Phytochemistry, 1972, Vol. 11, pp. 1881 to 1886. Pergamon Press. Printed in England.

THE EFFECT OF SODIUM CHLORIDE ON ENZYME ACTIVITIES FROM FOUR HALOPHYTE SPECIES OF CHENOPODIACEAE

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(*Received* 25 *November* 1971)

Abstract—The *in vitro* effect of sodium chloride on the enzyme acitivity of four halophytes, *Beta vulgaris* ssp. *maritima* (L.) Thell., *Halimione portulacoides* (L.) Aell., *Salicornia ramosissima* Woods and *Suaeda maritima* (L.) Dum. was investigated. The activity was, in general, affected by sodium chloride in a similar manner to that reported for salt sensitive species. The most notable exceptions were the sodium chloride stimulated ATPases of *Beta* and *Salicornia*.

INTRODUCTION

THE ACCUMULATION of salts by plants growing in saline environments enables the osmotic adjustment of the plant water potential and the maintenance of gradients of free energy from substrate to leaf. In the case of halophytes this may necessitate the deposition of substantial amounts of salt in the plant tops if a sufficient gradient is to be maintained. Although the enzymes of halphilic bacteria are clearly salt tolerant[1] a limited number of studies have indicated that enzymes isolated from plant halophytes may not be so:[2,3] the enzymes isolated from salt tolerant species were just as sensitive to salt added *in vitro* as were the same enzymes isolated from salt sensitive species.

It is of interest to ascertain if the salt sensitivity of the enzymes of halophytes is a general phenomenon and if so, what mechanisms exist to overcome the effects of these high concentrations of soluble salts in the plant tops on enzyme activity. Four species commonly found in saline habitats, *Salicornia ramosissima*, *Suaeda maritima*, *Halimione portulacoides* and the maritime subspecies of *Beta vulgaris* were used for the preparation of homogenates in which the activity of an enzyme characteristic of the Krebs cycle (malic dehydrogenase), oxidation (peroxidase), the pentose phosphate pathway (glucose 6 phosphate dehydrogenase) and ATP metabolism (ATPase) were assayed.

In general, the enzymes studied from all four species were as sensitive to salt as those isolated from *Pisum*,[2] which is a very salt sensitive plant.[2,11,12]

RESULTS

Malic Dehydrogenase

Malic dehydrogenase activity was inhibited by the higher levels of NaCl (0·33 M) in all cases (Table 1). The effects on *Suaeda* and *Beta* were similar at all the pH values used and

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[2] T. J. FLOWERS, *J. Exptl Bot.* in press.
[3] H. GREENWAY and C. B. OSMOND, *Plant Physiol.* in press.

TABLE 1. THE EFFECT OF SODIUM CHLORIDE ON MALIC DEHYDROGENASE ACTIVITY OF HALOPHYTES AT VARIOUS pH VALUES

		Suaeda			*Salicornia*		
	pH	6·4	7·1	7·8	5·8	7·0	7·7
NaCl (M)	0	3·05	3·40	3·71	0·53	0·61	0·41
	0·167	2·18 (71)	2·36 (69)	1·98 (53)	0·46 (87)	0·52 (86)	0·42 (103)
	0·33	1·44 (47)	1·33 (39)	1·13 (30)	0·29 (55)	0·34 (56)	0·23 (55)
LSD $p = 0·05$			0·12			0·07	
		Beta			*Halimione*		
	pH	5·5	7·0	7·6	5·8	7·0	7·7
NaCl (M)	0	1·27	1·68	1·39	3·26	4·45	3·24
	0·167	0·77 (61)	1·07 (64)	1·08 (78)	3·30 (101)	3·67 (82)	2·73 (84)
	0·33	0·46 (36)	0·62 (37)	0·61 (44)	2·64 (81)	2·30 (52)	1·77 (55)
LSD $p = 0·05$			0·22			0·51	

The activity is expressed as Δ A/min/mg protein in 3 ml of a medium containing Tris-maleate (25 mM), NADH (50 μM) and oxalacetate (100 μM). The values in parenthesis are relative % to those in the absence of salt.

TABLE 2. THE EFFECT OF SODIUM CHLORIDE ON THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY OF HALOPHYTES AT VARIOUS pH VALUES

		Suaeda			*Salicornia**		
	pH	6·4	7·6	8·1			
NaCl (M)	0	4·23	13·7	17·6			
	0·167	3·31 (78)	11·4 (83)	13·73 (78)			
	0·33	—*	8·04 (59)	9·46 (54)			
LSD $p = 0·05$			1·73				
			Beta			*Halimione*	
	pH	6·5	7·5	8·2	6·5	7·4	8·3
NaCl (M)	0	1·23	8·53	10·3	4·33	10·1	12·1
	0·167	—*	6·98 (82)	8·78 (85)	2·88 (67)	7·79 (77)	8·94 (74)
	0·33	—*	3·80 (45)	6·32 (61)	1·15 (27)	5·48 (54)	5·77 (48)
LSD $p = 0·05$			1·77			1·11	

The activity is expressed as Δ A × 10^3/min/mg protein in 3 ml of a solution containing Tris-maleate (17 mM), $MgCl_2$ (13 mM), NADP (200 μM) and glucose-6-phosphate (1·3 mM).

* No measurements possible as enzyme activity too low.

there was a mean inhibition of 61 % in the presence of 0·33 M sodium chloride. The enzyme activity in the homogenates of *Halimione* and *Salicornia* was, in general, more tolerant of NaCl. There was a mean inhibition of 45 % in the presence of 0·33 M NaCl, excepting the *Halimione* enzyme assayed at pH 5·8. In the latter case sodium chloride appeared to have a much less marked affect (19 % inhibition with 0·33 M NaCl).

Glucose-6-phosphate Dehydrogenase

Enzyme activity was not detectable in extracts of *Salicornia* even though a range of pH values, NaCl concentrations, and enzyme concentrations were used. Furthermore, activity at low pH values in both *Suaeda* and *Beta* was difficult to detect. However, in all instances illustrated in Table 2 sodium chloride was inhibitory at all pH values with a mean effect of 50 % in the presence of 0·33 M sodium chloride.

Peroxidase

Peroxidase activity was detected in all four species and in each case the most severe effect of sodium chloride occurred at the lowest pH value used (Table 3). As the pH was raised so the effect of sodium chloride was reduced, there generally being no significant effect of sodium chloride at a concentration of 0·167 M above pH 7·0 (with the exception of the *Salicornia* enzyme).

TABLE 3. THE EFFECT OF SODIUM CHLORIDE ON THE PEROXIDASE ACTIVITY OF HALOPHYTES AT VARIOUS pH VALUES

	Suaeda			*Salicornia*		
pH	4·8	5·4	7·2	4·8	5·3	7·1
NaCl (M) 0	4·77	6·36	1·92	6·36	6·94	1·20
0·167	2·10 (44)	4·61 (72)	1·85 (96)	2·97 (47)	3·48 (50)	0·83 (69)
0·33	1·56 (33)	3·23 (51)	2·27 (118)	1·72 (27)	3·24 (47)	0·86 (71)
LSD $p = 0·05$		0·54			0·24	
	Beta			*Halimione*		
pH	4·7	5·3	7·0	5·0	5·4	7·5
NaCl (M) 0	1·36	3·00	1·24	6·63	7·92	1·54
0·167	0·68 (50)	1·67 (56)	1·18 (95)	2·52 (38)	3·95 (50)	1·23 (80)
0·33	0·49 (36)	1·25 (42)	1·05 (85)	2·36 (36)	3·66 (46)	1·29 (84)
LSD $p = 0·05$		0·14			0·10	

The activity is expressed as Δ A/min/mg protein in 2·5 ml of a solution containing Tris-maleate (50 mM), *p*-phenylenediamine (3·7 mM) and hydrogen peroxide (1·2 μM).

Tris-maleate buffer was used in the enzyme assays and the pH in the absence of added sodium chloride is reported in Tables 1–4. Addition of sodium chloride to this buffer caused a decrease in the pH which was maximal at about pH 5·5 and approached zero at pH 8.[2] This sodium chloride induced pH shift, which under the assay conditions used was never greater than 0·4 pH unit, was too small to affect the malic dehydrogenase and glucose-6-

phosphate dehydrogenase activities which were assayed at relatively high pH values. Difficulty arises, however, in expressing the relative effects of sodium chloride on enzymes with sharp acid pH optima, such as peroxidase. In general, correction for the pH shift means the true inhibition is less than that indicated in Table 3. The important point to note, however, is that all the enzymes were affected in a similar manner by the addition of sodium chloride and that this effect was similar to that previously reported for a salt sensitive species.[2]

TABLE 4. THE EFFECT OF SODIUM CHLORIDE ON THE ATPase ACTIVITY OF HALOPHYTES AT VARIOUS pH VALUES

	Suaeda			*Salicornia*		
pH	4·7	5·2	6·7	5·5	6·9	8·5
NaCl (M) 0	127·2	151·0	125·2	47·2	49·3	15·0
0·167	92·2 (72)	149·0 (99)	114·1 (91)	63·6 (135)	48·6 (98)	17·2 (114)
0·33	54·8 (43)	116·1 (77)	79·1 (63)	65·4 (138)	46·4 (94)	12·9 (86)
LSD $p = 0·05$		4·8			13·8	
	Beta			*Halimione*		
pH	4·7	5·2	6·7	4·7	5·3	6·8
NaCl (M) 0	106·0	128·3	63·2	124·1	128·4	51·2
0·167	122·5 (116)	130·2 (102)	47·2 (75)	126·9 (102)	136·3 (106)	45·4 (89)
0·33	111·0 (105)	116·1 (91)	40·2 (64)	111·1 (89)	114·0 (89)	37·5 (73)
LSD $p = 0·05$		8·4			7·8	

The activity is expressed as nmol of Pi released/min/mg protein in 3 ml of a medium containing Tris-maleate (42 mM) together with ATP and $MgCl_2$ (both at 2 mM).

ATPase

The enzyme activities present in the extracts of *Suaeda*, *Beta* and *Halimione* all showed acid pH optima, with greater activity at pH 5 than at pH 7 which is characteristic of the acid phosphatases. The enzyme prepared from *Salicornia*, however, showed a more neutral pH optimum and the effect of sodium chloride was consequently assayed over a higher pH range.

The effect of sodium chloride on ATPase activity was more variable than its effect on the other enzymes assayed. The *Suaeda* enzyme was significantly inhibited by 0·167 M NaCl at pH 4·7 and by 0·33 M NaCl at all three pH values used. The *Halimione* enzyme was in general affected in like manner, although there was no significant effect of the lower salt concentration at any pH. The enzyme prepared from *Beta*, although having an acid pH optimum, was rather differently affected by sodium chloride. Activity was slightly stimulated by 0·167 M NaCl at low pH values while being inhibited at higher values. Similarly, the more neutral ATPase of *Salicornia* showed a marked sodium chloride stimulation at low pH (5·5): there was no significant effect of either sodium chloride level at the other pH values used. It is not clear why these differences should occur although there is a considerable discrepancy in the reported properties of isolated ATPases.[4]

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DISCUSSION

Salt tolerance may be defined in a number of ways and the definitions vary in the degree of emphasis placed either on the ability to persist in high levels of salt or on the productivity in given conditions of salinity.[5] Under natural conditions, however, it may be difficult to evaluate the relative salt tolerance of various species owing to the influence of factors other than salinity on the growth of the plants. In general, however, halophytes may most usefully be defined in terms of the former definition, as plants with an ability to persist under conditions of high salinity. The genus *Salicornia* is characteristic of saline habitats[6] and physiological studies have shown that these plants are among the most tolerant of the halophytes,[7–9] optimal growth generally occurring in about 0·2–0·3 M sodium chloride. *Suaeda maritima*, a species also characteristic of the lower levels of salt marshes,[10] has been shown to grow optimally in similar sodium chloride concentrations.[2] *Halimione* is also characteristic of the more saline regions of salt marshes although apparently its distribution is controlled, at least in part, by a requirement for a well drained situation.[13] *Beta* on the other hand is probably less salt tolerant and is to be found in the upper levels of salt marshes.

The effects of the *in vitro* additions of sodium chloride on the enzymes of the four species were in general similar, although there were differences in the sensitivities of the malic dehydrogenases and amongst the ATPases. The effects of the salt on the enzyme activity in *Suaeda* reported here were in all cases similar to those for laboratory grown *Suaeda*. These effects were similar to those on enzymes isolated from *Pisum* which is very salt sensitive: for example the mean inhibitions of malic and glucose-6-phosphate dehydrogenases by 0·33 M NaCl were 66% and 60% respectively. Thus although all four species used in this investigation are characteristic of highly saline environments and accumulate large amounts of salts in the plant tops,[2,6] their enzymes are on the whole very similar to those of *Pisum* in terms of sensitivity to sodium chloride. It may therefore be argued that salt tolerance in plants involves a very different mechanism from that conveying salt tolerance on bacteria. Presumably salt and enzymes are separated spatially in the plant cell such that the enzymes are not subjected to high concentrations *in vivo*. This is made possible in the halophyte by the presence of a large vacuole in which salts may be sequestered. The osmotic balance in the cytoplasm may be maintained by sugars, which have a lesser effect on enzyme activity.[14,15]

EXPERIMENTAL

Plants were collected from sites on the lower reaches of the river Ouse, in Sussex. During the summer months the *Suaeda*, *Salicornia* and *Halimione* plants were covered by water during the Spring tides, when the chlorinity approached that of full sea water (Sussex River Authority, personal communication). Following a high tide, the mean levels of Cl and of leachable Na were 0·54 and 0·58 meq/g of dry soil respectively: these corresponded to 0·84 and 0·83 N solutions based on the water content.

Enzyme activity was measured in a homogenate of the leaves of *Halimione* and *Beta* and of the leaves and stems of *Suaeda* and *Salicornia*, which was produced by grinding approx. 30 g of tissue in 25 ml of a solution

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containing sucrose (400 mM) and Tris-HCl (20 mM Tris, pH 7·0), centrifuging for 1 hr at 103 000 *g* and then dialysing for at least 22 hr against 4 l. of Tris-HCl (5 mM, pH 7·0). Malic dehydrogenase and glucose-6-phosphate dehydrogenase activities were assayed spectrophotometrically by the oxidation of NADH and the reduction of NADP respectively. Peroxidase was also assayed spectrophotometrically using *p*-phenylenediamine as H donor while the ATPase was assayed by determining the release of Pi from ATP. The methods have been described in more detail elsewhere.[2] Between 0·025 and 0·17 mg of protein were used per assay for the malic dehydrogenase; the figures for the glucose-6-phosphate, peroxidase and ATPase were 0·8–1·5 mg, 0·070–0·174 mg and 0·7–1·2 mg respectively. The effect of NaCl was assayed at pH values above, below and approximating to the pH optimum and all the assays were carried out approx. 24 hr after homogenizing.

Acknowledgements—I should like to thank Miss M. Viney for her technical assistance, Mr. T. Yeo for his analysis of the soil samples and Dr. J. L. Hall for reading the manuscript. The work was supported in part by a grant from the Science Research Council.

Key Word Index—Chenopodiaceae; halophytes; enzyme activity; sodium chloride effect; malate dehydrogenase; glucose-6-phosphate dehydrogenase; peroxidase; ATPase.