Notes

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Radiation-Inactivation of Aspartate Aminotransferase and Its Protection by 2-Mercaptoethylamine and 2-Aminoethylisothiuronium Bromide

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Aspartate aminotransferase from a water soluble fraction of pig heart muscle was irradiated with ^{60}Co γ-ray, 274 and 380 nm light in aqueous solutions with or without radioprotective amines, *i.e.* 2-mercaptoethylamine (MEA) and 2-aminoethylisothiuronium bromide (AET). For γ-irradiation, the enzyme was quite sensitive (D_{37}, 37.5 krad) and was effectively protected by the amines against the radiation-inactivation. The change in protein structure judged from gel electrophoretic pattern was also improved by MEA. The 380-nm irradiation caused inactivation, which was least protected by MEA, without serious changes in the protein structure. Photochemical degradation of pyridoxal phosphate was supposed to be responsible for the inactivation. MEA protects apoenzyme probably as a radical scavenger.

Keywords——aspartate aminotransferase; radiation-inactivation of an enzyme; protection against irradiation; pyridoxal; 2-mercaptoethylamine (MEA); 2-aminoethylisothiuronium bromide (AET)

It is well known that some amines protect mammals against damages caused by ionizing radiation.[2] These amines are 2-mercaptoethylamine, 2-aminoethylisothiuronium bromide (hereafter, abbreviated as MEA and AET, respectively), cysteine, serotonine, histamine, reserpine and so on. Many arguments have been made on the mechanism of the protection.[2,3] Pyridoxal enzymes were suggested to be one of the vital molecules which were possibly protected by these amines.[4] Matsushima and Akaboshi[5] discussed a correlation between the protective abilities and modes of reactions with pyridoxal on a variety of amines and amino acids.

These prompted us to investigate γ- and UV-irradiation on a pyridoxal enzyme and effects of the radioprotective amines on this system. The present paper describes the radiation-inactivation of pig heart aspartate aminotransferase (E.C. 2.6.1.1.)[6] in the presence and in the absence of MEA and AET.

Experimental

Enzyme——Aspartate aminotransferase was purified from a water soluble fraction of homogenate of pig heart muscle.[6] The purification procedures reported by Kagamiyama[7] were employed with a slight modification. The preparation used in the present study was homogeneous in disc gel electrophoresis.

1) Location: *3-1-1, Maidashi, Higashi-ku, Fukuoka-shi, 812, Japan.*
2) Z.M. Bacq and P. Alexander, "Fundamentals of Radiobiology," 2nd ed., Pergamon Press, London, U.K., 1961; A. Hanaki and S. Akaboshi, *Japan Analyst.*, **15**, 518 (1966).
3) A. Pihl and L. Eldjahn, *Pharmacol. Rev.*, **10**, 437 (1958).
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5) Y. Matsushima and S. Akaboshi, *J. Radiat. Res.*, **12**, 37 (1971).
6) This enzyme was conventionally called as soluble glutamic oxaloacetic transaminase and, hence, as s-GOT.
7) H. Kagamiyama, *Osaka Daigaku Igaku-Zasshi*, **18**, 171 (1966).

The enzyme activity was measured by the method of Reitman and Frankel,[8] in which oxaloacetate formed from aspartate, a substrate, was determined with 2,4-dinitrophenylhydrazine. Protein was determined by the method of Lowry, *et al.*[9]

Materials——MEA[10] and AET[11] were prepared according to the references cited. Other substances were obtained from commercial sources.

General Experimental Procedures——In 0.1M phosphate buffer (pH 7.5) was dissolved the enzyme (concentration; 1.2 mg/ml) with varying amounts of MEA or AET. Aliquots of the solutions were transfered to plastic test tubes placed in an ice-water bath and were submitted to ^{60}Co γ-irradiation in dose rate of 312 rad/min. Then, the irradiated as well as unirradiated solutions were assayed for the enzyme activity. The absorbed dose was measured by ferrous sulfate dosimeter.[12]

For the ultraviolet irradiation, the solutions were placed in quarz cells with a 1.0-cm light path. The monochromatic irradiation was carried out with a JASCO Model CRM-FA Spectro-Irradiator. Irradiated energy was calculated on the basis of data on energy-wavelength relationships in the Spectro-Irradiator, which had been elaborated by a measurement with a vacuum thermocouple.

Electrophoresis——Disc gel electrophoresis in 7.5% polyacrylamide[13] was employed. Bromphenol blue was used as a tracking dye. The protein was stained with Amide Black.

Results

Gamma Irradiation

Aspartic aminotransferase was quite sensitive for γ-irradiation. When irradiated in an aqueous solution (1.2 mg/ml), D_{37} value was estimated to be 37.5 krad (Fig. 1). MEA effectively protected the enzyme from the radiation inactivation.

Figure 1 shows dose-inactivation relationships in the presence or absence of MEA. Protection by AET was also eminent. After 56 krad γ-irradiation, 62 and 78% of the original activity was retained in the presence of 1 mM and 10 mM AET, respectively, while the residual activity was only 23% without the amine. Since residual activity was 71% in 10 mM MEA, AET seems to be slightly more effective in the same conditions. In the presence of 100 mM AET, the enzyme was slightly inhibited and a reproducible result was not obtained.

Ultraviolet Irradiation

The spectrum of the enzyme in an aqueous solution (pH 7.5) had two absorption bands in the UV region at around 274 and 370 nm. The 370-nm band is ascribed to the π—π^* transition of pyridoxal phosphate,[14] which binds the apoenzyme in an azomethine linkage.[15] The 274-nm band may be a π band of the coenzyme overlapped by an absorption due to the apoprotein.

Monochromatic light near the absorption maxima, 380 and 274 nm, was irradiated on the enzyme with or without MEA, in order to obtain information on effects of radiation in UV region. Results are summarized in Table I with that of γ-irradiation. The protection by MEA was most prominent in γ-irradiation. Inactivation by 380-nm irradiation was least protected by MEA.

Electrophoretic Study

In order to investigate effects of irradiation and the protective substances on the protein structure, the enzyme solution used in the irradiation studies were examined by polyacrylamide gel disc electrophoresis.

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TABLE I. Inactivation of Aspartate Aminotransferase by Gamma and Ultraviolet Radiations

Radiation	Dose[a)]	MEA concentration (M)	Activity after irradiation (%)
380-nm light	1160 J	0	41
		0.1	55
274-nm light	280 J	0	17
		0.1	38
^{60}Co gamma ray	56 krad	0	23
		0.1	76

a) Dose of the UV irradiation is expressed as total energy of light irradiated on every 1 ml of the solutions.

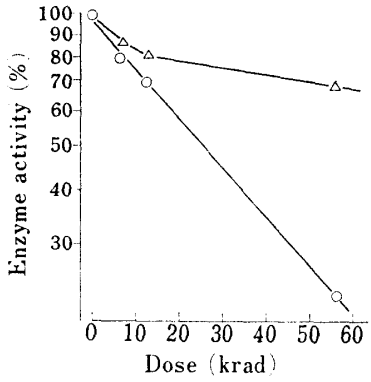

Fig. 1. Enzyme Activity after Gamma-Irradiation in Solutions Containing 0 (○) and 0.1M (△) MEA

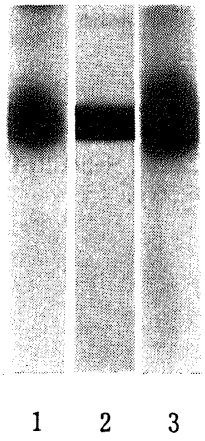

Fig. 2. Disc Electrophoretic Patterns of Aspartate Aminotransferase

1. ^{60}Co-irradiated (56 krad)
2. Unirradiated
3. 274-nm irradiated (280 J)

The enzyme irradiated 56 krad with ^{60}Co γ-ray showed a very diffused and less stained band. This was much improved in the samples irradiated in the presence of MEA or AET. In the 274-nm irradiated protein which retained only 17% of the original activity, electrophoretic pattern was also obscured. MEA fairly improved the electrophoretic pattern as well as the enzyme activity. No significant difference was found between the patterns of the unirradiated and 380-nm irradiated protein, though the activity of the latter was 41% of the former.

Irradiation of Pyridoxal

Effects of irradiation on pyridoxal in aqueous phosphate buffer (pH 7.5) were studied by the changes of UV-absorption spectra. Little change in the spectrum was detected in the γ-irradiated solution. On the other side, the 380-nm irradiation (1160 J) caused significant changes. Absorption bands at 316 and 250 nm ascribable to neutral species of pyridoxal disappeared and an obscured spectrum without any appreciable absorption at the longer wavelength region than 240 nm was obtained.

Discussion

The electrophoretic results indicate that γ-irradiation caused serious changes in protein structure in the enzyme. Destruction of the coenzyme may have taken place concomitantly.

Pyridoxal did not undergo serious decomposition by 56 krad irradiation. Then, it is plausible that the changes in protein structure contribute dominantly to the inactivation of the enzyme.

On the other hand, the 380-nm irradiation inactivated the enzyme without serious changes in the protein structure. Small changes in the apoprotein that are not detectable by electrophoresis might be the cause of the inactivation. This possibility can not be ruled out by the present findings. Pyridoxal phosphate is light sensitive and complete destruction of pyridoxal was achieved by 380-nm irradiation of the same dose. Therefore, it is more likely to assume that the photochemical degradation of the pyridoxal moiety is responsible for the inactivation. Karpeiskii, *et al.*[16] obtained a similar conclusion from the photochemical studies of the enzyme.

MEA protected the enzyme from γ-ray more effectively than from UV light. The fact, together with the assumption that the inactivation by γ-ray and 380-nm light is caused by the destruction of the apoenzyme and the coenzyme, respectively, suggests the following mechanism; the apoenzyme was protected effectively by MEA, which probably served as a radical scavenger. Protection of the coenzyme was not evident in the present experimental conditions.

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