

# Compatible Solutes and Inorganic Ions in the Mangrove Plant *Avicennia marina* and Their Effects on the Activities of Enzymes

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Naturally grown two-month-old seedlings of *Avicennia marina* contain high concentrations of Na<sup>+</sup> and Cl<sup>−</sup>. Our NMR studies revealed an accumulation of glycinebetaine, asparagine and stachyose in *A. marina*. The highest concentration of glycinebetaine was observed in young leaves, while the distribution of stachyose was restricted in stems and roots. Asparagine comprised more than 96% of total free amino acids in roots and 84% in leaves from two-year-old plants. Little or no accumulation of proline or polyols, which are proposed as compatible solutes in other plants, could be detected in *A. marina*. The activities of phosphofructokinase, pyrophosphate:fructose-6-phosphate 1-phosphotransferase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase (decarboxylating), phosphoenolpyruvate carboxylase and NAD:malate dehydrogenase from young leaves of *A. marina* were inhibited by NaCl, while the activity of fructose-1,6-bisphosphate aldolase was activated by 50–200 mM NaCl. There was little or no effect of high concentrations (up to 500 mM) of glycinebetaine on the activities of any of these enzymes. No significant protection by glycinebetaine was detected against NaCl inhibition of these enzymatic activities. Based on these results, possible mechanisms for the salt-resistance of *A. marina* cells are discussed.

## Introduction

Mangrove plants are classified as halophytes, because they grow primarily in saline habitats such

as subtropical and tropical coasts (Tomlinson, 1986). *Avicennia marina* is a mangrove found in the Iriomote Island, Okinawa, Japan (Niiri *et al.*, 1984). *A. marina* has salt glands and excess salts are excreted from the plant at leaf surfaces. The salt resistance of this plant seems to be based on three different mechanisms: (i) salt avoidance via the roots; (ii) the capacity to preserve normal metabolic activity in the presence of high intracellular salt levels; and (iii) recreation of some of the penetrating ions (Waisel *et al.*, 1986).

In the present study, in order to reveal the biochemical mechanism of salt resistance at cellular level, we determined the concentrations of major inorganic ions in *A. marina* using both ion chromatography and <sup>23</sup>Na NMR spectroscopy, and we screened possible compatible solutes for this plant using <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy and an amino acid analyzer. Finally, we examined the effect of NaCl and glycinebetaine, a possible com-

**Abbreviations:** ALD, fructose-1,6-bisphosphate aldolase (EC 4.1.2.13); F6P, fructose-6-phosphate; GABA,  $\gamma$ -aminobutyrate; G6PDH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); GDH, glycerol-3-phosphate dehydrogenase (EC 1.1.1.8); HEPES, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid]; LS medium, Linsmaier and Skoog (1965) medium; MDH, NAD-malate dehydrogenase (EC 1.1.1.37); NMR, nuclear magnetic resonance; PEPC, phosphoenolpyruvate carboxylase (EC 4.1.1.31); PFK, phosphofructokinase (EC 2.7.1.11); PFP, pyrophosphate: fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.90); 6PGDH, 6-phosphogluconate dehydrogenase (EC 1.1.1.44); PVP, polyvinylpyrrolidone; TPI, triose phosphate isomerase (EC 5.3.1.1); Tris, tris(hydroxymethyl)aminomethane.

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patible solute for this plant, on the activities of several enzymes extracted from leaves of *A. marina*. The results suggest that *A. marina* plants accumulate  $\text{Na}^+$  and  $\text{Cl}^-$  ions in the vacuoles, and produce some compatible solutes, such as glycinebetaine, in cytoplasm to adjust the osmotic balance between cytoplasm and vacuole.

## Materials and Methods

### *Plant materials*

Four-month-old seedlings (shoot length, ca. 85 mm) of *Avicennia marina* (Forstk.) Vierh. which were collected at Komi, Iriomote Island, Okinawa-ken, Japan in March were used for the analysis of compatible solutes. For determination of the levels of free amino acids, leaves and roots from two-year-old plants grown in a green house were also used. Levels of metabolites in *A. marina* were determined after the plants had been washed with distilled water. For the salt stress experiments, segments (200 mg fresh weight, 5 mm x 5 mm) of young leaves (ca. 300 mg fresh weight, ca. 40 mm long and 20 mm in width) from mature trees of *A. marina* were incubated with 2 ml of the LS medium (Linsmaier and Skoog, 1962), pH 5.7, supplemented with various concentrations of NaCl, and shaken at 27 °C for 18 hrs.

### *Measurement of inorganic ions and possible organic compatible solutes*

For the analysis of the various ions, segments of plant samples were homogenized in distilled water with a mortar and pestle. The homogenate was centrifuged at 20,000  $\times g$  for 20 min. The precipitate was washed with distilled water and centrifuged exactly as before. All supernatants were combined and used for analysis of ions. Cations and anions were determined with a Shimadzu ion chromatograph (type HIC-6A). In some experiments, the amount of  $\text{Na}^+$  was also determined by  $^{23}\text{Na}$ -NMR spectroscopy.

For the analysis of organic solutes, plant samples were homogenized in 80% ethanol and each homogenate was incubated at 70 °C for 10 min. After cooling, the homogenate was centrifuged at 20,000  $\times g$  for 10 min. The precipitate was re-suspended in 5 ml of 80% ethanol and centrifuged

again. These supernatants were combined and evaporated to dryness at 50 °C and used for analysis. Organic solutes were examined using NMR spectroscopy (Jones *et al.*, 1986; Larher, 1988).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded on a Varian Unity 500 spectrometer using  $\text{D}_2\text{O}$  as solvent. Free amino acids were also analyzed with a fluorometric amino acid analyzer (Shimadzu Co., Kyoto, Japan) as described elsewhere (Saito *et al.*, 1989; Ukaji and Ashihara, 1987).

### *Extraction and partial purification of enzymes*

Segments (ca. 1 g fresh weight) of young leaves (ca. 300 mg fresh weight, ca. 40 mm long and 20 mm in width) from mature tree of *A. marina* were homogenized in a chilled mortar and pestle with 50 mM imidazole-HCl buffer (pH 7.6) containing 2 mM  $\text{MgCl}_2$ , 1 mM EDTA, 0.1% (v/v) 2-mercaptoethanol, 0.5% sodium ascorbate and 5% (w/v) insoluble PVP. The homogenate was filtered through a layer of nylon cloth, and the resulting filtrate was centrifuged at 20,000  $\times g$  for 20 min at 2–4 °C. The supernatant was treated with finely ground, solid  $(\text{NH}_4)_2\text{SO}_4$ . The protein fractions that were precipitated by differing concentrations of  $(\text{NH}_4)_2\text{SO}_4$  were used for assay of enzymatic activity: 30–60% saturation for phosphofructokinase (PFK) and for pyrophosphate:fructose-6-phosphate-1-phosphotransferase (PFP); 35–60% for fructose-1,6-bisphosphate aldolase (ALD); 40–60% saturation for glucose-6-phosphate dehydrogenase (G6PDH) and for 6-phosphogluconate dehydrogenase (6PGDH); 35–55% for phosphoenolpyruvate carboxylase (PEPC). The fractions prepared by centrifugation (20,000  $\times g$ , 10 min) were dissolved in 2.5 ml of the extraction medium referred to above and desalted on a column (15 mm i.d. x 50 mm) of Sephadex G-25 that had been equilibrated with the same buffer. The eluted protein fractions (3.5 ml) were used immediately for the determination of activity. In the case of NAD-malate dehydrogenase (NAD-MDH), the leaf segments were homogenized in 50 mM imidazole-HCl buffer (pH 7.6) containing 2 mM  $\text{MgCl}_2$ , 2 mM  $\text{MnCl}_2$ , 2 mM EDTA, 5 mM dithiothreitol, 0.5% sodium ascorbate and 5% (w/v) insoluble PVP. The supernatant obtained after centrifuging was directly desalted and used as a enzyme source.

### Assays of enzymatic activity

The activities of the enzymes were measured spectrophotometrically by following the changes in absorbance at 340 nm. The measurements were made at 30 °C in a Hitachi double-beam spectrophotometer, type U-3200, fitted with accessories for enzyme analysis.

The assays for activities of individual enzymes were taken from previously published methods. The total volume of each reaction mixture was 1.0 ml. Concentrations of reagents used in assays were optimized for enzymes from *A. marina* as follows.

PFK (Ashihara *et al.*, 1972): 50 mM HEPES-NaOH buffer (pH 7.2), 5 mM fructose-6-phosphate (F6P), 1 mM ATP, 5 mM MgCl<sub>2</sub>, 0.2 mM NADH, 20 nkat ALD, 20 nkat glycerol-3-phosphate dehydrogenase (GDH) and 200 nkat triose phosphate isomerase (TPI).

PFP (Ashihara and Horikosi, 1987): 50 mM HEPES-NaOH buffer (pH 7.2), 5 mM F6P, 1 mM PPI, 1 μM fructose-2,6-bisphosphate, 5 mM MgCl<sub>2</sub>, 0.2 mM NADH, 20 nkat ALD, 20 nkat GDH and 200 nkat TPI.

G6PDH (Ashihara and Matsumura, 1977): 25 mM Tris-HCl buffer (pH 7.2), 2 mM glucose-6-phosphate, 5 mM MgCl<sub>2</sub>, 0.2 mM NADP<sup>+</sup> and 10 nkat 6PGDH.

6PGDH (Ashihara and Matsumura, 1977): 25 mM Tris-HCl buffer (pH 7.2), 0.1 mM 6-phosphogluconate, 5 mM MgCl<sub>2</sub> and 0.2 mM NADP<sup>+</sup>.

ALD (Ashihara and Matsumura, 1977): 25 mM Tris-HCl buffer (pH 7.2), 1 mM fructose-1,6-bisphosphate, 5 mM MgCl<sub>2</sub>, 0.2 mM NADH, 20 nkat GDH and 200 nkat TPI.

NAD-MDH (Nagano *et al.*, 1994a): 50 mM HEPES-NaOH buffer (pH 7.2), 1 mM oxaloacetate, 5 mM MgCl<sub>2</sub> and 0.2 mM NADH.

PEPC (Nagano *et al.* 1994b): 25 mM Tris-HCl buffer (pH 8.0), 1 mM phosphoenolpyruvate (PEP), 1 mM KHCO<sub>3</sub>, 5 mM MgCl<sub>2</sub>, 2 mM DTT, 0.2 mM NADH and 20 nkat MDH.

## Results

### Identification of compatible solutes in naturally grown *A. marina* seedlings

The <sup>23</sup>Na-NMR spectrum revealed that Na<sup>+</sup> ions were distributed at high concentrations within var-

ious parts of young seedlings of *A. marina* (130–200 μmol g<sup>-1</sup> fresh weight). Accumulation of organic compounds in leaves, stems and roots of young seedlings of *A. marina* was surveyed using <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopy. High concentrations of glycinebetaine, asparagine, and stachyose were found. As shown in Fig. 1, glycinebetaine was distributed in all parts of *A. marina* seedlings. Its concentration was highest in young leaves (180 μmol g<sup>-1</sup> fresh weight) and lowest in roots (19 μmol g<sup>-1</sup> fresh weight). By contrast, stachyose (39–76 μmol g<sup>-1</sup> fresh weight) was found only in the stem and roots (Fig. 1).

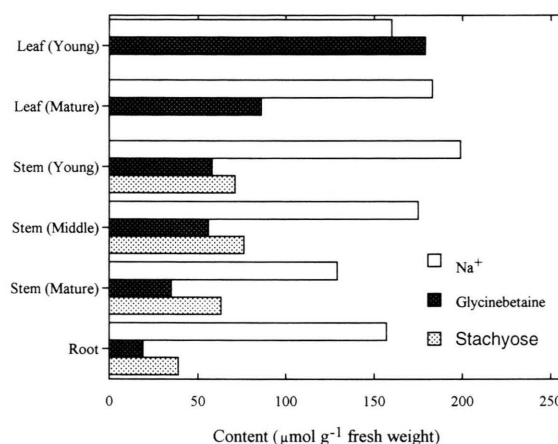


Fig. 1. Distribution of Na<sup>+</sup> ions, glycinebetaine and stachyose in naturally grown two-month-old seedlings of *Avicennia marina*. Concentrations are expressed as μmol g<sup>-1</sup> fresh weight and the values shown were obtained from a typical seedling.

Young leaf: 110 mg fresh weight, 25 mm long; mature leaf: 300 mg fresh weight, 42 mm long; young stem: 85 mg fresh weight, 25 mm long; middle stem: 200 mg fresh weight, 30 mm long; mature stem: 320 mg fresh weight, 30 mm long; roots: 460 mg fresh weight, 80 mm long.

We also analyzed the profiles of free amino acids using the amino acid analyzer (Table I). As the results of <sup>23</sup>NMR spectroscopy indicated, there was a remarkable accumulation of asparagine in both leaf (84% of total free amino acids) and roots (97%). The concentration of asparagine in roots (318 μmol g<sup>-1</sup> fresh weight) was more than five times higher than in leaves (57 μmol g<sup>-1</sup> fresh weight). A significant amount of glycerol was also accumulated in young leaves from mature trees of *A. marina* (Table II), although glycerol was not detected in any parts of 2-month-old seedlings. How-

Table I. Levels of free amino acids in leaves and roots from two-year-old *Avicennia marina* plants. Concentrations of amino acids are expressed as nmol g<sup>-1</sup> fresh weight with s.d. (*n*=3). The values in parentheses show the percentage of total free amino acids.

Amino acid	Leaf		Root	
Asp	2,207 ± 140	(3.2)	1,502 ± 207	(0.5)
Thr	47 ± 2	(-)	552 ± 90	(-)
Ser	122 ± 1	(-)	1,588 ± 247	(0.5)
Asn	57,294 ± 97	(84.0)	317,870 ± 28048	(96.5)
Glu	1,730 ± 173	(2.5)	164 ± 3	(-)
Gln	826 ± 104	(1.2)	472 ± 20	(-)
Pro	31 ± 6	(-)	60 ± 1	(-)
Gly	32 ± 2	(-)	138 ± 15	(-)
Ala	207 ± 17	(-)	2,336 ± 110	(0.7)
Val	103 ± 14	(-)	108 ± 12	(-)
Met	133 ± 13	(-)	32 ± 1	(-)
Ile	47 ± 5	(-)	29 ± 4	(-)
Leu	51 ± 8	(-)	32 ± 6	(-)
Tyr	58 ± 7	(-)	158 ± 35	(-)
Phe	60 ± 6	(-)	98 ± 3	(-)
His	313 ± 31	(-)	505 ± 38	(-)
Lys	564 ± 17	(0.8)	322 ± 40	(-)
Arg	2,497 ± 307	(3.7)	1,809 ± 303	(0.5)
P-Ser	132 ± 11	(-)	58 ± 7	(-)
GABA	613 ± 19	(0.9)	508 ± 4	(-)
Others*	1,143 ± 54	(1.7)	1,058 ± 24	(-)
Total	68,209 ± 507	(100)	329,400 ± 27261	(100)

\* Others contained five unidentified compounds.

ever, proline and polyols, which are often reported as compatible solutes in various plant species, could not be detected by NMR spectroscopic analysis in this plant.

#### *Effect of salt stress on the level of ions, glycinebetaine and glycerol in A. marina leaf segments*

Table II shows the levels of major ions and glycinebetaine in *A. marina* leaves, after leaf seg-

ments were incubated with the LS medium supplemented with 0, 250 and 500 mM NaCl for 18 hrs. A two fold increase in the concentrations of both Na<sup>+</sup> and Cl<sup>-</sup> ions was detected with 250 mM NaCl, but the leaves were saturated at 500 mM NaCl. Uptake of K<sup>+</sup> ion was also enhanced by NaCl, while the cellular levels of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> decreased.

The level of glycinebetaine was also increased by NaCl. An approximately twofold increase was detected with 250 mM NaCl. By contrast, the level of glycerol was decreased by NaCl.

#### *Effect of NaCl and glycinebetaine on enzymes from A. marina leaves*

The effect of NaCl and glycinebetaine on the activity of selected enzymes related to the carbohydrate metabolism was examined (Figs. 2 and 3). Activities of all enzymes were inhibited by high concentrations of NaCl, although the rates of inhibition varied greatly. Marked inhibition of the activity of PFP was observed; nearly 80% inhibition of the enzymatic activity was found at 100 mM NaCl. The NaCl concentrations which caused 50% inhibition of the enzymatic activity were 70 mM (PFP), 100 mM (6PGDH), 110 mM (PEPC), 260 mM (NAD-MDH), 320 mM (PFK), 350 mM (G6PDH) and 370 mM (ALD). Exceptionally, considerable enhancement of ALD activity by 50–200 mM NaCl was detected. By contrast, glycinebetaine had little or no inhibitory effect on the activity of these enzymes from *A. marina*.

Table II. Levels of major ions, glycinebetaine and glycerol in young leaf segments obtained from mature trees of *A. marina* after incubation with 0, 250 and 500 mM NaCl in LS medium for 18 hrs. Contents are expressed as μmol g<sup>-1</sup> fresh weight with s.d. (*n*=3). The values in parentheses show the percentage of control (0 mM).

Inorganic ions and compatible solutes	NaCl		
	0 mM	250 mM	500 mM
Na <sup>+</sup>	166.5 ± 3.5 (100)	373.0 ± 3.0 (224)	454.5 ± 3.5 (273)
K <sup>+</sup>	45.4 ± 7.4 (100)	74.6 ± 4.6 (164)	87.8 ± 12.1 (193)
NH <sub>4</sub> <sup>+</sup>	60.1 ± 2.6 (100)	42.0 ± 3.0 (70)	40.3 ± 2.4 (67)
Mg <sup>2+</sup>	14.2 ± 2.4 (100)	18.3 ± 0.4 (129)	18.7 ± 4.0 (132)
Ca <sup>2+</sup>	7.1 ± 0.6 (100)	6.6 ± 2.0 (93)	5.4 ± 0.3 (76)
Cl <sup>-</sup>	325.0 ± 31.3 (100)	665.2 ± 89.5 (205)	831.0 ± 72.8 (256)
NO <sub>3</sub> <sup>-</sup>	32.8 ± 3.2 (100)	17.0 ± 2.9 (52)	14.5 ± 1.0 (44)
SO <sub>4</sub> <sup>2-</sup>	8.1 ± 0.9 (100)	6.9 ± 0.1 (85)	7.3 ± 1.0 (90)
Glycinebetaine	62.2 ± 6.0 (100)	119.0 ± 0.2 (191)	119.4 ± 2.4 (192)
Glycerol	82.1 ± 0.9 (100)	70.1 ± 15.4 (85)	46.3 ± 3.8 (56)

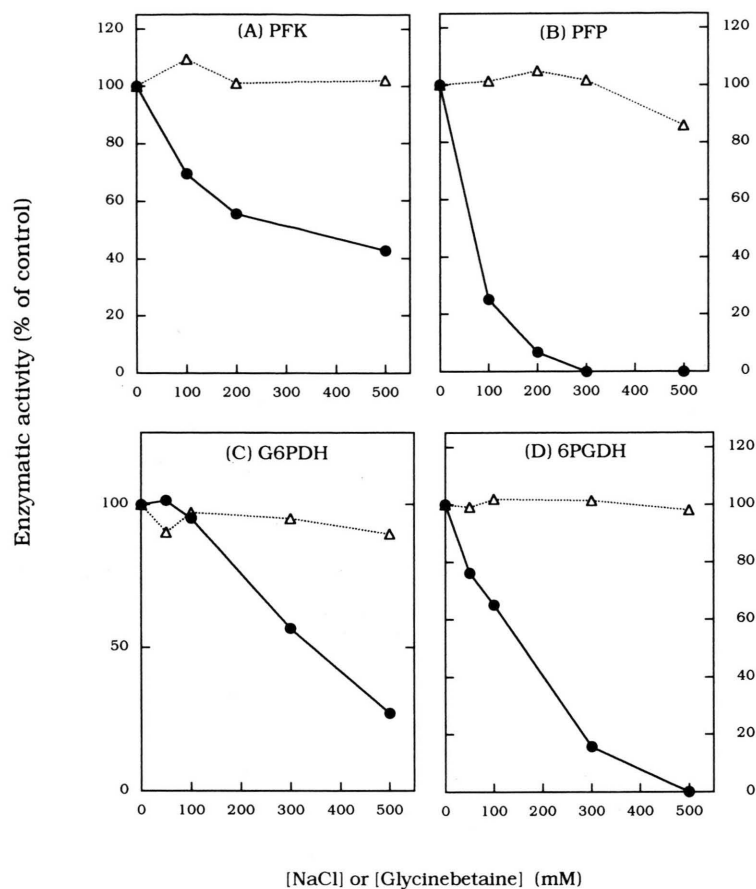


Fig. 2. Effects of NaCl (●) and glycinebetaine (Δ) on the activities of phosphofructokinase (A), pyrophosphate:fructose-6-phosphate 1-phosphotransferase (B), glucose-6-phosphate dehydrogenase (C) and 6-phosphogluconate dehydrogenase (decarboxylating) (D) from leaves of *Avicennia marina*. The values for enzymatic activity are expressed as a percentage of control activity (A: 220 pkat  $\text{mg}^{-1}$  protein; B: 440 pkat  $\text{mg}^{-1}$  protein; C: 120 pkat  $\text{mg}^{-1}$  protein; D: 143 pkat  $\text{mg}^{-1}$  protein).

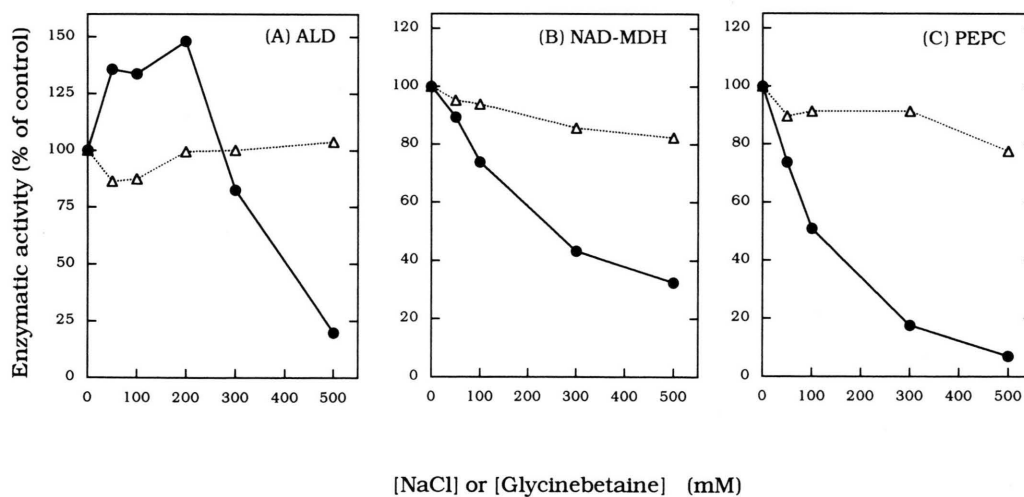


Fig. 3. Effects of NaCl (●) and glycinebetaine (Δ) on the activities of aldolase (A), NAD:malate dehydrogenase (B) and phosphoenolpyruvate carboxylase (C) from leaves of *Avicennia marina*. The values for enzymatic activity are expressed as a percentage of control activity (A: 1370 pkat  $\text{mg}^{-1}$  protein; B: 150 nkat  $\text{mg}^{-1}$  protein; C: 163 pkat  $\text{mg}^{-1}$  protein).



Protection by glycinebetaine against salt inhibition of enzymatic activity was not detectable for most of the enzymes examined here. Inhibition of the activities of G6PDH and NAD-MDH only, by 300 mM NaCl, was slightly relieved by 500 mM glycinebetaine (Fig. 4 A and B). The NaCl concentrations of 50% inhibition for G6PDH and NAD-MDH moved from 350 mM to 430 mM and from 260 mM to 340 mM respectively upon addition of 500 mM glycinebetaine.

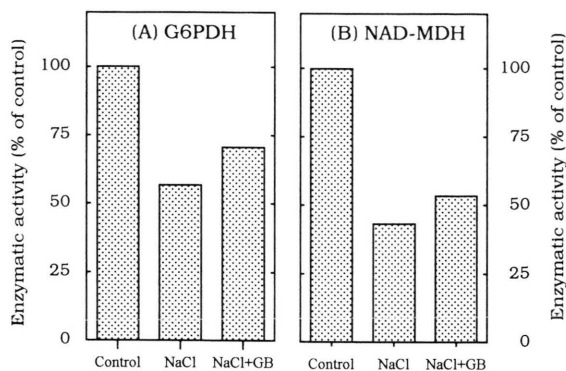


Fig. 4. Effect of addition of 500 mM glycinebetaine on inhibition of *A. marina* glucose-6-phosphate dehydrogenase (A) and NAD:malate dehydrogenase (B) by 300 mM NaCl. The values for enzymatic activity are expressed as a percentage of control activity (A: 120 pkat  $\text{mg}^{-1}$  protein; B: 150 nkat  $\text{mg}^{-1}$  protein). GB: glycinebetaine.

## Discussion

*A. marina* plants have salt glands and secrete salt from leaves. Nevertheless, the concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  in seedlings were very high, and these ions were distributed in all parts of the plants. The concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  in leaves of *A. marina* incubated with 0, 250 and 500 mM NaCl were similar to concentrations in leaves from seedlings of *A. marina* grown with 50, 250 and 500 mM NaCl (Ball and Farquhar, 1984a). In contrast to experiments with seedlings (Ball and Farquhar, 1984a), the  $\text{K}^+$  concentration in leaf disks also increased with increasing salinity of the incubation media (Table II), although the  $\text{Na}^+/\text{K}^+$  ratios increased from 3.7 (0 mM) to 5.0 (250 mM) and 5.2 (500 mM) if NaCl is added to the media (Table II). Inhibition by  $\text{Na}^+$  of the uptake of  $\text{K}^+$  was often observed in *A. marina* and other plants

(Rains and Epstein, 1967; Ball and Farquhar, 1984a; Ball *et al.*, 1987).

In the present study, we found some possible compatible solutes using NMR spectroscopy. These are glycinebetaine, asparagine, glycerol and stachyose. One of the most likely compatible solutes in *A. marina* is glycinebetaine. The concentration of this compound was high in leaves, especially young leaves (Fig. 1). The salt response of the level of glycinebetaine supports the hypothesis that this compound acts as a compatible solute in *A. marina*. Consistent with our results, the presence of methylated onium compounds in *A. marina* have been suggested by Popp *et al.* (1984a), who could not, however, identify this compound because they used the classical spectrophotometric method (Wall *et al.*, 1960; Storey and Wyn Jones, 1977).

Accumulation of asparagine in roots and leaves of *A. marina* was also detected by NMR spectroscopy; essentially the same values were obtained from amino acid analysis of free amino acids. The results were different from those reported by Popp *et al.* (1984b) in which arginine and GABA plus alanine were the major amino acids in young and old leaves of *A. marina*, respectively. The discrepancy may be due to the techniques used. Popp *et al.* noted that some amino acids in their samples, including asparagine, could not be determined correctly because of interfering substances. The role of accumulated asparagine in *A. marina* remains uncertain, but enhancement of NaCl tolerance in *Arabidopsis thaliana* by exogenously supplied asparagine has been reported by Lehle *et al.* (1992). In stems and roots of *A. marina*, we found accumulation of stachyose, a tetrasaccharide consisting of galactose-galactose-glucose-fructose. Stachyose and other oligosaccharides of the raffinose (galactose-glucose-fructose) family have been recognized as important transport carbohydrates in a large number of woody plants (See the review by Dey, 1985). However, we believe that this is the first detection of a significant accumulation of this sugar in *A. marina*.

The activity of most enzymes from *A. marina* was rigorously inhibited by NaCl. Therefore, in contrast to the salt tolerant enzymes from halophilic bacteria (Larsen, 1967), enzymes from *A. marina* seem to have similar properties to enzymes from salt-sensitive plants. For example, 50% inhi-

bition of the activity of G6PDH from *A. marina* is caused by 350 mM NaCl. A similar inhibitory effect by NaCl has been reported in G6PDH from halophytes and from a salt sensitive species and even in commercially available yeast G6PDH (Flowers, 1972; Greenway and Osmond, 1972). Osmond and Greenway (1972) reported that PEPC isolated from leaves of C4 plants, such as *Atriplex spongiosa* and *Zea mays*, was extremely sensitive to inorganic salts, whereas the enzyme extracted from leaves of C3 plants, such as *Atriplex hastata* and *Phaseolus vulgaris*, was much less sensitive. The activity of PEPC from *A. marina* was quite sensitive to NaCl (50% inhibition: 110 mM NaCl) and similar to PEPCs from C4 plants. Some details of the photosynthetic CO<sub>2</sub> fixation by *A. marina* are known. This plant has been classified as a C3 plant by Ball and Farquhar (1984b); however, Joshi *et al.* (1984) have suggested that a modified C4 pathway is operative in mangrove plants including *A. marina*.

Glycinebetaine had little or no inhibitory effect on the activity of any of the enzymes from *A. marina* examined in this study, even in high concentration. A similar observation has also been reported in some enzymes from barley (Pollard and Wyn Jones, 1979). Partial protection by glycinebetaine against salt inhibition was observed in

G6PDH and NAD-MDH from *A. marina* (Fig. 4). Pollard and Wyn Jones (1979) also demonstrated that glycinebetaine slightly but clearly alleviated the inhibition of the activities of barley embryo pyruvate kinase and barley leaf malate dehydrogenase (decarboxylating) by NaCl.

Since respiratory and related carbohydrate metabolism in leaves and roots of *A. marina* was not restrained in the presence of high concentrations of NaCl (Fukushima *et al.*, 1997), Na<sup>+</sup> and Cl<sup>-</sup> ions and these enzymes are probably mostly separated spatially. Na<sup>+</sup> and Cl<sup>-</sup> ions appear to be concentrated in vacuoles. Glycinebetaine did not inhibit enzyme activity and presumably accumulated in the cytoplasm. Thus, osmotic balance in the cytoplasm of *A. marina* cells may be maintained by glycinebetaine and the other organic solutes discussed above.

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