Effect of Trypsin on Native Glutamate Dehydrogenase of Eel Liver

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Effect of Trypsin on Native Glutamate Dehydrogenase of Eel Liver

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Abstract

Trypsin did limited proteolysis of native GDH as observed in the eel liver. GDH activity after trypsin treatment was increased. The limited proteolysis of native GDH was observed by polyacrylamide gel electrophoresis in the presence of sodiumdodecyl sultate and 2-mercaptoethaol (SDS-PAGE). The molecular weight of the product examined by SDS-PAGE was from 50,000 to 52,000, which was the same as that of GDH suffered in vivo limited proteolysis in the eel liver.

We revealed that leupeptin sensitive protease(s) caused limited proteolysis of native GDH in eel liver[1]. Trypsin is one of leupeptin sensitive proteases. Then the effect of trypsin on native GDH purified from eel liver was investigated. It was found that trypsin cleaved native GDH and the product was a protein with 50,000 to 52,000 dalton which was the same as that of limited proteolyzed GDH.

This report describes the effect of trypsin on the limited proteolysis and the activity of native GDH.

Materials and Methods

Materials

Trypsin was obtained from Wako Pure Chemical Industries. Phenylmethyl sulforyl-fluoride (PMSF) was obtained from Nakarai Chemical Ltd. Native GDH was prepared from the eel liver as described previously[2]. Other reagents were obtained from Wako Pure Chemical Industries and Nakarai Chemical Ltd.

Reaction by Trypsin

For the time course experiment reaction mixture was 1 m*l* of native GDH and 0.11 m*l* of trypsin. Native GDH solution was 1.02mg protein per m*l* of 50% glycerol - 0.025 M Tris-Cl (pH 7.5) - 0.5 mM EDTA and trypsin solution was 5mg (10 units) per m*l* of 0.05 M Tris-Cl

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(pH 7.5) - 1 mM EDTA. Reaction mixture was incubated at 55 ℃. A portion (200 μl) of the reaction mixture was taken into a test tube in which 20 μl of 11 mM PMSF was added previously and GDH activity was measured. The remained mixture after measurement of GDH activity was used for polyacrylamide gel electrophoresis in the presence of sodiumdodecyl sulfate and 2-mercaptoethanol (SDS-PAGE)[3].

Another experiment was performed as follows. Trypsin solutions of 10, 20, 40, 80, and 160 units per ml of 0.05 M Tris-Cl (pH 7.5) - 1 mM EDTA were prepared. Each of trypsin solution (20 μl) was added to 200 μl of native GDH solution, which was 1.02mg protein per ml of 50% glycerol-buffer. The mixture was incubated at 55 ℃ for 30 min, then the reaction by trypsin was terminated by addition of 20 μl of 11 mM PMSF. GDH activity of the mixture was determined and the remained mixture was used for SDS-PAGE.

Assay of GDH Activity

GDH activity was assayed as described previously[2].

Results and Discussion

Effect of Trypsin on Native GDH

When trypsin at a final concentration of 1 unit/ml was added to native GDH, GDH activity after 30 min incubation was increased by 182% as shown in Fig. 1. Furthermore, native GDH was suffered limited proteolysis by trypsin. The molecular weight of the

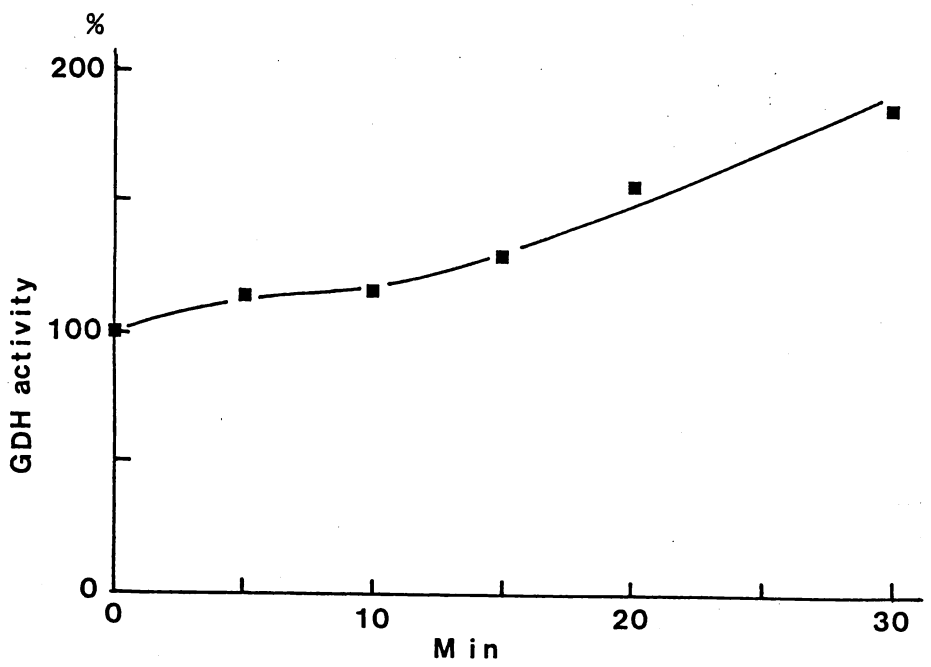

Fig. 1. Time course of GDH activity treated with trypsin at a final concentration of 1 unit/ml. Trypsin and native GDH were incubated at 55℃ and pH 7.5.

product examined by SDS-PAGE was from 50,000 to 52,000, which was the same as that of GDH suffered limited proteolysis in eel liver (Fig. 2).

When the final concentration of trypsin in the mixture of native GDH and trypsin was increased, the GDH activity was increased as shown in Fig. 3. After the digestion of native GDH by trypsin at a final concentration of 14.5 units/m*l*, the GDH activity increased about 4 times higher than that of undigested condition. The limited proteolysis of native GDH was also progressed (Fig. 4).

These results showed that trypsin did limited proteolysis of native GDH as observed in the eel liver. Therefore, it is assumed that the protease(s) in the eel liver to cause limited proteolysis of native GDH was trypsin-like protease(s).

Although limited proteolyzed GDH was purified from the acetone powder of the eel liver as described previously[1], there was the possibility of the contamination of trypsin itself in the acetone powder. Then the possibility of the contamination was investigated. The extract from the acetone powder of the eel liver was applied on a soybean trypsin-inhibitor column, then proteases on the column were eluted and the effect of the proteases on native GDH was investigated. The proteases eluted from the column had no effect on native GDH. This result denied the contamination of trypsin.

There were some similar reports on bovine liver GDH, which was activated by chymotrypsin[4,5]. Pyruvate oxidase[6] and aspartase[7] purified from *E. coli* were also activated by chymotrypsin and trypsin, respectively. All these reports were intended to reveal the structure and function of enzyme by artificial limited proteolysis. For the eel liver GDH trypsin is available for the investigation of its structure and function.

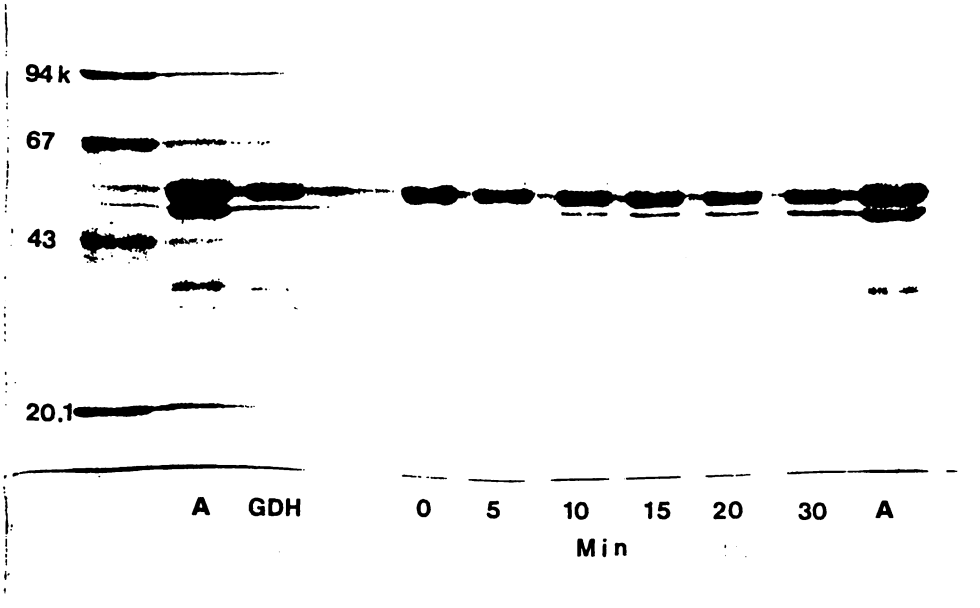

Fig. 2. Sodiumdodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) of GDH treated by trypsin. A : GDH suffered limited proteolysis in eel liver.

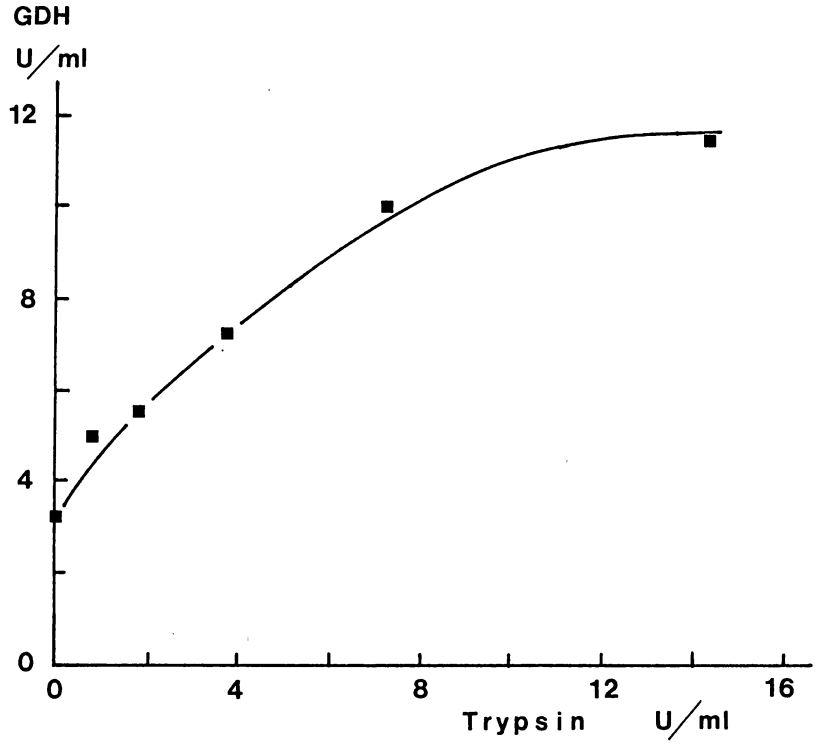

Fig. 3. Effect of trypsin at different concentration on GDH activity. Trypsin and native GDH were incubated at 55℃ and pH 7.5 for 30 min.

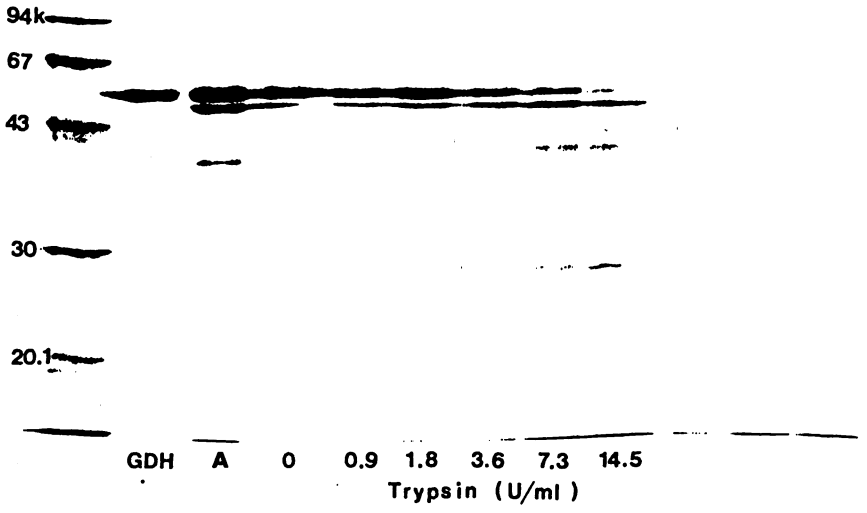

Fig. 4. SDS-PAGE of GDH proteolyzed by trypsin at different concentration.
A : GDH suffered limited proteolysis in eel liver.

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