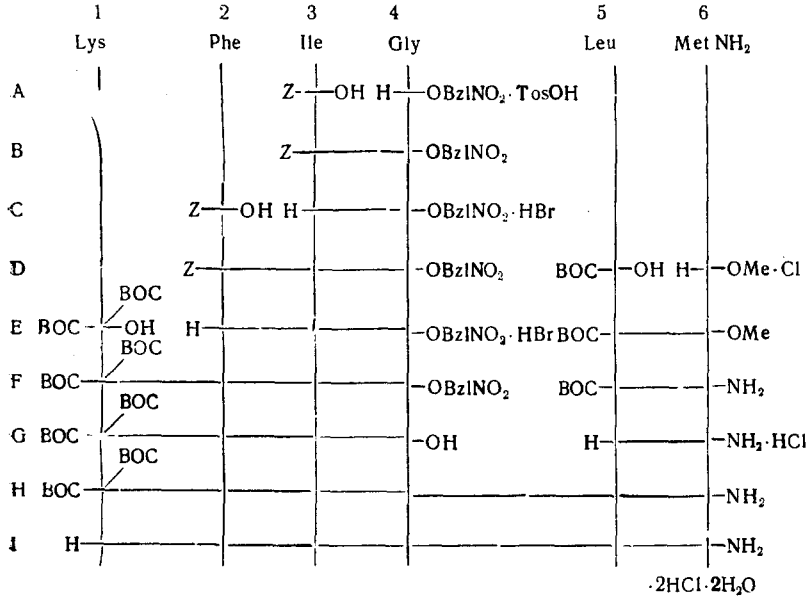
Scheme of the Synthesis



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LOCALIZATION OF THE ACTIVITY OF LEUCINE AMINOPEPTIDASE AFTER ELECTROPHORESIS IN ACRYLAMIDE GEL

A. Ya. Strongin, N. M. Azarenkova, T. I. Vaganova, E. D. Levin, and V. M. Stepanov

UDC 577.156-537.363

A method has been developed for localizing the activity of leucine aminopeptidase after electrophoresis in acrylamide gel with the aid of the substrate L-leucine p-nitroanilide [2].

The leucine aminopeptidase was isolated from "orizin" – a mixture of the proteins of Asp. oryzae (obtained in the Moscow enzyme factory) by precipitation with ethanol and chromatography on columns of DEAE-cellulose at pH 5.6 and 6.9 and hydroxylapatite. Disk electrophoresis was performed at pH 8.3 in tris-glycine buffer on a "Canalco-Europe" instrument (Holland), using an 11.2% acrylamide gel and 70×6 mm tubes. The duration of an experiment was 1.5 h at a current strength of 3 mA/gel and a temperature

All-Union Scientific-Research Institute of the Genetics and Breeding of Industrial Microorganisms.
Translated from Khimiya Prirodnykh Soedinenii, No. 6, p. 820, November-December, 1975. Original article submitted June 4, 1975.

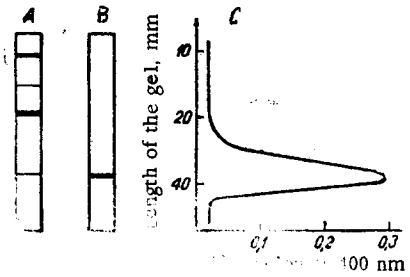

Fig. 1. Electrophoresis of leucine aminopeptidase in acrylamide gel: A) staining with Coomassie Blue (50 μg); B) localization with respect to L-leucine p-nitroanilide (5 μg); C) determination of the activity in eluates from segments of the gel (100 μG).

of 20°C. After the end of the experiment, the gel was stained for 1 h with a 0.3 solution of Coomassie Blue GL in 30% trichloroacetic acid. The excess of dye was eliminated with 7.5% acetic acid.

After electrophoresis, parallel gels were incubated at 37°C for 5-30 min in a mixture of 0.3 ml of a solution of L-leucine p-nitroanilide (1 mg/ml in 0.01 N HCl) and 0.6 ml of 0.05 M tris-HCl buffer, pH 8.0. The zone containing the active enzyme was colored yellow. This band became particularly noticeable in transmitted UV light with its maximum intensity at 360 mm (black band on the light background of the gel). Gels with such bands were frozen at −4°C to prevent the diffusion of the p-nitroaniline formed. Simultaneously, another series of parallel gels was cut into segments 2 mm long, and each segment was incubated with stirring at 37°C for 1 h in a mixture consisting of 2.5 ml of 0.05 M tris-HCl buffer and 0.5 ml of a solution of L-leucine p-nitroanilide in 0.01 N HCl. After incubation, the absorption of the eluate at 400 mm was determined.

Since the preparation of the leucine aminopeptidase of Asp. oryzae was not homogeneous, after staining, four protein fractions were detected in the gel. The amount of the most mobile fraction possessing leucine amino-peptidase activity was about 10% according to densitometry. When gels upon which 5, 11, and 30-50 μg of the protein of this preparation had been deposited were incubated, clearly visible bands appeared after 30, 15, and 5-10 min, respectively.

When 25 μg of electrophoretically homogeneous leucine aminopeptidase from cattle crystalline lens was deposited, a distinct zone appeared after 5 min.

On the basis of the results on the localization of the leucine aminopeptidase fraction from orizin by means of the direct measurement of the activity in eluates from the segment of the gel (Fig. 1) it may be considered that 50-100 μg of protein from this preparation must be deposited on the gel to obtain reliable results.

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