ELECTROPHORETIC INVESTIGATIONS OF ISOENZYMES OF SOME COTTON SEED DEHYDROGENASES

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We give here the results of a determination of the activity of the redox enzymes lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and glutamate dehydrogenase (GDH) in the total extract isolated from dormant cotton seeds of variety 108-F and give zymograms of the localization of their activity in a gel after electrophoresis. The combined extract was obtained by a previously described method [1]. The activity in the protein solution was determined by the spectrophotometric method by measuring the rate of oxidation of NAD · H_2 at 340 nm. The amount of enzyme causing a change in the optical density at 340 nm of 0.001 in 1 min was taken as the unit of activity, and the number of units of enzyme per mg of protein was taken as the specific activity. The following results were obtained:

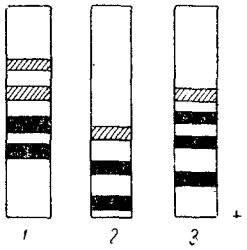

Fig. 1. Zymogram of the total extract of cotton seeds; 1) malate dehydrogenase; 2) lactate dehydrogenase; 3) glutamate dehydrogenase.

Enzyme	Specific activity
MDH	3600 units
LDH	155 units
GDH	93.5 units

To determine the isoenzyme composition of the above-mentioned enzymes, we carried out disc electrophoresis in polyacrylamide gel and determined the localization of the activity by the tetrazolium method. After electrophoresis, the gel was immersed in the reaction mixture and incubated in the dark at 37° C for 30 min [2]. The active fractions appeared

TABLE 1

Enzyme	Malic acid, 13 mg/ml	Lactic acid, 330 μg	Glutamic acid, 30 mg/ml	NAD, 7 mg/ml	NTB*, 3 mg/ml	PMS*, 5 mg/ml	0.1 M phosphate buffer, pH 7.4	Number of colored zones
MDH	0,5**	—	—	0,5	0,2	0,2	2,5	4
LDH	—	0,5	—	0,5	0,2	0,2	2,5	3
GDH	—	—	0,5	0,5	0,2	0,2	2,5	4

* NTB – the dye Nitrotetrazolium Blue; PMS – phenazine methosulfate.

** All the amounts of substances added are given in ml.

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in the form of colored bands (Fig. 1). The composition of the reaction mixtures used for incubation and the numbers of bands that appeared are given in Table 1.

Thus, in the water-soluble fraction of the protein of the seeds of the cotton plant of variety 108-F the malate dehydrogenase is the most active enzyme and is present as four isomeric forms, while lactate dehydrogenase is present in three and glutamate dehydrogenase in four isomeric forms.

LITERATURE CITED

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