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The Effect of Salinity on the Malic Dehydrogenase of Pea Roots[1]

Edna Hason-Porath and Alexandra Poljakoff-Mayber

Department of Botany, The Hebrew University of Jerusalem, Israel

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Abstract. **Effect of salinity on malate dehydrogenase activity was studied.**

Pea root tips contain 2 different malate dehydrogenases. One is located in the particulate, mitochondrial fraction, the other in the soluble, cytoplasmic fraction. Both can act when coupled with either NAD or NADP.

Growing plants in Na_2SO_4 salinated medium did not affect the pattern of the malate dehydrogenases in the root tips. Growing plants in NaCl salinated media resulted in the appearance of a new, third isoenzyme. This new isoenzyme was located in the cytoplasmic fraction.

Salinity of both types, when present in growth medium, induced increases in the NADP coupled activity of the mitochondrial malate dehydrogenase. The NAD coupled activity, however, was depressed except in the cytoplasmic fraction of plants grown in media salinated with NaCl to 1 atmosphere. Addition of either of the salts to assay media of enzymes, isolated from plants grown in non salinated substrate, did not have any significant effect.

In a previous paper (12) it was reported that increasing concentrations of either NaCl or Na_2SO_4 in growth medium, induced a progressive decrease of the specific activity of malate dehydrogenase located in the mitochondria isolated from pea roots and coupled to NAD. However, malate dehydrogenase activity was reported also in the supernatant fraction obtained after sedimentation of the mitochondria. Moreover, the activity of both enzymes may be coupled either to NAD or NADP (1, 7, 14, 16, 19, 20). The importance of the tricarboxylic acid cycle enzymes located in the cytoplasm has been mentioned in many papers in connection with the absorption of excess of cations (5, 8, 9, 17). It was decided therefore to investigate in more detail the activity of the malate dehydrogenase and how it is affected by salinity.

Materials and Methods

Pea plants (var. Laxton progress) were grown on vermiculite moistened with Hoagland solution and salinated with NaCl or Na_2SO_4 at concentration of 1 to 5 atm as previously described (12, 13). Root tips, 1 cm long, were collected from the main roots and homogenized in 0.25 M sucrose—0.1 M phosphate buffer, pH—7.0 containing 5 mM EDTA, 1 mM $MgSO_4$ and 1 mM $MnCl_2$.

The crude homogenate was centrifuged at 1000*g* for 10 min. The resultant supernatant was centrifuged again at 20,000*g* for 30 min.

The 20,000*g* supernatant was considered for this purpose as the cytoplasmic fraction since the behavior of malate dehydrogenase activity in this supernatant and in the supernatant obtained after centrifugation at 100,000*g*, was identical. The pellet sedimenting at 20,000*g* was resuspended in the same sucrose phosphate buffer and used as the mitochondrial fraction.

Malate dehydrogenase-isoenzymes were separated by starch gel electrophoresis and located on the gel according to Fine and Costello (3). Good separations were obtained at 13.0 $V/_{cm^2}$ and constant current of 24 to 27 ma in a 16 hr run at 4°. The protein on the starch gel was stained with amido black according to Smithies (15).

The assay mixture contained 3 μM $MnCl_2$, 3 μM $MgSO_4$, 10 μM phosphate (as buffer at pH 7.5), 30 μM K-malate. To this mixture the enzyme source was added, then 0.3 μM NAD or NADP and the activity of the enzyme measured as the increase in O.D. at 340 mμ using Beckman D.U. Spectrophotometer.

As enzyme source, either mitochondrial suspension equivalent to 15 to 25 μg protein, or cytoplasmic fraction equivalent to 50 to 70 μg protein, was used.

Addition of salt to the assay mixture was done immediately after the enzyme source; the mixture was then left for 5 min before the cofactor was added and the readings taken.

Protein was determined according to Lowry *et al.* (10).

Results and Discussion

A number of reports are concerned with the connection between salt absorption and the accumulation of acids of the tricarboxylic cycle (5, 8, 9, 17).

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Changes in the amount of acids in the cell, in response to the salinity of the medium, may affect the level of the enzymes connected with the metabolism of these acids. Many investigators (2, 4, 18), therefore, have looked for the effect of salinity on malate dehydrogenase activity. Most of them, however, dealt with the effect of salt present in the assay mixture, on the enzyme activity. From our work it seems that the effect of growing the plants in saline media is different from the direct effect of salt present in the assay mixture.

It was found that the enzymes in both the mitochondrial and the cytoplasmic fractions were active either with NAD or NADP as coenzymes. However, in both fractions the specific activity with NAD was higher than with NADP (tables I and II).

Growth of plants in saline media (chloride or sulfate) resulted usually in a decreased NAD coupled activity in both fractions. But, while in plants grown in NaCl salinated media the decrease in the mitochondrial fraction was gradual with increasing salinity, as previously reported (12), this was not the case in the sulfate salinated plants, either in the mitochondrial or in the cytoplasmic fractions. An exception were the plants grown on medium salinated with NaCl to 1 atm. In these plants an increase in the specific activity coupled to NAD was observed in the cytoplasmic fraction (table I).

In roots of plants exposed to both types of salinity, the specific activity coupled to NADP increased in the mitochondrial fraction and decreased in the cytoplasmic fraction.

An attempt was made to differentiate between the direct effect of the salt on the enzyme, and any indirect effect of salinity affecting the plant while growing in the saline substrate. To achieve this, in one case the plants were grown in saline media while the assay of enzymes was carried out in absence of salts (table I). In the other case the plants were grown in non-saline substrate while salt, in various concentrations, was added to the enzyme assay mixture (table II). In this case, the final salt concentrations in the assay mixture were identical with those in the growth media of the previous case.

Table I. *Effect of Salinity of Growth Medium on Malic Acid Dehydrogenase Activity in Pea Roots*

The enzyme was isolated from plants grown in media salinated either with NaCl or Na_2SO_4 as indicated in the table. The reaction medium contained no salt. Either NAD or NADP was used as a coenzyme. The coenzyme was added to the reaction mixture after 5 min of preincubation and then activity of enzyme was measured as described in Methods.

Type	Salinity concn.	Specific activity of malic acid dehydrogenase: Mitochondrial fraction, With NAD	Mitochondrial fraction, With NADP	Cytoplasmic fraction, With NAD	Cytoplasmic fraction, With NADP
	Atm	*Δ OD at 340 mμ per mg protein per min ± standard error*			
NaCl	0	4.34 ± 0.08	0.100 ± 0.01	1.46 ± 0.065	0.50 ± 0.17
	1	3.25 ± 0.14	0.130 ± 0.04	3.07 ± 0.13	0.14 ± 0.01
	3	1.63 ± 0.07	0.990 ± 0.13	0.51 ± 0.08	0.26 ± 0.04
	5	0.96 ± 0.018	0.900 ± 0.11	0.32 ± 0.01	0.22 ± 0.05
Na_2SO_4	0	4.34 ± 0.03	0.10 ± 0.04	1.46 ± 0.05	0.50 ± 0.03
	1	1.14 ± 0.05	0.76 ± 0.07	0.36 ± 0.01	0.22 ± 0.01
	3	2.50 ± 0.1	0.73 ± 0.05	0.71 ± 0.04	0.21 ± 0.05
	5	1.80 ± 0.1	1.13 ± 0.2	0.43 ± 0.08	0.24 ± 0.04

Table II. *Effect of Presence of Salt in Reaction Mixture on the Activity of Malic Acid Dehydrogenase*

The enzyme was isolated from plants grown in non-salinized Hoagland medium NaCl or Na_2SO_4 at concentrations indicated in the table was added to the reaction mixture. All other conditions as stated in legend to Table I.

Type	Salinity concn.	Specific activity of malic acid dehydrogenase: Mitochondrial fraction, With NAD	Mitochondrial fraction, With NADP	Cytoplasmic fraction, With NAD	Cytoplasmic fraction, With NADP
	Atm	*Δ OD at 340 mμ per mg protein per min ± standard error*			
NaCl	0	4.34 ± 0.08	0.100 ± 0.01	1.46 ± 0.065	0.50 ± 0.17
	1	4.56 ± 0.3	0.143 ± 0.03	1.47 ± 0.06	0.52 ± 0.17
	3	4.50 ± 0.2	0.176 ± 0.02	1.53 ± 0.21	0.52 ± 0.11
	5	4.47 ± 0.2	0.176 ± 0.06	1.55 ± 0.17	0.52 ± 0.13
Na_2SO_4	0	4.34 ± 0.03	0.100 ± 0.04	1.46 ± 0.05	0.50 ± 0.03
	1	4.13 ± 0.13	0.110 ± 0.05	1.06 ± 0.03	0.57 ± 0.05
	3	4.00 ± 0.10	0.130 ± 0.02	1.02 ± 0.01	0.66 ± 0.03
	5	3.57 ± 0.13	0.120 ± 0.02	0.95 ± 0.03	0.66 ± 0.04

Comparison of tables I and II shows clearly that the effect of salinity in the 2 cases is entirely different. Presence of NaCl in the assay mixture did not affect the NAD coupled activity at all in any of the fractions. The presence of Na_2SO_4, however, caused a slight decrease in activity of the mitochondrial fraction but did not affect the activity of the cytoplasmic fraction.

In cases where plants were exposed to salinity continuously during their growth, a significant effect on enzyme activity was observed as already described above.

The NADP coupled activity of malate dehydrogenase, in the mitochondrial fraction, was significantly increased in plants exposed to salinity during their growth (table I), while there was no significant effect on enzyme activity, by salt added to the assay mixture (table II).

In the cytoplasmic fraction, the NADP linked activity was not affected by NaCl but was slightly stimulated by Na_2SO_4 present in the reaction mixture (table II). This activity was significantly decreased in plants exposed to salinity during their growth.

In view of these findings, showing different effects of the 2 treatments and different effects of the 2 types of salinity, it was of interest to study the electrophoretic pattern of the enzyme. The results are shown in Fig. 1.

Two distinct bands showing malate dehydrogenase activity were found in the crude homogenate of the control plants (supernatant after 1000*g* for 10 min), 1 moving towards the cathode and 1 to the anode.

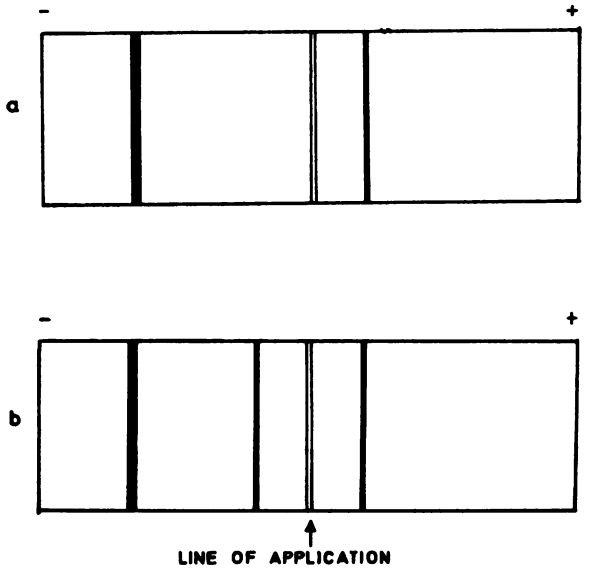

FIG. 1. Diagram of starch gel electrophoretic pattern of: (a) crude homogenate extracted from control plants; (b) crude homogenate extracted from chloride salinated plants.

In the mitochondrial fraction only 1 band, migrating about 1 cm from the line of application, towards the cathode was found. This band was identical with the cathodal band of the crude homogenate. The cytoplasmic fraction of the control plants, showed 1 band, moving to the anode, 3.5 cm from the line of application.

The solution used for localizing the enzyme activity on the starch gel contained either NAD or NADP. The same bands were obtained with both coenzymes, but the color of the bands was not of the same intensity, even when the concentration of protein and nucleotides was the same in both cases. The activity with NADP was much weaker than that with NAD.

The electrophoretic pattern from homogenates of plants grown under Na_2SO_4 salinity was the same as that of control plants, with either NAD or NADP.

The electrophoretic pattern of crude homogenate of plants grown under NaCl salinization (1 and 5 atm) was, however, different. There was 1 band on the cathodal side, corresponding to the enzyme from the mitochondria, but there were 2 bands on the anodal side. Both these bands were due to the cytoplasmic fraction; one migrating 3.5 cm from line of application as previously described, and a second new band, migrating 1 cm from line of application. Again all of these bands were active with both coenzymes. There was no difference in the intensity of color of the new band obtained from supernatants prepared from plants grown at different levels of salinity (the amount of protein applied being always the same).

Evans and Sorger (2), who studied the effect of salts on many enzymes, already mentioned malate dehydrogenase as a salt sensitive enzyme. Hiatt and Evans (4) investigated the effect of salt on the enzyme activity in a whole range of pH. They found the same response of the enzyme to different salts of the same concentration. They concluded that the effect was not ion specific. From results reported in tables I and II and Fig. 1, it seems that the effect of NaCl affecting the plant during its growth is rather specific. Growth of plants under sulfate salinity of the same osmotic potential, did not affect enzyme mobility on starch gel, although the effect on the activity of the enzyme was rather similar to that of NaCl.

It seems more probable that NaCl affects the secondary, or the tertiary structure of the enzyme protein, although, in view of findings reported by Kahane and Poljakoff-Mayber (6), effect on the primary structure cannot be completely excluded.

Weimberg (18) found that an isoenzyme of malate dehydrogenase isolated from pea seeds, was stimulated by NaCl if the concentration of the salt did not exceed 20 mM. Higher concentration of salts were inhibiting. It was shown in this investigation (table I), that the cytoplasmic malate dehydrogenase activity was stimulated in those plants

grown under 1 atm NaCl (24 mM) and inhibited in the plants grown under higher concentrations of NaCl.

On the basis of the results reported in this paper, and on the basis of the results reported previously (12, 13), it seems that a fundamental difference exists between the short-time effect of salt, when present only in the assay mixtures, and the continuous effect of salinity stress to which the plant is exposed during its growth. It is also evident that although the effect of NaCl and Na_2SO_4 salinities is similar, it is by no means identical.

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