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THE EFFECT OF BILAYER THICKNESS ON THE ACTIVITY OF $(Na^+ + K^+)$-ATPase

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Summary

The activities of purified $(Na^+ + K^+)$-ATPase supported by a series of phosphatidylcholines with monounsaturated (*cis*-9) fatty acyl chains (di(n : 1) phosphatidylcholine) varying in length from $n = 12$ to $n = 23$ were determined by the lipid titration technique. The ATPase activity at 20°C decreased from 2.9 to 0.1 μmol/min per mg protein as n was decreased from 16 to 12 and decreased from 2.9 to 1.0 μmol/min per mg protein as n was increased from 20 to 23. In further experiments, the di(n : 1) phosphatidylcholine-ATPase complexes were treated with increasing proportions of n-decane, which has been shown previously to increase the thickness of black lipid membranes. n-Decane caused a large increase (greater than 20-fold) in activity of the short-chain complexes (n = 12,13); for n = 14—18, the ATPase activity first increased and subsequently decreased as the proportion of decane was increased, and for n = 20 or 23 decane caused a progressive decrease in activity with increasing concentration. These effects confirm qualitatively that a major factor determining the activity in each bilayer is its thickness. This behaviour closely parallels that of the $(Ca^{2+} + Mg^{2+})$-ATPase of sarcoplasmic reticulum [1] and suggests that a major class of trans-membrane transport proteins may have a similar dependence on bilayer thickness.

We have recently determined the activities of the $(Ca^{2+} + Mg^{2+})$-ATPase from sarcoplasmic reticulum supported by a series of phosphatidylcholines with unsaturated (*cis*-9) fatty acyl chains (di(n : 1) phosphatidylcholine) varying in length from $n = 12$ to $n = 23$ and by mixtures of two di(n : 1) phospha-

tidylcholines [1]. The data indicated that the major factor determining ATPase activity in these complexes is the thickness of the bilayer with maximal activity at $n = 20$ and less than 1% of the maximal activity for $n = 12$. Further experiments in which the di(n : 1) phosphatidylcholine-ATPase complexes were treated with n-decane, which is known to increase bilayer thickness in black lipid membranes [2,3], confirmed that the major factor determining maximal activity in these phosphatidylcholine/decane bilayers is the thickness. Maximal activity for mixtures of each complex with n-decane was obtained at the same total proportion by weight of hydrocarbon (lipid alkyl chain and n-decane) per phosphatidylcholine molecule, equivalent to di(20 : 1) phosphatidylcholine without decane. In particular, the addition of optimal proportions of n-decane to di(12 : 1) phosphatidylcholine-ATPase complexes caused a 500-fold increase in activity at 37°C. This study was the first systematic examination of the effect of lipid chain length and bilayer thickness on the function of a transmembrane protein. Since a major class of membrane proteins, which probably includes all transport proteins, span the bilayer, it would be expected that the length of the lipid chains, and therefore the thickness of the bilayer, will affect the interactions of both the chains and the headgroups of the lipid molecules directly apposed to a trans-membrane protein. The data summarized for the $(Ca^{2+} + Mg^{2+})$-ATPase are consistent with the view that the range of bilayer thickness for the optimal function of this class of proteins will be limited. To determine whether this may be a general phenomenon, we have carried out similar experiments on the purified $(Na^+ + K^+)$-ATPase with di(n : 1) phosphatidylcholines and n-decane.

The $(Na^+ + K^+)$-ATPase from porcine kidneys was purified by using the method of Jørgensen [4] with 1 mM EDTA present at all stages. To determine the effect of phosphatidylcholine chain length, the purified $(Na^+ + K^+)$-ATPase was incubated with sonicated dispersions of di(n : 1) phosphatidylcholine ($12 \leqslant n \leqslant 23$) in potassium cholate. 1 vol. of $(Na^+ + K^+)$-ATPase (21.5 mg/ml) was added to 4 vol. of the freshly sonicated dispersion of di(n : 1) phosphatidylcholine (25 mg/ml) in potassium cholate (12.5 mg/ml) in a buffer containing 250 mM sucrose, 1 M KCl; 1 mM EDTA; 50 mM potassium phosphate; pH 8.0. The samples were incubated at 0°C and 4-μl aliquots were removed and assayed for $(Na^+ + K^+)$-dependent ATPase activity at 20 or 30°C by a coupled enzyme assay in 2.0 ml of assay medium which contained 130 mM NaCl, 20 mM KCl, 3 mM ATP, 3 mM $MgCl_2$, 0.1 mM EGTA, 30 mM histidine (pH 7.5), 0,5 mM phospho*enol*pyruvate, 0.15 mM NADH, 0.025 mg/ml pyruvate kinase and 0.050 mg/ml lactate dehydrogenase. It has been shown previously that the detergent-dilution technique results in the formation of bilayer structures incorporating intrinsic proteins [5,6]. The activity of the purified enzyme, without added di(n : 1) phosphatidylcholine or cholate, was about 3.3 μmol/min per mg protein.

The ATPase activities of these lipid titration complexes, determined at intervals after the start of the incubations, rapidly came to equilibrium at levels which depended on the chain length (Fig. 1). Vesicles prepared by the detergent-dilution technique under the conditions described have been shown previously to incorporate all of the intrinsic protein present, and are also freely permeable to small solutes in that they do not accumulate ions [5]. The

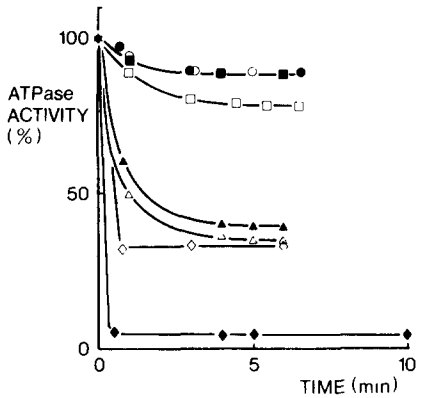

Fig. 1. The effect of phosphatidylcholine chain length on $(Na^+ + K^+)$-ATPase activity. For details of preparation of the titration complexes and assay see text. The activity of the purified $(Na^+ + K^+)$-ATPase was 3.3 μmol/min per mg protein at 20°C (100%). n = 12 (♦), 13 (△), 14 (▲), 15 (□), 16 (■), 18 (○), 20 (●), 23 (◇).

extent to which the added phosphatidylcholine and endogenous lipids of the purified ATPase had equilibrated was examined for the di(18 : 1) phosphatidylcholine complex, which was isolated by sucrose gradient centrifugation as described previously [5]. The lipids were extracted by $CHCl_3/CH_3OH$ (2 : 1, v/v) and the composition of the fatty acids determined by GLC after transesterification. It was found that 70% of the phospholipids associated with the ATPase were from the added di(18 : 1) phosphatidylcholine, compared with an expected level of 87% for complete equilibration of the two lipid pools, which has been shown to occur under comparable conditions for many lipid complexes with the $(Ca^{2+} + Mg^{2+})$-ATPase from sarcoplasmic reticulum [1,5]. The data for the lipid titration experiments with the $(Na^+ + K^+)$-ATPase should only be interpreted qualitatively, as any effect of the residual endogenous lipids on the ATPase activity is unknown. A further difference in the behaviour of the enzymes was that the low activity obtained by incubation with the short-chain members of the phosphatidylcholine series with the $(Na^+ + K^+)$-ATPase could only be partly reversed (30% or less) by back-titration with di(18 : 1) phosphatidylcholine [5]. This irreversible effect is probably due to interaction with the phosphatidylcholine rather than cholate, since considerable variation in the cholate concentration used to equilibrate the lipid pools (±40%) did not affect the final level of ATPase activity in the complexes. With the above reservations, we conclude that the activity of the $(Na^+ + K^+)$-ATPase is very probably sensitive to changes in bilayer thickness in a similar way to the $(Ca^{2+} + Mg^{2+})$-ATPase, as compared in Fig. 2a and b.

It can be seen from Fig. 2 that maximal activity for the $(Na^+ + K^+)$-ATPase was obtained for n = 16—20, and that the activity decreased substantially for $n \leqslant 14$ or $n = 23$. Qualitatively similar results were obtained at both 20 and 30°C. This is important in that the di(23 : 1) phosphatidylcholine has been shown by light-scattering or spin-label assay to have a phase transition between 18 and 24°C [1]. The substantial inhibition observed in di(23 : 1) phosphatidylcholine-ATPase complexes at 30°C cannot therefore be attributed to a gel state of the phosphatidylcholine, and strongly suggests that the inhibition

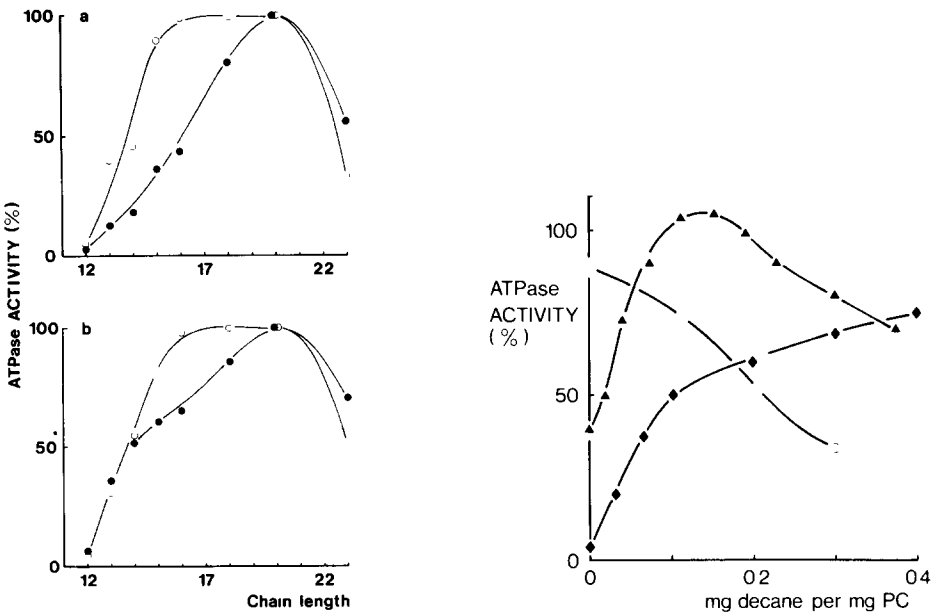

Fig. 2. Comparison of the effect of phosphatidylcholine chain length on $(Na^+ + K^+)$-ATPase (○) and $(Ca^{2+} + Mg^{2+})$-ATPase activity (●) at (a) 20°C and (b) 30°C. The activities are compared with those of the corresponding di(20 : 1) phosphatidylcholine-ATPases (100%) which were 2.9 and 3.2 I.U. at 20°C, and 9.3 and 8.8 I.U. at 30°C, respectively.

Fig. 3. The effect of *n*-decane on the activity of the di(*n* . 1) phosphatidylcholine-$(Na^+ + K^+)$-ATPase titration complexes (see text for details or preparation). Di(20 : 1) phosphatidylcholine-ATPase (○); di(14 : 1) phosphatidylcholine-ATPase (▲) and di(12 : 1) phosphatidylcholine-ATPase (◆). The activity of the purified $(Na^+ + K^+)$-ATPase was 3.3 μmol/min per mg protein at 20°C (100%). PC, phosphatidylcholine.

is due to the effect of an overthick bilayer on the function of the enzyme.

The effects of *n*-decane on the activities of the complexes were determined as described for the preparation of the di(*n* : 1) phosphatidylcholine-ATPases, except that varying proportions of *n*-decane were cosonicated with the di(*n* : 1) phosphatidylcholine/cholate dispersions before incubation with the purified $(Na^+ + K^+)$-ATPase. Constant ATPase activities were obtained within 4 min, and are plotted for three of the complexes (n = 12, 14, or 20) in Fig. 3 as a function of *n*-decane concentration. With short-chain phosphatidylcholines (n = 12 or 13), the ATPase activity increased to the highest concentration of *n*-decane used. For n = 14—18, the ATPase activity first increased and subsequently decreased as the proportion of decane in the bilayer was increased, although for n = 16 or 18 there was only a marginal increase in activity before the onset of inhibition by *n*-decane. Addition of decane to di(20 : 1) phosphatidylcholine-ATPase caused inhibition of activity at all concentrations. All decane effects were completely reversible by the addition of defatted bovine serum albumin to a final concentration of 10 mg/ml in the assay medium, as described previously for the $(Ca^{2+} + Mg^{2+})$-ATPase-lipid complexes [1]. Binding of all the *n*-decane to bovine serum albumin corresponded to 0.75 molecules of *n*-decane per molecule of bovine serum albumin at the highest *n*-decane

concentration used. The effects of other *n*-alkanes on the activity of di(n : 1) phosphatidylcholine-ATPase complexes were consistent with their known ability to thicken black lipid membranes. Thus, *n*-octane had effects similar to *n*-decane, whereas *n*-hexadecane had no effect on the ATPase activity of any of the titration complexes, because it has a very low partition coefficient into the bilayer [3].

It seems clear from the general similarities between these results for the $(Na^+ + K^+)$-ATPase and the more detailed study of the $(Ca^{2+} + Mg^{2+})$-ATPase that a major factor in determining the activity of the enzymes is the thickness of the bilayer. Although the relative activities supported by phospholipids of different headgroup structures are different for the two enzymes, with negatively charged lipids supporting the highest activities of the $(Na^+ + K^+)$-ATPase [7] and zwitterionic lipids for the $(Ca^{2+} + Mg^{2+})$-ATPase [8], the same general model for the effect of bilayer thickness on activity can be applied to both enzymes. We have proposed that the optimal protein conformation for activity is maintained by lipids with chain lengths which match the dimensions of the hydrophobic surface of the trans-bilayer part of the protein structure, leaving the polar headgroups appropriately apposed to the more polar parts of the protein structure at the surface of the bilayer. Overthickening a bilayer of optimal thickness by the selective partitioning of *n*-decane at the centre of the bilayer displaces both the phospholipid chains and the headgroups from their optimal interaction with the protein, and results in inhibition of activity. In contrast, the addition of decane to a bilayer which is too thin to support activity displaces the short-chain lipids towards their optimal interaction with the protein when the bilayer is thickened.

This model is qualitatively consistent with the mechanism proposed by Haydon et al. [9] for the local anaesthetic action of alkanes on the Na^+ channel in the excitable membrane of nerves in that it demonstrates that the function of an Na^+-transport protein is affected by the thickness of the bilayer in which it is operating. The sensitivity of the $(Na^+ + K^+)$-ATPase to changes in bilayer thickness is, however, smaller than that estimated by Haydon et al. [9] for the Na^+ channel.

We note that the assays described here correspond to the rate of consumption of ATP rather than ion movement. There is no evidence at present to suggest that the ATPase activity of the enzyme does not actually represent the molecular functioning of the ATPase as a pump: i.e., Na^+ and K^+ may move with respect to the protein in exactly the same way in unsealed membrane fragments as in the sealed vesicles required to observe the ion fluxes which measure pumping action. Whether the ATPase activity measured here is a sufficient parameter to define the pumping activity of the protein remains an open question.

Finally, we note that changes in membrane protein activities caused by extraneous agents (anaesthetics, etc.), are frequently attributed to changes they induce in bilayer fluidity. Agents which increase bilayer fluidity will often be preferentially distributed towards the centre of the bilayer where the structure is most liquid-like and the entropy is relatively high. They are therefore likely to increase the thickness of the bilayer as well as its fluidity, and the

relative importance of these two parameters in modulating membrane protein functions remains to be assessed.

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