A NEW ENZYME THAT SPECIFICALLY INACTIVATES APO-PROTEIN OF NAD-DEPENDENT DEHYDROGENASES

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SUMMARY

The existence of a new inactivating enzyme for NAD-dependent enzymes was recognized in small intestine of niacin-deficient rats. The increase of the enzyme activity was in parallel with the degree of niacin deficiency. The enzyme splits apo-NAD dependent enzymes to smaller molecular compounds. The inactivating reaction is prevented by the presence of NAD.

INTRODUCTION

In an accompaying paper(1) we have reported that a new enzyme was found in the small intestine and skeletal muscle which specifically inactivated apo-enzymes of pyridoxal enzymes and that the activity of this enzyme increased under the condition of B_6 deficiency. When rats were rendered niacin-deficient, another inactivating enzyme was found in small intestine which reacted with apo-enzymes of lactic dehydrogenase (LDH) and glutamic dehydrogenase (GDH). The activity of this enzyme was not induced under the B_6-deficient condition. The inactivation of apo-LDH and apo-GDH was protected by the addition of NAD; that is, the inactivating enzyme is unable to react with holo-enzymes.

Since the inactivating enzyme for NAD dependent enzymes which is induced by niacin deficiency and that for pyridoxal enzymes which is induced by B_6-deficiency behave differently in ammonium sulfate fractionation, these two enzymes seem to be different proteins.

The present paper describes the existence of a specific inactivating enzyme for group of enzymes which require NAD as the coenzyme.

MATERIALS AND METHODS

Niacin deficient rats

35 mg/100 g body weight of acetyl pyridine was injected intraperitonially

daily into young rats which were fed a niacin-free 9 % casein diet for 2-3 weeks(2). LDH activity in the small intestine of these rats decreased to less than 50 % of the normal value.

Preparation of inactivating enzyme

The small intestines of niacin-deficient rats were homogenized with an equal volume of 0.05 M potassium phosphate buffer, pH 7.5. The homogenate was sonicated at 10 kilocycles for 2 min, then centrifuged for 10 min, at 10,000 g. The supernatant was centrifuged at 105,000 g for 60 min. After centrifugation, the supernatant was fractionated by ammonium sulfate precipitation and 35-55 % fractions were passed through Sephadex G-25 equilibrated with the buffer described above. The protein fraction was further fractionated by acetone. The 40-70 % acetone fraction represented 6-10 fold purification over the original homogenate and was used as the source of inactivating enzyme for NAD-dependent enzymes.

Assay conditions for inactivating enzyme

The reaction mixtures contained in a final volume of 0.5 ml, 0.05 M potassium phosphate buffer, pH 7.5, 2-3 mg of apo-LDH or apo-GDH and various amounts of the inactivating enzyme, and were incubated at 37°C. The reaction was terminated by 10-20 fold dilution by cold buffer, and the remaining activity of LDH or GDH was assayed by the decrease of NADH. In some experiments, trichloroacetic acid (TCA) was added to the reaction mixture to a final concentration of 10 %. Remaining enzyme protein was determined in the precipitate by the biuret method and ninhydrin reaction was performed on the acid soluble fraction.

Enzymes as substrate

Crystalline apo-GDH from bovine liver and apo-LDH from rabbit muscle were obtained commercially.

RESULTS

The time course of the enzyme reaction is shown in Fig. 1. The inactivating enzyme preparation was incubated with apo-LDH or apo-GDH for the indicated period. After the incubation, the remaining LDH or GDH activities were deter-

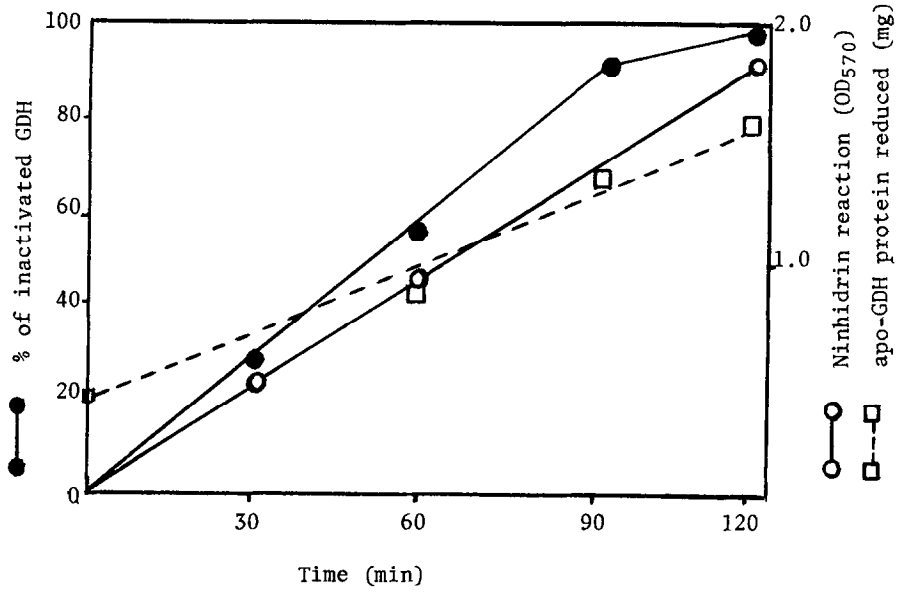

Fig. 1. Time course of the inactivating enzyme reaction. Reaction mixtures contained 2 mg of apo-GDH as substrate and 0.4 mg of the inactivating enzyme concentrated by acetone fractionation. Assay conditions for the inactivating enzyme are described in the text. Reduced amount of apo-GDH protein in the TCA precipitate during incubation was indicated in the ordinate at right hand. After TCA was added to the reaction mixture, the increases of ninhidrin reaction (OD_{570}) in TCA soluble fractions were also estimated.

mined by observing the decrease of NADH. The quantity of GDH protein precipitated by 10 % TCA in each reaction mixture was determined by the biuret method and at the same time the TCA soluble ninhydrin-positive products were assayed. In the course of enzyme reaction, GDH protein precipitate progressively decreased and the ninhydrin-positive compounds increased in TCA soluble fraction correspondingly. On increasing the enzyme concentration, the extent of LDH inactivation was also increased and corresponding increases in the production of ninhydrin-positive compounds in the TCA soluble fraction were observed (Fig.2). The major part of inactivating enzyme activity for apo-NAD dependent enzymes is precipitated by 35-55 % ammonium sulfate, while the inactivating enzyme for pyridoxal enzymes is concentrated in the 50-65 % fraction. The inactivating enzyme for NAD enzymes is heat labile and is inactivated completely by treatment at 55°C for 2 min. The inactivation of apo-NAD dependent enzymes

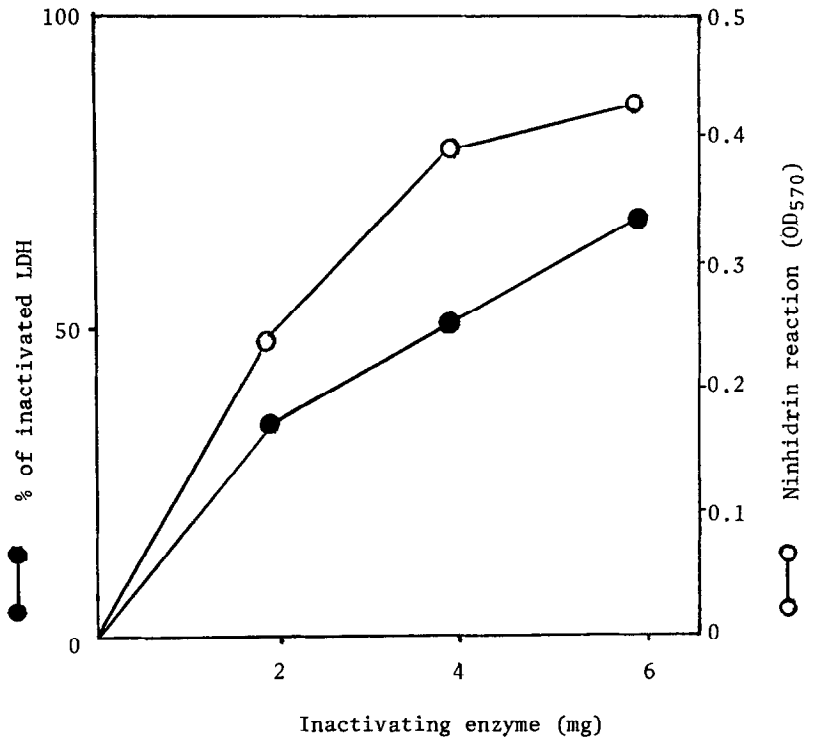

Fig. 2. Dose response curve of the inactivating enzyme. Reaction mixtures contained apo-LDH as substrate and inactivating enzyme concentrated by acetone fractionation. After the addition of TCA, ninhidrin reaction in TCA soluble fraction was assayed.

by the inactivating enzyme is protected by the addition of NAD. The relationship between the appearance of the activity of the inactivating enzyme and the duration of niacin deficiency are plotted in Fig. 3. Correspondence of the induction of this inactivating enzyme and decrease of LDH activity in small intestine as an index of the degree of niacin deficiency was observed. It should be pointed out that the inactivating enzyme for pyridoxal enzymes does not increase in the niacin deficient condition. Inactivating enzyme for pyridoxal enzymes purified from B_6-deficient animals (200-400 fold purified) does not show any inactivation of apo-NAD dependent enzymes. Thus it is quite probable that these two group-specific inactivating enzymes are different enzymes.

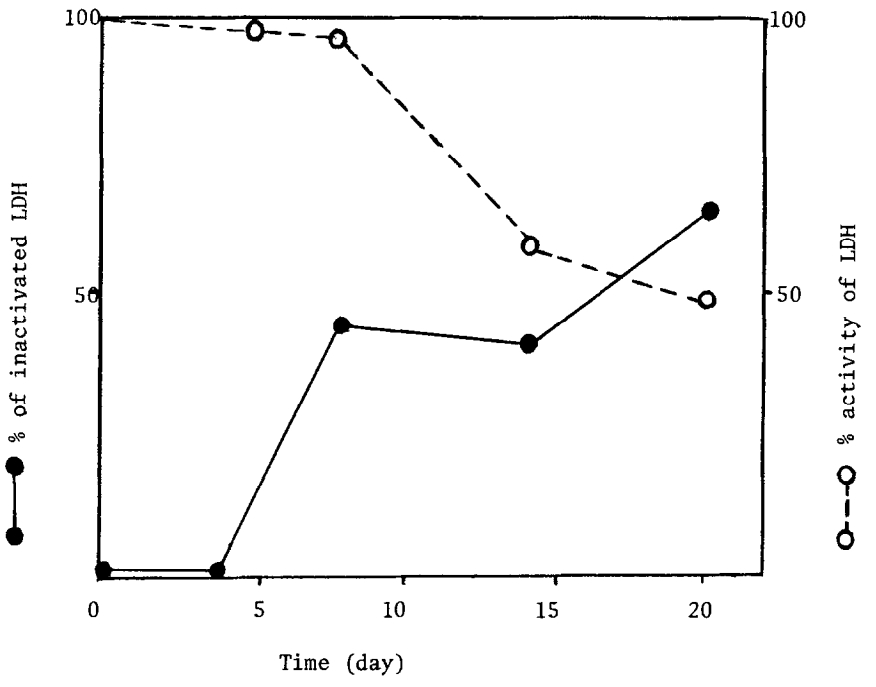

Fig. 3. Relationship between the appearance of the inactivating enzyme activity and the decrease of LDH activity in small intestine during niacin deficiency.
Male rats of wister strains, weighing 180-200 g were fed on niacin deficient diet for indicated period. Acetyl pyridine was also injected daily by the intraperitoneal route. The activity of LDH and of inactivating enzyme in small intestine was assayed. Each point represents the mean of 2-4 rats.

DISCUSSION

Since the inactivating enzyme for NAD dependent enzymes is not sufficiently pure, it is not clear whether all the TCA soluble ninhydrin-positive compounds are direct products of the inactivation reaction or secondarily formed by contaminating non-specific peptidase. But it might be considered that the initiation of the degradation of NAD dependent enzymes is due to the action of this specific inactivating enzyme. As we have reported in an accompany paper, the inactivating enzyme for pyridoxal enzymes is now obtained in sufficiently pure form, free from non-specific proteinases or peptidases and the product of the reaction is demonstrated to be a homogeneous and smaller protein and oligo-peptides. We may assume that the mechanism of action of the inactivating enzyme

for NAD dependent enzymes is similar to the inactivating enzyme for pyridoxal enzymes. Induction mechanisms for the enzyme in the condition of niacin deficiency are still unknown, but we may offer following hypothesis: the action of this enzyme helps to supply NAD from small intestine to more essential organs in the case of NAD deficiency. It is also possible that the enzyme participates in the regulation of the rate of the decay of NAD enzymes.

REFERENCES

1. N. Katunuma, E. Kominami and S. Kominami, Biochem. Biophys. Res. Commun. (1971).
2. D.W. Woolley, Ann. New York Acad. Sci., 52, 1235 (1950).