THE ISOELECTRIC FOCUSING OF THE L-ASPARAGINASE OF E. coli IN POLYACRYLAMIDE GEL

M. R. Buka, M. A. Geidan, and R. A. Zhagat

UDC 577.150.7:577.155.3

The present paper gives the results of a study of the electrophoretic behavior of the highly purified freeze-dried L-asparaginase of E. coli with a specific activity of 250 MU/mg of protein. We performed the disc electrophoresis [1] of the enzyme in tris–glycine buffer solution, pH 8.3 at 5°C in 8% polyacrylamide gel. The time of the process was 2.5 h. Three electrophoretically different components possessing enzymatic activity were obtained (Fig. 1, curve a).

The disc isoelectric focusing was performed by Catsimpoolas's method [2] in columns of 5% polyacrylamide (6.5 × 75 mm) at 5°C for 4 h. During the electric-focusing process, the initial current strength (3.5 mA) fell to 0.65 mA in the column. The enzyme (50-150 μg) was deposited in a 10% solution of sucrose (0.05-0.15 ml) at the cathode end of the column, above which a protective layer was established – 0.1 ml of a 1% solution of Ampholine in 5% sucrose solution. After electric focusing, the zones were fixed with 12% trichloroacetic acid and were stained with a 0.25% solution of Coomassie Blue. After decoloration by intensive washing, the gels were densitometered on a "Chromoscan" instrument. The L-asparaginase was separated by disc isoelectric focusing into five fractions (Fig. 1b).

Isoelectric focusing in a thin layer of 6% acrylamide gel containing 3% of Ampholine (100 × 140 × 1 mm) was performed by the method of Awdeh et al. [3]. The initial voltage (100 V) was gradually raised to 300 V over 2 h. The current strength at the beginning of the process was 5.2 mA, and at the end 0.6 mA. The time of the process was 48 h. To determine the pH gradient, pieces of the gel (3 × 9 mm) were eluted with 1 ml of double-distilled water, and the pH was measured with an LPU-01 instrument. The plate of gel was stained with a 0.25% solution of Coomassie Blue. By isoelectric focusing in a thin layer of poly-

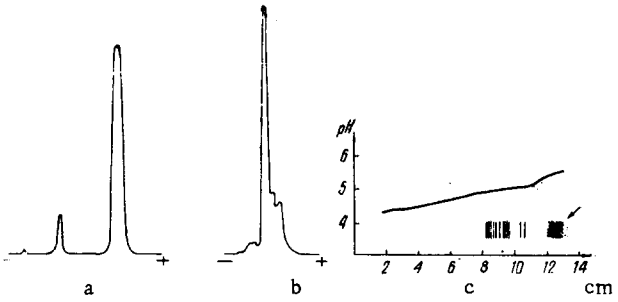

Fig. 1. Photometry curves and scheme of the electric focusing of the L-asparaginase of E. coli on polyacrylamide gel: a) disc electrophoresis; b) disc isoelectric focusing; c) electric focusing in a thin layer (the arrow denotes the position of application).

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 248-249, March-April, 1972. Original article submitted October 25, 1971.

acrylamide, the enzyme was separated into eleven components located in the pH range from 4.8 to 5.1 (Fig. 1e).

LITERATURE CITED

1. B. J. Davis, Ann. N. Y. Acad. Sci., 121, 404 (1964).
2. N. Catsimpoolas, Anal. Biochem., 26, No. 3, 480 (1968).
3. Z. L. Awdeh, A. R. Williamson, and B. A. Askonas, Nature, 219, 66 (1968).