ISOLATION AND PURIFICATION OF L-ASPARAGINASE FROM Escherichia coli 671

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Several methods for obtaining L-asparaginase from various sources are known [1-5]. The present paper describes a new and modified method for obtaining the enzyme L-asparaginase from E. coli 671.

The proposed method of isolation includes the following purification stages: extraction of the enzyme from an acetone powder of the microorganism with water, acid-thermal denaturation of impurities, precipitation of the active protein with acetone, fractionation with polyethyleneglycol, and chromatography on DEAE-cellulose. In this sequence of operations, from 10 g of acetone powder is obtained an enzyme with a specific activity of 209.5 MU/mg of protein (initial activity 2.5 MU/mg of protein) with a yield of 19.6% with respect to activity and 0.24% with respect to protein.

The purity and homogeneity of the isolated enzyme were shown by disk electrophoresis in polyacrylamide gel (Fig. 1). The positions of the main protein and enzyme bands coincide, and the purified enzyme contains only traces of other proteins.

EXPERIMENTAL

We obtained the producting agent of L-asparaginase – the strain E. coli 671 – from S. A. Grannikova (N. F. Gamaleya Institute of Epidemiology and Microbiology of the Academy of Medical Sciences of the USSR). The culture of the microorganism was grown on a medium containing 3% of maize extract (dry weight), 1% of peptone, 0.5% of NaCl, and 0.1% of asparagine, pH 7.0-7.2. Fermentation was performed in a glass fermenter (50 liters) under aerobic conditions at 27-28°C for 10-12 h. The biomass was separated from the medium on a FS-45 supercentrifuge. Yield: 400-450 g of moist biomass (35-37% of dry residue) with a specific activity of 850 MU/g of dry residue or 2.5 MU/mg of protein.

The biomass was twice suspended in cold acetone (1 : 10), the cells were separated by centrifuging, and the product was dried in a desiccator until the solvent has been eliminated completely. The yield of acetone powder was 20% of the crude biomass (specific activity 750-800 MU/g of dry powder).

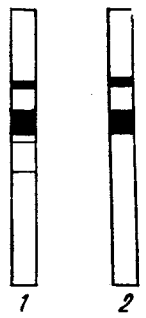

Fig. 1. Electrophoregram of L-asparaginase: 1) protein; 2) enzyme.

To isolate the L-asparaginase, 10 g of the acetone powder was suspended in 250 ml of distilled water and was extracted at room temperature for 2 h, and the extract was separated by centrifuging (30 min, 20,000 rpm). Then 3% of glycine was added, the pH of the extract was brought to 4.5 with 2 N hydrochloric acid, and it was heated on the water bath to 55°C and was rapidly cooled to 0°C. After 30 min, it was centrifuged (20 min, 6000 rpm), after which the supernatant was brought to pH 5.5 with 5 M KOH.

Then it was fractionated with cold acetone (−10°C) in a ratio of 1:1.8 (by volume). The precipitate was separated by centrifuging (10 min, 6000 rpm) and was dissolved in 30 ml of potassium phosphate buffer (0.05 M, pH 8.0).

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The resulting protein solution was acidified with 2 N acetic acid to pH 5.5, polyethyleneglycol with a molecular weight of 1500 was added to 20% saturation, and the mixture was left in the refrigerator for 24 h. The precipitate was separated by centrifuging (20 min, 6000 rpm), and the concentration of polyethyleneglycol was brought to 50% saturation. After a day (4°C), the precipitate was separated off by centrifuging and was dissolved in 5 ml of water.

The L-asparaginase was purified further on DEAE-cellulose. For this purpose, the protein was precipitated with a threefold amount of acetone, the precipitate was dissolved in 0.01 M potassium phosphate buffer, pH 8.5 (concentration of protein 0.2-0.3 mg/ml), and the proteins were adsorbed on DEAE-cellulose ("Reanal"). Under these conditions, 65.5% of the total protein and 85.3% of the total L-asparaginase activity were adsorbed.

The desportion of the L-asparaginase was performed with 0.065 M potassium phosphate buffer, pH 8.0. As a result, 47.1% of the adsorbed protein and 90.3% of the adsorbed L-asparaginase activity were desorbed. The specific activity of the preparation obtained was 209.5 MU/mg of protein.

To the enzyme solution was added 1% of glycine and it was freeze-dried (activity 200 MU/mg of protein).

The activity of the L-asparaginase was determined by direct Nesslerization [6] and the protein content by Lowry's method [7]. Disk electrophoresis in polyacrylamide gel was performed by Davis's method [8]. The L-asparaginase activity was determined by staining in the gel by a method previously proposed by us [9].

CONCLUSIONS

A modified method for obtaining purified L-asparaginase from E. coli 671 is proposed. The purification includes extraction with water from an acetone powder, acid-thermal treatment, fractionation with acetone and with polyethyleneglycol, and volume chromatography on DEAE-cellulose.

The specific activity of the L-asparaginase was 200 MU/mg of protein, and the yield of enzyme 19.6%. Its purity was shown by electrophoresis in polyacrylamide gel.

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