NADP-MALATE DEHYDROGENASE: PHOTOACTIVATION IN LEAVES OF PLANTS WITH CALVIN CYCLE PHOTOSYNTHESIS

Hilary S. Johnson

Botany Department, University of Queensland, St. Lucia., Queensland, Australia. 4067.

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SUMMARY: Several properties of NADP-malate dehydrogenase in leaves of plants with Calvin cycle photosynthesis have been studied. The enzyme is similar to NADP-malate dehydrogenase in leaves of maize, a plant utilizing the C_4-dicarboxylic acid pathway of photosynthesis, in the following respects: (i) the activity of the enzyme is regulated by light in intact leaves., (ii) activation of the enzyme is accomplished by dithiothreitol *in vitro*., (iii) the enzyme is physically distinct from NAD-malate dehydrogenase and appears to exist in at least two molecular weight forms. These results suggest that NADP-malate dehydrogenase may have a common role in both Calvin cycle and C_4-photosynthesis.

INTRODUCTION: The presence of an NADP-specific malate dehydrogenase in leaves of both Calvin cycle plants (C_3-plants) and plants in which the C_4-dicarboxylic acid pathway of photosynthesis is operative (C_4-plants) was first reported by Hatch and Slack (1). A major role for this enzyme in C_4-photosynthesis was indicated by the higher activities of NADP-malate dehydrogenase in leaves of C_4-plants as compared to C_3-plants (1), and its location in the mesophyll chloroplasts (1,2). Studies by Johnson and Hatch (3) on NADP-malate dehydrogenase in maize leaves showed that this enzyme was physically distinct from the NAD-specific enzyme and that its activity was regulated by light *in vivo* and by dithiothreitol *in vitro*.

In this communication, the photoactivation and physical characteristics of NADP-malate dehydrogenase in leaves of the C_3-plants spinach and barley are

described and discussed, and a common role for this enzyme in both Calvin cycle and C_4-photosynthesis, proposed.

MATERIALS AND METHODS: Leaves of spinach (Spinacea oleracea) and barley (Hordeum vulgare) were obtained from plants grown at 23° at a day length of 14 hrs. under artificial illumination of 16,000 lux. Other materials were obtained as previously (3).

Conditions for extraction and assay of NADP- and NAD-specific malate dehydrogenases were identical with those given previously (3) except that the temperature of assay was 30°. Chlorophyll was estimated by the method of Arnon (4).

RESULTS: Light dependent regulation of NADP-malate dehydrogenase in intact leaves. Leaves of intact plants were exposed to a particular light intensity for 10 to 30 mins. and then homogenized at 0° and the activity of NADP-malate dehydrogenase measured within 3 mins. (3). To obtain an accurate measure of NADP-malate dehydrogenase in leaves at a specific light intensity it was absolutely essential that the leaves were homogenized at the light intensity to which the plants were exposed. The activity of NADP-malate dehydrogenase in leaves exposed to saturating light intensities (in excess of 16,000 lux) was greater than 75% of the total activity revealed after incubation of the leaf extract with 5mM-dithiothreitol. The relatively lower activities which were obtained for plants exposed to lower light intensities or darkness could be increased to maximum values by incubation with dithiothreitol. The results for barley and spinach are given in Table 1 and are compared to those reported previously for maize leaves (3). On a chlorophyll basis, the activities of NADP-malate dehydrogenase for barley and spinach were 1.5 and 3.0 μmoles per min. per mgm chlorophyll respectively. These values are somewhat higher than those reported for other C_3- plants (1).

Table 1: Light intensity and the activity of NADP-malate dehydrogenase in leaves.

Light intensity (lux)	Activity as % of potential activity*		
	spinach	barley	maize+
50,000-120,000	80	86	98
16,000	74	79	-
4,200	-	-	33
3,200	15	13	-
0	0	9	2

*Potential activity was the activity obtained after incubation of the leaf extract with 5mM-dithiothreitol at 30°. The activity was maximal within 15-20 mins.

+Results for maize leaves were those reported previously by Johnson and Hatch (3) and are included for comparison.

Dithiothreitol mediated activation of NADP-malate dehydrogenase in leaf extracts. Spinach leaf extracts which were prepared in the presence of dithiothreitol and then treated on a small column of Sephadex G-25 (fine grade) to remove the thiol, gradually lost NADP-malate dehydrogenase activity. At 30°, 84% of activity was lost within the chromatography time of 20 mins. with a further 5% loss in activity by 30 mins. If this column effluent was now incubated with either 5mM-dithiothreitol or 5mM-dithiothreitol and 5mM-$MgCl_2$, the enzyme activity increased to that initially present in the leaf extract applied to the column. Appropriate corrections for column dilution were made. The rate of reactivation of the enzyme was slightly faster when $MgCl_2$ was also present. These results are similar to those reported for maize leaf NADP-malate dehydrogenase (3).

Inactivation and activation of NADP-malate dehydrogenase in spinach leaf extracts was also followed at 0^{o}. In this case, only 30% of the activity was lost during the 40 min. required for removal of thiol on the Sephadex column, but a further decline to 16% of the initial activity occurred during the next 70 mins. An increase in time for inactivation at 0^{o} as compared to 30^{o} was observed earlier for the maize leaf enzyme (3). However, results for reactivation at 0^{o} of the spinach leaf enzyme inactivated at 0^{o}, differed from those reported for the maize leaf enzyme. For the maize leaf enzyme only 25% to 30% reactivation occurred with 5mM-dithiothreitol and 50% to 60% reactivation occurred when $MgCl_2$ was also present, the rate of reactivation with $MgCl_2$ being faster. With the spinach leaf enzyme, complete reactivation occurred at 0^{o} with either dithiothreitol or, at a faster rate, dithiothreitol and $MgCl_2$.

Partial purification of spinach leaf NADP-malate dehydrogenase.

The distribution of NADP-malate dehydrogenase and NAD-malate dehydrogenase in successive ammonium sulphate fractions of spinach leaf was similar to that reported for maize leaves (3). The majority of NADP-malate dehydrogenase appeared in the protein fractions precipitating between 50% to 55% and 55% to 65% ammonium sulphate, whereas the majority of NAD-malate dehydrogenase precipitated at ammonium sulphate concentrations exceeding 55%. In the 50% to 55% ammonium sulphate cut, a ten fold purification of NADP-malate dehydrogenase was effected in the ratio of the NADP- to the NAD-specific enzyme. In crude leaf extracts the ratio of NADP-malate dehydrogenase to NAD-malate dehydrogenase could vary between 1:50 and greater than 1:200.

Gel filtration on Sephadex G-200 (superfine grade) of the 50% to 55% ammonium sulphate fraction gave an elution profile for NADP- and NAD-malate dehydrogenases almost identical to that published for maize leaves (3) (Table 2).

Table 2: Relative mobilities of malate dehydrogenases on Sephadex G-200.

Elution volume / Column volume	spinach*	maize+
NADP-malate dehydrogenase		
High molecular weight	0.09	0.07
Low molecular weight	0.15	0.16
NAD-malate dehydrogenase	0.25	0.25

+After Johnson and Hatch (3). The column dimensions were 68cms x 2.5 cms. Gel filtration was conducted at 22° and 15mm pressure. The elution buffer used contained, 25mM-Tris-HCl, pH 8.0, 2.5mM-dithiothreitol and 2mM-EDTA.

*The column dimensions were 23 cms x 1.9cms. Gel filtration was conducted at 0° and 15mm pressure. The elution buffer was the same as that used for the maize leaf fractionation.

Evidence for at least two molecular weight species of NADP-malate dehydrogenase was also obtained in spinach leaves. The peaks of activity of NADP-malate dehydrogenases were clearly separated from NAD-malate dehydrogenase.

DISCUSSION: The similar photoactivation of NADP-malate dehydrogenase in leaves of maize (C_4-plant) and spinach (C_3-plant) suggests that this enzyme, which from gel filtration data appears similar in the two sources, is operative in both Calvin cycle and C_4-photosynthesis. In C_4-plants where NADP-malate dehydrogenase is located in the mesophyll chloroplasts and where its activity well exceeds the photosynthesis rate of the leaves (1, 2, 3) it has been suggested that this enzyme is responsible for the reduction of oxaloacetate, the product of β-carboxylation in C_4-photosynthesis (1, 3). A second and possibly more general function of NADP-malate dehydrogenase in photosynthesis is indicated by the significant levels of this enzyme in leaves of C_3-plants, where the rate of CO_2 fixation by β-carboxylation is considered to be small (5).

Heber (6, 7), has proposed a shuttle between the chloroplasts and the cytoplasm mediated by NAD-malate dehydrogenase regulating the movement of reducing power between the two compartments. Evidence for oxaloacetate reduction in chloroplasts was the oxaloacetate-dependent stimulation of oxygen evolution by isolated spinach chloroplasts (7). However, there is some controversy as to whether or not NAD-malate dehydrogenase ascribed to the chloroplasts (8, 9) is actually contamination by peroxisomal NAD-malate dehydrogenase (10). The results presented in this communication suggest that NADP-malate dehydrogenase rather than NAD-malate dehydrogenase is the enzyme responsible for oxaloacetate reduction in spinach chloroplasts, and may also be the enzyme involved in the shuttle proposed by Heber regulating the distribution of reducing power between the chloroplasts and the cytoplasm. In support of this proposal, it appears from a sucrose density gradient fractionation that in spinach leaves, the majority of NADP-malate dehydrogenase is located in the chloroplasts (Ting, personal communication), as it is in plants with C_4-photosynthesis (1, 3).

The necessity for photoregulation of NADP-malate dehydrogenase is not immediately obvious. However, $NADPH_2$ produced by metabolic transformations in the chloroplast in the dark, could be used for oxaloacetate reduction which would result in a continual movement of reducing power from the chloroplast to the cytoplasm by the proposed shuttle system. In this regard, it has been observed that the oxidative pentose phosphate pathway in the chloroplasts is activated in the dark (11), and that this is controlled through dark activation of glucose-6-phosphate dehydrogenase (12). Light activation and dark inactivation of NADP-malate dehydrogenase would thus provide a means by which the movement of reducing power from the chloroplasts to the

cytoplasm might be governed by the activity of the photosynthetic electron transport chain rather than by the availability of substrates.

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