SUMMARY

Preparative methods have been worked out for the bromination of unprotected deoxy-AMP, deoxy-GMP, and deoxy-CMP and methods have been devised for the isolation and purification of the bromine derivatives.

Some physicochemical characteristics (UV spectra, R_f values on thin-layer and paper chromatography, coefficients of millimolar extinction) of the bromine derivatives of the DNA components obtained have been determined.

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ACTIVATION OF TRYPSINOGEN BY AN ACID PROTEINASE FROM *Aspergillus awamori*

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UDC 577.156

The activation of trypsinogen can take place autocatalytically — under the action of trypsin — and with the participation of a number of other proteolytic enzymes [1], especially those of microbiological origin [1, 2]. We have investigated the possibility of the activation of the trypsin precursor by acid proteinases — pepsin, rennin, and the enzyme produced by the mold fungus *Aspergillus awamori*.

Pepsin did not activate tripsinogen either at pH 3.0 or at pH 5.0, which is in harmony with the results of other authors [2]. In our experiments, rennin also showed no capacity for activating trypsinogen. A high activating capacity was shown only by the proteinase from *A. awamori*. We have found that the optimum pH for the activation of trypsinogen by this enzyme is 3.0 (0.057 M CH_3COOH). This excludes the autoactivation of trypsinogen, which takes place readily at higher pH values in the presence of even traces of trypsin [3]. On the other hand, at pH 3.0 the proteinase from *A. awamori* possesses a fairly high proteolytic activity, ensuring a high rate of activation and, moreover, trypsin is most stable at this pH value.

In contrast to the autoactivation of trypsinogen, the presence of Ca^{2+} ions is not essential for the activation of the zymogen by the proteinase from *A. awamori*, since we obtained similar results when using 0.05 M $CaCl_2$ and in the absence of Ca^{2+}.

At a ratio of trypsinogen to the *A. awamori* proteinase of 1000:1 (Fig. 1a), activation is practically complete in 1 h. The enzyme from *A. awamori*, in addition to bringing about the specific cleavage of trypsinogen with the formation of trypsin, also performs the nonspecific proteolysis of the protein, which leads to a loss of trypic activity. Thus, at a ratio

M. V. Lomonosov Moscow State University. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 528-530, July-August, 1976. Original article submitted November 19, 1975.

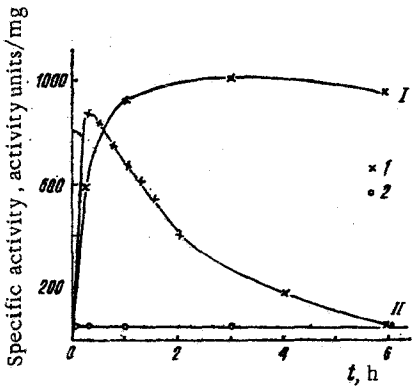

Fig. 1

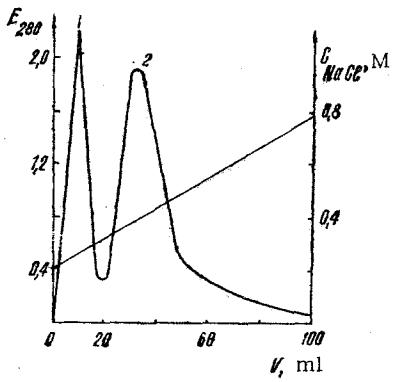

Fig. 2

Fig. 1. Activation of trypsinogen by the acid proteinase from *Aspergillus awamori* at pH 3.0 (0.057 M CH_3COOH) and 37°C: I) ratio of trypsinogen to proteinase 1000:1; II) 100:1 (1 — tryptic activity of the trypsinogen + proteinase system; 2 — tryptic activity of a control solution of trypsinogen).

Fig. 2. Chromatography of the products of the activation of trypsinogen by the acid proteinase from *Aspergillus awamori* on SP-Sephadex G-25. 1) Inactive protein; 2) active trypsin. Conditions: 50 mg of protein; column 1.0 × 3.0 cm; temperature 20°C; 0.057 M CH_3COOH, pH 3.0, linear gradient of NaCl (from 0.2 to 0.8 M); rate of elution 30 ml/h, volume fraction 4 ml. E_{280} — optical density at 280 nm.

of trypsinogen to proteinase of 100:1, after 6 h the tryptic activity has practically ceased (see Fig. 1b). However, the rate of activation is far higher than the rate of nonspecific proteolysis, and therefore with a smaller excess of proteinase it is possible to obtain 100% activation (see Fig. 1a).

Chromatography of the products of the activation of trypsinogen by the *A. awamori* proteinase on the cation-exchange resin sulfopropyl-Sephadex G-25 (SP-Sephadex) at pH 3.0 (Fig. 2) enabled us to obtain a highly active preparation of trypsin without loss of activity.

In the activation of trypsinogen by the *A. awamori* proteinase, the Lys^6—Ile^7 bond in the zymogen is split, as in the classical case. The possibility is not excluded of an additional cleavage of a peptide bond at another point of the polypeptide chain, since forms of trypsin are known which differ by the number of polypeptide chains and the nature of the N-terminal amino acids [4].

On analyzing the results obtained, it may be concluded that acid proteinases of different origins, with all their similarity, may differ in the specificities, the specificity of the acid proteinase from *A. awamori* being broader than the specificities of the pancreatic enzymes of animals — porcine pepsin and calf rennin. The acid proteinase from *A. awamori* is probably close to the microbial enzymes that are capable of activating trypsinogen (for example, aspergillopeptidase A from *A. saitoi* and peptidase A from *Penicillium janthinellum*).

EXPERIMENTAL

Enzymes. Crystalline bovine trypsinogen; the acid proteinase from *A. awamori*, purified on ECTEOLA-cellulose [5], having a specific activity with respect to hemoglobin of 35 activity units/mg; porcine pepsin, purified on DEAE-cellulose [6], having a specific activity with respect to hemoglobin of 55 activity units/mg; and rennin, purified on biospecific sorbents [7] having a specific milk-clotting activity of 9900 activity units/mg.

Determination of Tryptic Activity. The activity of the trypsin was determined on a synthetic substrate — the hydrochloride of the p-nitroanilide of N-benzoyl-D,L-arginine [8] — at 37°C. The specific activity was expressed in nmole of substrate cleaved by 1 mg of the enzyme in 1 min (activity units/mg).

Activation of Trypsinogen by the Acid Proteinase from *A. awamori*. An aliquot of the proteinase solution was added to a solution of the zymogen (1 mg/ml) in 0.057 M CH_3COOH, pH 3.0, thermostatted at 37°C. After predetermined intervals of time, samples were taken of the solution undergoing incubation, and their tryptic activities were determined. A con-

trol solution of trypsinogen without the addition of proteinase was set up in parallel. After the end of activation, the solution was freeze-dried.

Chromatography of the Products of the Activation of Trypsinogen by the *A. awamori* Proteinase. A solution of the protein in 0.057 M CH_3COOH, pH 3.0, was deposited on a column of SP-Sephadex G-25 equilibrated with the same buffer. Elution was performed in a linear gradient of NaCl (from 0.2 to 0.8 M) in 0.057 M CH_3COOH (see Fig. 2). The active fraction was desalted on Sephadex G-25 equilibrated with 0.057 M CH_3COOH, pH 3.0, and was then freeze-dried. There was practically no loss of activity on drying. A highly active trypsin preparation with a specific activity of 1660 activity units/mg was obtained (the activity of a preparation of trypsin from the firm of Worthington, according to our observations, was 1260 activity units/mg).

SUMMARY

1. It has been shown that the acid proteinase from the mold fungus *Aspergillus awamori* is capable of activating trypsinogen at pH 3.0.

2. Conditions have been proposed for the chromatography of the products of the activation of trypsinogen on SP-Sephadex G-25 which exclude autolysis and enables a highly active trypsin preparation to be obtained.

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