparently related to its temperature effect on changes in the internal relationships of the atoms in the 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	225.1	0	225.1
CO_2	191.0	33	158.0
O_2	117.2	0	117.2
H_2	103.2	0	103.2
NH_3	92.0	0	92.0

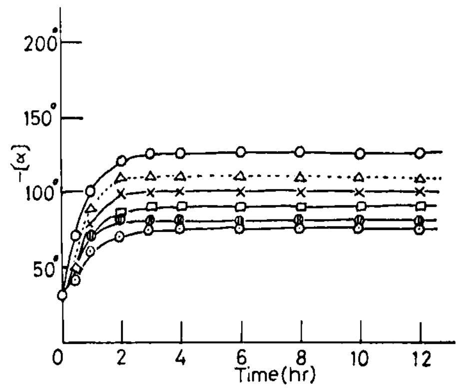

FIG. 1. Specific rotation vs time in various irradiation atmospheres: (○) N_2, (△) air, (×) CO_2, (□) O_2, (◍) H_2, and (⊙) NH_3. Conditions: 2% albumin in 7 M urea, 10^3 R, 30°C.

DISCUSSION

The changes in the internal relationships of the atoms in protein molecule are estimated from the changes in optical rotation as shown in Figs. 1-4.

The relation between the change in optical rotation and the energy

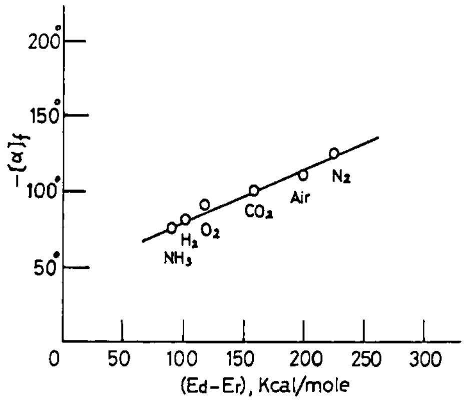

FIG. 2. Specific rotation as a function of energy difference of gases in the irradiation atmosphere. Conditions: 2% albumin in 7 M urea, 10^3 R, 30°C.

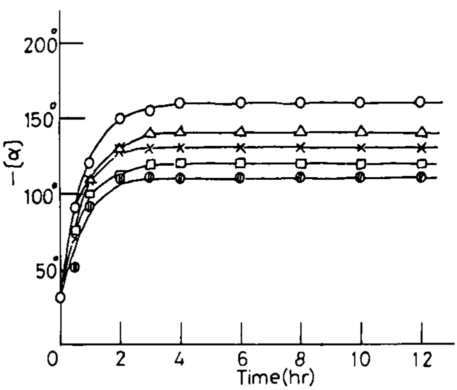

FIG. 3. Specific rotation vs time at various temperature: (○) 8°C, (△) 50°C, (×) 18°C, (□) 40°C, and (◍) 30°C. Conditions: 2% albumin in 7 M urea, 10^3 R.

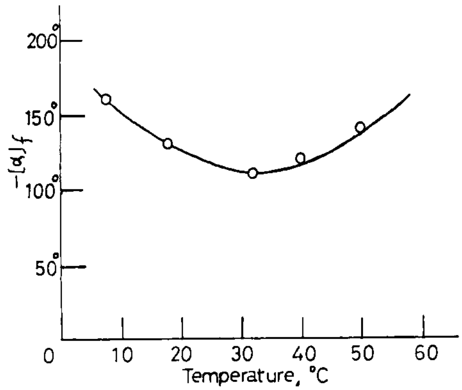

FIG. 4. Specific rotation as a function of temperature. Conditions: 2% albumin in 7 M urea, 10^3 R.

difference of the gases composing the irradiation atmospheres is related to the change in the internal relationships of the atoms in the protein molecule and its effect. When the concentration of protein and urea and the radiation dose are constant, a change of the energy difference results in a change of activation energy required for the change in the internal relationships of the atoms in the protein molecule (see Fig. 2). 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 group in the initial position in the vicinity of the asymmetric carbon atoms in the ablumin molecule, P* is the activated position in the vicinity of the asymmetric carbon atoms in the irradiated albumin molecule, X–X is the molecule of gases composing the irradiation atmosphere, X* is the activated molecule of gases composing the irradiation atmosphere, and $E_d - E_r$ is the energy difference for the gases composing the irradiation atmosphere. The activation step is Process (3), which means that the observed optical rotation is related to the energy difference for the gases composing the irradiation atmosphere. Therefore the response of the internal relationships of the atoms in the protein molecule to the irradiation atmosphere can be determined by measuring the specific rotation.

The phenomena, then, will be treated in terms of a molecular mechanism. If in the system the activation rate by the energy difference for the gases composing irradiation atmosphere d(P)/dx is a, then one obtains

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

If the activation rate by the energy difference d(P)/dx is proportional to the rate of change in optical rotation d[α]f/dx, then

$$d(P)/dx = d[\alpha]f/dx \tag{5}$$

From Eqs. (4) and (5)

$$d[\alpha]f/dx = a \tag{6}$$

Integration of Eq. (6) yields

$$[\alpha]f = a\,X + b \tag{7}$$

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

With an increase in the temperature, there is first a decrease, then a minimizing, and finally an increase in the final specific rotation of the irradiated albumin molecule in urea solution that is inferred to be related to the temperature dependence on the changes in the internal relationships of the atoms in the albumin molecule.

The changes of the internal relationships of the atoms in the albumin molecule irradiated are rendered more stable with respect to the temperature effect on minimizing the specific rotation.

If T is the reaction temperature expressed in °C; T_0 is the temperature of $[\alpha]f = c$ (a minimum); and a, b, and c are adjustable constants, then

$$[\alpha]f = a(T - T_0)^2 + b(T - T_0) + c \quad (8)$$

This formula agrees with the experimental data which describe the curve in Fig. 4.

This optical rotation behavior shows a similar dependence on irradiation atmosphere and temperature as was shown in earlier viscosity experiments [1, 2].

ACKNOWLEDGMENTS

The author wishes to thank Professor T. Kagiya of Kyoto University for many helpful suggestions on this subject and Dr. T. Hirano of Tokyo University of Fisheries for the use of their 1500 Ci of ^{60}Co γ-ray source.

REFERENCES

[1] M. Nisizawa, J. Appl. Polym. Sci., 12, 2183 (1968).
[2] M. Nisizawa, J. Macromol. Sci.–Chem., A5, 925 (1971).
[3] M. Nisizawa, J. Appl. Polym. Sci., 13, 2265 (1969).

Accepted by editor February 14, 1974
Received for publication March 1, 1974