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BRIEF COMMUNICATION

Amino acids response of glutamate dehydrogenase from light and dark treated roots and shoots of maize

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Abstract

The *in vitro* activity of glutamate dehydrogenase (NADH-GDH), from dark-treated root segments of maize seedlings responded differently to amino acids threonine, glutamate and methionine than that from light-treated root segments, and to the amino acid methionine in dark- and light-treated shoot segments. In most cases amino acids inhibited GDH activity, the inhibition increased with amino acid concentration. However, methionine activated GDH from dark-treated roots and light-treated shoots, while aspartate had little effect on enzyme activity.

The activity of glutamate dehydrogenase (L-glutamate NAD-oxidoreductase, GDH E.C. 1.4.1.2.2-4), which is regulated by a variety of exogenous and endogenous factors, seems to be important in the assimilation of ammonium under certain environmental conditions (Srivastava and Singh 1987). Glutamate and most other amino acids inhibit GDH activity in pea (Sahulka 1972, Joy 1973) and maize roots (Singh and Srivastava 1983), although a few of them increased the activity of GDH in tea roots (Takeo 1979). In *Lemna minor* alanine, aspartate, glycine and serine had no effect on GDH activity (Stewart and Rhodes 1977). In maize tissues, the inhibitory effect of cysteine was more pronounced than that of most other amino acids (Singh and Srivastava 1983). Thus the nature of amino acid and/or species seem to be determining the response of the enzyme. One of the important environmental factors regulating NADH-GDH activity is the light and dark regime (Srivastava and Singh 1987). Generally enzyme activity is increased in the darkness, which is believed to be due to *de novo* synthesis of a new isozyme (Puranik and

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Srivastava 1986). We have studied the effects of several amino acids on GDH activity in light- and dark-treated maize roots and shoots with the view to investigate whether the effects of amino acids differ according to type of tissue and environmental conditions.

Seeds of *Zea mays* L. cv. Ganga Safed-2 used in the present investigation were obtained from National Seed Corporation, New Delhi. They were surface sterilized with 0.1 % bleaching powder ($CaOCl_2$) for about 5 min and then washed thoroughly with distilled water. The seedlings were raised on moist (half strength Hoagland's solution without nitrogen) filter paper in Petri plates in a growth chamber at 25 + 2 ^oC and 12 h photoperiod (approximately 60 W m^{-2}) for 5 d. In each case samples from uniformly grown seedlings were taken for further treatment and analysis. Excised segments of roots and shoots from seedlings grown without nitrogen were incubated in half strength Hoagland solution containing 5 mM NH_4NO_3 as sole N source for 24 h in the light or in the darkness. NADH specific GDH activity was assayed as described by Singh and Srivastava (1982). Amino acids were dissolved in the assay buffer and the required quantity of solution was added to the assay mixture, so as to give the desired concentration of the amino acid. The protein in the enzyme extract was precipitated by using equal volumes of 20 % trichloracetic acid and then estimated by the method of Lowry *et al.* (1951). In a few experiments, the enzyme was partially purified by employing $(NH_4)_2SO_4$ precipitation and dialysis but the response to amino acids was the same as observed by using crude enzyme preparations. The data presented are means of at least three independent experiments in duplicate and *t*-test was applied to test significance between light- and dark-treatment.

The effect of amino acids, aspartate, cysteine, glutamate, methionine and threonine was dependent upon the concentration of amino acid, light or dark conditions during enzyme induction and the type of tissue. In roots (Table 1) 1 to 10 mM cysteine inhibited GDH activity, the activity of GDH decreased with the increase of cysteine concentration in both light- and dark-treated plants. A similar effect of threonine was seen, although the inhibition was more pronounced in light- than in dark-treated plants. Glutamate, on the other hand, at concentrations 1.0 to 5.0 mM increased GDH activity in dark-treated plants and had no effect in light-treated plants and only in concentration 10.0 mM slight inhibition of GDH activity. Methionine increased GDH activity in dark-treated plants, while it had little effect on light-treated plants. Aspartic acid had little effect on GDH activity. The GDH in shoots (Table 2) responded to the amino acids similarly as GDH in roots. Only, in contrast to roots, the effects of various concentrations of threonine and glutamate on GDH in light- and dark-treated plants was not significantly different and the stimulatory effect of methionine was observed only in light-treated plants and the stimulation decreased with the increase in methionine concentration. Further, aspartic acid had no effect at concentration 1.0 mM but inhibited GDH activity at higher concentrations.

As indicated earlier (Singh and Srivastava 1983) cysteine appears to be a specific regulator of GDH, both in the roots as well as in shoots. *In vitro* inactivation of nitrate reductase, another enzyme of nitrogen assimilation pathway, by addition of cysteine was also observed (Tischner *et al.* 1986).

Table 1. Effect of some amino acids on *in vitro* activity of GDH enzyme from light and dark treated root tissues. The enzyme activity in control was 20.7±3.1 μmol (NADH oxidised) s^{-1} kg^{-1} (f.m.) in dark and 20.5±2.9 μmol (NADH oxidised) s^{-1} kg^{-1} (f.m.) in light which has been indicated as 100 in the Table. Other values are relative to this control value. L = Light, D = Dark

Conc.	Cysteine		Threonine		Glutamic acid		Methionine		Aspartic acid	
[mM]	D	L	D	L	D	L	D	L	D	L
0.0	100	100	100	100	100	100	100	100	100	100
1.0	80	57	93	68	116	101	113	101	101	101
2.0	49	34	85	66	126	103	118	102	109	102
5.0	30	27	75	65	132	107	143	104	94	97
10.0	13	12	69	63	92	84	153	111	92	95
t-value	2.02		3.48*		4.55*		3.69		0.11	

*Differences between light and dark significant at P= 0.05.

Table 2. Effect of some amino acids on *in vitro* activity of GDH enzyme from light and dark treated root tissues. The enzyme activity in control was 45.6±1.3 μmol (NADH oxidised) s^{-1} kg^{-1} (f.m.) in dark and 42.0±0.4 μmol (NADH oxidised) s^{-1} kg^{-1} (f.m.) in light which has been indicated as 100 in the Table. Other values are relative to this control value.

Conc.	Cysteine		Threonine		Glutamic acid		Methionine		Aspartic acid	
[mM]	D	L	D	L	D	L	D	L	D	L
0.0	100	100	100	100	100	100	100	100	100	100
1.0	98	99	99	94	112	102	102	149	101	102
2.0	87	62	97	93	114	107	101	124	90	94
5.0	34	28	68	89	124	108	92	121	89	89
10.0	22	21	86	87	90	94	83	111	87	83
t-value	1.92		1.09		1.93		6.05*		0.15	

*Differences between light and dark significant at P= 0.05.

These effects of cysteine may have physiological significance in integration of sulphur and nitrogen metabolism in plants. The differences in the response of GDH to some amino acids in light- and dark-treated plants show the possibility that GDH induced in dark might be different than that induced in light. However, other possibilities may not be ignored. Pyruvate dehydrogenase complex, another mitochondrial enzyme, has been recently shown to be inactivated by light possibly by phosphorylation of the enzyme (Budde and Randall 1990).

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