Pertanika 6(1), 63–66 (1983)

Properties of glutamate dehydrogenase in developing legume fruit. II. The effects of divalent cations.

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Key words: Glutamate dehydrogenase, developing seeds; legume; manganese.

RINGKASAN

Aktiviti glutamate dehydrogenase dari ekstrak kasar bijian Lupinus anguistifolius *yang direncat oleh EDTA boleh dipulih kembali samada separa atau sepenuhnya oleh kation-kation berdwivalensi yang ditambah ke dalam campuran pengeraman. Mn^{2+} dan Ca^{2+} memberi rangsang yang tinggi sekali masing-masing 67% dan 66% dari kontrol, diikuti oleh Zn^{2+} (59%), Co^{2+} (55%), Mg^{2+} (53%) dan Cu^{2+} (49%). Enzim yang tak cukup manganum adalah lebih sensitif terhadap manganum dan lebih teransang pada kepekatan manganum yang rendah dari enzim yang cukup manganum. Tetapi enzim yang tak cukup manganum mencapai aktiviti maksima sebanyak 91% pada 1.4 mM Mn^{2+} sementara enzim yang cukup manganum mencapai aktiviti maksima 105% pada 2.0 mM Mn^{2+}.*

SUMMARY

The activity of EDTA-inhibited glutamate dehydrogenase from a crude seed extract of Lupinus angustifolius *could be partially or fully restored by the addition of divalent cations to the incubation mixture. Mn^{2+} and Ca^{2+} gave the highest stimulations of 67% and 66% of the control, respectively; followed by Zn^{2+} (59%), Co^{2+} (55%), Mg^{2+} (53%) and Cu^{2+} (49%). The Mn-deficient enzyme was more sensitive to manganese and more stimulated at a lower manganese concentration than the Mn-sufficient enzyme. However, Mn-deficient enzyme achieved a maximum activity of 91% at 1.4mM Mn^{2+} while Mn-sufficient enzyme achieved a maximum activity of 105% of control at 2.0mM.*

INTRODUCTION

The importance of glutamate dehydrogenase (GDH) in amino acid metabolism especially in developing legume seeds has been widely emphasised. One of the interesting characteristics of GDH is its response to certain metals. The EDTA-inactivated GDH can either can be fully or partially reactivated by several metal ions, and GDH from different plant sources seem to respond to different metals (Pahlich and Joy, 1971); Yamasaki and Suzuki, 1969; Sheid *et al.*, 1980).

In the previous paper, a detailed study of GDH inhibition by EDTA was reported. The following study was conducted to investigate the effects of metal ions on GDH activity from developing seeds of *Lupinus angustifolius*. Knowledge on the metal ion response by the enzyme will provide a useful parameter in studying metal requirements in plants and perhaps the interaction of different metals, especially the trace elements.

MATERIALS AND METHODS

General procedure

Plant materials and chemicals

Mn-deficient seeds were obtained from plants grown where no manganese fertilisers were applied and the soil is inherently deficient in manganese. Similarly, the Mn-sufficient seeds from plants grown from plots with added manganese fertilisers.

The use and supply of chemicals were as in the previous paper (Marziah, 1982).

Experimental details

The crude extract preparations and the enzyme assays have been described elsewhere (Marziah, 1982). In order to prevent contamination from exogenous metalic cations all buffers and solutions were treated with Chelex-100. Glassware was soaked and washed with 0.2M EDTA and thoroughly rinsed with double-deionised water.

Crude Enzyme Studies

The effects of metal ions on GDH activity

The crude enzyme extract was preincubated with 1mM EDTA and the respective metal ions for 30 min. The final concentration of metal ions, used as chlorides and adjusted to pH 7.0 in the reaction mixture, was 1mM.

Stimulation of GDH activity by manganese

The stimulation studies were carried out both with EDTA-treated and Chelex-treated enzyme extracts, Mn^{2+} was preincubated with the enzyme extracts for 30 min. before NH_4Cl was added to initiate the reaction.

Responses of GDH activities in Mn-deficient and Mn-sufficient seed extract to manganese.

The manganese concentration used ranged from 0.6mM – 3.0mM. The study was made on EDTA-preincubated enzyme extracts.

RESULTS AND DISCUSSION

NADH-GDH could be partially activated by metal ions (Table 1) maximum stimulation was achieved with Mn^{2+} (67%) and Ca^{2+} (66%) and to a lesser extent Zn^{2+} (59%), Co^{2+} (55%) and Mg^{2+} (53%). Cu^{2+} caused stimulation of only 49%. The results were consistent with those of previous workers who reported that GDH was activated by divalent cations. (Ehmke and Hartmann, 1976; 1978; Fawole and Boulter, 1977; King and Wu, 1971; Pahlich and Joy, 1971; Yamasaki and Suzuki 1969).

Table 1
The effects of divalent cations on NADH-GDH activity in the seeds of *L. augustifolius* cv. UniCrop.

Treatment (Final concentration)	NADH-GDH activity (Expressed as % of control)
Control	100
Control + 1.0 mM EDTA	20
1.0 mM Mn^{2+} + 1.0 mM EDTA	67
1.0 mM Ca^{2+} + 1.0 mM EDTA	66
1.0 mM Zn^{2+} + 1.0 mM EDTA	59
1.0 mM Co^{2+} + 1.0 mM EDTA	55
1.0 mM Mg^{2+} + 1.0 mM EDTA	53
1.0 mM Cu^{2+} + 1.0 mM EDTA	49

Mn^{2+} and Ca^{2+} seemed to activate the enzyme from both the crude and purified extracts of most of the tissues studied (Fawole and Boulter, 1977, Chou and Splitstoesser, 1972, Ehmke and Hartmann, 1978).

The stimulation of GDH activity by Mg^{2+}, Co^{2+} and Zn^{2+} has also been reported (Yamasaki and Suzuki 1969). Chou and Splitstoesser (1972) reported full GDH stimulation by Cu^{2+} in a pumpkin cotyledon extract.

In Table 2, NADH–GDH activity from EDTA-pretreated enzyme was greatly stimulated by Mn^{2+}. The activity was 76% of the control when the EDTA-treated enzyme was incubated with 1mM Mn^{2+}. A higher Mn^{2+} concentration caused inhibition. It is interesting to note that the NADPH-activity was not stimulated by Mn^{2+}, or by other metal ions examined by earlier workers (Fawole and Boulter, 1977; King and Wu, 1970).

Table 2
The effects of manganese concentrations on GDH activities on EDTA-pretreated enzyme extract from seeds of *L. angustifolius* cv. UniCrop.

Treatment (Final concentration)	Activity (Expressed as % of control)	
	NADH-GDH	NADPH-GDH
Control	100	100
Control + 1.0 mM EDTA	6	30
0.25 mM Mn^{2+} + 1.0 mM EDTA	36	30
0.50 mM Mn^{2+} + 1.0 mM EDTA	58	33
1.00 mM Mn^{2+} + 1.0 mM EDTA	76	30
2.00 mM Mn^{2+} + 1.0 mM EDTA	67	34
3.00 mM Mn^{2+} + 1.0 mM EDTA	24	30

Mn^{2+} was supplied as chloride and was pre-incubated with the EDTA-pretreated enzyme extract for 30 min. before reaction was initiated.

The Chelex-treated enzyme, however, was stimulated by more than 100% by all the Mn^{2+} concentrations used (Table 3). The marked increase of the enzyme activity was probably not only due to the stimulation effects of metal ions but also due to the removal of divalent cationic inhibitors as proposed earlier.

Table 3
The effects of manganese concentrations on NADH-GDH activity (after Chelex-treated) obtained from seed extract of *L. angustifolius* cv. UniCrop.

Treatment (Final concentration)	NADH-GDH activity (Expressed as % of control
Control	100
Control + Chelex 100	95
0.50 mM Mn^{2+} + Chelex 100	100
1.00 mM Mn^{2+} + Chelex 100	146
2.00 mM Mn^{2+} + Chelex 100	171
3.00 mM Mn^{2+} + Chelex 100	114

Mn^{2+} was supplied as chloride and was pre-incubated with Chelex-treated enzyme extract for 30 min.

The precise mechanism of metal-stimulation on EDTA-inhibited enzyme is still not understood, though there is general agreement that EDTA does not remove the essential metal components of the enzyme (Steid *et al.*, 1980; Pahlich and Joy, 1971; King and Wu, 1971).

Yamasaki and Suzuki (1969) suggested that EDTA complexes with the metal ions of the enzyme protein, while Pahlich and Joy (1971) further suggested that divalent cations stabilise a particular enzyme conformation and EDTA chelates only the stabilising metal ions but not the essential ions at the active site. An extensive study was made of Ca^{2+} – activation on EDTA-treated enzyme by various workers. (Ehmke and Hartmann, 1976; 1978).

Joy (1969) first proposed that Ca^{2+} is involved in the aggregation or disaggregation of the enzyme. Ehmke and Hartmann (1976; 1978 proposed that the metal ion Ca^{2+}, governs only the NADH-reaction involving an equilibrium of two forms, and this is achieved by the relatively weak bonds between Ca^{2+} and the enzyme. They further suggested that the removal of Ca^{2+} and the addition of Ca^{2+} may involve the alteration in the binding character or binding requirements of the substrate NH_4^+. Sheid *et al.*, (1980) suggested that the role of Ca^{2+} is in governing a reversible equilibrium between catalytically active and inactive forms. It is likely that Mn^{2+} and other metal ions may be involved in similar mechanisms as proposed by Sheid *et al.*, (1980).

It should be emphasised that in all the metal ion activation studies, no Mn^{2+} or other metal ions was added to the extraction medium or to the pre-assay mixture. It is highly likely that the GDH activity would be higher than what was obtained in the control if the addition of metal ions, such as Mn^{2+} or Ca^{2+}, were added. Generally, it appears that any metal that is present in the GDH molecule is not easily dissociable and consequently a higher concentration of EDTA is required to inactivate the enzyme.

Fig. 1 showed that the NADH-GDH activity from Mn-deficient seeds achieved a maximum stimulation with the addition of 1.0mM – 1.4mM Mn^{2+}, while enzyme preparation from Mn-sufficient seeds achieved maximum stimulation with the addition of 1.6mM – 2.0mM Mn^{2+}. The stimulation was more gradual compared to the Mn-sufficient enzyme, and the activity of more than 100% was obtained with 2.0mM Mn^{2+}. However, although the Mn-deficient seeds extract was easily stimulated at a lower manganese concentration the maximum stimulation was 91% with 1.4mM Mn^{2+}. However, it is not known how much of the actual free Mn^{2+} concentration in the EDTA-treated assay mixture is available to the enzyme, but it can be assumed that it is less than the amount supplied.

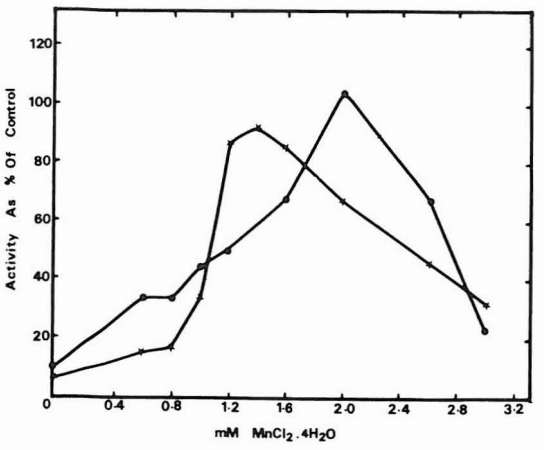

Fig. 1. Responses of NADH-GDH activity in Mn-deficient and Mn-sufficient seeds extracts to different concentrations of Mn^{2+} (supplied as chloride). Mn^{2+} and EDTA were preincubated for 30 min with the extracts before assay. Mn-sufficient (●——●), Mn-deficient (★——★).

In general, the experiment showed that GDH from Mn-deficient seeds is more sensitive to Mn^{2+} stimulation than the enzyme extract from Mn-sufficient seeds. It appears that the

Mn-deficient enzyme, because of the low manganese availability in the extract, has to be efficient in its utilization or requirement of Mn^{2+}. The case of stimulation at lower manganese concentration shows that the seeds that are Mn-deficient have conditioned themselves to a low manganese availability, thus keeping its requirement to the minimum. Full stimulation of the enzyme activity, however, was not achieved because of the limited Mn^{2+} supply. The situation was not true in Mn-sufficient seeds.

CONCLUSION

The EDTA-inactivated NADH-GDH in a crude extract from developing lupin seeds was highly stimulated by Mn^{2+} and Ca^{2+} followed by Zn^{2+}, Co^{2+} and Mg^{2+}. The NADPH-GDH, however, was not at all stimulated. The Mn-deficient enzyme appeared to be more sensitive and stimulated than the Mn-sufficient enzyme. It is highly likely that the Mn-deficient enzyme has conditioned itself to low manganese availability and it is more efficient in its usage of manganese.

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(Received 25 February 1982)