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Radiation Chemical Studies of Protein Reactions: Effect of Amino Acids on Optical Rotation

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Synopsis

Monosodium 1-glutamate, disodium inosine-5′-monophosphate, disodium guanosine-5′-monophosphate, calcium *d*-pantothanate, 1-arginine, and 1-aspartic acid were found to protect the changes in the internal relationships of the atoms in the protein molecule from radiation damage. The behavior of the optical rotation was determined. The empirical equation for the protective effect is given by $[\alpha]_f = b - a \log X$, where $[\alpha]f$ is the final specific rotation of the solution, X is the concentration of the amino acids, and a and b 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] and also, that the changes in the internal relationships of the atoms in the protein molecule are accelerated by gamma irradiation.[2] On the other hand, some amino acid such as sodium glutamate and some benzene-series hydrocarbon such as sodium benzoate protect the unfolding of polypeptide chains folded in the protein molecule from an activation effect of gamma radiation.[3] Since monosodium 1-glutamate, disodium inosine-5′-monophosphate, disodium guanosine-5′-monophosphate, calcium *d*-panthothanate, 1-arginine, and 1-aspartic acid are well-known amino acids, it was thought desirable to see, first, whether it showed such a protective property and, second, what would be the effect of its concentration on the position changes of the groups in the vicinity of the asymmetric carbon atoms in the protein molecule.

The urea denaturation of protein was selected as the changes in the internal relationships of the atoms in the protein molecule, since it was described in a previous paper.[2] The determination can be followed conveniently by measuring the optical rotation of the solution as a function of the concentration of the amino acid.

EXPERIMENTAL

Materials

Albumin, urea, and monosodium 1-glutamate used in this work were commercial materials produced by Kanto Chemical Co., Inc. L-arginine was a commercial material produced by Tokyo Chemical Industry Co., Ltd. L-aspartic acid was a commercial material produced by Junsei Pure Chemicals Co., Ltd. Calcium *d*-panthothanate was a commercial material produced by Daiichi Pure Chemicals Co., Ltd. Disodium inosine-5′-monophosphate and disodium guanosine-5′-monophosphate were a gift from Dr. Y. Komata of the Ajinomoto Central Research Laboratory.

Apparatus and Procedure

An irradiation source containing about 300 curies of ^{60}Co was used. The dose rate in this work was 1.7×10^3r/hr. The solid albumin was irradiated in air at room temperature. The irradiated solid albumin was dissolved with distilled water and mixed with the urea solution containing the amino acid. Then the optical rotation was measured.[2]

RESULTS

The changes, with time, in optical rotation of albumin and amino acid (monosodium 1-glutamate, disodium inosine-5′-monophosphate, disodium guanosine-5′-monophosphate, calcium *d*-panthothanate, 1-arginine, or 1-aspartic acid) were studied with 2% albumin in 7 *M* urea, 10^3r, and 30°C.

The results are shown in Figures 1–6. The relation between the values of the final specific rotation and of the percentage of amino acid are shown in Figures 7–12. From these it is clear that the specific rotation does not go to an infinite value, but approaches a limiting value. The decrease of the final specific rotation with increasing percentages of amino acid on the logarithmic scale indicates that the effect of amino acid on the optical rotation is apparently related to its inhibition of the position changes of the

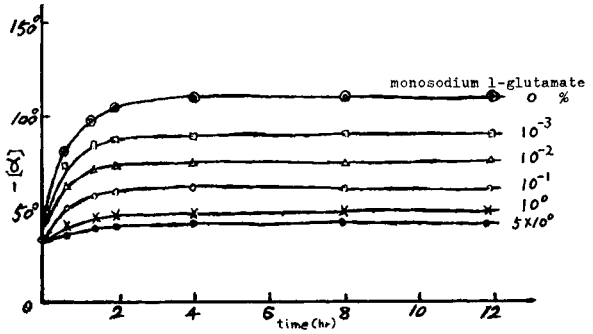


Fig. 1. Specific rotation versus time; 2% albumin in 7 *M* urea, 10^3r, and 30°C, in the presence and in the absence of monosodium 1-glutamate.

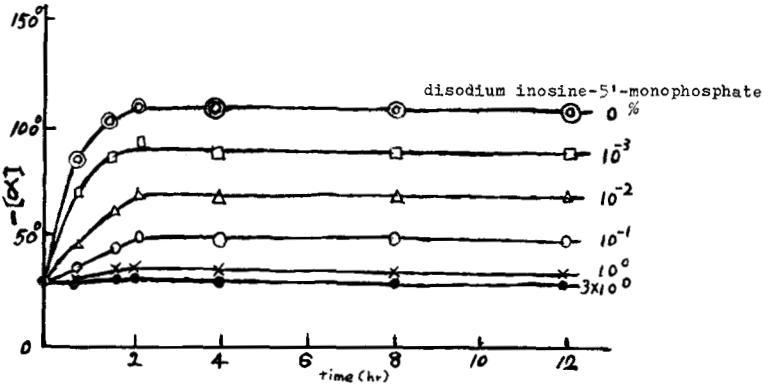


Fig. 2. Specific rotation versus time; 2% albumin in 7 *M* urea, 10^3r, and 30°C, in the presence and in the absence of disodium inosine-5'-monophosphate.

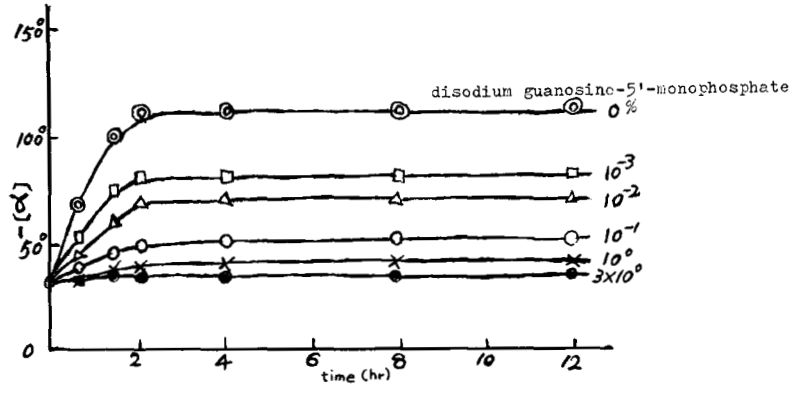


Fig. 3. Specific rotation versus time; 2% alubumin in 7 *M* urea, 10^3r, and 30°C, in the presence and in the absence of disodium guanosine-5-monophosphate.

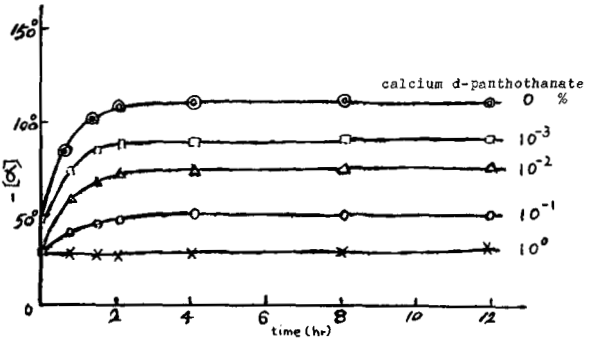


Fig. 4. Specific rotation versus time; 2% albumin in 7 *M* urea, 10^3r, and 30°C, in the presence and in the absence of calcium *d*-panthothanate.

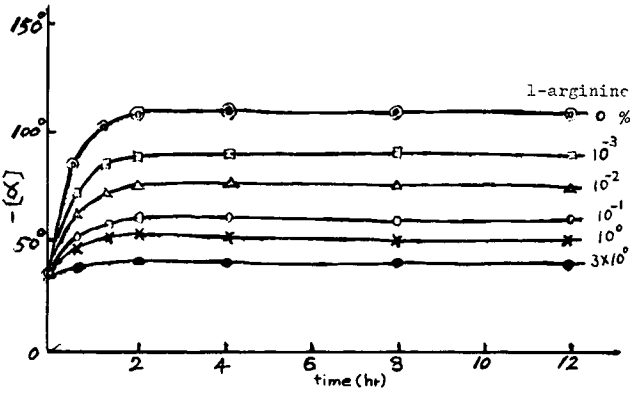


Fig. 5. Specific rotation versus time, 2% albumin in 7 *M* urea, 10^3r, and 30°C, in the presence and in the absence of l-arginine.

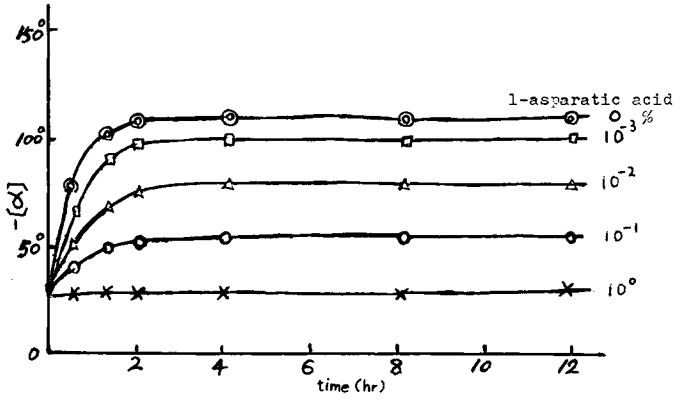


Fig. 6. Specific rotation versus time; 2% albumin in 7 *M* urea, 10^3r, and 30°C, in the presence and in the absence of l-aspartic acid.

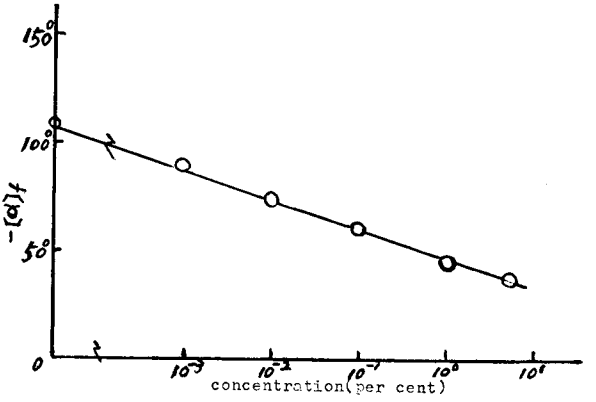


Fig. 7. Dependence of protective effect on the concentration of monosodium l-glutamate; 2% albumin in 7 *M* urea, 10^3r, and 30°C.

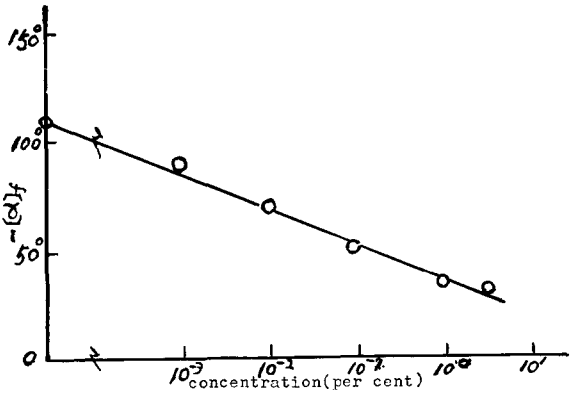


Fig. 8. Dependence of protective effect on the concentration of disodium inosine-5′-monophosphate; 2% albumin in 7 *M* urea, 10^3r, and 30°C.

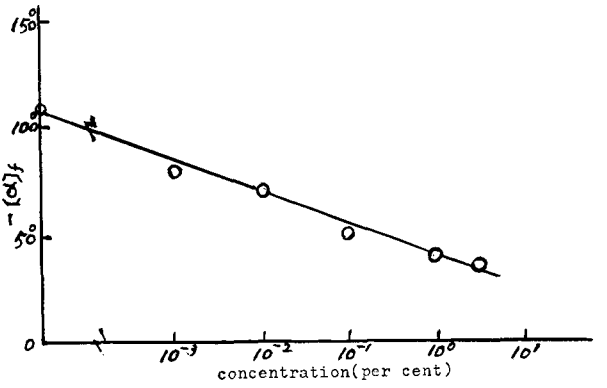


Fig. 9. Dependence of protective effect on the concentration of disodium guanosine-5′-monophosphate; 2% albumin in 7 *M* urea, 10^3r, and 30°C.

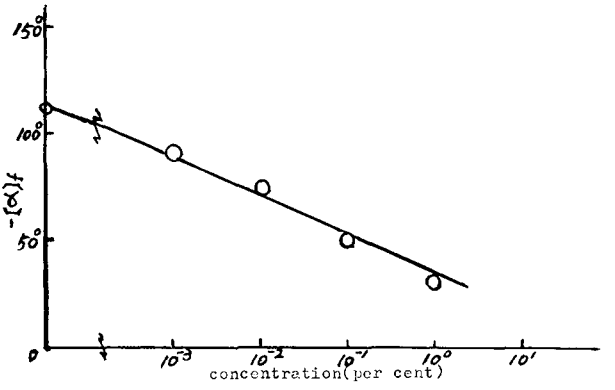


Fig. 10. Dependence of protective effect on the concentration of calcium *d*-pantothanate; 2% albumin in 7 *M* urea, 10^3r, and 30°C.

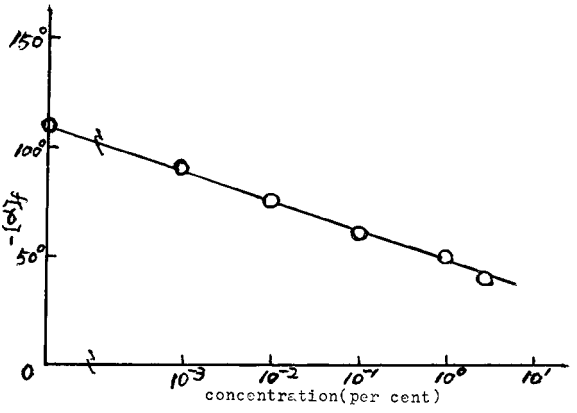


Fig. 11. Dependence of protective effect on the concentration of l-arginine; 2% albumin in 7 *M* urea, 10^3r, and 30°C.

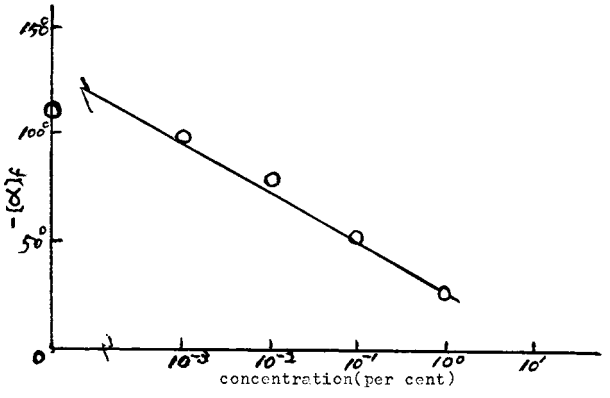


Fig. 12. Dependence of protective effect on the concentration of l-aspartic acid; 2% albumin in 7 *M* urea, 10^3r, and 30°C.

groups in the vicinity of the asymmetric carbon atoms in the protein molecule.

DISCUSSION

As stated above, it is known that the changes in the shape of the external envelope of the protein molecule are accelerated by gamma irradiation[1] and also, that the changes in the internal relationships of the atoms in the protein molecule are accelerated by gamma irradiation.[2] On the other hand, some amino acid such as sodium glutamate and some benzene-series hydrocarbon such as sodium benzoate protect the unfolding of polypeptide chains folded in the protein molecule from an activation effect of gamma radiation.[3] A discussion of the effect of amino acid such as monosodium l-glutamate, disodium inosine-5′-monophosphate, disodium guanosine-5′-

monophosphate, calcium *d*-panthothanate, l-arginine, and l-aspartic acid on the position changes of the groups in the vicinity of the asymmetric carbon atoms in the protein molecule follows. The position change of the groups in the vicinity of the asymmetric carbon atoms in the protein molecule is estimated from the change in optical rotation and this change is given in Figures 1–12. The relation between the change in optical rotation and the concentration of the amino acid is related to that between the change in the internal relationships of the atoms in the protein molecule and its inhibition. When the concentration of protein and of urea and the radiation dose all are constant, a change in the concentration of amino acid results in the change in optical rotation required for the internal relationships of the atoms in the protein molecule; see Figures 7–12. The reaction mechanism must, therefore, depend on the concentration of the amino acid. If the main processes in the protective action are assumed to be

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

$$\text{P}^* + \text{P}^* \rightarrow \text{P–P} + \text{Ea} \tag{2}$$

$$Ea + S \rightarrow S^* \tag{3}$$

$$S^* \rightarrow S + Ea \tag{4}$$

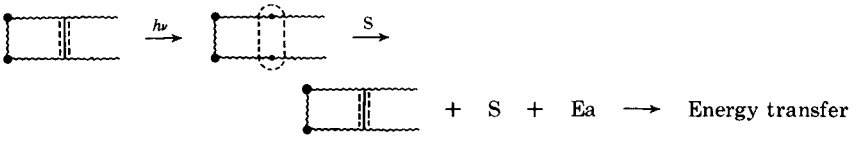
where P–P is the group in the initial position in the vicinity of the asymmetric carbon atoms in the albumin molecule, P^* is the activated group in the activated position in the vicinity of the asymmetric carbon atom in the irradiated albumin molecule, Ea is the activation energy of the gamma rays, S is the amino acid, and S^* is the activated amino acid, then the protective step is reaction (3), which means that the observed protective effect is expressed as a linear line, a logarithmic abscissa being the concentration of the amino acid. Therefore the response of the position change of the groups in the vicinity of the asymmetric carbon atoms in albumin molecule to the amino acid may be determined by measuring the specific rotation.

If in the system X is the concentration in per cent, and a and b are adjustable constants; then one gets

$$[\alpha]_f = b - a \log \text{X} \tag{5}$$

This formula agrees with the experimental data plotted in Figures 7–12.

The following mechanism was considered for the protective effect of amino acid against radiation;



In this mechanism these amino acids may protect the position changes of the groups in the vicinity of the asymmetric carbon atoms in the protein molecule by energy transfer of gamma rays.

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References

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