

ISOENZYME COMPOSITION OF SOLUBLE MALATE  
DEHYDROGENASE FROM COTTON SEEDS

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The presence of three forms of soluble malate dehydrogenase (MDH) in dormant seeds of the cotton plant *Gossypium hirsutum* has been reported previously [1]. We have studied the isoenzyme composition of the soluble MDH from cotton seeds of variety 108-F.

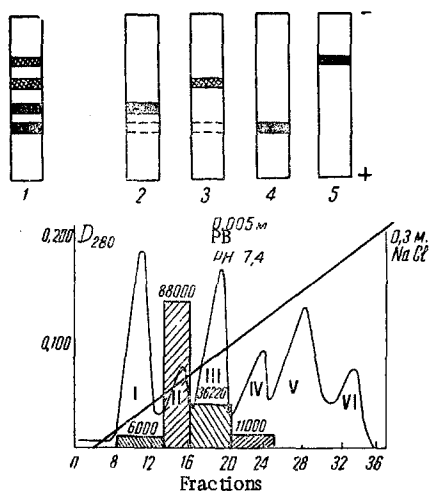


Fig. 1. Zymograms of the isoenzymes of the malate dehydrogenase of the combined fraction (1) and of the fractions separated on a column of DEAE-cellulose (2-5).

An acetone powder was extracted with 0.005 M phosphate buffer, pH 7.4 (1:10) containing 0.005 M of EDTA and 0.005 M of cysteine. The extract was centrifuged at 18,000 rpm for 30 min. The supernatant liquid was salted out with 95%  $(\text{NH}_4)_2\text{SO}_4$ . The precipitate was dissolved in the minimum amount of 0.005 M phosphate buffer (pH 7.4) and was desalted on a column (2.5 × 45 cm) of Sephadex G-25. The desalted protein solution was passed through a column of DEAE-cellulose (1.5 × 30 cm, rate of elution 18 ml/h) equilibrated with the above-mentioned buffer. The protein was eluted from the column with a linear gradient of from 0 to 0.3 M NaCl (Fig. 1). The fractions corresponding to the individual peaks were combined and their protein contents were determined by the Warburg-Christian method [2] and their enzymatic activity by a spectrophotometric method [3]. Information on purification is given in Table 1.

The original extract and the fractions obtained after ion-exchange chromatography were studied by disc electrophoresis with the determination of the localization of the activity in the gel by the tetrazolium method [4]. The original extract produced four colored zones, while the active frac-

TABLE 1.

Stage of purification	Total volume, ml	Protein content, mg/ml	Activity		Yield on activity, %	Degree of purification
			Specific, $\mu\text{mole NAD}/\text{min}/\text{mg}$ of protein	total		
Initial extract	4	69	2057	567732	—	—
Precipitation and desalting on Seph. G-25	5	34	3294	559980	98	1,5
Separation on DEAE-cellulose						
I	27	0,2	6000	32400	5,7	3
II	18	0,15	88000	316800	42	44
III	27	0,18	39220	176029,2	31,4	18
IV	22	0,14	11000	33880	6,04	5
V	54	0,16	—	—	—	—
VI	49	0,16	—	—	—	—

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tions after separation on DEAE-cellulose each gave a single colored zone.

Thus, on the basis of the results of electrophoresis and ion-exchange chromatography, dormant cotton seeds of variety 108-F contain four active forms of soluble MDH.

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