How Lipids Interact with an Intrinsic Membrane Protein: the Case of the Calcium Pump

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Abstract

The Ca-ATPase has been purified from skeletal muscle sarcoplasmic reticulum and reconstituted into phospholipid bilayers of defined chemical composition. These studies show that the effects of phospholipid structure on the activity of the Ca-ATPase are complex and these and related studies on simple model peptides show that both phospholipids and proteins distort to optimize lipid-protein interactions.

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Effects of Lipid Structure on the Function of a Membrane Protein are Complex

We have been studying the interactions between phospholipids and the Ca^{2+}-ATPase purified from skeletal muscle sarcoplasmic reticulum (SR). The Ca^{2+}-ATPase is an enzyme, coupling the hydrolysis of one molecule of ATP to the transport of two Ca^{2+} ions across the membrane. Mixing the purified ATPase with lipid in detergent followed by removal of the detergent allows the reconstitution of the ATPase into lipid bilayers of defined composition. If the ATPase is reconstituted into membrane fragments, ATPase activity (hydrolysis of ATP) can be measured in the absence of net accumulation of Ca^{2+}, removing any problems associated with leak of Ca^{2+} across the membrane.

We have shown that the function of the Ca^{2+}-ATPase depends on the chemical structure and physical phase of the lipids surrounding it in the membrane. ATPase activity is highest in bilayers of dioleoylphosphatidylcholine (di(C18:1)PC) and activities are low in bilayers with shorter (C14) or longer (C22) fatty acyl chains so that fatty acyl chain length, and thus bilayer thickness, is important for the proper functioning of the

ATPase (Starling et al., 1993). Activities in bilayers of phosphatidylethanolamine are the same as in phosphatidylcholine so that the exact structure of a zwitterionic phospholipid headgroup appears not to be important (Starling et al., 1996a). However, activities are low in bilayers of phosphatidylserine or phosphatidic acid so that a negatively charged headgroup supports low activity (Dalton et al., 1997). Activities in gel phase lipid are very low, and activities in phosphatidylethanolamines under conditions where the phosphatidylethanolamine is in the hexagonal H_{II} phase are also low (Starling et al., 1995a, 1996a). Thus a liquid crystalline bilayer of the appropriate thickness is required for high activity; these experiments provide no evidence that the exact phospholipid composition of the native SR membrane is in any way special, at least as far as ATPase activity is concerned.

We have also shown that there is no one common mechanism that explains the effects of all phospholipids on the function of the ATPase; the activity observed in a bilayer of any one particular phospholipid depends on the unique conformational state of the ATPase in that particular bilayer. This can be illustrated by studies of the effects of bilayer thickness on the ATPase. In short (C14) or long (C24) chain phosphatidylcholines, the stoichiometry of Ca^{2+} binding changes from the usual 2 Ca^{2+} ions bound per ATPase molecule to 1 Ca^{2+} ion bound; the rate of dephosphorylation of E2P decreases; the E1/E2 equilibrium shifts towards E1 in di(C14:1)PC but not in di(C24:1)PC; and the rate of phosphorylation decreases in di(C14:1)PC but not di(C24:1)PC (Michelangeli et al., 1991; Starling et al., 1993, 1994, 1995b). The observed effects of chain length on Ca^{2+} binding and on the rate of dephosphorylation are distinct; in di(C22:1)PC the stoichiometry of Ca^{2+} binding is normal, but the rate of dephosphorylation is decreased (Starling et al., 1996b).

An effect of bilayer thickness on Ca^{2+} binding is, perhaps, not surprising since the Ca^{2+} binding sites on the ATPase are located between trans-membrane α-helices embedded in the lipid bilayer (Figure 1). However, what is then surprising is that Ca^{2+} binding is unaffected by lipid head group structure (Dalton et al., 1997) or by a lipid phase transition from the liquid crystalline into the gel or hexagonal H_{II} phases (Starling et al., 1995a, 1996a). Indeed, phosphorylation and dephosphorylation of the ATPase are more sensitive to lipid structure than is Ca^{2+} binding in that changes are seen in phosphorylation and dephosphorylation not only with changes in phospholipid chain length but also with changes in lipid phase and lipid headgroup structure. Thus in gel phase lipid the rate of phosphorylation becomes very slow (Starling et al., 1995a) and in hexagonal H_{II} phase lipid, although the rate of phosphorylation is normal, the rate of dephosphorylation becomes slow (Starling et al., 1996a). Phosphorylation and dephosphorylation of the ATPase occur on the cytoplasmic domain of the ATPase, located a considerable distance above the bilayer surface (Baker et al., 1994). Changes in bilayer thickness must be sensed by the trans-membrane α-helices, since these are the parts of the ATPase in contact with the bilayer. Changes in helix packing in response to changes in bilayer thickness must then be linked to changes in the nucleotide binding and phosphorylation domains of the ATPase.

These effects follow from distinct effects on the conformational states of the ATPase. Lipid fluidity is not involved; as described, changes in a number of equilibrium properties of the ATPase have been observed on changing lipid structure, and changes in fluidity (a dynamic property of the system) cannot result in a change in an equilibrium property (Lee, 1991). Experimentally, no correlation is observed between lipid order parameter and ATPase activity (East et al., 1984). Changes in the aggregation state of the ATPase are not involved in effects of chain length or lipid phase; low ATPase activities are observed in short or long chain phospholipids or in gel phase lipids when the ATPase is reconstituted into sealed vesicles containing isolated, single ATPase molecules, where aggregation is not possible (Starling et al., 1995c). However, low activities observed in anionic lipids follow in large part from a decrease in the proportion of the ATPase able to bind ATP, and could result from formation of dimers (in phosphatidylserine) or trimers (in phosphatidic acid) with only one ATPase molecule per oligomer being able to bind ATP (Dalton et al., 1997).

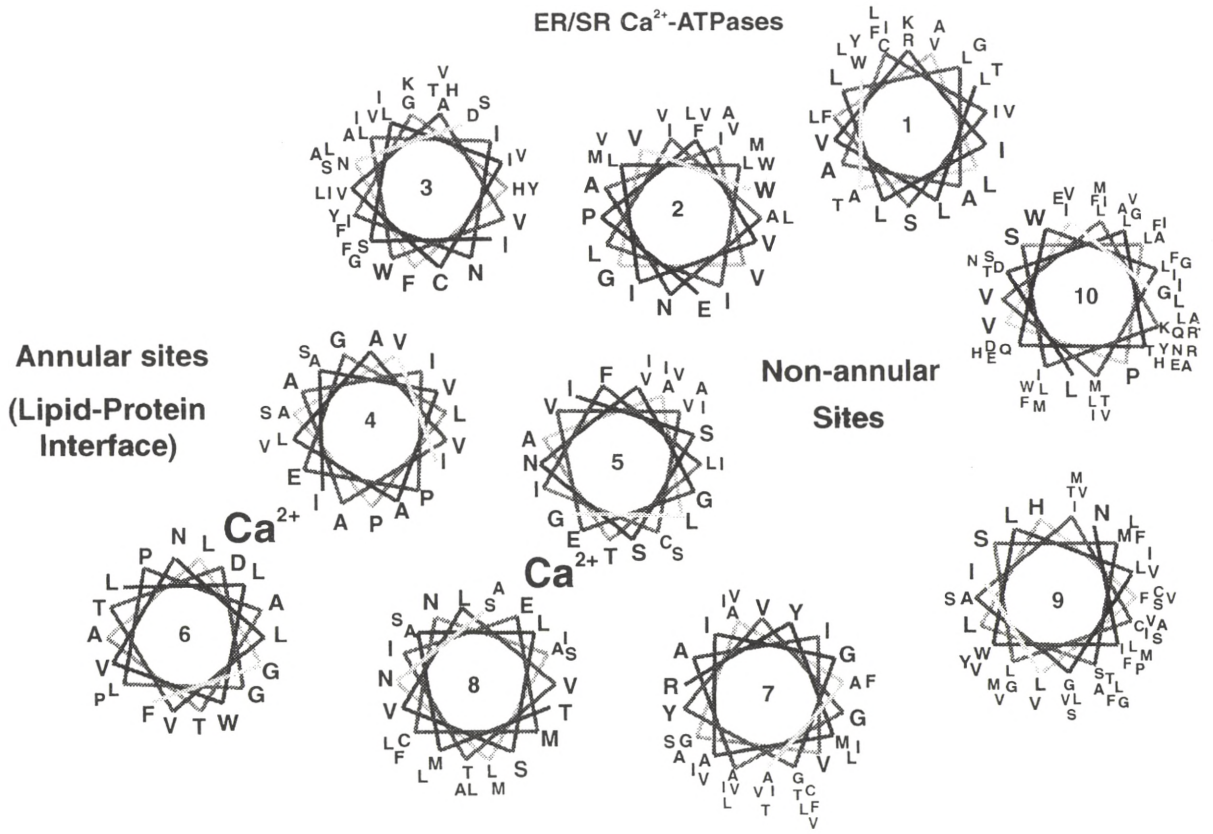

Figure 1. Possible packing of the 10 transmembrane helices of the Ca^{2+}-ATPase. The model is based on that of Stokes et al. (1994). Residues conserved amongst the ER/SR Ca^{2+}-ATPases are shown in large capitals. Residues not conserved are shown by small capitals. Ca^{2+} binding sites are located in a channel between helices 4, 5, 6 and 8. Non- specific binding sites for the bulk phospholipids in the membrane are proposed to exist around the outer surface of the structure (annular sites). Specific binding sites for a small number of "special" phospholipids could exist in the regions between helices, marked as non-annular sites.

Both Lipids and Proteins Distort to Optimise Lipid-protein Interactions

These marked effects of phospholipid structure on ATPase activity presumably follow from changes in the conformational state of the ATPase. There must be an energetic cost to these conformational changes, which would be expected to be reflected in different binding constants for phospholipids of different structure. In fact, phosphatidylcholines with chain lengths between C14 and C24 bind to the ATPase with equal strengths (East and Lee, 1982). However, although the energetics of the interaction of one phospholipid with the ATPase may change little with chain length, the ATPase is surrounded by about 30 phospholipid molecules in the membrane (East et al., 1985) and the total effect of all these phospholipids could be sufficient to result in significant conformational effects; consistent with this interpretation, the effects of chain length on the function of the Ca-ATPase have been

Table 1. Relative phospholipid binding constants for peptides P_{16} and P_{22}

Lipid	Bilayer Thickness (Å)[a]	Emission Maximum (nm)		Relative Binding Constant[b]	
		P_{16}^{c}	P_{22}^{d}	P_{16}^{c}	P_{22}^{d}
di(C14:1)PC	22.8	328	330	0.4	0.9
di(C16:1)PC	26.3	325	328	0.8	0.7
di(C18:1)PC	29.8	323	325	1.0	1.0
di(C20:1)PC	33.3	322	323	0.7	1.8
di(C22:1)PC	36.8	-	323	-	2.0
di(C24:1)PC	40.3	-	323	-	1.5

[a] Bilayer hydrophobic thickness d calculated from $d = 1.75(n - 1)$ where n is the number of carbon atoms in the fatty acyl chain (Sperotto and Mouritsen, 1988).
[b] Binding constant relative to that for di(C18:1)PC calculated from equations 1 and 2 with $n = 2.7$ for P_{16} and $n = 2.3$ for P_{22}.
[c] Estimated hydrophobic length 27 Å.
[d] Estimated hydrophobic length 36 Å.

shown to be highly cooperative (Starling et al., 1993).

Figure 1 shows a possible arrangement for the transmembrane helices of the Ca^{2+}- ATPase, based on the EM studies of David Stokes (Stokes et al., 1994). The bulk, solvent, or annular lipids interact with the outside surfaces of this bundle of helices (Lee et al., 1995). The interaction is short lived, as shown by the rapid rate of lipid exchange at these sites (East et al., 1985). It is relatively non-specific, since it is independent of chain length, as already described, and is unaffected by methyl branching of the chains (Froud et al., 1986). Binding of phosphatidylethanolamines is a factor of 2 weaker than binding of phosphatidylcholines (East and Lee, 1982), but anionic lipids bind as strongly as phosphatidylcholines (Dalton et al., 1997). Binding of gel phase lipid is a factor of 20 weaker than binding of lipids in the liquid crystalline phase (East and Lee, 1982), presumably as a result of poor van der Waals contact between rigid chains and the rough protein surface. We conclude that phospholipids are not bound at distinct 'sites' around the ATPase but rather should be pictured as interacting with the surface of the ATPase.

The conformational changes induced in the ATPase by changes in phospholipid structure presumably follow from changes in the packing of the trans-membrane α-helices embedded in the bilayer; changes in the structures of the α-helices themselves are unlikely. One obvious change enabling the ATPase to respond to changes in the thickness of the bilayer is a tilting of the helices. We have investigated this process using simple peptides of the type Ac-K_2-G-L_m-W-L_n-K_2-A-amide (P_{m+n}) containing a central Trp residue to act as a fluorescence reporter group and a pair of Lys residues at each end to anchor the peptide across the lipid bilayer (Webb et al., 1997). The peptides have been incorporated into bilayers of phosphatidylcholines with chain lengths between C14 and C24 by mixing peptide and excess phospholipid in organic solvent, removing the

solvent, and hydrating the mixture to give a bilayer containing the peptide. The peptide P_{22} ($m = 10, n = 12$) incorporates into all bilayers but P_{16} ($m = 7, n = 9$) does not incorporate into bilayers when the fatty acyl chain length is C24, and only partly incorporates into bilayers where the chain length is C22. This asymmetry follows because a too-long peptide can be matched to a too-thin bilayer both by stretching the lipid and by tilting the peptide. However, a too-thin peptide can only be matched to a too-thick bilayer by compression of the lipid, which becomes energetically unfavourable when the difference between the bilayer thickness and the peptide length exceeds about 10 Å. In the region of hydrophobic mismatch where the peptide still incorporates into the bilayer, there will be an energetic cost associated with stretching or compressing the lipid fatty acyl chains, which will be reflected in values of relative lipid binding constants. As shown in Table 1, strongest binding of lipid to P_{16} is observed for di(C18:1)PC and for P_{22} strongest binding is observed with di(C22:1)PC, as expected for optimal matching. However, it is clear that effects of a too-thin bilayer are relatively small, and level out at a factor of 2- 2.5 reduction in binding constant at a mismatch of about 6 Å.

These experiments with simple peptides therefore show that peptides can reorient in a lipid bilayer to help achieve an optimal match between the hydrophobic thickness of the lipid bilayer and the hydrophobic length of the peptide. It is not yet clear how these ideas should be extended to effects of bilayer thickness on a membrane protein such as the Ca^{2+}- ATPase containing a large number of trans-membrane α-helices. In a too-thin bilayer helices in the Ca^{2+}-ATPase could tilt to match the bilayer thickness, this resulting in changes in the conformation of the ATPase and changes in activity. The response to a too-thick bilayer is much less clear. The problem here is that, for the Ca^{2+}-ATPase, a too-thick bilayer is defined in a functional sense as one in which activity is less than in a bilayer of optimal thickness (provided by C18 chains). In terms of hydrophobic mismatch, it could be that the lengths of the helices in the Ca^{2+}-ATPase match the thickness of a C24 bilayer, explaining the observation that the Ca^{2+}-ATPase incorporates normally into bilayers of di(C24:1)PC. Some tilting of the helices would then be observed in di(C18:1)PC, and it would then have to follow that this degree of tilting corresponded to a conformational state for the ATPase which showed maximal ATPase activity. Finally, in di(C14:1)PC the degree of tilt would have become too extreme, giving a conformational state of low ATPase activity.

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