A NEW ENZYME THAT SPECIFICALLY INACTIVATES APO-PROTEIN OF PYRIDOXAL ENZYMES

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SUMMARY

We found a new inactivating enzyme in small intestine and skeletal muscles, which specifically reacts with apo-proteins of pyridoxal enzymes. This inacti vating enzyme does not react with all the non-pyridoxal enzymes tested. The inactivation by this enzyme is prevented by the addition of PALP. The enzyme splits apo-pyridoxal enzymes into smaller protein and oligopeptides. The activity of inactivating enzyme increases from 10-20 fold only in the case of B_6 deficiency compaired with that of normal rats.

INTRODUCTION

When rats were placed on pyridoxal deficient condition, rapid and marked decrease of ornithine transaminase (OTA) in small intestine was observed(1).

We have studied the mechanism of the decrease of OTA activity in small intestine and discovered a new enzyme which specifically inactivates OTA and other pyridoxal enzymes in their apo-forms. Pyridoxal phosphate (PALP) was found to protect the inactivation of pyridoxal enzymes by the inactivating enzyme. It was also found that all the non-pyridoxal enzymes tested were unaffected by the inactivating enzyme. The inactivating enzyme splits apo-pyridoxal enzymes into smaller protein and oligopeptides.

This new inactivating enzyme is only detected in small intestine and skeletal muscle among the organs studied and the activity of this enzyme increases 10 to 20 fold in B_6 deficient rats from the level of normal rats. The inducing mechanism of the inactivating enzyme in the B_6 deficient condition has not been clarified and the reason why the increase of the enzyme is only confined in small intestine and skeletal muscle is also unknown. But it is possible that this enzyme may play an important role in the regulation of the rate of degradation of pyridoxal enzymes.

MATERIALS AND METHODS

Vitamin B_6 deficient rats

B_6 deficient rats were obtained by feeding on vitamin B_6-free, 20 % casein diet for 4 weeks. In some cases, 20 mg/100 g of deoxypyridoxine were mixed in B_6-free diet to accelerate the deficiency.

Preparation of inactivating enzyme

The small intestines of B_6 deficient rats were homogenized with 4 volumes of 0.05 M potassium phosphate buffer, pH 7.5. The homogenates were sonicated at 10 kilocycles for 2 min, then centrifugated for 10 min at 8,500 g. The supernatant was passed through Sephadex G-25, equilibrated with the buffer described above, and the protein fractions were collected. The conbined fractions were usually used as the source of inactivating enzyme for pyridoxal enzymes. In some experiments, a more purified preparation was used. The purification steps include ammonium sulfate fractionation (250-400g/l), acetone fractionation (40-70 % v/v) and DEAE-cellulose column chromatography. The active fraction was eluted with 0.2 M phosphate buffer (pH 7.5). These purification steps resulted in 200-400 fold increase in specific activity as compared with the crude extract.

Assay conditions and enzyme unit for the inactivating enzyme

Reaction mixtures contained 0.05 M potassium phosphate buffer, pH 7.5, 150-200 units of apo-OTA and different amounts of inactivating enzyme in a final volume of 0.3 ml, and were incubated at 37°C. After incubation, reaction was stopped by standing at 0°C. Subsequently, the remaining activity of OTA was assayed in the presence of pyridoxal phosphate (5 γ/ml) after the reaction mixture was diluted 10-20 folds. One unit of the inactivating enzyme was defined as the amount inactivating 50 % of 150-200 units of OTA in 30 min.

Preparation and assay method of ornithine transaminase

Crystallization of OTA from rat liver and converstion to apo-form were carried out according to the method of Matsuzawa et al(2). Activity of OTA was assayed by the method of Katunuma et al.(2)

RESULTS

The inactivating enzyme was incubated with apo-OTA in the presence or in

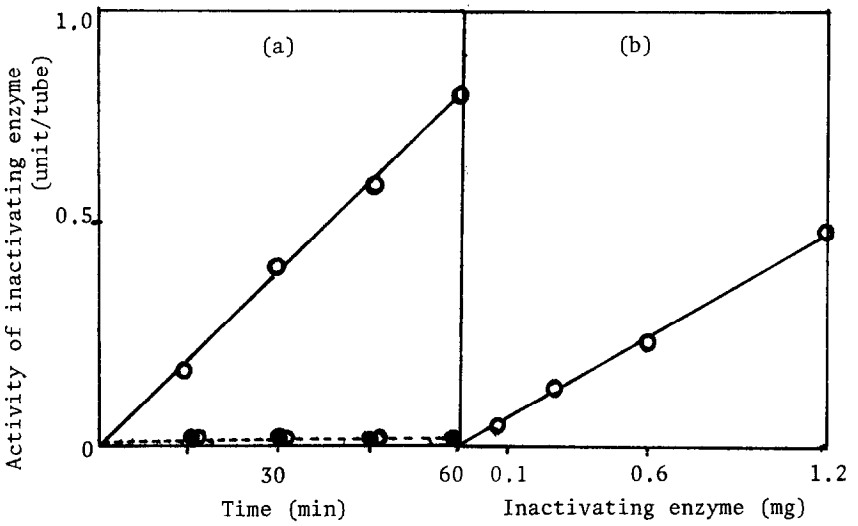

Fig. 1. Time course(a) and dose responce curve(b) of inactivation by the inactivating enzyme.
Reaction mixture contained apo OTA(O—O) or holo OTA(O···O) as substrate and 1.2 mg of the inactivating enzyme in Fig. 1(a). (●···●) indicates reaction of apo OTA with boiled inactivating enzyme. In Fig. 1(b), incubation was performed for 30 min.

the absence of PALP. After incubation for indicated periods, OTA activity remaining in each reaction mixture was assayed by the addition of PALP. Irreversible inactivation of OTA as shown in Fig. 1(a). Fig. 1(b) shows the relation between the enzyme concentrations and the amount of OTA inactivated. Small intestine and skeletal muscle contain considerable amounts of the inactivating enzyme but practically no activity was detected in brain, heart, liver or kidney. In the experiments to be published elsewhere, apo-enzymes of serine dehydratase and tyrosine transaminase were also inactivated by the same enzyme preparation to various degrees. But lactic dehydrogenase, glutamic dehydrogenase and glucose oxidase were not inactivated at all. These observations indicate that the inactivating enzyme specifically reacts with apo-pyridoxal enzymes as substrates, but does not react with all the non-pyridoxal enzymes. The following gel filtration experiments were designed in order to elucidate the nature of products of this inactivation reaction. The pattern of Sephadex G-100 in Fig. 2(a) shows the case in which holo-OTA was used as substrate.

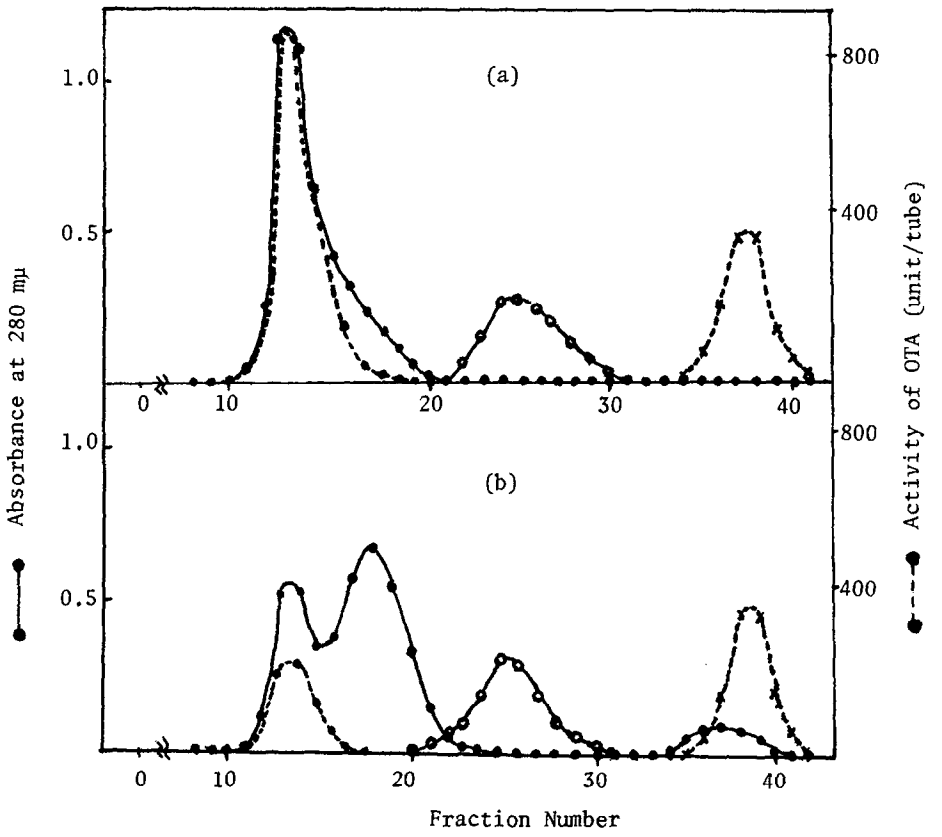

Fig. 2. Chromatography of the reaction products containing OTA and the inactivating enzyme on sephadex G-100.
Sephadex G-100 column (1.8 x 50 cm) was equilibrated with 0.05 M potassium phosphate buffer, pH 7.5 containing 10^{-3}M mercapto-ethanol. The flow rate was adjusted to 8.0 ml/hr. 12 mg of crystalline holo-OTA(a) or apo OTA(b) was incubated with 2.0 mg of the partially purified inactivating enzyme for 12 min. After the reaction was stopped by standing at 0°C, the reaction mixture was applied to the chromatography. NH_2SO_4 was mixed in applied mixture as a marker.
o——o activity of inactivating enzyme
✱----✱ concentration of ammonia

All the OTA activity added was recovered in the first peak and no other products were detected. After incubation of apo-OTA with the inactivating enzyme, the reaction mixture was developed by Sephadex G-100 as shown in Fig. 2(b). The first peak of solid line indicates remaining OTA and the second peak of solid line shows the product from OTA by the enzyme reaction. No OTA activity was observed in the second peak and the molecular weight of this material is smaller than the intact OTA. The protein product is acid insoluble and the

electrophoretic pattern of this product on cellulose acetate membrane appears to be a homogeneous protein. The inactivating enzyme itself appears in the third peak and the forth small peak seems to be oligopeptides. Since these products do not react against the antibody for OTA, it might be considered that OTA is split by the inactivating enzyme into a smaller protein and oligopeptides. The other point of biological interest is the fact that the inactivating enzyme activity increases 10 to 20 fold only in the case of B_6 deficient rats compaired with that of normal rats, but no increase was observed in other conditions, for instance, niacin deficiency, high protein diet, low protein diet or fasting. Fig. 3 shows the induction of the inactivating enzyme in B_6 deficient condition, but in even in B_6 deficient rats, no increase of the inactivating enzyme was detected in brain, heart, liver or kidney.

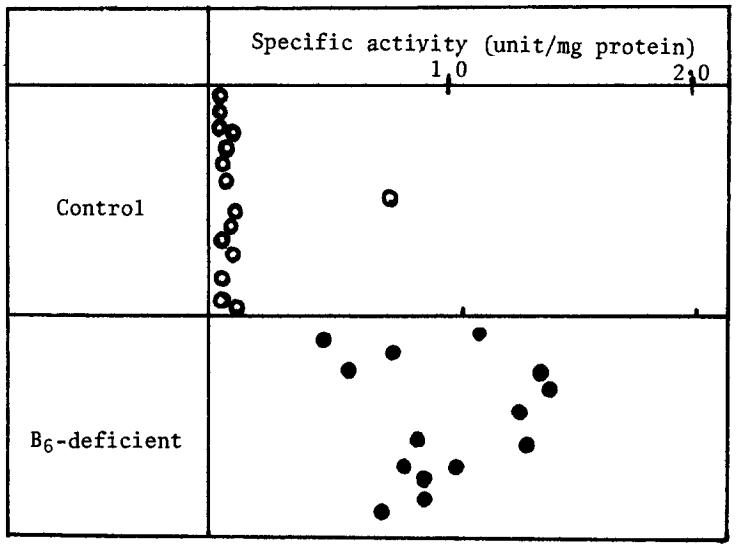

Fig. 3. Comparison of activity of the inactivating enzyme in small intestine between B_6-deficient and control rats.

DISCUSSION

We may speculate that the inactivating enzyme attacks pyridoxal enzymes near the binding site of PALP from the following evidences; 1) The enzyme reacts only with pyridoxal enzymes. 2) PALP completely protects the inacti-

vation by the enzyme. The induction of the enzyme in small intestine and skeletal muscles under the condition of B_6 deficiency may play a role in the biological economy of PALP utilization. In order to protect the decreases of PALP in more important organs like brain, heart, liver and kidney in B_6 deficient condition, the induction of this enzyme in small intestine and muscle may bring about the mobilization of PALP from these organs.

REFERENCES

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2. T. Matsuzawa, T. Katsunuma and N. Katunuma, Biochem. Biophys. Res. Commun. 32, 161 (1968).