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

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There is no literature information on the C-terminal amino acids of malate dehydrogenase (1.1.1.37 L-malate NAD oxidoreductase) of plant origin. For the enzyme isolated from ox cardiac muscle it has been established by the methods of hydrazinolysis and the action of carboxypeptidase A that the C-terminal amino acid is alanine [1].

To determine the C-terminal amino acid of the malate dehydrogenase obtained from cottonseed [2], we have used the method of splitting it off with carboxypeptidase A (Reanal, Hungary). The latter was first purified by three recrystallizations [3] and was activated [4] before incubation ($D_{280} = 0.7$).

The enzyme (35 mg) was dissolved in 6 ml of water, and the pH was brought to 7.8 with caustic soda, after which 1 ml of a solution of carboxypeptidase A was added. The mixture was incubated at 27°C, 1-ml samples being taken after the required intervals of time (from 5 to 120 min). In the samples taken, the reaction was stopped by acidification to pH 1-2 with 1 N hydrochloric acid, and the resulting protein precipitate was separated by centrifuging at 3000-4000 rpm. For the complete extraction of the amino acids split off, the precipitate was washed three times with water and was centrifuged. The aqueous solutions were evaporated in a rotary evaporator.

In the dry residue (corresponding to 5 mg of protein) the amount of amino acids was determined on an amino-acid analyzer of type 620 A. The results are given in Table 1.

In cottonseed malate dehydrogenase, the carboxypeptidase A first split off valine and then phenylalanine, leucine, and tyrosine. Consequently, valine is the C-terminal amino acid of this enzyme, and it was also identified by chromatography in a thin layer of cellulose.

TABLE 1.

Time of incubation, min	Amino acids split off, moles*			
	Val	Pha	Leu	Tyr
5	0,30	—	—	—
30	3,90	Tr.	—	—
60	3,98	0,30	—	—
120	3,90	2,20	0,30	Tr.

*The yield of amino acids was calculated in moles per mole of protein (mol. wt. 140,000 ± 5000).

LITERATURE CITED

1. C. Wolfenstein, S. Englard, and I. Listowsky, J. Biol. Chem., 244, 23, 6415 (1969).
2. K. Davranov, M. A. Kuchenkova, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 374 (1972).
3. N. Green and H. Neurath, in: Proteins (ed. Neurath and Bailey), 2nd ed., Academic Press, New York (1963-70).
4. V. I. Stepanov and T. I. Greil', Biokhimiya, 28, No. 3, 540 (1966).

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