Malic Dehydrogenase from Tamarix Roots

EFFECTS OF SODIUM CHLORIDE IN VIVO AND IN VITRO

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ABSTRACT

Soluble and mitochondrial malic dehydrogenases (MDH) were isolated from root tips of the halophyte Tamarix tetragyna L. grown in the presence and absence of NaCl. The activity of the enzymes isolated from root tips grown in the presence of NaCl was lower than that of the enzymes isolated from roots grown in absence of NaCl. The mitochondrial MDH was much more sensitive to salinity than the soluble MDH. The soluble enzyme from roots grown in NaCl had a higher Km for malate and lower Km for NAD than enzyme from the control roots. Addition of NaCl in vitro at 72 mM significantly stimulated the reductive activity of soluble MDH, while higher NaCl concentrations (240 mM and above) depressed enzyme activity. The inhibition of enzyme activity by various salts was found to be in the order MgCl₂ > NaCl = KCl > Na₂SO₄. Mannitol at equiosmotic concentrations had no effect. Substrate inhibition, typical for oxaloacetate oxidation, was not observed at high NaCl concentrations in vitro and high substrate concentrations neutralized the inhibitory effect of NaCl. Increased coenzyme concentrations had no effect. In vitro NaCl increased the Km for malate and oxaloacetate already at relatively low concentrations. At the same time NaCl decreased the Km for NAD and NADH. The inhibitory effect of NaCl on enzyme activity seems not to be due to the effect on the Km alone. Soluble and mitochondrial MDH had different responses to pH changes, mitochondrial MDH being more sensitive. Mitochondrial MDH released from the particles had a similar response to that of the entire particles. Changes of pH modified the effect of NaCl on enzyme activity. It was postulated that NaCl apparently induces conformational changes in the enzyme.

Many plants are capable of growing in saline substrate. This adaptation is usually accompanied by decreased internal osmotic potential, in order to maintain a gradient for water uptake. The mechanism for this osmotic adaptation involves increased ion uptake and changes in the balance between the inorganic and organic ions in the cell sap (3, 4). However, the increased ionic concentration and the lower osmotic potential may affect the structure and function of enzyme proteins (5, 16).

Halophytes are plants that are naturally adapted to grow in saline substrate. This group apparently consist of plants with diverse mechanisms of adaptation, the common denominator being the ability to endure the adverse conditions of a saline habitat. In many cases, the halophytes show typical morphological and anatomical features; many of them have very low osmotic potentials in their cell sap; some are able to regulate the internal salts concentration, either by salt excretion or by accumulation of excessive water in their cells, i.e. succulence. However, they usually show considerable tolerance to high internal osmotic concentrations. The whole problem was recently reviewed by Waisel (29). He concluded that no satisfactory explanation exists for the mechanism of this tolerance.

Another group of organisms showing a very high tolerance to substrate salinity is the group of the halophilic bacteria. The enzymes of these organisms are apparently adapted to function in the presence of high ionic concentrations and are activated by them (1, 2, 6, 13-16, 21, 22). Holmes and Halverson (13) studied the effect of NaCl on malic dehydrogenase from obligatory halophilic bacteria, facultative halophilic bacteria, and animal tissue. The enzyme from the animal tissue showed maximal activity in the absence of NaCl and the activity decreased with increasing NaCl concentration. The enzyme isolated from obligatory halophilic bacteria, on the other hand, showed the highest activity at the high NaCl concentrations (up to 4 mM) and its activity decreased with decreasing NaCl concentrations. Hubbard and Miller (14, 15) showed similar to halophilic bacteria, or whether the enzyme was similar. It was of interest, therefore, to examine whether an enzyme of a halophytic plant showed salt requirement for its activity, similar to halophilic bacteria, or whether the enzyme was similar to that of the glycophytes. Malic dehydrogenase (I-malate:NAD oxidoreductase EC 1.1.1.37) isolated from Tamarix roots was examined.

MATERIALS AND METHODS

Cuttings of Tamarix tetragyna L. were collected from a tree from Mediterranean coastal salt marshes in the Naaman area and were rooted and grown in vermiculite moistened either with half strength Hoagland’s solution (12) or with Hoagland’s solution containing 0.12 mM NaCl (osmotic potential = −π = −5 atm). When the cuttings began to root, salinity was stopped up to final salinities of 0.12 mM, 0.24 mM, 0.36 mM, and sometimes 0.48 mM NaCl (corresponding to π = −5, −10, −15, and −20 atm, respectively) and higher. Loss of water was compensated daily.

Isolation of Mitochondria and Preparation of Enzyme. Root tips, 2 cm long, were collected and blotted with filter paper. Two g of root were ground with sand and 0.5 g of Polyclar AT in 0.1 M tris-HCl buffer (pH 7.4) containing 0.5 mM sucrose, 5 mM EDTA, and 1 mM dithioerythritol (buffer I in Fig. 1) and treated as outlined in Figure 1.

For kinetic studies, the soluble and the solubilized enzymes were partially purified by precipitation with solid (NH₄)₂SO₄,
Results

More than 70% of the total activity of the crude preparation was located in this fraction. The activity of malic dehydrogenase was followed spectrophotometrically at 340 nm in both directions—oxidation of malate and reduction of OAA.

All the preparatory stages were absolutely necessary, otherwise appreciable activity was not achieved. Substitution of Polyclone by Na-ascorbate was not successful.

Protein was estimated according to Lowry et al. (18).

sMDH and mMDH enzymes were found in the roots of Tamarix. The activity of both isoenzymes was studied in roots grown at different levels of salinity in the presence or absence of NaCl in the assay mixture. The results are given in Table I.

Growing the roots in saline media resulted in decreased malic dehydrogenase activity, specific and total, mitochondrial and soluble. The inhibition was significant only at concentrations higher than 120 mm (τ = approximately −5 atm), and the higher the salinity the stronger was the effect. This effect was more pronounced on the mitochondrial than on the soluble enzyme. At the two higher levels of salinity, inclusion of NaCl, at equivalent concentrations, in the assay mixture made the inhibition even higher.

Salinity may affect the enzyme in two ways. (a) NaCl in growth medium may depress or repress the protein synthesis, thus less enzyme will be synthesized. This will be a strictly in

The fraction precipitating between 55 and 75% saturation was collected. The fraction was dissolved in 50 mM tris-HCl buffer (pH 8.0) and passed through a column of Sephadex G-25 column.

Table I. Malic Dehydrogenase Activity in Mitochondrial and Soluble Fractions Isolated from Tamarix Roots Grown at Different Levels of Salinity

The reaction followed NADH oxidation in the presence of OAA at 340 nm. Reaction mixture contained in 3 ml: 1 µmole of OAA, 0.2 µmole of NADH, 3 µmole of EDTA, 1 µmole of cysteine-HCl, 300 µmole of tris-HCl, pH 7.5. The results are means of at least three replicates. In part A, no NaCl was present in the assay mixture. In part B, NaCl was present in the assay medium at the same concentration as in growth medium.

<table>
<thead>
<tr>
<th>NaCl in Growth Medium</th>
<th>mMDH</th>
<th>sMDH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spec. activity</td>
<td>Control</td>
</tr>
<tr>
<td>A. No NaCl in assay medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−0.5</td>
<td>0.78</td>
<td>100</td>
</tr>
<tr>
<td>−0.5</td>
<td>0.73</td>
<td>94</td>
</tr>
<tr>
<td>−0.5</td>
<td>0.49</td>
<td>63</td>
</tr>
<tr>
<td>−15.5</td>
<td>0.31</td>
<td>40</td>
</tr>
<tr>
<td>B. NaCl present in assay medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−0.5</td>
<td>0.78</td>
<td>100</td>
</tr>
<tr>
<td>−0.5</td>
<td>0.72</td>
<td>92</td>
</tr>
<tr>
<td>−0.5</td>
<td>0.38</td>
<td>48</td>
</tr>
<tr>
<td>−15.5</td>
<td>0.19</td>
<td>24</td>
</tr>
</tbody>
</table>

1 Change in absorbance/mg protein·min.
2 Change in absorbance/g fresh roots·min.

Abbreviations: OAA: oxaloacetate; sMDH: soluble malic dehydrogenase; mMDH: mitochondrial malic dehydrogenase; DTT: dithiothreitol.
vivo effect. (b) High salt concentration in the cell may affect the structure of the enzyme; conformational changes may occur and thus affect the catalytic activity and the ability to bind the substrate or the coenzyme or both. This type of effect could operate in vivo and in vitro. It was expected to differentiate between these two types of effects mainly with aid of a kinetic analysis.

Kinetic studies were carried out on the soluble enzymes derived from control roots and roots grown at 360 mM NaCl. The reaction studied was that of malate oxidation. No NaCl was present in the reaction mixture. Apparent $K_m$ values were calculated for malate and NAD. The $K_m$ for L-malate was 11.7 mM for the enzyme from salt-treated plants as against 7.6 mM for the control. The parallel $K_m$ values for NAD were 1.4 for the salt-treated and 2.4 for the controls. These data and other data not reported here on the response of the enzyme to salinity suggested the possibility of conformational changes in the enzyme molecule, which may have been induced by the continuous presence of NaCl in the cell. If this is the case, it should be possible to duplicate the effect in vitro.

The effect of NaCl in vitro on the malic dehydrogenase was therefore studied. NaCl was added to the assay mixture for the mMDH and sMDH, isolated from control roots and roots exposed to salinity (Fig. 2). The assay was run in a rather high buffer concentration but preliminary experiments showed that in the absence of NaCl in the reaction medium the activity of the enzyme was the same at 0.1, 0.05, or 0.025 M buffer. The addition of NaCl (or other salts) to any of these, changed the activity.

As shown in Figure 2, enzyme isolated from plants grown in nonsaline medium was stimulated in vitro by relatively low NaCl concentrations. The most effective concentration being 80 to 100 mM. The stimulation was most pronounced with the soluble enzyme in the oxidation reaction (line 1), and least apparent in the soluble enzyme in the reduction reaction where the effect was practically nonsignificant (line 4). The reduction reaction by the mitochondrial enzyme from roots grown in the absence of NaCl (line 2, Fig. 2) was less sensitive to NaCl in vitro than the soluble enzyme. These results are similar to those reported by Hiatt and Evans (11) and by Greenway and Osmond (10). Weimberg (30) has reported stimulation of sMDH from imbibed pea seeds by 10 and 25 mM NaCl, higher concentrations had an inhibitory effect. In our experiments, the in vitro stimulatory effect was usually larger for the soluble enzyme from control roots (Fig. 2, line 1) than for that isolated from roots exposed to 360 mM NaCl during growth (line 3), but the difference was not always reproducible.

The effect of sodium and chloride ions on the enzyme activity was studied also in combination with other ions. Effects of NaCl, KCl, Na$_2$SO$_4$, and MgCl$_2$ added in vitro to the reaction mixture are shown in Figure 3. The effect of isosmotic
Table II. Effect in Vitro of Various Salts on Malic Dehydrogenase Activity of Entire Mitochondria from Control Roots and Roots Grown in NaCl

Results are given as percentage of the activity (OAA reduction) in reaction mixture without salt. The activity in the mitochondria from the NaCl-treated roots was 40%, lower than that of the mitochondria from control roots.

<table>
<thead>
<tr>
<th>Salts in Reaction Mixture</th>
<th>Mitochondria from Control Roots</th>
<th>Mitochondria from Roots Grown in 360 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl</td>
<td>KCl</td>
</tr>
<tr>
<td>% inhibition</td>
<td>NaCl</td>
<td>KCl</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------</td>
<td>-----</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>48</td>
<td>95</td>
<td>90</td>
</tr>
<tr>
<td>120</td>
<td>89</td>
<td>85</td>
</tr>
<tr>
<td>192</td>
<td>81</td>
<td>81</td>
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<tr>
<td>240</td>
<td>74</td>
<td>76</td>
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<tr>
<td>288</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>360</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>432</td>
<td>52</td>
<td>56</td>
</tr>
<tr>
<td>490</td>
<td>48</td>
<td>51</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of substrate concentration on the inhibition of mMDH activity by 480 mM NaCl. The results are expressed as percentage inhibition compared with reaction mixture without NaCl.

Fig. 5. Double reciprocal plot for the activity of sMDH with changing malate concentration (A) or NAD concentration (B). NAD concentration at A was 1 mM and malate concentration at B was 10 mM. Reaction mixture without NaCl (○); 480 mM NaCl in reaction mixture (●). Plants were grown in sand. (**P < 0.01, *P < 0.05).
added in vitro the apparent \( K_m \) and the \( V_{max} \) for both the substrate (OAA) and the coenzyme (NADH) are affected (Fig. 8, A and B). A similar effect could be observed also for the soluble enzyme. Figure 8, C and D, shows that any addition of NaCl, whether in stimulatory or inhibitory concentration, increases the \( K_m \) but the effect on \( V_{max} \) differs with the two NaCl concentrations. Results reported by Weimberg (30) for sMDH from imbibed pea seeds show that salt increased the apparent maximal velocity of the reaction but decreased the affinity for OAA. He used much lower salt concentration than were used by us.

The effect of NaCl on the activity of the enzyme at different pH values was studied. Siegel and Englard (25, 26) and Sulebele and Silverstein (27) have shown that mMDH and

Fig. 6. Effect of NaCl at changing substrate concentrations on the reductive activity of mMDH. Plants were grown in nonsaline medium. NaCl (0.5 M) was added into the reaction mixture. Composition of the reaction mixture in \( \mu \)moles: OAA, 1; NADH, 0.2; tris-HCL, 300; EDTA, 3; DTT, 0.6. Results are expressed as percentage of maximal activity of the control.

Fig. 7. Effect of NaCl and various substrate concentrations on the reductive activity of sMDH. Reaction in absence of NaCl (▲); 72 mM NaCl (●); 480 mM NaCl (■). A: Plants grown in absence of NaCl; B: plants grown in 360 mM NaCl and then 480 mM or 72 mM NaCl were added to the reaction mixture. The reaction mixture contained 600 mM NaCl (□); 480 mM NaCl (■); 72 mM NaCl (●); no NaCl (▲).
sMDH have different pH requirements. Figure 9 shows that, indeed, this is the case for the reaction of OAA reduction. In the soluble enzyme, the rate of reduction decreased with increasing pH from 7.5 to 10 (Fig. 9B). For the mitochondrial enzyme when inside the particle, a broad peak between pH 5.7 and 8.5 was found, but if the reaction was studied with solubilized mitochondrial enzyme, the pH optimum was at 8.7 and the peak was more defined (Fig. 9A).

**DISCUSSION**

The data show that malic dehydrogenase isolated from roots of the halophyte Tamarix tetragyna L. had no obligatory requirement for salt. Although low NaCl concentrations did stimulate enzyme activity, concentrations above 100 mM had a strong inhibitory effect. This is similar to the effect of NaCl on the enzymes isolated from glycophytes. However, obligatory

![Fig. 9. Effect of pH on the activity of malic dehydrogenase from control roots. The reaction mixture contained in mm: glycine-KOH, 100; EDTA, 2; NAD, 1; malate, 10, or NADH 0.2; OAA, 0.3; DTT, 0.1. Results are given in arbitrary units calculated on the basis of maximal activity for each substrate. A: Mitochondrial enzyme: liberated enzyme (----); enzyme inside the particles (---). B: Soluble enzyme: malate oxidation (●); OAA reduction (●).](#)

![Fig. 10. Effect of NaCl on the specific activity of sMDH at different pH values. Results are given as percentage of the maximal control value. A: Oxidation of malate; B: reduction of OAA. Reaction mixtures are the same as in legend to Fig. 5. Numbers on the right-hand side (A and B) indicate mM NaCl added into the reaction mixture. Plants were grown in absence of NaCl.](#)

![Fig. 11. Effect of NaCl on the activity of mMDH at different pH values. Results are given as percentage of the maximal control value. Grown and reaction in absence of NaCl-control (●); grown in presence of 360 mM NaCl reaction without NaCl (□); grown in presence of 360 mM NaCl in growth medium and 480 mM NaCl in reaction medium (●).](#)
requirements for high salt concentrations (to 4 m) are well known for enzymes of halophilic bacteria (13).

The data in Figure 3 and in Table II support the notion that the effect of salinity on the malic dehydrogenase is not a simple effect of ionic strength. There certainly are specific effects of the different ions. The order of the salts for the effect was

\[ \text{MgCl}_2 > \text{KCl} > \text{NaCl} > \text{NaSO}_4 \]

This order is different from that reported for halophilic bacteria, where considerable differences were found between the effects of NaCl and KCl (1).

An attempt was made to analyze the effect of NaCl on the kinetics of the malic dehydrogenase. Duporique and Kun (8) found that increase in phosphate concentration from 50 to 100 mm increased 8-fold, the apparent Km value for OAA. They concluded that phosphate behaved as a competitive inhibitor of OAA. The effect of chloride was considered to be similar to that of phosphate. Also Weiberg (30) suggested that NaCl at inhibitory concentrations had a competitive effect. Hiatt and Evans (11) found that in the presence of 67 mm NaCl the Km for OAA and for NADH increased 2-fold, but they concluded that the enzyme was activated at this NaCl concentration. These findings suggested that the inhibition is not strictly competitive. Kinetic analysis of our experiments (Figs. 5 and 8) show that NaCl in vitro caused an increase in the Km for the substrate (either malate or OAA) and a decrease in the Km for the co-enzyme; the latter is contrary to the findings of Hiatt and Evans (11) for the enzyme from spinach leaves.

The kinetic behavior of malic dehydrogenase is often explained by formation of a ternary complex. First there is a binding of the enzyme and the co-enzyme, then the binding of the substrate to the enzyme occurs. Raval and Wolfe (23, 24) suggest four steps in the reaction:

\[ \text{MDH} + \text{NAD} \xrightarrow{K_1} \text{MDH-NAD} \]
\[ \text{MDH-NAD} + \text{Malate} \xrightarrow{K_3} \text{MDHXY} \]
\[ \text{MDHXY} \xrightarrow{K_5} \text{MDH-NAD} + \text{OAA} \]
\[ \text{MDH-NAD} \xrightarrow{K_7} \text{MDH} + \text{NADH} \]

The fourth step seems to be the rate-limiting one—the dissociation of the complex MDH-NAD (K7). The increased affinity for the coenzyme induced by salinity, as reported in this paper, will cause the increase in rate of step 1, but it will also stabilize the complex of “enzyme-reduced coenzyme” and thus will reduce considerably the rate of step 4. Increase of the Km for the substrate, in this course of events, will have an inhibitory effect. The rate of the over-all reaction will depend on the compulsory sequence of the above four steps.

The graphs of 1/v versus 1/s for malate oxidation (Fig. 5) and for OAA reduction (Fig. 8) indicate the possibility that NaCl exerted partially mixed noncompetitive inhibition and possibly inhibited the dissociation of the reaction products from the enzyme. At the same time, an increased Km indicated the possibility of disturbances in binding the substrate and competition with the substrate. On the other hand, the curves for the coenzyme (Figs. 5B and 5B) suggested coupling or noncompetitive inhibition, i.e., NaCl increased the affinity for the coenzyme but at the same time inhibited the rate of reaction as suggested above.

Table III shows the apparent kinetic data for the effect of NaCl on malic dehydrogenase. Apart from the changes in Km already mentioned above, Ki and a for malate, for both isoenzymes sMDH and mMDH, were practically identical. This was not the case for the Ki for OAA of the two isoenzymes. A great similarity was found between the data (OAA reduction) for the soluble enzyme from control roots and roots grown in presence of NaCl (Km, K'm, Ki, a). The similarity was less apparent between the mitochondrial enzymes from the two sources.

An interaction was found between substrate concentration and salinity level. Salinity counteracted substrate inhibition but an increasing substrate concentration neutralized the inhibitory effect of NaCl (Figs. 4 and 6).

In vitro NaCl seems to have three types of effects on the soluble enzyme (Fig. 8, C and D). (a) Change in affinity to the substrate that is manifested by changes in the Km; this required comparatively low NaCl concentrations, as seen for the soluble enzyme (Fig. 8, C and D) where the lines for both NaCl concentrations intercept the axis at the same place. (b) Effect on rate of reaction: low NaCl concentrations increased Vmax of the soluble enzyme, high NaCl concentrations decreased Vmax, thus Vmax/Vmax was > 1. Vmax is apparently not directly dependent on Km and is not affected by the effect of NaCl on the affinity between substrate and enzyme only. (c) Counteracting substrate inhibition: this may be caused by NaCl either in vivo (Fig. 7B) or in vitro (Fig. 7A). In vitro NaCl did not affect the Km for OAA (Table III), but it did affect the Km for malate and NAD.

It was shown that the NaCl inhibition of the enzyme activity depends on substrate concentration; increased substrate concentration counteracted the damaging salinity effect and thus may have a protective function under saline conditions. It would appear that the internal pH conditions may have a similar effect. Ting (28) and Zschoche and Ting (31) showed that OAA substrate inhibition was affected by pH. The soluble and mitochondrial enzymes showed different pH requirements (Fig. 9). Similar findings were reported previously by Siegel and Englard (25, 26) for enzymes isolated from beef hearts: the soluble enzyme was less sensitive to pH changes than the

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**Table III. Kinetic Data for Effect of NaCl on Activity of Malic Dehydrogenase**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>NaCl Concentration in Growth Medium</th>
<th>Substrate</th>
<th>Km (mM)</th>
<th>Vmax/Vmax</th>
<th>Km (mM)</th>
<th>Ki (mM)</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>μM</td>
<td>m</td>
<td>μM</td>
<td>μM</td>
<td>μM</td>
<td>μM</td>
</tr>
<tr>
<td>sMDH</td>
<td>0</td>
<td>Malate</td>
<td>7,450</td>
<td>480</td>
<td>1.65</td>
<td>13,300</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>NAD</td>
<td>1,820</td>
<td>480</td>
<td>2.67</td>
<td>910</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>OAA</td>
<td>52</td>
<td>480</td>
<td>3.02</td>
<td>134</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>OAA</td>
<td>53</td>
<td>480</td>
<td>2.96</td>
<td>139</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>NADH</td>
<td>25</td>
<td>480</td>
<td>3.50</td>
<td>11,600</td>
<td>0.42</td>
</tr>
<tr>
<td>mMDH</td>
<td>0</td>
<td>Malate</td>
<td>11,600</td>
<td>600</td>
<td>2.18</td>
<td>23,100</td>
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<td>0</td>
<td>OAA</td>
<td>33</td>
<td>480</td>
<td>2.50</td>
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<tr>
<td></td>
<td>360</td>
<td>OAA</td>
<td>37</td>
<td>480</td>
<td>1.90</td>
<td>74</td>
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<td>360</td>
<td>NADH</td>
<td>25</td>
<td>480</td>
<td>3.50</td>
<td>11,600</td>
<td>0.42</td>
</tr>
</tbody>
</table>

1 pH 9.5: When malate concentration was changed, NAD was 1.9 mM. When NAD concentration was changed, malate concentration was 9 mM.

2 pH 7.5: When OAA concentration was changed, NAD concentration was 67 μM. When NAD concentration was changed, OAA concentration was 0.33 mM.
mitochondrial enzyme. At pH 8.0 the mitochondrial enzyme had practically no oxidative activity (Fig. 9A), while the reductive activity showed half of its maximal activity at this pH (Fig. 9B). The reductive activity showed a reversed trend; the optimal pH for the mitochondrial enzyme was at pH 8.5, while the soluble enzyme had its optimal activity around pH 7 to 7.5. There was very little difference between the mitochondrial enzyme inside the particles and when solubilized.

Addition of NaCl in vitro to the soluble enzyme resulted in decreased sensitivity to pH changes (Fig. 10). The effect was less pronounced for the mitochondrial enzyme (Fig. 11). The similarity of the effect of the same NaCl concentration in vivo and in vitro suggests that also inside the cell, under saline conditions, the mitochondrial enzyme is exposed to the effect of NaCl.

Flowers (9) postulates that the tolerance of the halophytes to salinity is due to their ability to accumulate the ions inside the vacuole and counteract their osmotic effect by accumulation of sugars in the cytoplasm. It was shown that in Tamarix the mitochondrial enzyme is somehow exposed to the effect of NaCl in the cell as an in vivo effect was demonstrated; therefore, the compartmentalization cannot be complete.

The results suggest that the halophyte can counteract the harmful effect of salinity by several mechanisms, among them possible changes of pH and, mainly, changes in metabolic pathways resulting in accumulation of substrate. This may have an osmotic balancing effect as postulated by Flowers (9), but also may result in reduction of the inhibition of enzyme activity caused by salinity.

Our data would indicate that NaCl has very similar effects on the enzyme in vivo and in vitro. No evidence was presented in this paper that NaCl depresses enzyme synthesis, but the evidence suggests very strongly that conformational changes occur in the enzyme due to salinity. This view is supported by the findings showing the effect of NaCl on K_m of substrate and coenzyme, and by the finding that the effect of NaCl on enzyme activity is not due solely to the effect on the K_m. Similar views that the effect of salinity on enzyme is through conformational changes were expressed by Duporque and Kun (8), Datta (6), and by Lieberman and Lanyi (16), but it has not yet been proven beyond doubt.

LITERATURE CITED