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Radiation Chemical Studies of Protein Reactions: Dependence of Restorative Amino Acids on Radiation Dose

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ABSTRACT

Changes of specific rotation of protein irradiated with Co-60 gamma-rays were restored by some amino acids, such as monosodium 1-glutamate. Loss of this recovery effect in some amino acids by irradiation was studied by polarimetry and infrared spectometry. Optical rotation of protein-amino acid solutions increased with an increasing radiation dose given to the amino acids. Changes in optical rotation with irradiation may be due to the loss of the recovery effect of amino acids for the irradiated protein molecule. Changes in infrared spectra increased with an increasing radiation dose given to the amino acids. Such changes in infrared spectra with irradiation may be due to destruction of some radicals in the amino acid molecule.

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INTRODUCTION

Structural changes in protein occurred by ionizing radiation [1-5]. Some amino acids, such as monosodium 1-glutamate, recovered the structural changes in protein due to radiation damage [6, 7]. Since the effect of the restorative amino acid on the radiation-induced structural changes in protein is a problem of general interest, it was decided to investigate the radiation-dose dependence of the restorative amino acid for radiation-induced structural changes in protein. The radiation-induced optical rotation of protein was selected as the structural change in protein because it has been described in previous papers [2, 7].

The determination was made by measuring the optical rotation of the solution as a function of the radiation dose, the elapsed time after irradiation, and the concentration of the amino acid.

EXPERIMENTALS

Materials

The albumin, urea, monosodium 1-glutamate, 1-aspartic acid, and 1-arginine used in this work were the same as those described in a previous paper [7].

Apparatus and Procedure

Irradiation

An irradiation source containing about 1500 Ci of Co-60 was used. The dose rates in this experiment were 1.2×10^4 to 3.0×10^5 R/hr. Solid albumin and solid amino acids (such as monosodium 1-glutamate, 1-aspartic acid, and 1-arginine) were irradiated in air at room temperature.

Polarimetry

The irradiated solid albumin (10^3 R) was dissolved with distilled water and mixed with urea solution containing the irradiated amino acid (0 to 10^7 R). Then the optical rotation of the solution was measured [2, 7].

Infrared Spectrometry

The irradiated (10^3 R) or nonirradiated solid albumin and the irradiated (10^7 R) or nonirradiated solid amino acid were molded into 1.5% pellets with KBr, and then the infrared spectra were measured.

RESULTS AND DISCUSSION

Effects of Irradiation and Postirradiation on Protein

The changes in optical rotation of albumin at different times after irradiation in the presence or absence of the restorative amino acids were studied with 2% albumin, 0.1% monosodium 1-glutamate, 7 M urea, 10^3 R, and 30°C.

Figure 1 shows the relationships between the values of the final specific rotation and the time after irradiation in the presence or absence of the amino acid (monosodium 1-glutamate). From these results it is clear that the decrease in the specific rotation with lapse of time after irradiation on the logarithmic scale indicates a post-irradiation effect in the protein molecule.

The relationship between the change in optical rotation and the time after irradiation is related to that between the changes of internal relationships of the atoms in the protein molecule and the time after irradiation [8]. At constant concentration of protein and urea and radiation dose, a change with time after irradiation results in a change in the optical rotation required for a change of the internal relationships of the atoms in the protein molecule. Also, in the presence of the amino acid the optical rotation of the protein solution decreases remarkably (see Fig. 1). This behavior shows the amino acid recovered vs changes from radiation damage in the internal relationships of the atoms in the protein molecule [7].

The infrared spectra of irradiated protein (10^3 R) do not change

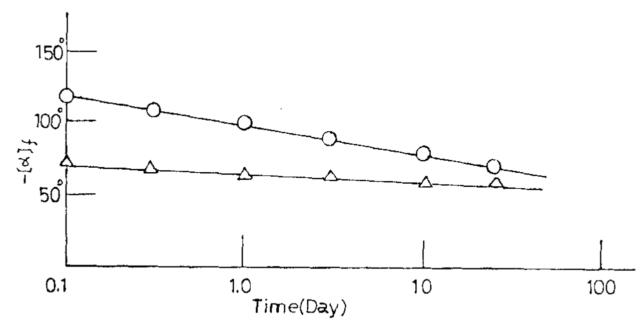


FIG. 1. Final specific rotation vs time after irradiation in the presence (△) and absence (○) of restorative amino acid (0.5% monosodium 1-glutamate). Conditions: 2% albumin in 7 M urea, 10^3 R, and 30°C.

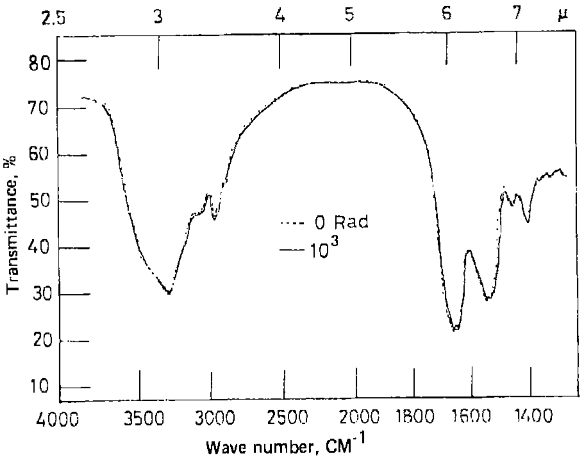


FIG. 2. IR spectra of nonirradiated and irradiated (10^3 R) protein: (- -) 0 R and (—) 10^3 R. Conditions: 1.5% albumin in KBr pellet at 15°C.

much (Fig. 2). From this it is clear that the chemical structure of protein molecule does not change significantly due to 10^3 R irradiation.

Effect of Radiation Dose on Restorative Amino Acids

Changes in optical rotation of the irradiated albumin (10^3 R) in the presence of irradiated amino acid (0 to 10^7 R) were studied with 2% albumin and 0.1% amino acid in 7 M urea at 30°C.

The restorative amino acids used were monosodium 1-glutamate, 1-aspartic acid, and 1-arginine (because they had previously been used to study the structural changes in protein) [7].

Figure 3 shows the relationships between the values of the final specific rotation and the radiation dose of the amino acid. The increase of the specific rotation with increasing radiation dose of the amino acid on the logarithmic scale indicates that the radiation dose affects the restorative action of the amino acid for the radiation-induced structural changes in protein. From these it is clear that the radio-resistance of the restorative amino acid is apparently related to their molecular structure. These radio-resistances are in the following order of radiation dose dependency: 1-arginine > monosodium 1-glutamate > 1-aspartic acid.

The relationship between the changes in optical rotation and the amino acids is related to that between the changes in the internal

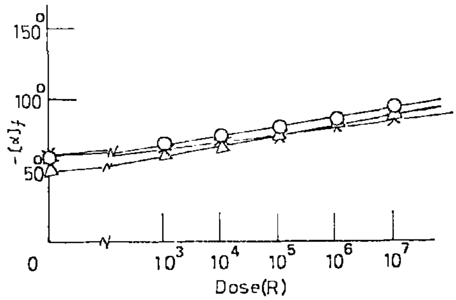


FIG. 3. Dependence of radiation dose on amino acid as restorative (0.1%): (○) monosodium 1-glutamate, (△) 1-arginine, and (×) 1-aspartic acid. Conditions: 2% albumin in 7 M urea, 10^3 R, and 30°C.

relationships of the atoms in the protein molecule and the restorative effect [7]. At constant concentrations of protein (10^3 R), amino acid (0 to 10^7 R), and urea, an increase in the radiation dose of the amino acid results in a decrease in the restorative effect required for changes in the internal relationships of the atoms in the protein molecule (see Fig. 3).

For the present system the observed optical rotation is expressed as a linear line, a logarithmic abscissa being the radiation dose of the amino acid.

$$[\alpha]f = a + b \log R$$

This formula agrees with the experimental data plotted in Fig. 2. The radiation dose dependency is related to the molecular structure of the amino acid (see Table 1). The b-values in the formula are in inverse proportion to the radio-resistance of the restorative amino acid.

The infrared spectra of the irradiated amino acid (10^7 R) changed remarkably (Figs. 4a and 4b). From these results it is clear that the chemical structure of the amino acid molecule (COO^-) changed remarkably due to 10^7 R irradiation.

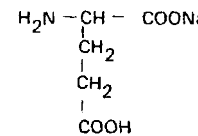
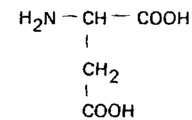
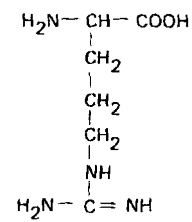
Effect of Concentration on Restorative Amino Acids

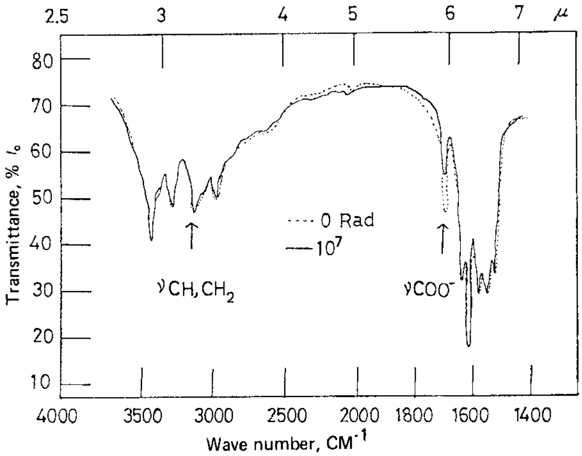
Changes in the optical rotation of the irradiated albumin (10^3 R) with the concentration of irradiated (10^7 R) or nonirradiated amino acid were studied with 2% albumin and 0.1% monosodium 1-glutamate in 7 M urea at 30°C.

Figure 5 shows the relationship between the values of the final specific rotation and the concentration of irradiated or nonirradiated

TABLE 1.

b-values of amino acid used as restorative

Amino acids	Formula	Structure	b-value
Monosodium 1-glutamate	$C_5H_8O_4NNa$		6.25
1-aspartic acid	$C_4H_7NO_4$		7.50
1-arginine	$C_6H_{14}N_4O_2$		5.00



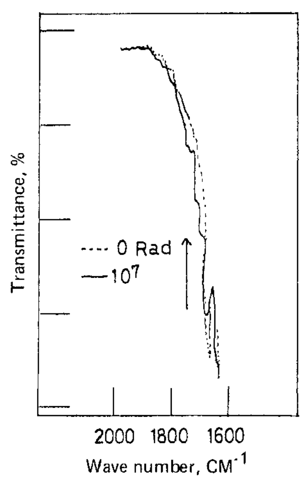


FIG. 4a. IR spectra of nonirradiated or irradiated (10^7 R) amino acid (monosodium 1-glutamate): (- -) 0 R and (—) 10^7 R. Conditions: 1.5% monosodium 1-glutamate in KBr pellet at 15°C.

FIG. 4b. See Fig. 4a legend.

amino acid (monosodium 1-glutamate). From these results it is clear that the decrease of the specific rotation with increasing concentration of the amino acid on the logarithmic scale indicates a restorative effect of the amino acid for the radiation-induced structural changes in protein. The relationship between the change in optical rotation and the concentration of the amino acid is related to that between the changes of internal relationships of the atoms in the protein molecule and its recovery [7]. At a constant concentration of protein and urea

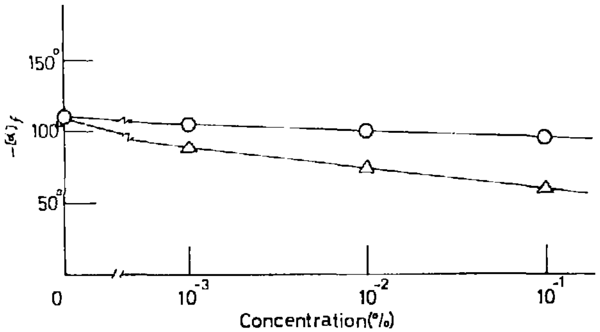


FIG. 5. Dependence of restorative effect on the concentration of nonirradiated and irradiated (10^7 R) amino acid (monosodium 1-glutamate): (△) 0 R and (○) 10^7 R. Conditions: 2% albumin in 7 M urea, 10^3 R, and 30°C.

and radiation dose, an increase in the concentration of irradiated or nonirradiated amino acid results in the decrease in optical rotation required for changes of the internal relationships of the atoms in the protein molecule. Also, in the presence of irradiated amino acid, the optical rotation of protein solution does not decrease remarkably (see Fig. 5). This behavior indicates that the irradiated amino acid does not recover from changes due to radiation damage in the internal relationships of the atoms in the protein molecule.

In this mechanism the restorativity of the amino acid may be lost by ionizing radiation damage, and the recovery effect for the radiation-induced structural changes in protein molecule may be depressed.

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