

Na,K-ATPase: THE ROLE OF STATE OF LIPIDS AND Mg IONS IN ACTIVITY REGULATION

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1. Introduction

The non-linearity of Arrhenius plots for multi-step reactions are usually explained by a change of the limiting step resulting in a change of the apparent energy of activation. The second reason for this phenomenon can be the temperature-dependent shift between two conformers being characterized by different temperature dependences. Both of these explanations have been used at different times for Na⁺, K⁺-activated, Mg²⁺-dependent adenosine triphosphatase, Na,K-ATPase [1–3], but at present neither of these seems to be correct.

The working mechanism of this enzyme's operation is not multistep according to latest data [4,5], thus a change with temperature variation of the limiting step of the overall reaction is unlikely. On the other hand, the interconversion of two forms of the enzyme with different molecular activities must lead to a sharp break in Arrhenius plots of activity [6,7]. In reality the discontinuity has a gradual character, and linear approximation of individual parts of the graph is permissible only as a method for calculation of thermodynamic parameters (fig. 1a).

Recently a third interpretation of the non-linear character of Arrhenius plots for the enzyme under consideration was proposed. It follows from an analogy between temperature dependence of enzyme activity and viscosity of lipids in membrane preparations of Na,K-ATPase. According to this interpretation, changes in the state of membrane lipids regulate the activity of the enzyme [8–13].

However, there is some contrary evidence to this view [14,15]: in some cases Na,K-ATPase has a linear temperature dependence in Arrhenius plots.

For this reason we have re-investigated the role of the structural change of lipids on Na,K-ATPase activity.

2. Materials and methods

Na,K-ATPase was isolated from ox brain [16], and enzyme activity was estimated in terms of P_i production. In EPR experiments we used spin labels of three types: lipid labels, a protein label and a label for ATPase itself (spin-labelled substrate), see figs. 2–6. Spectral parameters of these labels were measured in a Varian E-4 spectrometer. Data presented are averages of five or more experiments performed in duplicate.

3. Results and discussion

The Arrhenius plot of maximal enzyme activity versus absolute temperature (fig. 1a) is actually similar to the temperature dependence of the rotational freedom of the spin-labelled androstane analogue (fig. 2) and other hydrophobic spin labels. The critical temperature range in all cases is 20°C ± 2°C.

The spectral parameters ΔH_m and $I_{+1}/(I_{+1})_0$ for maleimide spin label, covalently bound to SH-groups of the protein molecule, have the same character of temperature dependence (see fig. 3). This means that the changes in freedom of the rotational motion of the spin label bound to protein correspond to the structural changes (phase transition or phase separation) in lipids.

Na,K-ATPase used in our experiments has a high affinity to ATP [16]. Using the spin-labelled analogue of ATP allowed us to detect conformational changes

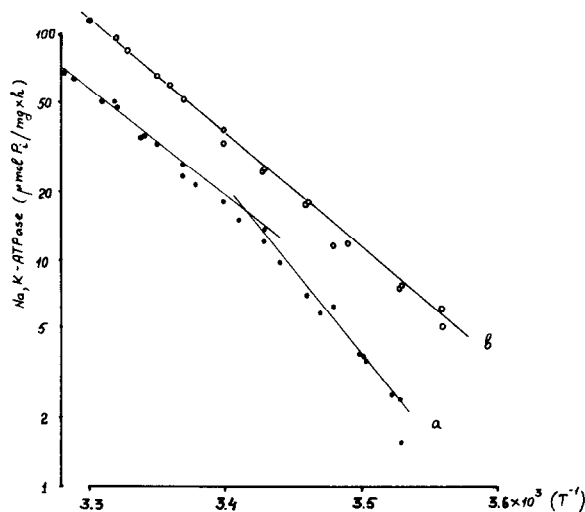


Fig. 1. Temperature dependence of Na,K-ATPase activity: (a) ●—●, ATP 3 mM, MgCl_2 1 mM, NaCl 130 mM, KCl 20 mM, imidazole 30 mM, pH 7.4 ± 0.1 ; (b) ○—○, ATP 1 mM, MgCl_2 5 mM, other conditions as in (a).

in the region of the active centre of Na,K-ATPase.

As is shown in fig. 4, the Arrhenius plot of correlation time (τ) versus T^{-1} for this spin label is also non-linear.

We believe the controlling role of lipids for Na,K-ATPase activity to be proved by this comparison of the temperature dependence of enzyme activity with the temperature behaviour of the spectral parameters for

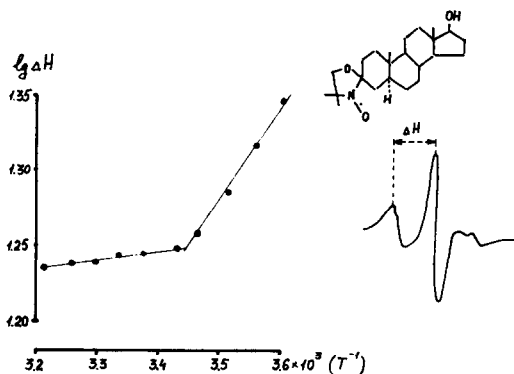


Fig. 2. Temperature dependence of the spectral parameter ΔH (characterizing the rotational freedom of the spin label) for an androstane analogue. Spin label 10^{-4} M, protein 4 mg/ml.

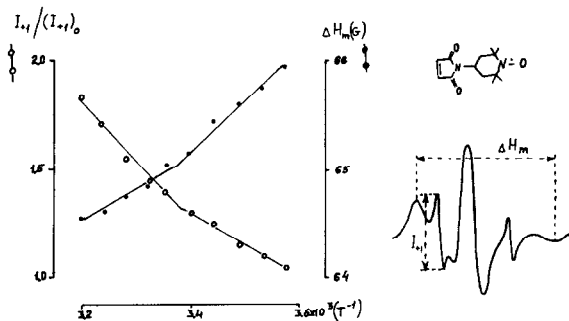


Fig. 3. Temperature dependence of the spectral parameters for a spin-labelled maleimide analogue. ΔH_m — for strongly immobilized spin label, $I_{+1}/(I_{+1})_0$ — for weakly immobilized spin label. Activity of spin-labelled enzyme was 50–60% from control. $(I_{+1})_0$ — at 0°C .

the spin labels used. In some cases, however, Arrhenius plots for Na,K-ATPase are linear (see table 1).

Analysis of data in table 1 shows that there are two situations when linearity is observed: with sarcolemmal ATPase and with a high Mg^{2+} concentration (compared to ATP concentration).

In the first case the absence of break point can be explained by the high cholesterol content in sarcolemmal membranes [19]. The correlation between the temperature dependence of enzyme activity and the lipid composition of these membranes proves once

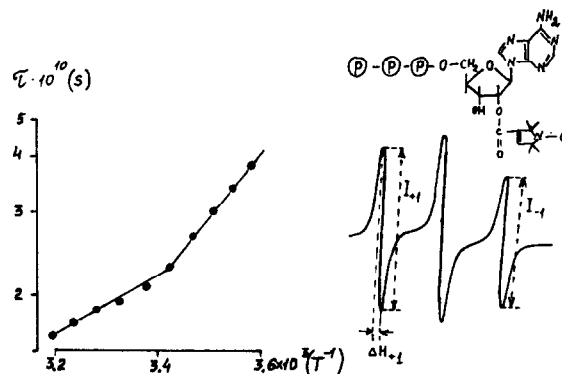


Fig. 4. Temperature dependence of the correlation time τ for spin-labelled ATP analogue. SL 25–100 mM, protein 3 mg/ml.

$$\tau \approx 6.6 \times 10^{-10} \Delta H_{+1} \left(\sqrt{\frac{I_{+1}}{I_{-1}}} - 1 \right) \text{ sec}$$

Table 1
Apparent activation energy for hydrolysis of different substrates by Na,K-dependent ATPase

Enzyme from	Substrate (mM)	MgCl ₂ (mM)	NaCl (mM)	KCl (mM)	kcal/mole		Break position	Ref.
					Above 20°C	Below 20°C		
Rat brain	ATP, 3	4	120	80	20.3 ± 3.2	41.7 ± 7.5	~ 20°C	17
Rat brain	ATP, 3	15	75	15	23.2	23.2	no break	14
Ray brain	ATP, 3	3	100	20	13.0	24.0	~ 20°C	1
Sheep kidney	ATP, 3	3	100	10	15.2	32.6	~ 20°C	9
Rabbit kidney	ATP, 4	4	80	20	13.8 ± 0.7	28.5 ± 1.5	~ 20°C	2
The same after re-activation by phosphatidylserine	ATP, 3	3	100	10	13–15	30–35	~ 17°C	18
	ATP, 3	10	100	10	13–15	13–15	no break	18
Muscle sarcolemma	ATP, 1	1	80	70	22.8 ± 1.2	22.0 ± 0.8	no break	15
Muscle sarcolemma	ATP, 1	3	80	70	15.9 ± 1.3	15.0 ± 1.5	no break	15
Ox brain (own data)	ATP, 3	3	130	20	16.7 ± 1.8	32.8 ± 2.1	~ 20°C	
	ATP, 3	1	130	20	21.0 ± 1.6	31.6 ± 6.7	~ 20°C	
	ATP, 1	5	130	20	21.6 ± 1.2	22.6 ± 3.9	no break	
	UTP, 1.5	1.5	154	—	11.8	11.8	no break	
	UTP, 1.5	1.5	144	10	11.2	11.2	no break	
	UTP, 1.5	1.5	34	120	11.5	11.5	no break	
	pNPP, 10	10	—	100	12.8	13.0	no break	
	pNPP, 10	20	—	100	12.4	12.6	no break	
	pNPP, 10	20	10	90	14.4 ± 0.6	14.3 ± 0.6	no break	
	pNPP, 10	30	70	10	14.4	14.3	no break	
Acetylphosphate,	4	20	15	135	12.2 ± 2.2	14.5 ± 3.0	no break	
	4	20	130	20	14.2	14.2	no break	
	4	1	—	150	13.0	13.0	no break	
	4	1	50	100	13.0	13.0	no break	
	4	1	145	5	11.5	11.5	no break	

again the controlling role of lipids in enzyme activity. It is interesting that a change in lipid environment shifts the break position in the ATPase Arrhenius plots. That is why 17°C instead 20°C was found as a break point for Na,K-ATPase reactivated by phosphatidylserine (table 1).

The fact that Mg²⁺ ions remove the break position from the temperature region investigated corresponds to the displacement of the transition point for artificial lipid bilayers to a higher temperature region by magnesium [20]. So it was important to compare the influence of Mg²⁺ ions on the ATPase activity and

parameters of EPR spectra for hydrophobic spin labels. We have used in these experiments spin labels $I_{(12,3)}$ and $I_{(1,14)}$ — see figs. 1,5 and 6.

We found that high concentration of Mg²⁺ leads to increasing of the order parameter S for $I_{(12,3)}$ — see fig.5. This means a tighter packing of lipids in the surface layer. One can see that an increase in Mg²⁺ concentration straightens the curves in Arrhenius plots both of ATPase and of the order parameter S (cf. figs. 1 and 5).

On the other hand, the parameter A/B for $I_{(1,14)}$ characterizing solubility of this spin label in the

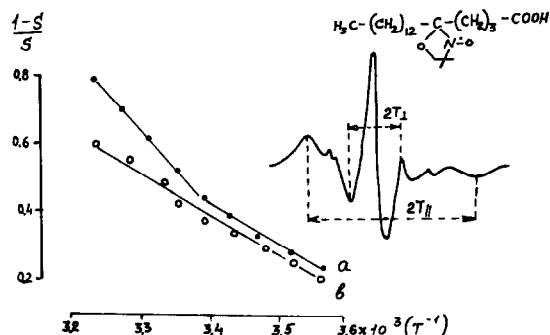


Fig.5. Temperature dependence of $(1-S)/S$ for a spin-labelled fatty acid $I_{(12,3)}$. Spin label 10^{-4} M, protein 2–3 mg/ml. (a) ●—●, without $MgCl_2$; (b) ○—○, 20 mM $MgCl_2$.

$$S = \frac{T_{\parallel} - T_{\perp}}{T_{xx} - T_{zz}}, T_{xx} - T_{zz} \approx 25 \text{ G}$$

hydrophobic region is increased by increasing the Mg^{2+} concentration (fig.6). Probably, high magnesium concentration activates, Na,K-ATPase by interaction with lipid phase. This interaction results in tightness of the surface of the lipid bilayer and an increase of the effective hydrophobic volume, that makes conformational changes of the enzyme molecule easier, especially at lower temperatures [21].

Thus in normal conditions the Arrhenius plot of Na,K-ATPase activity is non-linear for membranes

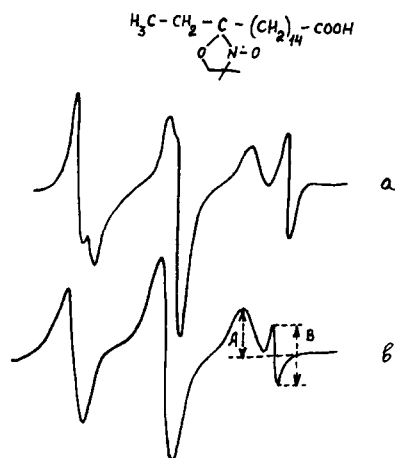


Fig.6. EPR spectra of a spin-labelled fatty acid $I_{(1,14)}$. Spin label 10^{-4} M, protein 3–4 mg/ml. (a) 0.1 mM $MgCl_2$; (b) 7 mM $MgCl_2$.

that have structural changes in lipid phase. This discontinuity, as can be seen in table 1 and [12], does not depend of Na/K ratio in the incubation medium. Table 1 indicates also that the enzyme has linear Arrhenius plots for UTP, pNPP and acetylphosphate hydrolysis. A comparison of the temperature dependences of the activity for these substrates and ATP is important, because ATP is the only substrate the hydrolysis of which is accompanied by ion translocation. When the hydrolytic process is uncoupled from ion transport (with UTP, pNPP and acetylphosphate — see ref. [22–24]), only one line is present in the Arrhenius plots. This linearity takes place at both high and low Mg^{2+} concentration and also does not depend of Na/K ratio. Thus we can conclude that structural changes in membrane lipids control the ion translocation process rather than the phosphate group hydrolysis during Na-pump operation.

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