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Short Communication

Glutamate Dehydrogenase Changes in *Lemna* Not Due to Enzyme Induction

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In an earlier paper (3) I reported that the levels of the enzymes nitrate reductase, nitrite reductase, and the NADH-dependent glutamate dehydrogenase of *Lemna minor* changed when the nitrogen source was altered. The increases of nitrate and nitrite reductases in response to addition of nitrate appeared to be due to the induction of the enzymes: the response was rapid and was prevented by addition of the protein synthesis inhibitor cycloheximide. Glutamate dehydrogenase increased when cultures grown on amino acids (hydrolyzed casein) were transferred to ammonia; the response was very slow and the effect of protein synthesis inhibitors was not investigated. However, enzyme increase was proportioned to ammonia concentration, and it was assumed that induction was taking place. Subsequent work indicates that this assumption was incorrect and emphasizes the danger of interpretation of enzyme level changes as induction on the basis of only one type of experiment.

In most of the previous work (3), EDTA had been included in the medium used for extraction of enzymes from *Lemna,* as it had been shown that EDTA had a beneficial effect on extraction of nitrate reductase, and had, for *nitrate grown Lemna,* no adverse effect on nitrite reductases or glutamate dehydrogenase. However, experiments with glutamate dehydrogenase from pea roots indicated that the NADH-dependent activity of the purified enzyme was sensitive to inhibition by EDTA (4). Further examination of the enzyme from

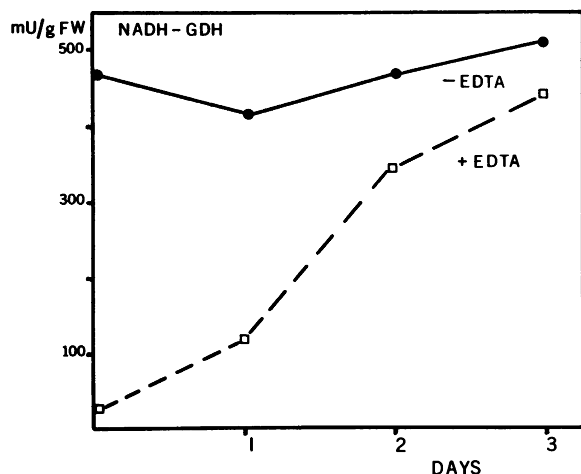

Fig. 1. Levels of NADH-dependent glutamate dehydrogenase assayed in extracts of *Lemna minor,* following transfer of cultures from amino acid to ammonia medium, showing changes in effect of EDTA (in extraction medium) on measurement of the enzyme. Enzyme extracted in 50 mM phosphate buffer, with or without 10 mM EDTA.

Table I. *Effect of EDTA on Extraction and Assay of NADH-dependent Glutamate Dehydrogenase from Amino Acid-grown Lemna and Effect of Storage on EDTA Inhibition*

Activities expressed as percentage of activity of preparations freshly extracted in 50 mM phosphate buffer, pH 7.5 (calculated from milliunits/gram fresh weight figures).

Extraction Medium and Treatment	NADH-GDH Activity		
	%		
Phosphate	100		
Phosphate + 10 mM EDTA	4		
	Freshly Extracted	Enzyme Stored 18 hr at −20 C	Enzyme Stored 18 hr at 4 C
		%	
Phosphate extracted; EDTA in assay system:			
0	100	79	61
10 mM	18	...	50
1 mM	12	49	49
0.1 mM	17	63	51

Lemna, by the methods already described (2, 3), has shown that cultures grown with amino acids as nitrogen source contain NADH-dependent glutamate dehydrogenase which is extremely sensitive to EDTA inhibition. As shown in Figure 1, the sensitivity to inhibition decreased progressively when the plants were transferred to ammonia as a nitrogen source. When extraction was carried out in the presence of 10 mM EDTA, the curve suggests that an increase in level of glutamate dehydrogenase occurred, whereas extraction without EDTA showed that the enzyme level was initially high and was relatively unchanged with time. The NADPH activity of the enzyme was at all times relatively insensitive to EDTA inhibition.

The sensitivity to EDTA inhibition in amino acid grown *Lemna* could be demonstrated not only by its presence during the extraction procedure, but also by addition of EDTA to the enzyme assay system (Table I). Table I also shows that much of the EDTA sensitivity of the glutamate dehydrogenase was lost with storage overnight.

The factors controlling the sensitivity of the enzyme to EDTA have not been elucidated, but may be related to presence of organic acids or the cation-anion changes linked to nitrogen uptake. When the ammonium ion is accompanied by a number of organic anions the change does not occur and the enzyme remains sensitive to EDTA even after several days in the presence of ammonia (Table II). Gamborg and

Table II. *Effect of Ammonia and Accompanying Organic Anions on Inhibition of NADH-dependent Glutamate Dehydrogenase by EDTA*

Lemna plants were grown on amino acids, then transferred for 3 days to ammonia medium (with 4 mM ammonium chloride). Enzyme preparations were made in phosphate buffer, with or without 10 mM EDTA, from replicate samples of plants. Results expressed as percentage of activity of ammonia only sample, extracted without EDTA (calculated from milliunits/gram fresh weight figures).

Conditions during Final 3 Days of Growth	NADH-GDH Activity	
	−EDTA	+EDTA
	%	
Amino acid control	136	4
Ammonium chloride medium	100	87
plus 4 mM sodium potassium tartrate	127	0
plus 4 mM sodium acetate	45	4
plus 4 mM sodium citrate	87	9

Shyluck (1) have shown that the presence of organic acids with ammonia can influence growth and activity of a number of enzymes of cultured plant cells. The EDTA sensitivity in *Lemna* also varies with age and decreases in older amino acid grown cultures.

DISCUSSION

The properties of NADH-dependent glutamate dehydrogenase in crude extracts from *Lemna* vary with nitrogen source of the culture, in that the enzyme from nitrate-grown cultures is insensitive to inhibition by EDTA, whereas the enzyme from amino acid-grown plants is readily inhibited by EDTA. Transfer of plants from amino acids to ammonia causes a change in enzyme, from EDTA sensitive to EDTA insensitive. There is no evidence to suggest that enzyme synthesis is involved in this change.

The nature of the alteration in EDTA sensitivity is not clear, but may involve a change in the enzyme protein structure. The glutamate dehydrogenase of pea roots can apparently exist in different forms with different catalytic activities (4). Alternatively, changes may occur in the cell components which then directly or indirectly influence the enzyme in the crude extracts which were used in this work. However, the EDTA sensitivity of the enzyme was lost on storage, suggesting that either an effector acting on the enzyme or the sensitive state of the enzyme itself is labile once the intact cell is destroyed. Investigation of more purified enzyme preparations may help to clarify the problem.

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