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BRIEF COMMUNICATION

Effect of DTNB on glutamate dehydrogenase activity in root and shoot extracts of maize seedlings

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Abstract

NADH specific glutamate dehydrogenase (GDH) activity was examined in roots and shoots of maize seedlings grown in half-strength Hoagland's solution containing NH_4NO_3 as sole nitrogen source under irradiance of 60 W m^{-2} and temperature of 25 ± 2 °C. When 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was supplied to the assay mixture, it inhibited NADH-GDH activity in both roots and shoots, irrespective of whether the enzymes were extracted from light- or dark-treated roots and shoots. In each case the inhibition increased with the increase in DTNB concentration. At the maximum concentration of DTNB used (20 μM) the inhibition of shoot NADH-GDH was more pronounced than inhibition of root enzyme. This indicated differences in shoot and root NADH-GDH.

Key words: 5,5'-dithio-bis (2-nitrobenzoic acid), dithiotreitol, *Zea mays*

The activity of glutamate dehydrogenase (L-glutamate NADH-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). Several light dependent enzymes are known to be regulated by thiol compounds (Anderson *et al.* 1978, Wolosiuk and Buchanan 1977, Puranik and Srivastava 1986). Glutamate dehydrogenase (GDH) from various plant species is also modulated by thiol binding agents (Ramirez *et al.* 1977, Puranik and Srivastava 1986). Among the various agents

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tested 5,5'-dithio-bis (2-nitrobenzoic acid) appeared to be the most effective enzyme inhibitor in *Agave* (Ramirez *et al.* 1977) and in bean (Puranik and Srivastava 1986). Generally enzyme activity is increased in the darkness which is believed to be due to *de novo* synthesis of a new isozyme (Puranik and Srivastava 1986). In a preliminary investigation, we found that NADH-GDH from dark-grown root and shoot differed from the GDH from light-grown root and shoot in some regulatory aspects. In this study, we analyzed the differences in the sensitivity of the enzymes from the two sources, *i.e.* root and shoot, to DTNB.

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 throughly with distilled water. The seedlings were raised on moist (5 mM NH_4NO_3-half strength Hoagland's solution) filter paper in continuous darkness or light (irradiance of 60 W m^{-2} supplied by fluorescent tubes combined with incandescent bulbs) at temperature 25 ± 2 °C in a growth chamber. 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's solution containing 5 mM NH_4NO_3 as sole nitrogen source for 24 h in the light or in the darkness.

The enzyme was extracted using pestle and mortar from the freshly harvested root and shoot in the extraction medium containing 0.05 M NaPi buffer, 2 mM EDTA, 1 mM $CaCl_2$, 0.1 % mercaptoethanol and 0.5 % Triton-X 100. The ratio of the plant material to extraction medium was 1:4. The homogenate was centrifuged at 20 000 *g* for 15 min. Solid $(NH_4)_2SO_4$ was added to the supernatant to bring it to 30 %. The protein precipitation was removed by centrifugation and more $(NH_4)_2SO_4$ was added to the supernatant to 55 %. The precipitate thus obtained was removed by centrifugation and dissolved in 1 cm^3 of 0.05 M NaPi buffer (pH 7.4) containing 2 mM EDTA. The protein solution was then filtered through a column (25 × 1 cm) of Sephadex-G-100 and eluted by the same buffer. The two fractions (3^{rd} and 4^{th}) containing maximum enzyme activity were pooled and used as enzyme preparation.

NADH specific GDH activity was assayed as described by Singh and Srivastava (1982). The protein in the enzyme extract was precipitated by using equal volumes of 20 % trichloroacetic acid and then estimated by the method of Lowry *et al.* (1951). The data presented are means of at least three replicate experiments ± S.E.

In vitro effects of some thiol modulation compounds on NADH-GDH activity in root and shoot samples from either light- or dark-grown seedlings were studied, to evaluate their possible involvement in light/dark effects (Tables 1 and 2). Dithiothreitol (DTT) had little effect on the GDH activity in dark while it slightly increased the activity of it in light. DTNB, on the other hand, inhibited activity in both conditions and the inhibition was complete at 20 μM concentration of DTNB. At concentrations lower than this, the inhibition in light-treated segments was higher than that of dark-treated segments.

Supply of 1 to 20 μM DTNB to assay mixture inhibited enzyme activity in both roots and shoots and irrespective of whether the enzyme was extracted from light- or dark-treated roots and shoots (Table 2). In each case, the inhibition increased with

the increase in DTNB concentrations. But at the maximum concentration of DTNB used, the inhibition of shoot enzyme was more pronounced than that of root. The inhibition of enzyme activity by DTNB was strongly dependent upon NADH concentration in the assay mixture. As the concentration of NADH increased the inhibition also increased (Table 3). At each concentration of NADH, the inhibition of GDH from light-treated segments was higher than from dark-treated segments.

Table 1. Effect of thiol modulating compounds (DDT or DTNB) on the *in vitro* NADH-GDH activity in root and shoot samples from light- or dark-grown seedling. Seedlings were raised in half-strength Hoagland's solution containing 5 mM NH_4NO_3 as sole nitrogen source for 5 d in light or dark at 25 ± 2 °C. The extracted GDH from these samples was treated with the indicated concentration of the inhibitor. The control activity of the root GDH was 15.2 ± 1.2 and 8.2. ± 0.2 and that of the shoot GDH 12.4 ± 1.5 and 7.6 ± 1.2 nmol (NADH oxidized) mg^{-1}(protein) s^{-1} for dark- and light-treated segments, respectively.

Inhibitor	[μM]	Enzyme activity relative to control [%]			
		root		shoot	
		dark	light	dark	light
DTT	1	92 ± 8.0	108 ± 6.5	90 ± 7.5	104 ± 8.5
	2	97 ± 6.5	114 ± 6.5	95 ± 6.5	112 ± 7.5
	10	85 ± 6.0	102 ± 5.5	80 ± 6.0	102 ± 7.0
	20	80 ± 4.5	98 ± 4.0	76 ± 5.0	96 ± 6.5
DTNB	1	97 ± 6.0	92 ± 9.0	92 ± 9.0	90 ± 9.0
	2	91 ± 4.0	87 ± 8.0	88 ± 3.5	84 ± 8.0
	10	61 ± 8.0	63 ±12.0	55 ± 8.0	60 ± 7.5
	20	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

Table 2. Effect of DTNB on *in vitro* NADH-GDH activity in excised root and shoot samples from light- and dark-treated tissues (24 h) in the presence of ammonium nitrate. Seedlings were raised as indicated in Table 1.

Inhibitor	[μM]	Enzyme activity relative to control [%]			
		root		shoot	
		dark	light	dark	light
DTNB	1	92 ± 6.0	97 ± 6.0	94 ± 8.0	95 ± 5.0
	2	87 ± 5.0	91 ± 6.5	85 ± 8.0	96 ± 5.0
	10	72 ± 7.0	70 ± 7.0	73 ± 6.0	70 ± 6.0
	20	63 ± 7.0	61 ± 8.0	54 ± 7.0	52 ± 7.0

The experiments provide some scope for discussion of the difference in enzyme level in light/dark conditions. In non-inducing condition (no nitrogen during treatment), GDH activity is either unaffected (in roots) or decreased (in shoots) slightly in dark as compared to that in light. However, under inducing conditions (Table 1) the activity of GDH is generally higher in dark than in light in both roots as

well as shoots. Dark-increase or light-decrease of enzyme induction has been observed in many other systems also (Srivastava and Singh 1987). In most cases, decreased activity of enzyme in light has been explained in terms of increased carbohydrate level in the tissue, as exogenously supplied saccharose and some other sugars inhibit enzyme activity (Sahulka and Lisá 1980). Alternatively dark has been implicated to induce a more active isoenzyme of GDH (Srivastava and Singh 1987). This would mean that the enzyme induced in dark will have some different properties to that induced in light. This difference is exhibited in the form of higher inhibition of light-induced enzyme by DTNB than that of dark one (Puranik and Srivastava, 1986). However, in the present study the *in vitro* effect of DTNB on enzyme inhibition was almost the same, whether the enzyme was extracted from light-induced or dark-induced root or shoot tissues. This difference may by either because of different tissues or different plant species used. Also tissue specific differences seem to be apparent in the present study.

Table 3. *In vitro* inhibition of NADH-GDH from roots of either dark- or light-grown seedlings by DTNB at different concentration of NADH. NADH-GDH activity in the roots from light or dark grown seedlings (raised as in Table 1) was assayed in the absence and presence of 0.16 mM DTNB at various concentrations of NADH using saturating concentrations of ammonium sulphate (200 mM) and 2-oxoglutarate (6.66 mM).

NADH [μM]	Enzyme activity [nmol(NADH oxidized) mg^{-1}(protein) s^{-1}] dark			light		
	-DTNB	+DTNB	inhibition [%]	-DTNB	+DTNB	inhibition [%]
16.0	6.4 ± 1.1	5.2 ± 1.0	14	4.0 ± 1.2	2.5 ± 0.6	38
32.0	9.5 ± 1.2	6.0 ± 1.4	34	7.0 ± 1.8	3.0 ± 0.8	54
64.0	15.0 ± 1.4	7.2 ± 1.6	50	8.8 ± 1.0	2.8 ± 1.0	65
128.0	16.5 ± 1.0	5.0 ± 1.2	70	9.0 ± 1.0	1.2 ± 0.8	85

The shoot enzyme was more depressed than root enzyme at 10 μM concentration of DTNB. The results suggest that the enzyme contains free sulfhydryl groups at the active centre (Anderson *et al.* 1978) which are apparently more abundant in the light-induced enzyme than in dark-induced enzyme. The inhibition by DTNB does not seem to be time dependent, as addition of DTNB to the enzyme preparation inhibits the enzyme activity immediately. Percent inhibition of both enzymes by DTNB is little affected by enzyme protein concentration. It is likely that the number of SH residues affected by the presence of DTNB is limited and the concentrations of DTNB used are able to bind with all of them even at high concentration of the protein. However, inhibition of both enzymes by DTNB increase with NADH concentration in the assay mixture. It may be postulated that the enzyme first forms a complex with NADH and then this complex is acted upon by DTNB to block free sulfhydryl groups. Generally the degree of inhibition will depend upon the availability of enzyme-NADH complex, which would increase with increase in NADH concentration for a given enzyme level.

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