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THE TEMPERATURE DEPENDENCE OF ACTIVATION BY PHOSPHATIDYLSERINE OF THE SODIUM PUMP ADENOSINE TRIPHOSPHATASE

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SUMMARY

1. Treatment of rabbit brain homogenates with deoxycholate reduced ouabain-insensitive ATPase sixfold and subsequently adding phosphatidylserine had no effect. Ouabain-sensitive ATPase was made entirely latent but it was fully restored on adding phosphatidylserine.

2. Temperature and pH were varied to see if the reconstituted system resembled that in the original membranes. Linear Arrhenius plots were always obtained with the homogenate, and the activation energy was higher for the ouabain-sensitive than for the ouabain-resistant enzyme.

3. A break at about 15° C was found in the Arrhenius plot of the reconstituted enzyme, but there was no break without added phosphatidylserine or when ouabain was added. The break suggests that the conformation and catalytic activity of the enzyme protein depended on the physical state of phosphatidylserine.

INTRODUCTION

Cell membranes contain large quantities of phospholipid which appear to be needed for the sodium pump ATPase. Thus, treatment of membranes from ox brain with sodium deoxycholate (DOC) removes lipid, and the ATPase is then no longer activated by sodium (Tanaka & Strickland, 1965). Phosphatidylserine appears to be specific in restoring sodium activation (Fenster & Copenhaver, 1967; Wheeler & Whittam, 1970), and the question arises whether the characteristics of the reconstituted enzyme are the same as those of the original enzyme. In view of the dependence on temperature of enzyme activity and the physical properties of lipids the response of ATP hydrolysis to temperature was examined in the original membranes and in the lipid-depleted preparation both before and after adding phosphatidylserine.

A short report of some of this work has been made (Priestland & Whittam, 1970).

METHODS

A soluble ATPase preparation was made as previously described (Wheeler & Whittam, 1970) except that the starting material was an homogenate of rabbit brain cortex instead of ox brain microsomes. ATPase activity was measured in a medium containing: 3 mM-Na ATP, 3 mM-$MgCl_2$, 100 mM-NaCl, 10 mM-KCl, 20 mM imidazole-HCl (pH 7·6). Inorganic phosphate (P_i) and protein were determined by the methods of Fiske & Subbarow (1925) and Lowry, Rosebrough, Farr & Randall (1951) respectively. Phosphatidylserine was obtained from Koch Light Ltd, Colnbrook, Buckinghamshire. Other chemicals were of Analar grade wherever possible.

RESULTS

Reversible changes in ouabain-sensitive ATPase activity following DOC treatment

The ATPase activity (in μmole P_i/mg protein per hr; $\pm$S.E. of mean; n = number of observations) at 37° C of rabbit brain homogenate was $9{\cdot}1 \pm 0{\cdot}5$ ($n = 7$) and of this $3{\cdot}1 \pm 0{\cdot}5$ was inhibited by ouabain. After treatment with DOC the total activity was reduced to $0{\cdot}77 \pm 0{\cdot}05$ and was not raised by sodium and potassium, or inhibited by ouabain. Addition of phosphatidylserine made the preparation sensitive to sodium, the total activity being raised to $3{\cdot}8 \pm 0{\cdot}3$ ($n = 11$); ouabain decreased this activity to $0{\cdot}99 \pm 0{\cdot}07$, the latter value being equal to the activity found without added phospholipid. These results show that DOC treatment made the sodium-activated ATPase latent but this activity could be restored by phosphatidylserine.

The different responses to temperature of the ouabain-sensitive and insensitive ATPases

ATP splitting by the original homogenate and the reconstituted enzyme was examined between 21 and 55° C. The values show marked differences between the ouabain-sensitive and ouabain-insensitive ATPases at the higher temperatures (Fig. 1). The ouabain-resistant activity in the homogenate increased linearly from 2 to 11 on increase of temperature between 21 and 55° C. The same activity with the soluble enzyme was much smaller and much less temperature dependent, the increase being only from 0·5 to 1·5 (Fig. 1). The ouabain-sensitive ATPase was similar in showing an increase to 45° C, when the activity was about 5, but there was then a fall and very little activity at 55° C. The results show that the ouabain-insensitive ATPase remained active at high temperatures (50–55° C) at which the ouabain-sensitive enzyme was inactive.

Change in temperature response by phosphatidylserine. ATP hydrolysis was analysed in more detail in the range 0–45° C. The Arrhenius plot for ouabain-resistant hydrolysis by the homogenate is a straight line equivalent to an activation energy of 16 kcal/mol (Fig. 2). Straight lines also describe the results for the soluble enzyme without ouabain-sensitive activity. Thus, the straight line for results in the absence of phosphatidylserine represents an activation energy of 15 kcal/mole. Similarly, when phosphatidylserine was added together with ouabain a straight line was again obtained (activation energy, 11 kcal/mole).

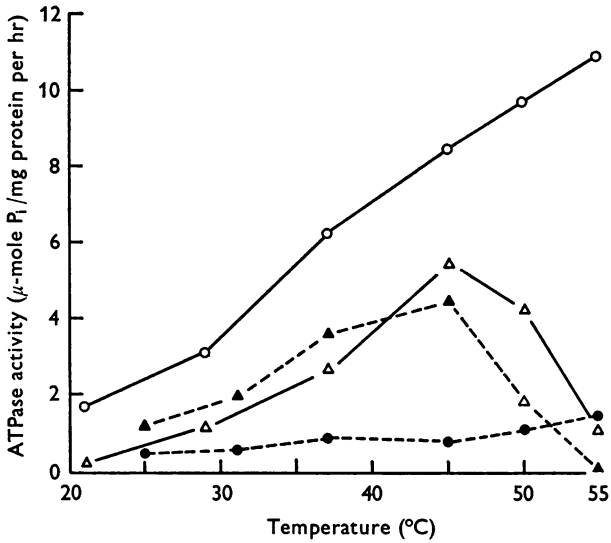

Fig. 1. The response to temperature of ATP hydrolysis. The ATPase activity of homogenate and soluble enzyme plus phosphatidylserine (0·5 μmole P/ml.) were compared. △ = ouabain-sensitive, ○ = ouabain-insensitive ATPase activity of untreated homogenate; ▲ = ouabain-sensitive, ● = ouabain-insensitive ATPase activity of soluble enzyme. Ouabain concentration was 0·1 mM.

On the other hand, when the ouabain-sensitive activity was examined there was a difference between the homogenate and the soluble enzyme. The homogenate results give a straight line with an activation energy of 27 kcal/mole (Fig. 3). In contrast, values for the reconstituted enzyme could not be fitted to a single straight line, and this feature was found repeatedly with six enzyme preparations (see Table 1). In order to describe the points according to the Arrhenius equation two straight lines intersecting at between 12 and 19° C were needed, the activation energies being 15 kcal/mole between 20 and 45° C and 34 kcal/mole below 20° C.

Dependence of activity on pH

A further way of investigating the two kinds of hydrolysis was to measure ATP splitting over a range of pH values (between pH 5·5 and 10·5). The ouabain-resistant ATPase of the homogenate showed a symmetrical response between pH 5·5 and 10·5, with a broad plateau between pH 7 and 9, when the activity was about 5·5 (Fig. 4). The soluble enzyme

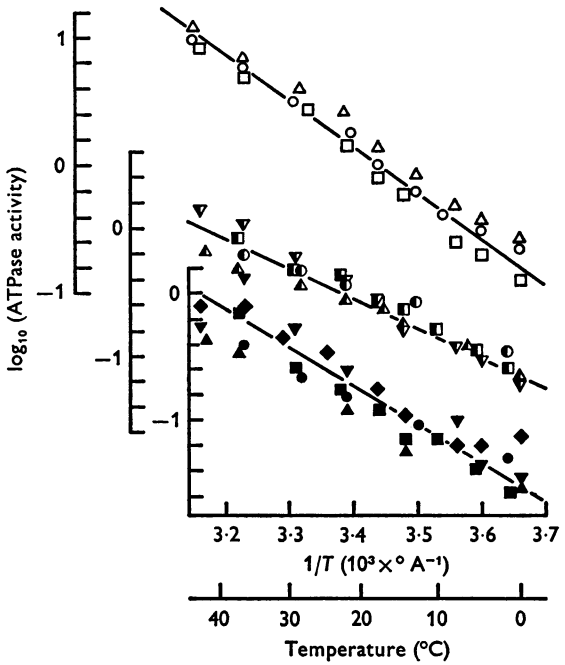

Fig. 2. Arrhenius plots for the ouabain-insensitive ATPase of homogenate and soluble enzyme. Top, ATPase of untreated homogenate in the presence of 0·1 mM ouabain; middle, ATPase of soluble enzyme in the presence of 0·1 mM ouabain and phosphatidylserine (0·4 μmole P/ml.); bottom, ATPase of soluble enzyme. The values of $\log_{10}$ (ATPase activity) are indicated by the scales of the three ordinates. The same symbols within each group indicate the results from the same preparation.

had very low ouabain-insensitive activity, and there was little effect of pH. A different profile was found with the ouabain-sensitive ATPase of both homogenate and reconstituted enzyme, each of which gave optimum values at pH 6·5–7·0. Above pH 7·0 there was a fall which was rather steeper with the soluble enzyme than with the homogenate. As was seen with the response to high temperature, addition of phosphatidylserine did not confer as much resistance to extreme conditions as was found with the native enzyme before treatment with DOC. These results show a difference

between the ouabain-sensitive and insensitive ATPase in that the ouabain-resistant ATPase remained active in alkaline solutions which markedly reduced hydrolysis by the sodium-pump ATPase.

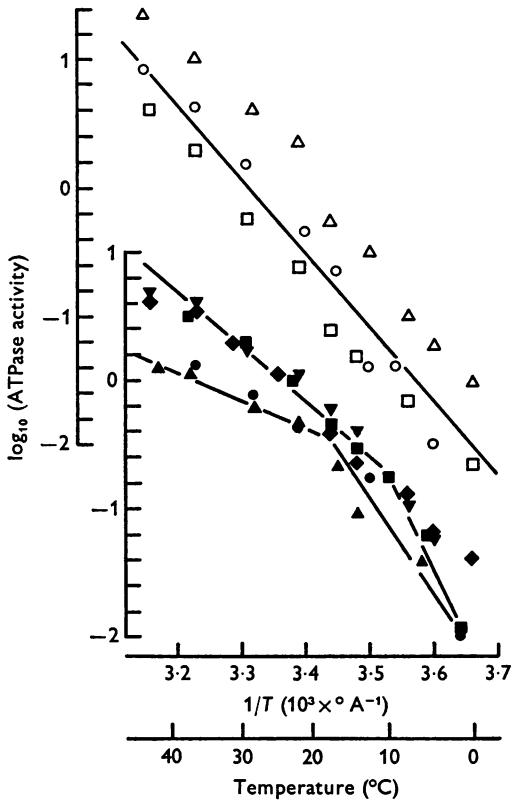

Fig. 3. Arrhenius plots for the ouabain-sensitive ATPase of brain homogenate and reconstituted soluble enzyme. Two sets of results are shown; upper, ouabain-sensitive ATPase of untreated homogenate; lower, ouabain-sensitive ATPase of reconstituted enzyme with phosphatidylserine (0·4 μmole P/ml.). The values of $\log_{10}$ (ATPase activity) are shown by the scales of the two ordinates. Within each group the different symbols indicate the results from the same preparation. Symbols in the upper group (untreated homogenate) correspond with values shown with the same symbols in the upper group of Fig. 2. There is a similar correspondence between symbols in the lower group (soluble enzyme), and the two lower groups of Fig. 2.

The values were obtained by difference between incubations in the absence and presence of 0·1 mM ouabain.

TABLE 1. The activation energies of the ATPases of rabbit brain

	Untreated homogenate			DOC-treated homogenate				
					With phosphatidylserine			
						Ouabain sensitive		
Expt.	With ouabain (kcal/mole)	Ouabain sensitive (kcal/mole)	Expt.	Without phosphatidylserine (kcal/mole)	With ouabain (kcal/mole)	Lower temperatures (kcal/mole)	Temperature of break in Arrhenius plot (° C)	Higher temperatures (kcal/mole)
1	17·2	26·1	1	25·7	14·6	50·2	14	16·6
2	15·6	26·5	2*a*	11·8	8·9	20·2	19	17·4
3	16·0	27·1	*b*	—	9·7	20·5	18	14·6
	—	—	3	16·0	12·8	30·9	14	16·1
	—	—	4	14·4	10·8	49·5	12	18·8
	—	—	5	10·7	8·1	40·2	13	15·2
	—	—	6*a*	11·4	8·7	24·1	13	8·7
	—	—	*b*	11·3*	—	—	—	—
Mean	16·3	26·6		14·5	10·5	33·7	15	15·3
S.E. of mean	±0·5	±0·30		±2·0	±0·9	±4·9	±1	±1·2

* With ouabain.

The ATPase activity of rabbit brain homogenate before and after DOC treatment was determined between 0 and 45° C with or without added phosphatidylserine (0·4 μmole P/ml.) and in the absence or presence of 0·1 mM ouabain. Ouabain-sensitive activity was determined by difference. In Expts. 3 and 4 ATPase activity was determined from the ^{32}P liberated from γ-labelled [^{32}P]ATP (Glynn & Chappell, 1964). Arrhenius plots of the results were prepared and the slopes of these calculated using the regression analysis of Ezekiel & Fox (1959). The activation energies were calculated from these slopes according to the equation: Activity $= k.e^{-E/RT}$ where E is the activation energy (kcal/mole), R is the gas constant (1·987 cal/mole per degree), T is the temperature (° A) and k is a constant.

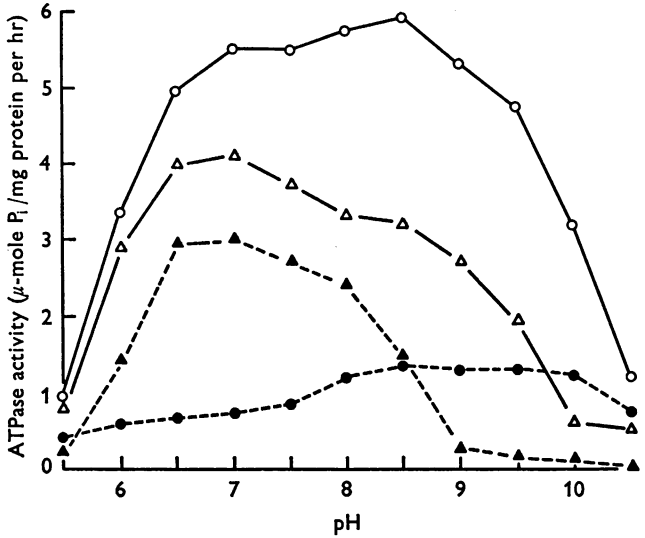

Fig. 4. The response to pH of ATP hydrolysis. The ATPase activity at 37° C of untreated homogenate is compared with that of the soluble enzyme plus phosphatidylserine (0·5 μmole P/ml.). △ = ouabain-sensitive, ○ = ouabain-resistant ATPase activity of untreated homogenate; ▲ = ouabain-sensitive, ● = ouabain-resistant ATPase of soluble enzyme. Ouabain concentration was 0·1 mM.

DISCUSSION

The results show that DOC treatment of rabbit brain homogenates preferentially removed the ouabain-insensitive ATPase and made the ouabain-sensitive enzyme latent but amenable to activation by phosphatidylserine. The acid insoluble phosphorus (representing phospholipid) was reduced from about 0·7 to 0·1 μmole P/mg protein (unpublished observations; also see Hegyvary & Post, 1969). After reconstitution, the specific activity for ouabain-sensitive hydrolysis was the same as initially in the homogenate. There was no activation by sodium or inhibition by ouabain unless phosphatidylserine was added in agreement with earlier work (Fenster & Copenhaver, 1967; Wheeler & Whittam, 1970).

The present results with DOC are not the only ones which have been described with this surface-active agent. According to the concentration it can either enhance or inhibit the ouabain-sensitive ATPase without affecting the ouabain-resistant component (Jørgensen & Skou, 1971). Again, there can be enhancement of ouabain-sensitive ATPase such that the total activity remains unchanged (Järnefelt, 1964; Ellory & Smith, 1969). There is some evidence that DOC increased the turnover rate of the

active sites of the transport ATPase, and the possibility has been excluded that the two enzymes for ATP hydrolysis are inter-convertible (Ellory & Smith, 1969). The present results with DOC treatment and the responses to temperature and pH provide further support for the view that the two components of ATP hydrolysis arise from the activity of two separate enzymes, and not from an interconvertible single enzyme as previously claimed (Järnefelt, 1964; Smith & Ellory, 1971).

A detailed study of the temperature response of ATP hydrolysis showed that the ouabain-insensitive component of both homogenate and soluble enzyme had similar features and gave linear Arrhenius plots (activation energies, 10–17 kcal/mole). In contrast, with the ouabain-sensitive ATPase, although there was a straight line with the homogenate the activation energy was doubled, in agreement with results with other particulate enzymes (Gruener & Avi-Dor, 1966; Swanson, 1966; Robinson, 1967). A further difference was the break at about 15° C in the Arrhenius plot with the phosphatidylserine reconstituted enzyme. Similar breaks were found for the ATPase of the intestinal mucosa of goldfish at temperatures which could be related to the lipid composition of the membranes (Smith, 1967; Kemp & Smith, 1970).

Several explanations have been given for breaks in Arrhenius plots (Dixon & Webb, 1964), but the one most applicable to the present results is that the enzyme configuration and activity change abruptly at a particular temperature in a way related to a change in the physical state of the lipid (Gruener & Avi-Dor, 1966; Kumamoto, Raison & Lyons, 1971). Abrupt changes in surface film packing occur between 0 and 20° C with fatty acids having chain lengths similar to those in phosphatidylserine (Adam & Jessop, 1926). Phosphatidylserine clearly allows a protein in the DOC preparation to become enzymically active and the most likely way is by a change in protein configuration. It is not surprising that the phospholipoprotein produced is more labile in its response to temperature than the native enzyme, but in other respects the reconstituted soluble complex closely resembles the original membrane-bound ATPase.

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