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POTATO ATPase SENSITIVITY TO HYDROSTATIC PRESSURE OF A COLD LABILE FORM

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SUMMARY

Activity of a cold sensitive form of potato ATPase (ATP phosphohydrolase, EC 3 6 1 3) increased with hydrostatic pressure till an optimum was reached at 5 atm applied pressure

Cold lability of ATPase (ATP phosphohydrolase, EC 3 6 1 3) isolated from beef-heart mitochondria has been demonstrated by RACKER[1] as well as cold lability of a Ca^{2+}-dependent ATPase isolated from chloroplasts by MCCARTY AND RACKER[2] Both enzymes are soluble proteins and both are required for oxidative phosphorylation and photophosphorylation respectively

Table I shows the sensitivity of a soluble ATPase isolated from potato tubers

TABLE I

COLD LABILITY OF POTATO ATPASE AS AFFECTED BY THE CONCENTRATION OF THE TRIS BUFFER, THE PRESENCE OR ABSENCE OF ATP (1 2 μmole) OR Mg^{2+} (1 μmole) AND pH DURING THE COLD PERIOD

Activity of the enzyme was measured at 30° for 30 min immediately after the treatment It is expressed as nmoles P_i per mg protein per h The negative values reflect side reactions

Line	*Treatment*					*Activity*	*pH*
	(Time min)	*Temp*	*ATP*	*Mg^{2+}*	*Buffer concn (M)*		
1	90	−10°	+	−	0 1	+101	7
2	90	−10°	+	−	0 05	+ 97	7
3	90	−10°	+	−	0 01	+ 7	7
4	90	−10°	+	−	0 001	− 3	7
5	90	−10°	−	−	0 001	+100	7
6	15	0°	+	−	0 001	− 6	7
7	30	0°	+	−	0 001	− 6	7
8	60	0°	+	−	0 001	− 7	7
9	60	0°	+	+	0 001	+ 96	7
10	0	0°	+	−	0 001	+ 20	4
11	0 5	0°	+	−	0 001	− 1	4
12	1	0°	+	−	0 001	− 3	4
13	2	0°	+	−	0 001	− 5	4

to freezing temperatures (−10°, lines 1 to 5 and to cold (0°, lines 6 to 13) ATPase was isolated and purified from potato tubers as described by KRISHMAN[3] A commercial preparation from Sigma, St Louis, Mo (U S A) showed similar properties as regard cold lability and sensitivity to pressure The reaction mixture consisted of 4 ml enzyme solution (1 mg/ml water), 1 ml buffer solution, 1 2 μmoles ATP (0 3 ml) and, if mentioned, 1 μmole $MgCl_2$ (1 ml) The buffer solution was Tris–HCl (pH 7) or potassium succinate (pH 4) The reaction was terminated with 1 ml trichloroacetic acid (20%, w/v) and inorganic phosphate was measured colorimetrically with added ammonium molybdate–sulfite reagents The requirements for cold lability were low buffer concentration (Table I, line 4), a protein concentration of less than 1 mg/ml, presence of ATP (lines 4 and 5), absence of divalent cations (line 9), and a fresh enzyme preparation In addition, sensitivity to cold was increased at low pH (lines 10 to 13) These requirements for cold lability of soluble potato ATPase strongly resembled those of the ATPase isolated from the beef-heart mitochondria[1]

Under conditions inducing cold lability, the enzyme activity was increased by application of pressure (Table II) After addition of ATP to the above reaction mix-

TABLE II

STIMULATION OF POTATO ATPASE BY APPLICATION OF HYDROSTATIC PRESSURE AS AFFECTED BY BUFFER CONCENTRATION AND PRESENCE OR ABSENCE OF Mg^{2+} (1 μmole)

Activity of the enzyme is given as nmoles P_i released per mg protein per h, measured at 20° and pH 4 for 45 min

Applied pressure (atm)	Mg^{2+}	*Buffer concn (M)*	*Activity (mean ± S E)*	%	*Significance (95% level)*
0	—	0 001	12 5 ± 4 6	100	
2 5	—	0 001	23 5 ± 4 4	188	Significant
5	—	0 001	48 6 ± 4 5	389	Significant
7 5	—	0 001	26 1 ± 4 5	209	Significant
10	—	0 001	19 9 ± 5 0	159	Not significant
20	—	0 001	16 6 ± 4 0	133	Not significant
5	—	0 1	12 0 ± 4 3	96	Not significant
5	+	0 001	14 0 ± 5 2	112	Not significant

ture the enzyme solution or the control solution without enzyme were exposed to atmospheric pressure or to an applied pressure which varied from 2 5 to 20 atm in the different experiments The temperature was maintained at 20° during the experiment No differences were observed if nitrogen gas or compressed air were used Activity of the enzyme solution increased with applied pressure till an optimum was reached at 5 atm, while at higher pressures again a decrease in activity was observed, though a noticeable but statistically not significant stimulation of the enzyme even at 20 atm pressure was still measured Under conditions which protect the enzyme against cold, high buffer concentration or addition of Mg^{2+} (Table II, lines 7 and 8), no stimulation of enzyme activity by applied pressure was observed At 10° and 15° the degree of stimulation by pressure was much less than at 20° Undoubtedly the interaction between the enzyme molecule and the structure of its surrounding water phase plays a decisive role in its sensitivity to both cold and applied pressure Possibly this ATPase fraction is also sensitive to cold and pressure in the intact cell[4]

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