# TRYPSIN-CATALYZED ACTIVATION OF ASPARTASE*

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Received September 22,1975

Aspartase [EC 4.3.1.1] of *Escherichia coli* is several-fold activated by treatment with trypsin. The activation requires a few minutes to attain a maximal level, and hereafter the enzyme activity gradually decreases resulting in a complete inactivation in about 4 hours. Prior or intermediate addition of soybean trypsin inhibitor results in an immediate cessation of any further change in the enzyme activity. No appreciable change is detected in the molecular weight of the subunits upon trypsin-mediated activation as judged from dodecyl sulfate-polyacrylamide gel electrophoresis, indicating that the structural alteration of the enzyme associated with the activation is a minor one. Kinetic properties of aspartase are also compared before and after the trypsin-activation.

Aspartase [EC 4.3.1.1] of *Escherichia coli* catalyzes the reversible deamination of L-aspartate to yield fumarate and ammonia, and is composed of four subunits of identical molecular weight (1). The enzyme contains approximately 9 sulfhydryl groups per enzyme subunit, one or two of which has been found to be essential for the activity (2). In an aim to elucidate the nature and the role of the sulfhydryl groups in the enzymatic reaction, a limited proteolysis of the enzyme protein was attempted and unexpectedly a marked activation was observed by treatment with trypsin [EC 3.4.4.4].

## MATERIALS AND METHODS

*Materials* ——— Bovine pancreatic trypsin (twice crystallized) was obtained from Worthington and treated with diphenyl carbamyl chloride (3). Soybean trypsin inhibitor was a product of Sigma.

*Enzyme Preparation and Assay of Its Activity* ——— Aspartase was purified from *E. coli* W cells as described previously (1). The purified enzyme was homogeneous as judged from ultracentrifugation and polyacrylamide gel disc

* This investigation was supported in part by research grants from the Ministry of Education of Japan, No. A-838036 and No. 010408.

Abbreviation used is: SDS, sodium dodecyl sulfate.

electrophoresis. The activity of aspartase was routinely determined spectrophotometrically by measuring the formation of fumarate following the increase in absorption at 240 nm at 30°C with a Hitachi 124 recording spectrophotometer equipped with a constant temperature cell housing. The assay system contained, in a total volume of 1.0 ml, 0.1 M sodium L-aspartate (pH 7.0), 2 mM $MgCl_2$, 0.1 M Tris-HCl buffer, pH 7.0, and the enzyme.

*Other Determinations* ——— Polyacrylamide gel disc electrophoresis was carried out according to the method of Davis (4) using 7.5% acrylamide gels. Electrophoresis in the presence of SDS was performed as described by Laemmli (5) using 10% acrylamide gels. Protein was determined by the method of Lowry *et al.* (6).

## RESULTS

Fig. 1A shows the effect of trypsin on the activity of aspartase. The

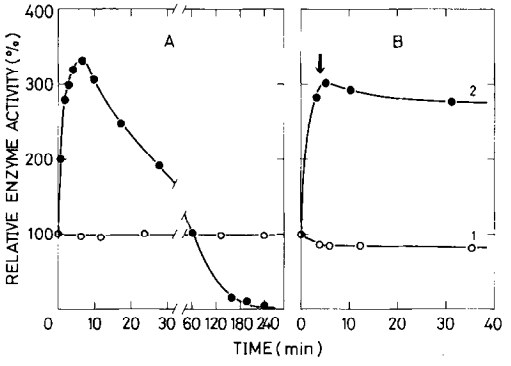


Fig. 1. Trypsin-catalyzed alteration of aspartase activity. A, The reaction mixture contained 0.1 M Tris-HCl buffer, pH 7.4, 180 µg of aspartase, and 10 µg of trypsin (—●—) or in its absence (—○—) in a total volume of 1.0 ml. The reaction was carried out at 30°C and the enzyme activity was determined at designated time intervals by using small aliquots of the reaction mixture as described in "MATERIALS AND METHODS". B, Curve 1 (—○—); Aspartase (20µg) was incubated with 1.0 µg of trypsin and 0.1 M Tris-HCl buffer, pH 7.4 in the presence of trypsin inhibitor (10 µg) in a total volume of 0.20 ml at 30°C. Curve 2 (—●—); Aspartase was incubated as indicated for Curve 1, except that trypsin inhibitor was omitted. After a 4-minute of incubation trypsin inhibitor (10 µg) was added (at the *arrow*) to the reaction mixture. The enzyme activity was determined as described in A.

activity was 3.3-fold increased 7-minute after the addition of trypsin at a neutral pH. Hereafter the activity was gradually decreased and the incubation for 4 hours resulted in an almost complete inactivation. As the concentration of trypsin was increased, the rates of both activation and inactivation markedly increased, while the extent of the maximal activation was not affected. In contrast, when trypsin had been inactivated by heat treatment in boiling water

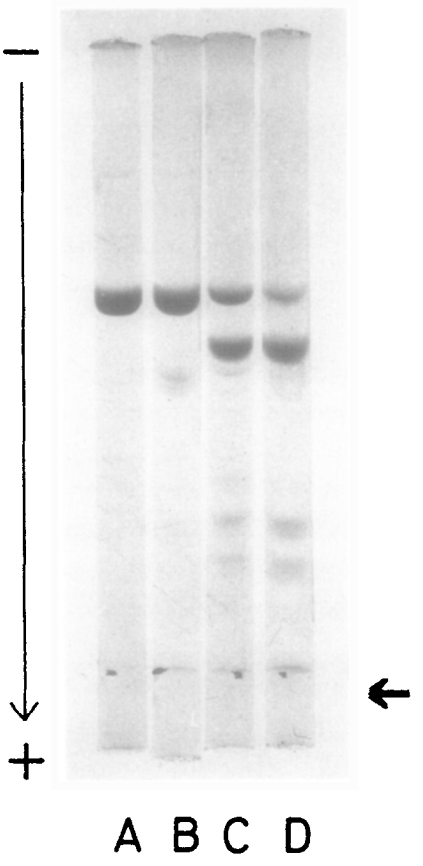


Fig. 2. SDS-polyacrylamide gel electrophoresis. Electrophoresis was carried out as described in "MATERIALS AND METHODS". The sample proteins were 18 μg (A, B, and C) or 27 μg (D). Electrophoresis was carried out at a constant current of 3 mA per gel for 7 hours. Direction of migration was from *top* to *bottom*. The *arrow* indicates the position of bromphenol blue used as a marker. The gels were stained with Coomassie brilliant blue. A, native enzyme. B, C, and D, trypsin-treated enzyme (see below). Aspartase was incubated with trypsin as described in the legend to Fig. 1A. After 1 minute and 20 seconds (B), 65 minutes (C), and 160 minutes (D), 0.1 ml-portions of the incubated mixture were transferred into test tubes containing 0.1 ml of 0.0625 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.001% bromphenol blue. Relative enzyme activities were A, 100%; B, 250%; C, 103%; D, 17%.

for 20 minutes at pH 9.0 prior to the addition, neither an activation nor an inactivation of aspartase was observed. When soybean trypsin inhibitor was added to the reaction mixture containing aspartase prior to the addition of trypsin, activation did not occur (Fig. 1B). The addition of trypsin inhibitor at intermediate stages of aspartase activation or inactivation halted any further change in the activity also as shown in Fig. 1B. A slight decrease in the activity observed a little after the addition of trypsin inhibitor as seen in the same figure appeared to be due to an inhibitory effect of trypsin inhibitor

on aspartase. These results are consistent with the possibility that the trypsin-mediated activation of aspartase is associated with a cleavage of the enzyme peptide bond.

In order to examine the magnitude of the structural alteration of aspartase, SDS-polyacrylamide gel electrophoresis was carried out. The results are shown in Fig. 2, which indicate that the subunits of the trypsin-activated enzyme preparation have nearly the same molecular weight as those of the native enzyme (A and B). Although faster moving faint bands are seen in the gel B, it is not certain whether the activation or the partial inactivation is responsible for release of the fragments. In contrast, prolonged incubation yielded a significant amounts of faster moving components involving one dominant and several minor fractions as seen in C and D, concomitant with the decrease in the enzyme activity. Inspection of the results of polyacrylamide gel disc electrophoresis also exhibited that the trypsin-activated enzyme preparation had the same mobility as the native enzyme at pH 8.9.

Kinetic properties of aspartase were compared before and after the trypsin activation. The value of the half-saturation concentration ($S_{0.5}$) for L-aspartate was slightly increased at pH 7.0, while it was 7-fold increased at pH 8.5 as shown in Table I. The $V_{max}$ value increased both at pH 7.0 and pH 8.5, although increment at pH 8.5 was smaller than that at pH 7.0. The optimum pH for the activity was not significantly altered by the trypsin treatment in comparison with that of the native enzyme (1), being in the range between pH 8.5 and pH 9.0. When kinetic properties were determined using the enzyme system to which trypsin inhibitor and trypsin were added in this order, virtually the same features as those of the native enzyme were obtained.

## DISCUSSION

Some enzymes are known to be synthesized as zymogen, an inactive precursor, activation of which involves limited proteolysis of peptide bonds. However, only few examples have been reported concerning proteinase-catalyzed activation of the native (active) enzyme. Recently, Kuczenski reported that tyrosine hydroxylase [EC 1.14.3.2] of rat brain was activated by treatment with trypsin (7). In his case, however, a marked alteration of the molecular weight was concomitantly observed. The shift of the optimum pH for the activity is sometimes responsible for the activation. Fructose 1,6-bisphosphatase [EC 3.1.3.11] of rabbit liver was found to be several-fold activated at pH 9.2, with a smaller decrease in the activity tested at pH 7.5 by subtilisin (8) or liver lysosomes (9). These changes in catalytic properties were accompanied by the appearance of a smaller subunit. In the present investigation,

Table I. Effects of trypsin-treatment on kinetic parameters of aspartase

Aspartase (150 μg) was incubated with 4.0 μg of trypsin and 0.1 M Tris-HCl buffer, pH 7.4 at 30°C in a total volume of 0.8 ml. After a 3-minute of incubation trypsin inhibitor (20 μg) was added and the test tube was transferred into an ice bath. This preparation was used as the trypsin-activated enzyme. The relative enzyme activity of this preparation was 320% and remained unchanged throughout the determination of the kinetic parameters.

| pH | | Native enzyme | Trypsin-activated enzyme |
|---|---|---|---|
| 7.0 | $S_{0.5}$ | 10 mM | 12 mM |
| | $V_{max}$* | 0.20 | 0.63 |
| 8.5 | $S_{0.5}$ | 3.1 mM | 21 mM |
| | $V_{max}$ | 0.71 | 1.1 |

* $\Delta A_{240}$/min

however, neither an appreciable change in the molecular weight nor a shift of the optimum pH for the activity was detected upon trypsin-catalyzed activation, although a time-dependent release of smaller peptides was obviously recognized as the inactivation proceeded. These results indicate that the structural change in aspartase associated with the activation is considerably a small one. Analysis of released small peptides and amino acids, if any, upon trypsin-catalyzed activation of aspartase and elucidation of the activation mechanism are currently under investigation.

The authors wish to express their sincere gratitude to Prof. Hirohiko Katsuki of this Department for continuous encouragement and valuable discussions during this investigation. Thanks are also due to Prof. Kyozo Hayashi of this University for valuable suggestions and discussions.

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