DETERMINATION OF THE N-TERMINAL AMINO ACID OF COTTONSEED MALATE DEHYDROGENASE

K. Davranov, M. A. Kuchenkova,
and P. Kh. Yuldashev

UDC 577.158.4

For the malate dehydrogenase (MDH) isolated from animal sources [1, 2] and from Neurospora crassa [3], the N-terminal amino acid is alanine, and for the enzyme obtained from porcine cardiac muscle it is leucine or isoleucine [4].

There is no information on the terminal amino acids of the enzyme isolated from plant sources.

We have determined the N-terminal amino acid of cottonseed malate dehydrogenase by the dinitrophenylation method [5]. The enzyme was obtained by a procedure described previously [6].

The MDH preparation (4 mg) was dissolved in 0.1 ml of water in a test tube and 0.2-0.3 ml of a 1% solution of $NaHCO_3$ solution was added, followed by 0.02 ml of fluorodinitrobenzene (FDNB) and 0.3 ml of ethanol. The reaction was performed at 37°C for 4 h, and then the excess of FDNB was eliminated by repeated (4-5 times) extraction with peroxide-free ether. The aqueous layer was acidified to pH 1 and was evaporated to dryness. The residue was treated with 0.4 ml of 6 N HCl and was hydrolyzed in a tube sealed under vacuum at 105°C for 18 h. Then the hydrolyzate was diluted with a sixfold volume of water, and the DNP-amino acids were extracted 5-6 times with ether. The ethereal extract was combined and washed with water (3 × 3 ml), and the ether was distilled off to dryness. The byproduct of the reaction, DNP-OH was sublimed off in vacuum over KOH. Then the residue was dissolved in the minimum amount of acetone and deposited on a plate with a thin layer of polyamide (10 × 10 cm [7]). Two-dimensional chromatography was performed in systems 1) benzene–acetic acid (4 : 1) and 2) formic acid–water (13 : 12).

The plates were dried and examined in UV light, and the DNP-amino acid (alanine) was cut out and transferred to a centrifuge tube, covered with 5 ml of a 1% solution of sodium bicarbonate, and centrifuged at 6000 rpm for 10 min. The amount of DNP-alanine was determined in the clear solution quantitatively by spectrophotometry [8] from the absorption at 360 nm. The blank was the pure polyamide from an area equal to that of the DNP-alanine spot which had been treated in the same way. The amount of DNP-alanine obtained was 0.111 μmole which, calculated to the weight of the sample of protein taken, corresponds to 3.88 moles per mole of protein. The DNP-alanine was identified chromatographically with a synthetic marker.

Thus, the N-terminal amino acid of cottonseed MDH is alanine. The quantitative determination showed that 1 mole of enzyme has 4 moles of N-terminal alanine.

LITERATURE CITED

1. F. C. Grim and D. G. Doherty, Biochem. Biophys. Acta, 57, 381 (1962).
2. C. J. R. Thorne, Biochem. Biophys. Acta, 59, 624 (1962).
3. K. D. Munkres and F. M. Richards, Arch. Biochem. Biophys., 109, 457 (1965).
4. T. Deventyi, S. J. Rogers, and R. G. Wolfe, Nature, 210, 489 (1966).
5. F. Sanger, Biochem. J., 39, 507 (1945).
6. K. Davranov, M. A. Kuchenkova, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 373 (1972).
7. K. R. Words and C. T. Wong, Biochem. Biophys. Acta, 133, 369 (1967).
8. Collection of Methodical Procedures for Special Practical Work on the Chemistry of Proteins and Nucleic Acids [in Russian], Moscow (1971).

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 658-659, September-October, 1972. Original article submitted February 1, 1972.