

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 $ZnSO_4$ 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 $ZnSO_4$ 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~~ 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. K_m was found to decrease while V_{max} 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 V_{max} without a change in K_m 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	3.7×10^{-5} M	6333
2M NaCl-grown culture + 0.2M NaCl	3.7×10^{-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).

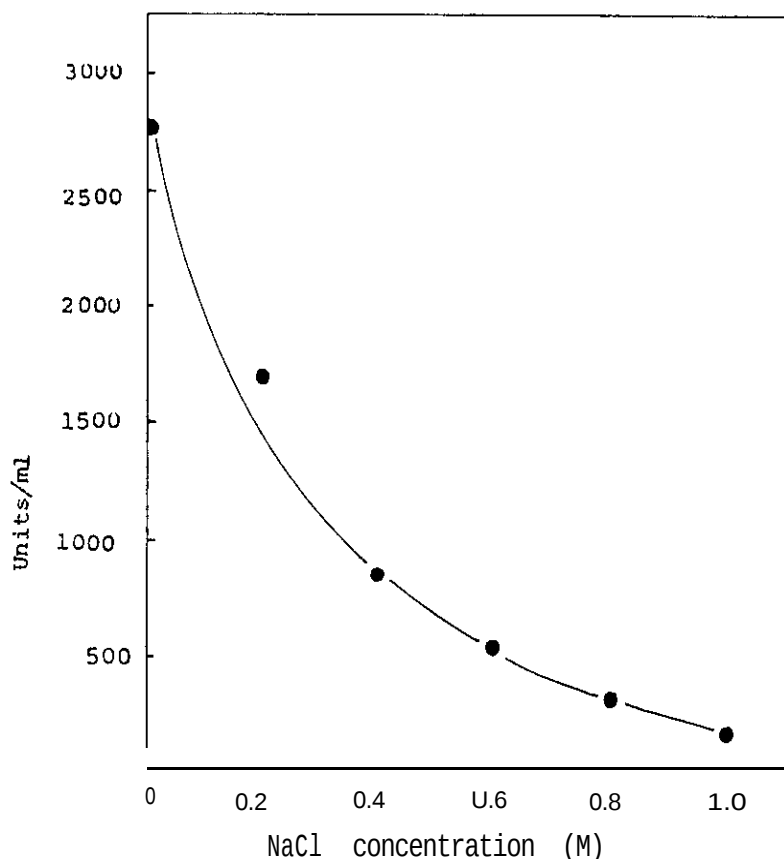


Fig. 1: In vitro effect of NaCl on the activity of G6PDH from A. sydowii

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% (NH ₄) ₂ SO ₄ precipitates of B	2455	-
F) A + 0.2 ml of B (lipase treated) ^{ab}	1863	24.0
G) A + 0.2 ml of B (lysozyme treated) ^{ab}	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 ZnSO ₄	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 Zn²⁺) 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 10⁻⁴ M Zn²⁺.

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 G6PDH, while a higher accumulation of Zn²⁺ in the control-grown cells could be one of the factors responsible for lowered G6PDH activity.

This work was supported by a Senior Research Fellowship to Trilok Parekh from the Council of Scientific and Industrial Research, New Delhi, India. - - - Department of Microbiology, Faculty of Science, M.S. University of Baroda, Baroda 390 002, India.