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Regulation of glucose-6-phosphate dehydrogenase under salt-stress condition in Aspergillus sydowii

Physiological responses of organisms to particular stresses are well understood in only a few cases (Bachofen, R. 1986 Experientia 42:1179-1182). Most of the salt-stress in nature is due to sodium salts, particularly NaCl (Strongonov, B.P. 1973. Structure and function of plant cells in saline habitats. Halsted Press, New York). Little information is available regarding the effect of salt on the regulation of enzymes in marine fungal systems. In our studies efforts have been made to understand the salt-mediated regulation of glucose-6-phosphate dehydrogenase (G6PDH) in halotolerant Aspergillus sydowii.

Aspergillus sydowii was isolated from salt pans. The growth conditions employed were the same as described earlier (Karlekar et al. 1985 J. Biosci 9:197-201) except that casemino acid and yeast extract were replaced by asparagine (1.0%). $Zn^{2+}$was added to the synthetic medium as ZnSO4 at the desired concentration. Zinc deficient, zinc suboptimal, zinc optimal and zinc supraoptimal conditions indicate no addition, addition of 0.1 mg, 1 mg and 10 mg per 100 ml of ZnS04 to the above growth medium, respectively. Methods for the preparation of cell-free extract, assay of G6PDH and protein were the same as described earlier (Savant et al. 1982 Experientia 38:310-311). Mycelial ash was prepared by heat drying the mycelia at 800°C for 5 hours.

Earlier studies with A. sydowii grown in the presence of 2M NaCl showed significantly higher levels of G6PDH compared with control cultures. Km was found to decrease while Vmax increased when NaCl was added to the growth medium (Parekh and Chhatpar 1986 In: Contemporary themes in biochemistry (Kon et al. eds.) ICSU Press Cambridge pg. 334-335).

Further studies on the in vitro effect of NaCl on kinetic constants of G6PDH in cell-free extracts of a 2M NaCl grown culture showed a decrease in Vmax without a change in Km values, suggesting a non-competitive type of inhibition of G6PDH by NaCl (Table 1). Various possibilities for regulation of G6PDH are; (a) Higher Na^+ accumulation in the culture grown in the presence of 2M NaCl condition might be responsible for increasing the activity of G6PDH; (b) A different pattern of accumulation of other electrolytes may also be responsible for altering enzyme activity; and/or (c) The control culture may be synthesizing or accumulating inhibitor(s) of G6PDH.

Table 1: Effect of NaCl on kinetic constants of G6PDH from A. sydowii grown in the presence of 2M NaCl.

Cell-free extract	Km	Vmax
2M NaCl-grown culture	3.7 x 10^-5 M	6333
2M NaCl-grown culture + 0.2M NaCl	3.7 x 10^-5 M	400

A. sydowii showed greater accumulation of Na+ when grown in the presence of 2M NaCl (Parekh and Chhatpar 1986). Addition of NaCl to the growth medium caused an increase in intracellular Na+ as well as G6PDH activity. On the contrary, in vitro addition of NaCl resulted in a significant inhibition of G6PDH activity (Fig. 1). Many enzymes from halophytes and glycophytes have been shown to be inhibited by salt concentration greater than 100 mM (Flowers et al. 1977 Ann. Rev. Plant Physiol. 28:89-121).

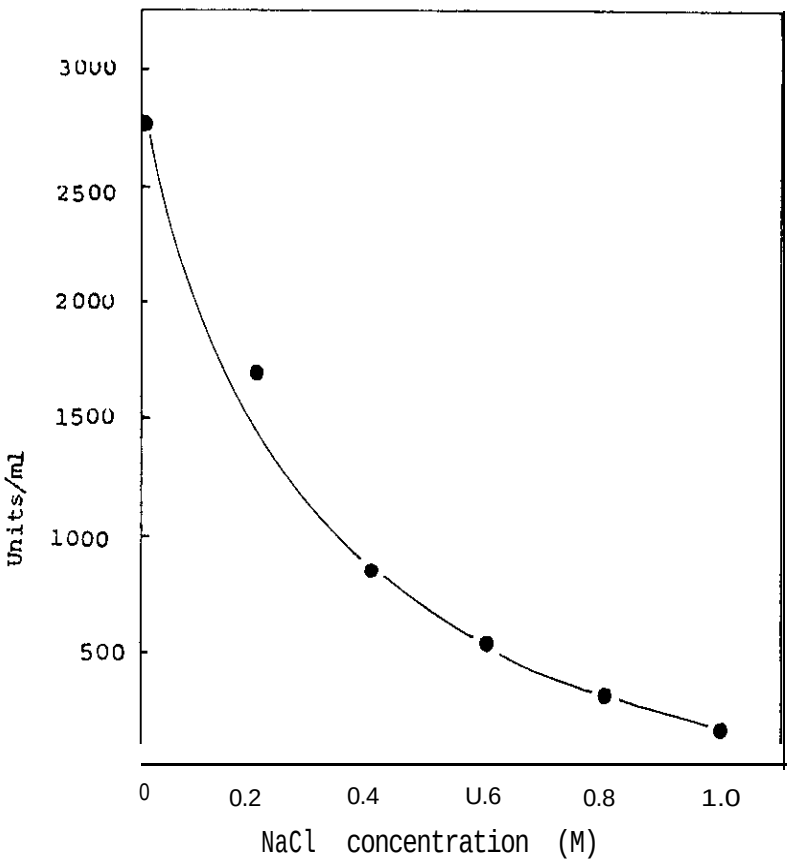

Fig. 1: In vitro effect of NaCl on the activity of G6PDH from A. sydowii

These observations suggested that there might be some factor(s) which significantly contribute to decreased activity of G6PDH in control cultures or increased activity in 2M the NaCl-grown condition. To determine if an inhibitor was present in control cultures, cell-free extract from a control culture was mixed with cell-free extract of a 2M NaCl-grown culture and the activity of G6PDH was measured. The observed significant decrease in the activity of G6PDH suggested the possibility of the presence of inhibitor(s) in cell-free extracts of the control culture (Table 2). This inhibitory activity was lost by dialyzing the control cell-free extract but not by boiling it for 10 minutes. The non-lipid and non-proteinic nature of the putative inhibitor was suggested from the observations that the inhibitory activity was not affected after lipase treatment or removal of proteins with (NH4)2 SO4 precipitation. Treatment with lysozyme of control cell-free extract also did not affect the inhibitory activity. Moreover, inhibitory activity was regained when dialysate of control cell-free extract concentrated appropriately and added in the assay system. Addition of ash prepared from control grown culture to the 2M NaCl-grown culture cell-free extract also showed inhibition, when added into the assay system (Table 2).

Table 2: Characterization of inhibitor of G6PDH from A. sydowii grown in the absence of NaCl.

	Cell-free extract	G6PDH activity (U/ml)	% Inhibition/ decrease of G6PDH
A)	2M NaCl-grown culture^a	2450	-
B)	Control-grown culture	490	80.0
C)	A + 0.2 ml of B	2107	14.0
D)	A +0.2ml of B (boiled for 10 min)	2009	18.0
E)	A + 100% (NH4)2 SO4 precipitates of B	2455	-
F)	A + 0.2 ml of B (lipase treated)^b	1863	24.0
G)	A + 0.2 ml of B (lysozyme treated)^b	1812	26.0
H)	A + 0.2 ml of B (dialyzed)	2450	-
I)	A + dialysate of B	1960	20.0
J)	i) A + 2 mg mycelial ash^c of A	2452	-
	ii) A + 2 mg mycelial ash of B	1617	34.0
K)	A + 100 ug of ZnSO4	1568	36.0

On the basis of these experiments it was inferred that the inhibitor accumulated in the control-grown culture is inorganic in nature and that it is heat stable. As reported earlier, control grown A. sydowii showed a 7-8 fold higher accumulation of Zn2+ (Parekh and Chhatpar 1985). Out of inorganic ingredients of the growth medium tested viz. ZnSO4, FeSO4, MgSO4, MnCl2, CaCl2, ammonium molybdate and borax (data not shown), Zn2+ was found to be responsible for regulation of G6PDH activity (Table 3).

Table 3: Effect of Zn2+ on G6PDH activity from A. sydowii grown in the presence of 2M NaCl

Growth condition	G6PDH activity (U/mg protein)
Zinc deficient	320
Zinc suboptimal	979
Zinc optimal	927
Zinc supraoptimal	520

These results indicated that higher accumulation of zinc in the control condition might be responsible for the reduced level of G6PDH. However, the possibility of the presence of other inhibitor(s) cannot be ruled out, since addition of 2 mg ash (approx. 0.15 ug of $Zn^{2}+$) to the assay systems gave 34% inhibition of G6PDH, which was the same as observed by the addition of 100 ug of ZnSO4 (approx. 23 ug of Zn2+). Earlier, Rouxel et al. (1987 Physiol. Plantarum 69:330-336) demonstrated that RNase from the halophyte Suaeda was not affected by the salinity of the growth medium but was totally inhibited by $10mMZn^{2}+$.

The results presented in Table 3 illustrate the influence of zinc (in the growth medium) on the level of G6PDH in A. sydowii. A lower level of G6PDH was observed when growth medium was supplemented at a supraoptimal concentration of Zn2+. However, a low concentration (suboptimal) of Zn2+ was required for the optimum activity of G6PDH. 2M NaCl-grown cells allow less accumulation of Zn2+ in the cytoplasm which may increase the activity of GGPDH, while a higher accumulation of Zn2+ in the control-grown cells could be one of the factors responsible for lowered G6PDH activity.

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