

Interactions of Phospholipids with the Potassium Channel KcsA

Ian M. Williamson, Simon J. Alvis, J. Malcolm East, and Anthony G. Lee

Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton, Southampton SO16 7PX, United Kingdom

ABSTRACT The potassium channel KcsA from *Streptomyces lividans* has been reconstituted into bilayers of phosphatidylcholines and fluorescence spectroscopy has been used to characterize the response of KcsA to changes in bilayer thickness. The Trp residues in KcsA form two bands, one on each side of the membrane. Trp fluorescence emission spectra and the proportion of the Trp fluorescence intensity quenchable by I^- hardly vary in the lipid chain length range C10 to C24, suggesting efficient hydrophobic matching between KcsA and the lipid bilayer over this range. Measurements of fluorescence quenching for KcsA reconstituted into mixtures of brominated and nonbrominated phospholipids have been analyzed to give binding constants of lipids for KcsA, relative to that for dioleoylphosphatidylcholine (di(C18:1)PC). Relative lipid binding constants increase by only a factor of three with increasing chain length from C10 to C22 with a decrease from C22 to C24. Strongest binding to di(C22:1)PC corresponds to a state in which the side chains of the lipid-exposed Trp residues are likely to be located within the hydrocarbon core of the lipid bilayer. It is suggested that matching of KcsA to thinner bilayers than di(C24:1)PC is achieved by tilting of the transmembrane α -helices in KcsA. Measurements of fluorescence quenching of KcsA in bilayers of brominated phospholipids as a function of phospholipid chain length suggest that in the chain length range C14 to C18 the Trp residues move further away from the center of the lipid bilayer with increasing chain length, which can be partly explained by a decrease in helix tilt angle with increasing bilayer thickness. In the chain length range C18 to C24 it is suggested that the Trp residues become more buried within the hydrocarbon core of the bilayer.

INTRODUCTION

Intrinsic membrane proteins must have co-evolved with the lipid component of the membrane to give optimal function, within the constraints imposed by the role of lipids in the general physiology of the cell and by the requirements of the biosynthetic machinery for translation and insertion of proteins into membranes. The rules describing the relationship between the lipid and protein components of the membrane are still being defined. One important property of the membrane is its hydrophobic thickness, defined as the separation between the glycerol backbone regions of the two leaflets making up the bilayer. The cost of exposing hydrophobic groups to water is high, so that the hydrophobic lengths of protein α -helices spanning the membrane would be expected to be equal to the hydrophobic thickness of the bilayer around the helices; this could be equal to the bulk thickness of the lipid bilayer in the absence of protein, or the bilayer could be distorted around the protein to give a thicker or thinner bilayer.

The thickness of a lipid bilayer in the liquid crystalline phase will be constantly fluctuating as a consequence of the molecular motion of the lipids. This is shown, for example, by the width of the Gaussian distributions representing the positions of groups in a liquid crystalline bilayer (Wiener and White, 1992). Molecular dynamics simulations also emphasize the roughness of the membrane surface resulting

from lipid motions with occasional lipid molecules protruding from the surface of the bilayer (Tieleman et al., 1997). Further, biological membranes contain a wide variety of lipid species with different fatty acyl chains so that lateral diffusion of lipid molecules within the plane of the membrane will result in fluctuating local thicknesses for the membrane.

Activities of a number of membrane proteins are sensitive to the thickness of the lipid bilayer, with the optimal thickness usually corresponding to that of a bilayer of dioleoylphosphatidylcholine (di(C18:1)PC) (Caffrey and Feigenson, 1981; Lee, 1998). One mechanism to reduce the effect of fluctuating bilayer thickness on the function of a membrane protein could be to reduce bilayer fluctuations in the vicinity of the protein. Indeed, the mobility of lipid molecules is reduced when they interact with the relatively immobile surface of a membrane protein, as shown by the presence of an “immobile” fraction of lipid in electron spin resonance (ESR) studies with spin-labeled lipid molecules (for example, see East et al., 1985; Marsh, 1995). Anchoring the protein firmly into the lipid bilayer could also reduce effects of changing bilayer thickness. It has been suggested that aromatic residues at the ends of transmembrane α -helices could achieve this by acting as “floats” at the interface; aromatic residues, particularly Trp, are found preferentially at the ends of transmembrane α -helices (Landolt-Marticorena et al., 1993; Wallin et al., 1997).

The exact location of Trp residues at the ends of transmembrane α -helices relative to the surrounding lipid bilayer is uncertain. Small water soluble analogues of Trp have been shown to bind in the glycerol backbone and lipid headgroup region of a lipid bilayer, stabilized partly by location of the aromatic ring in the electrostatically complex

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Address reprint requests to Prof. A. G. Lee, Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton, Southampton SO16 7PX, U.K. Tel.: 44-23-8059-4331; Fax: 44-23-8059-4459; E-mail: agl@soton.ac.uk.

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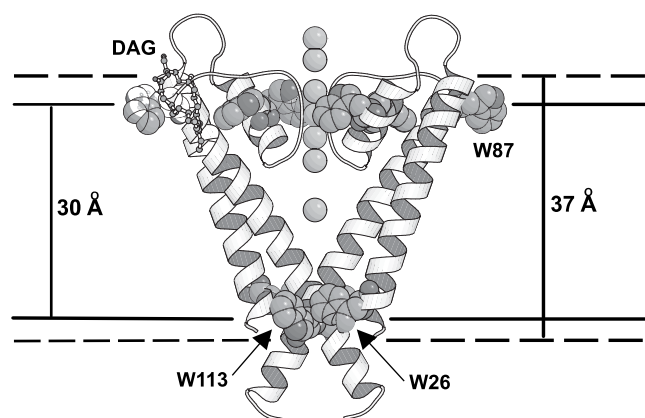


FIGURE 1 Structure of KcsA. For clarity only two of the monomers making up the tetramer are shown. Trp residues are shown in space-fill format. The lipid molecule modeled as a diacylglycerol (DAG) is shown in ball-and-stick format. Also shown are the positions of the K^+ ions. The figure was prepared using Bobscript (Esnouf, 1999) and the coordinates in PDB 1K4C (Zhou et al., 2001).

environment provided by this region of the bilayer and partly by exclusion of the flat, rigid ring system from the hydrocarbon core of the bilayer for entropic reasons (Yau et al., 1998). In contrast, aromatic residues in small peptides binding to the surfaces of lipid bilayers have been shown to penetrate into the hydrocarbon core region of the bilayer (Jacobs and White, 1989; Brown and Huestis, 1993). In the crystal structure of the bacterial photosynthetic reaction center the majority of the Trp residues are found near the ends of transmembrane α -helices with detergent molecules covering some of the Trp residues but not others (Roth et al., 1991). It is also likely that only some of the Trp residues in the photosynthetic reaction center will be located in the hydrocarbon core of a lipid bilayer. The hydrophobic thickness of a bilayer of a typical lipid such as di(C18:1)PC is 30 Å, which is insufficient to cover all of the Trp residues in the photosynthetic reaction center, so that some Trp residues would necessarily be located in the headgroup regions of the bilayer. In the potassium channel KcsA of *Streptomyces lividans* (Doyle et al., 1998; Zhou et al., 2001) the distribution of Trp residues is particularly clear (Fig. 1). The Trp residues form bands on the two sides of the membrane with the rings of the Trp residues being almost parallel to the surface of the membrane. On the periplasmic side of the membrane, Tyr residues also form a clear band “above” the band formed by the Trp residues. Two partial lipid molecules are seen in the x-ray structure, one modeled as nonan-1-ol and the other as a diacylglycerol with one C14 and one C9 chain (Zhou et al., 2001). As shown in Fig. 1, the diacylglycerol molecule is located close to Trp-87 with the Trp ring system just below the glycerol backbone of the diacylglycerol.

Of the five Trp residues in KcsA, Trp-26 and Trp-113, at the intracellular ends of transmembrane α -helices M1 and M2, respectively, are exposed to the lipid bilayer. At the

extracellular end of M2, Trp-87 is also exposed to the lipid bilayer, but Trp-67 and Trp-68 are located away from the lipid-protein interface as part of the short pore helix that points into the intracellular cavity. The fluorescence emission of Trp residues is environmentally sensitive (Lakowicz, 1999) so that major changes in the location of the Trp groups in KcsA relative to the lipid bilayer would be expected to be reflected in major changes in fluorescence emission spectra. Trp residues can also be used in fluorescence quenching experiments to determine lipid binding constants for a membrane protein. The experiments make use of brominated phospholipids such as dibromostearoylphosphatidylcholine (di(Br₂C18:0)PC). Di(Br₂C18:0)PC behaves much like a conventional phospholipid with unsaturated fatty acyl chains, because the bulky bromine atoms have effects on lipid packing that are similar to those of a *cis* double bond, but the presence of bromine atoms close to a Trp residue leads to quenching of the fluorescence of the Trp residue (East and Lee, 1982). If KcsA is reconstituted into bilayers containing a mixture of brominated and nonbrominated phospholipids, the degree of quenching of the tryptophan fluorescence of the KcsA depends on the fraction of the surrounding phospholipids that are brominated and thus on the strength of binding of the nonbrominated phospholipid to KcsA.

EXPERIMENTAL PROCEDURES

Didecanoylphosphatidylcholine (di(C10:0)PC) and dilauroylphosphatidylcholine (di(C12:0)PC) were obtained from Sigma (St. Louis, Mo). Dimyristoleoylphosphatidylcholine (di(C14:1)PC), dipalmitoleoylphosphatidylcholine (di(C16:1)PC), dioleoylphosphatidylcholine (di(C18:1)PC), dieicosenoylphosphatidylcholine (di(C20:1)PC), dierycophosphatidylcholine (di(C22:1)PC), dinervonylphosphatidylcholine, and (di(C24:1)PC) were obtained from Avanti Polar (Alabaster, AL). Phospholipids were brominated as described in East and Lee (1982) to give brominated analogues designated di(Br₂CN_c:0) in which N_c is the number of carbon atoms in the fatty acyl chains.

Purification of KcsA and reconstitution

A plasmid containing the *kcsA* gene (Schrempf et al., 1995) with a poly-His epitope at the N terminus was the generous gift of Professor Schrempf. *Escherichia coli* XL1 transformants carrying the pQE32 plasmid (Quiagen) with the *kcsA* gene were grown to midlog phase and then induced for 2 h in the presence of isopropyl- β -D-thiogalactopyranoside (0.5 mM). KcsA was purified using the protocol described by Schrempf et al. (1995). The cells were washed and resuspended in phosphate-buffered saline buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄), and lysed by sonication. The sample was spun at 100,000 \times g

for 30 min, and the membrane pellet was solubilized in phosphate-buffered saline containing 40 mM Mega-9 (Calbiochem) at 4°C for 3 h. The sample was spun at $8000 \times g$ for 20 min and the supernatant applied to a Ni-NTA column (Quiagen) and KcsA was eluted with 300 mM imidazole. Some samples of imidazole were found to contain a fluorescent impurity. This was removed by diluting the KcsA into phosphate-buffered saline thus lowering the concentration of Mega-9 below its critical micelle concentration, allowing KcsA to be pelleted at $100,000 \times g$ (1 h). The sample was stored at -80°C until use. Homogeneity of KcsA was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, using the method of Laemmli (1970).

Purified KcsA was reconstituted into lipid bilayers by mixing lipid and KcsA in cholate, followed by dilution into buffer to decrease the concentration of cholate below its critical micelle concentration, the method used previously for reconstitution of Ca^{2+} -ATPase of sarcoplasmic reticulum (East and Lee, 1982) and diacylglycerol kinase (Pilot et al., 2001). Phospholipid (0.6 μmol) was dried from a chloroform solution onto the walls of a thin glass vial. Buffer (400 μL , 20 mM Hepes, 1 mM EGTA, pH 7.2) containing 5 mM cholate was added, and the sample was sonicated to clarity in a bath sonicator (Ultrawave). KcsA (100 μg) was then added and the suspension left at room temperature for 15 min, followed by incubation on ice until use. Fifty microliters of the sample was then diluted into 3 mL of buffer (20 mM Hepes, 1 mM EGTA, pH 7.2) and the fluorescence recorded on an SLM 8000C fluorimeter with excitation at 290 nm.

For experiments in which KcsA was reconstituted into a mixture of two different phospholipids, separate solutions of the two lipids were prepared in cholate-containing buffer as described. These were then mixed in the appropriate proportions, incubated at 35°C for 30 min, and then mixed with KcsA, again as described above. This procedure gave the same results as when lipids were first mixed in chloroform solution, dried down and then dissolved in cholate, followed by reconstitution.

Quenching of Trp fluorescence was studied by addition of an aliquot of a stock solution of potassium iodide (KI) (1 M) in buffer containing $\text{Na}_2\text{S}_2\text{O}_3$ (100 mM) to KcsA (0.01 mg) in buffer (20 mM Hepes, 1 mM EGTA, pH 7.2) containing KCl, the total concentration KI + KCl being maintained constant at 0.83 M.

Phospholipid analysis

Lipid was extracted from purified KcsA with chloroform/methanol, using the procedure of Bligh and Dyer (1959). Lipid phosphorous was determined using the procedure of Bartlett (1959).

Analysis of fluorescence results

To obtain accurate values for wavelengths of maximum fluorescence emission intensity (λ_{max}), fluorescence spectra were fitted to skewed Gaussian curves (Rooney and Lee, 1986) over the wavelength range $F > 0.75 F_{\text{max}}$:

$$F = F_{\text{max}} \exp\{-(\ln 2)[\ln(1 + 2b(\lambda - \lambda_{\text{max}})/\omega_\lambda)/b]^2\} \quad (1)$$

in which F and F_{max} are the fluorescence intensities at wavelengths λ and λ_{max} , respectively, b is the skew parameter, and ω_λ is the peak width at half height.

Quenching of Trp fluorescence by brominated phospholipids was fitted to a lattice model for quenching (Caffrey and Feigenson, 1981; London and Feigenson, 1981; O'Keeffe et al., 2000). The probability that any particular Trp residue will give rise to fluorescence is proportional to the probability that none of the n lattice sites close enough to the residue to cause quenching are occupied by a brominated lipid so that

$$F = F_{\text{min}} + (F_o - F_{\text{min}})(1 - x_{\text{Br}})^n \quad (2)$$

in which F_o and F_{min} are the fluorescence intensities for KcsA in nonbrominated and in brominated lipid, respectively, and F is the fluorescence intensity in the phospholipid mixture when the mole fraction of brominated lipid is x_{Br} . This can be extended to the case of quenching when the brominated and nonbrominated lipids have different affinities for KcsA. The fraction of sites f_{Br} occupied by brominated lipid is given by:

$$f_{\text{Br}} = Kx_{\text{Br}}/(Kx_{\text{Br}} + [1 - x_{\text{Br}}]) \quad (3)$$

in which K is the binding constant of the brominated lipid relative to that of the nonbrominated lipid. Fluorescence quenching then fits to the equation:

$$F = F_{\text{min}} + (F_o - F_{\text{min}})(1 - f_{\text{Br}})^n \quad (4)$$

Eqs. 2 and 4 were fitted to the experimental data using the nonlinear least-squares routine in the SigmaPlot package.

RESULTS

Phospholipid content of purified KcsA

Lipid was extracted from the purified KcsA into chloroform/methanol using the method of Bligh and Dyer (1959). Analysis of the phosphorous content of the extracted lipid using the method of Bartlett (1959) showed that the phospholipid content of the purified KcsA was 0.2 mol phospholipid per mole KcsA.

Reconstitution of KcsA

KcsA was reconstituted into phospholipid bilayers of the required structure by mixing purified KcsA with lipid in cholate, followed by dilution into buffer to decrease the

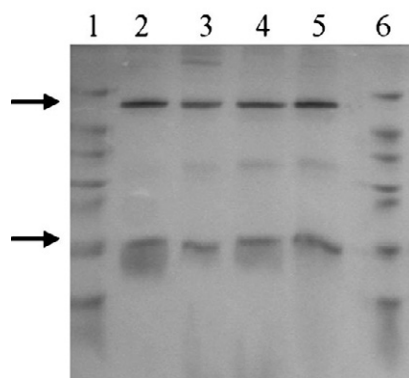


FIGURE 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of native and reconstituted KcsA. Lane 2 contains unreconstituted KcsA and lanes 3 to 5 contain KcsA reconstituted in di(C14:1)PC, di(C18:1)PC, and di(C24:1)PC, respectively. Lanes 1 and 6 contain molecular weight markers; the lower and upper arrows show the expected positions for monomeric and tetrameric KcsA, respectively.

concentration of detergent below its critical micelle concentration. KcsA was also reconstituted using dialysis overnight at 4°C to remove the detergent, giving identical results. Unless otherwise stated the molar ratio of phospholipid to KcsA was 100:1. To confirm that reconstitution did not result in denaturation of KcsA, we made use of the observation that native KcsA runs as a mixture of monomer and tetramer in sodium dodecyl sulfate gels while KcsA denatured by heating or by high pH runs as a monomer (Heginbotham et al., 1997). Fig. 2 compares sodium dodecyl sulfate gels for unreconstituted KcsA and for KcsA reconstituted into bilayers of di(C14:1)PC, di(C18:1)PC, and di(C24:1)PC. In all cases the major species seen is the tetramer with smaller amounts of monomer.

Fluorescence properties of KcsA

The fluorescence emission spectrum of KcsA reconstituted in di(C18:1)PC is shown in Fig. 3. The emission spectrum is centered at 324 nm, indicating a very hydrophobic environment for the Trp residues. The fluorescence emission spectrum for Trp in water is centered at 360 nm (Fig. 3), whereas that of a Trp residue in the transmembrane region of a peptide incorporated into a lipid bilayer is centered at 323 to 330 nm, depending on bilayer thickness and peptide length (Webb et al., 1998). Ladokhin et al. (2000) have shown that the relationship between the wavelength of maximum emission and the width of the fluorescence emission spectrum measured at half maximum peak height depends on the nature of the environment of the Trp residues in a protein and on the heterogeneity of the environment. For a single Trp shielded from water, fluorescence emission centered at ~325 nm would be expected to show a spectrum of width ~47 nm (Ladokhin et al., 2000). For KcsA in di(C18:1)PC the spectral width is 50 nm (Fig. 3), suggesting

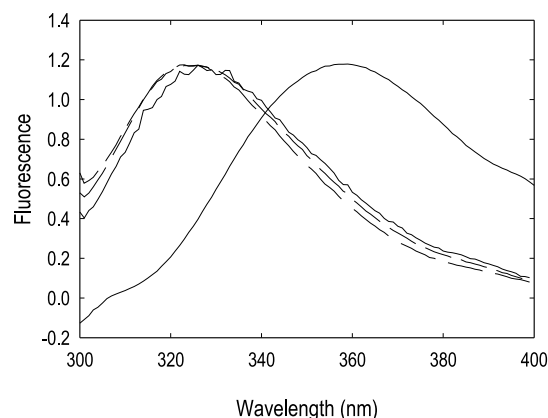


FIGURE 3 Fluorescence emission spectra for KcsA and tryptophan. Fluorescence emission spectra are shown for free tryptophan and for KcsA reconstituted into bilayers of: (solid line) di(C10:0)PC; (broken line) di(C18:1)PC; (dashed line) di(C24:1)PC. Wavelengths of maximum fluorescence emission intensity are listed in Table 1. The concentration of free tryptophan was 1.2 μ M and of KcsA was 0.24 μ M at a molar ratio of lipid to KcsA was 100:1. The buffer was 20 mM Hepes, 1 mM EGTA, pH 7.2.

a very similar, hydrophobic environment for all Trp residues, despite their different positions within the protein as shown in Fig. 1.

Any changes in fluorescence emission spectra on reconstituting into bilayers of phosphatidylcholine with chain lengths between C10 and C24 are very small (Fig. 3; Table 1) showing that over a bilayer thickness range of 24 Å the Trp residues in KcsA maintain a hydrophobic environment.

Fluorescence quenching by brominated phospholipids

When KcsA was reconstituted into mixtures of phosphatidylcholines with two monounsaturated fatty acyl chains and the corresponding phosphatidylcholine with two dibrominated fatty acyl chains, the fluorescence intensity decreased with increasing mole fraction of the brominated lipid (Fig. 4). The data fit to Eq. 2 with values

TABLE 1 Fluorescence properties of KcsA reconstituted into bilayers of phosphatidylcholine

Fatty acyl chains	Emission max (nm)	<i>n</i>
C10:0	327	-
C12:0	327	-
C14:1	327	1.55 ± 0.09
C16:1	326	1.76 ± 0.30
C18:1	324	1.66 ± 0.12
C20:1	326	1.99 ± 0.11
C22:1	326	1.49 ± 0.11
C24:1	325	1.69 ± 0.21

n is the number of lattice sites close enough to a Trp residue to cause quenching when occupied by a brominated lipid and was obtained by fitting the data in Fig. 4 to Eq. 2.

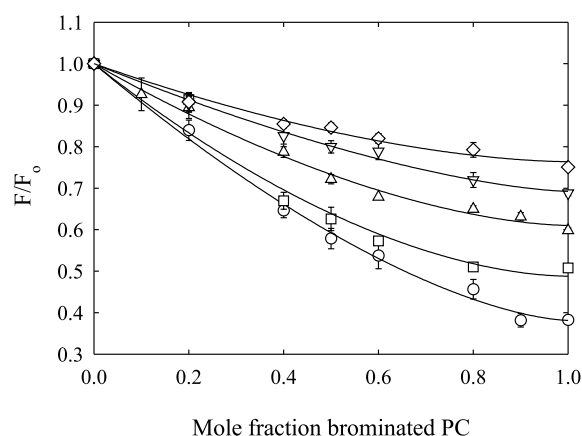


FIGURE 4 Quenching of KcsA fluorescence by brominated phosphatidylcholines. KcsA was reconstituted into bilayers containing mixtures of nonbrominated lipid and the corresponding brominated lipid. Fluorescence intensities are expressed as a fraction of the fluorescence for KcsA reconstituted in the nonbrominated lipid. Chain lengths were as follows: (○) C14; (□) C16; (△) C18; (▽) C22; (◇) C24. The solid lines show fits to Eq. 2 giving the values for n listed in Table 1. The concentration of KcsA was $0.24 \mu\text{M}$ and the molar ratio of lipid to KcsA was 100:1.

quenched (Table 1). The values for n are, within experimental error, independent of fatty acyl chain length. The average value for n is 1.69, very similar to the value (1.6) determined for quenching of the fluorescence of Ca^{2+} -ATPase by brominated phospholipids (East and Lee, 1982) but significantly less than the value of 2.5 determined for quenching of OmpF (O'Keeffe et al., 2000).

Analysis of the phospholipid content of the purified KcsA showed that the residual phospholipid content was very low, 0.2 mol phospholipid per mole of KcsA. It was confirmed in a number of ways that the presence of this small amount of residual lipid did not affect the results of reconstitution presented here. First, when KcsA was reconstituted with $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$ or a 1:1 mixture of $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$ and $\text{di}(\text{C18:1})\text{PC}$, the same levels of fluorescence quenching were observed at molar ratios of added phospholipid to KcsA of 100:1 or 1000:1 (Table 2). If significant levels of residual lipid had been present in the preparation of KcsA then higher levels of fluorescence quenching would have been observed at a molar ratio of added phospholipid to KcsA of 1000:1 than at 100:1, and this was not observed (Table 2). Second, it was shown that the purified KcsA preparation did not contain a mixture of fast-exchanging and slow-exchanging lipids by studying the time course of reconstitution with $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$. As shown in Table 3, maximum levels of fluorescence quenching were obtained after ~ 1 min incubation in 5 mM cholate and incubation with $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$ in cholate for up to 300 min at 25°C did not result in any change in the level of quenching. Third, reconstitution using 45 mM octylglucoside as detergent

TABLE 2 Effect of lipid:protein molar ratio on the level of fluorescence quenching of KcsA by $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$

Molar ratio $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$: $\text{di}(\text{C18:1})\text{PC}$	Molar ratio added phospholipid: KcsA	
	100:1	1000:1
1:1	0.74 ± 0.01	0.72 ± 0.02
1:0	0.61 ± 0.02	0.62 ± 0.03

The table gives the values of F/F_0 in which F_0 and F are fluorescence intensities for KcsA reconstituted in $\text{di}(\text{C18:1})\text{PC}$ and in the mixture containing $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$, respectively.

gave the same level of fluorescence quenching with $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$ as with 5 mM cholate (Table 3).

The maximal level of quenching of KcsA decreases with increasing chain length (Fig. 4). Quenching of Trp fluorescence by dibrominated quenchers has been shown to fit to a Forster-type energy transfer mechanism (Bolen and Holloway, 1990; Mall et al., 2001) with a value for R_0 , the distance at which energy transfer is 50% efficient, of 9 \AA for quenching by a dibrominated phospholipid (Bolen and Holloway, 1990). Quenching of Trp fluorescence by brominated phospholipids in a lipid bilayer needs to account for the distribution of bromine quenchers in the plane of the membrane. A convenient analysis of the problem is that of Dewey and Hammes (1980) that treats the fluorescence donors and acceptors as being distributed randomly on two planes a distance h apart. Because the Trp residues in KcsA are indeed located in two planes, one on each side of the bilayer (Fig. 1), the model of Dewey and Hammes (1980) is suitable for analyzing the observed quenching of KcsA fluorescence. Further, because the R_0 distance for the Trp-dibromolipid pair is very short, energy transfer only needs to be considered from the Trp residues on one side of the bilayer to the brominated lipids on that side of the bilayer.

TABLE 3 Effects of time and detergent on the level of fluorescence quenching of KcsA by $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$

Reconstitution conditions	Incubation time (min)	F/F_0
5 mM cholate	1	0.57
	60	0.61
	300	0.61
45 mM octylglucoside	30	0.62
	120	0.62

KcsA was reconstituted by incubation in the given detergent with $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$ at a molar ratio of $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$: KcsA of 100:1 at 25°C . After the given incubation times, samples were diluted into buffer and fluorescence intensities recorded as described in Experimental Procedures. F and F_0 are the fluorescence intensities in $\text{di}(\text{Br}_2\text{C18:0})\text{PC}$ and $\text{di}(\text{C18:1})\text{PC}$, respectively.

TABLE 4 Fluorescence quenching of KcsA in dibrominated phospholipids as a function of fatty acyl chain length

Fatty acyl chains	Position of <i>cis</i> double bond	Distance from double bond to bilayer surface (Å)	F/F_o	Calculated separation h (Å) Dewey-Hammes	Calculated separation h (Å) Koppel et al.
C14:1	9	7	0.38	9.9	10.2
C16:1	9	7	0.51	11.4	11.4
C18:1	9	7	0.61	12.7	12.3
C20:1	11	8.8	0.60	12.6	12.2
C22:1	13	10.5	0.69	14.1	13.0
C24:1	15	12.3	0.75	15.2	13.6
C14:1*	—	—	0.36	—	—
C18:1*	—	—	0.59	—	—

F_o and F are fluorescence intensities for KcsA reconstituted in nonbrominated phospholipid and the corresponding brominated phospholipid, respectively, measured at pH 7.2. The distance d of the double bond from the bilayer surface was calculated using $d = 0.875(n_c - 1)$ in which n_c is the number of carbons between the double bond and the carbonyl group. The separation h was calculated from F/F_o using Eqs. 5 and 6.

*Measured at pH 4.0.

Dewey and Hammes (1980) showed that the extent of fluorescence quenching is given by:

$$F/F_o = (A_2 + A_3)/2.0 \quad (5)$$

in which

$$A_2 = \left[1 + 0.4 \left(\frac{R_o}{h} \right)^6 \right] \times \left[1 + 0.4 \left(\frac{R_o}{h} \right)^6 + \pi \sigma \left(\frac{R_o^2}{2} \right) \left(\frac{R_o}{h} \right)^4 \right]^{-1}$$

and

$$A_3 = \left[1 + \frac{\pi \sigma R_o^2}{2} \left(\frac{R_o}{h} \right)^4 + 0.625 \left(\frac{R_o}{h} \right)^6 \right] \times \left(\left[1 + \frac{\pi \sigma R_o^2}{2} \left(\frac{R_o}{h} \right)^4 \right]^2 + \left[1 + \frac{\pi \sigma R_o^2}{2} \left(\frac{R_o}{h} \right)^4 \right] \times 0.625 \left(\frac{R_o}{h} \right)^6 - \frac{\pi \sigma R_o^2}{5} \left(\frac{R_o}{h} \right)^{10} \right)^{-1}$$

in which σ is the surface density of dibrominated fatty acyl chains, calculated assuming surface areas of 70 and 1200 Å² for lipid and KcsA, respectively. It should be noted that this equation cannot be used to describe quenching as a function of the mole fraction of brominated lipid in mixtures of brominated and nonbrominated lipids; the distribution of brominated chains within the membrane cannot be described by a simple average distribution because each brominated phospholipid molecule contains two brominated fatty acyl chains.

An alternative approach is that of Koppel et al. (1979) who showed that energy transfer could be represented by the equation:

$$\frac{F_o}{F_o - F} = 1 + \sigma^{-1.1} \left[\frac{0.62}{\pi R_o^2} \exp(-0.34r + 1.63r^2)^{1.1} \right] \quad (6)$$

in which r is defined as

$$r = h/R_o \quad (7)$$

The relative fluorescence intensities for KcsA reconstituted into brominated phosphatidylcholines are given in Table 4, together with the separation distances h calculated using Eqs. 5 and 6. The calculated distances between the planes of the Trp residues and the planes of the dibromine quenchers increase with increasing fatty acyl chain length although the relationship is complicated by differences in the position of the dibromine group within the fatty acyl chains. For chains of length C14 to C18, the double bond from which the brominated derivative is prepared is at the 9 position, but for the longer chains the double bonds are at later positions with the chain (Table 4). The KcsA channel is opened at pH 4.0 with significant changes in the orientations and tilts of the transmembrane α -helices detectable by ESR of spin-labeled KcsA (Liu et al., 2001). As shown in Table 4, values for the maximum fluorescence quenching observed at pH 4.0 are identical to those observed at pH 7.2 so that acid pH results in no significant movement of the Trp residues relative to the dibromo quenching groups.

Quenching by KI

Quenching of KcsA fluorescence by KI (Fig. 5) fits to a modified Stern-Volmer quenching equation:

$$F/(F_o^a + F_o^b) = F_o^b + F_o^a/(1 + K_a[I]) \quad (8)$$

in which F is the fluorescence intensity in the presence of I , the fluorescence intensity in the absence of I is ($F_o^a + F_o^b$), F_o^a and F_o^b are the fluorescence intensities quenchable and

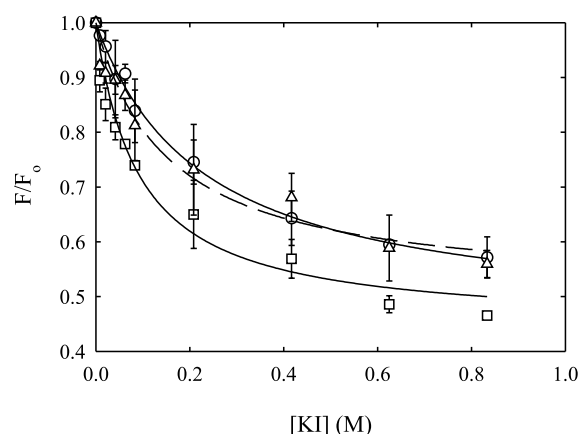


FIGURE 5 Quenching of KcsA fluorescence by KI. Data show quenching of KcsA in: (□) di(C10:0)PC; (○) di(C18:1)PC; (△) di(C24:1)PC. The lines show fits to Eq. 8 giving the parameters listed in Table 3.

nonquenchable by I, respectively, and K_a is the Stern-Volmer constant (Lakowicz, 1999). The fraction of KcsA fluorescence intensity quenchable by I is $\sim 50\%$ and is the same for KcsA in di(C10:0)PC, di(C18:1)PC, and di(C24:1)PC (Table 5). The Stern-Volmer quenching constant is significantly greater in di(C10:0)PC than in the other lipids (Table 5), suggesting greater accessibility of the quenchable Trp residues in di(C10:0)PC than in the other lipids. The fractional quenching caused by 0.83 M KI for KcsA in di(C18:1)PC and in di(Br₂C18:0)PC are very similar (Table 6) suggesting that di(Br₂C18:0)PC results in similar quenching for all classes of Trp residues present in KcsA.

Relative lipid binding constants

Fluorescence quenching curves for KcsA in mixtures of dibromostearoylphosphatidylcholine (di(Br₂C18:0)PC) and phosphatidylcholines of chain lengths C10, C16, C18, and C22 are shown in Fig. 6. Fluorescence quenching is more marked in mixtures of di(C10:0)PC and di(Br₂C18:0)PC at intermediate mole fractions of di(Br₂C18:0)PC than in mixtures of di(C18:1)PC and di(Br₂C18:0)PC (Fig. 6). This shows that the lipid-KcsA interaction is chain length dependent and that di(C10:0)PC binds to KcsA less strongly than does di(C18:1)PC. In contrast, quenching in mixtures of di(C22:1)PC and di(Br₂C18:0)PC is slightly less at inter-

TABLE 5 Quenching of KcsA fluorescence by KI

Phospholipid	Fraction of quenchable fluorescence	Stern-Volmer quenching constant K_a (M ⁻¹)
di(C10:0)PC	0.55 ± 0.05	10.9 ± 2.6
di(C18:1)PC	0.55 ± 0.02	4.4 ± 0.4
di(C24:1)PC	0.49 ± 0.03	6.5 ± 1.5
Free Trp	1	14.2 ± 0.8

Constants obtained by fitting the data in Fig. 5 to Eq. 8.

TABLE 6 Fractional quenching of KcsA by KI in di(Br₂C18:0)PC

	di(C18:1)PC	di(Br ₂ C18:0)PC
—	1	0.62 ± 0.03
+ 0.83 M KI	0.62 ± 0.02	0.42 ± 0.03
$\Delta F/F_0$	0.38	0.32

$\Delta F/F_0$ is the fractional quenching of fluorescence intensity observed on addition of 0.83 M KI for KcsA in di(C18:1)PC or di(Br₂C18:0)PC.

mediate mole fractions of di(Br₂C18:0)PC than in mixtures of di(C18:1)PC and di(Br₂C18:0)PC, showing that di(C22:1)PC binds slightly more strongly to KcsA than does di(C18:1)PC. Analysis of the data using Eq. 5 with the average value for n of 1.69 gives the relative binding constants listed in Table 7.

Fig. 7 shows fluorescence quenching curves for the reverse experiment in which KcsA is reconstituted in mixtures of di(C18:1)PC with brominated phospholipids with chain lengths of C14, C18, C22, and C24. Results in this case are not so obvious visually because of the different extents of quenching caused by the different brominated phospholipids, but the data can still be analyzed using Eq. 4, giving the relative binding constants listed in Table 7. For the longer chain brominated phospholipids where the maximum level of quenching is relatively low, the accuracy of the determinations is less than in the experiments using di(Br₂C18:0)PC, but relative binding constants determined from Figs. 4 and 5 are in good agreement, as shown in Table 7.

The experiments described above were performed at 25°C. We have determined the phase transition temperature of di(C24:1)PC to be 26°C, using the partitioning of the fluorescence probe 1-anilinonaphthalene-8-sulphonic acid as a marker for the transition between the liquid crystalline and gel phases (data not shown). We could detect no transition for the equivalent brominated lipid di(Br₂C24:0)PC in

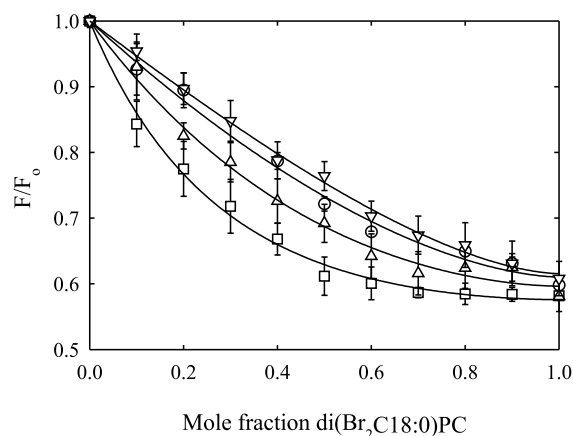


FIGURE 6 Quenching of KcsA in mixtures with di(Br₂C18:0)PC. KcsA was reconstituted into mixtures of di(Br₂C18:0)PC and (□) di(C10:0)PC; (△) di(C16:1)PC; (○) di(C18:1)PC; (▽) di(C22:1)PC. Solid lines show best fits to Eq. 4 giving the relative binding constants listed in Table 3.

TABLE 7 Relative lipid binding constants for KcsA

Fatty acyl chains	Hydrophobic thickness (Å)	Relative binding constant measured using di(Br ₂ C18:0)PC	Relative binding constant measured using di(C18:1)PC
C10:0	15.8	0.41 ± 0.03	—
C12:0	19.3	0.60 ± 0.02	—
C14:1	22.8	0.56 ± 0.06	0.77 ± 0.05
C16:1	26.1	0.70 ± 0.06	0.87 ± 0.05
C18:1	29.8	1	1
C20:1	33.3	0.95 ± 0.16	1.09 ± 0.10
C22:1	36.8	1.20 ± 0.06	1.02 ± 0.15
C24:1	40.3	0.92 ± 0.12	0.67 ± 0.13
C14:1*	—	0.73 ± 0.04	0.92 ± 0.06
C24:1*	—	0.65 ± 0.08	—
C24:1†	—	—	0.80 ± 0.03*

Relative lipid binding constants were calculated from quenching data for KcsA in mixtures of di(Br₂C18:0)PC with nonbrominated lipid or di(C18:1)PC with brominated lipid, at pH 7.2. Hydrophobic thickness was calculated from $D = 1.75(n - 1)$ in which n is the number of carbon atoms in the fatty acyl chains (Lewis and Engelman, 1983; Sperotto and Mouritsen, 1988).

*At pH 4.0 with a value for n of 2.7.

†At 37°C.

the temperature range 10°C to 60°C and so the transition temperature for this lipid must be below 10°C. The presence of membrane proteins lowers and broadens the phase transition temperatures for long chain lipids (Lee, 1977) so that di(C24:1)PC and all the other phospholipids used, when reconstituted with KcsA at a molar ratio of lipid:protein of 100:1, would be expected to be in the liquid crystalline phase at 25°C. To confirm that di(C24:1)PC was not present in the gel phase in these experiments, we repeated the determination of the relative lipid binding constant for di(C24:1)PC at 37°C (Table 7). As shown, the relative lipid

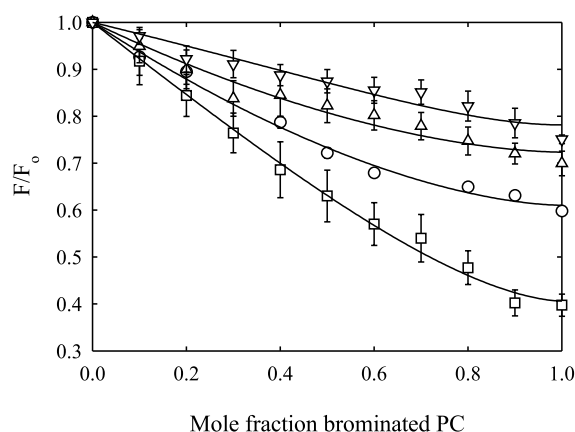


FIGURE 7 Quenching of KcsA in mixtures of di(C18:1)PC with brominated phospholipids. KcsA was reconstituted into mixtures of di(C18:1)PC and (□) di(Br₂C14:0)PC; (○) di(Br₂C18:0)PC; (△) di(Br₂C22:0)PC; (▽) di(Br₂C24:0)PC. Solid lines show best fits to Eq. 4 giving the relative binding constants listed in Table 3.

binding constant determined at 37°C agreed within experimental error with that determined at 25°C.

Relative lipid binding constants were also determined for di(C14:1)PC and di(C24:1)PC at pH 4.0. Quenching in mixtures of di(C18:1)PC and di(Br₂C18:0)PC fit to a value of n of 2.75 ± 0.16 (data not shown). Using this value for n , the values for the relative binding constants given in Table 7 are obtained; the values are very similar to those obtained at pH 7.2.

DISCUSSION

Hydrophobic matching in KcsA

Trp residues are found at the ends of transmembrane α -helices in many membrane proteins. This arrangement is particularly clear in the potassium channel KcsA (Fig. 1). In KcsA all the Trp residues are located close to the membrane surface; three Trp residues in each monomer making up the tetrameric channel are exposed to the lipid bilayer, and two are located within the protein as part of the short pore helix that points into the intracellular cavity (Doyle et al., 1998; Zhou et al., 2001). The location of the Trp residues at the membrane surface makes KcsA an attractive protein with which to study effects of lipid bilayer thickness on membrane protein structure; fluorescence emission spectra of Trp are very sensitive to environment (Lakowicz, 1999) and so can report on any changes in the regions around the Trp residues following from changes in the thickness of the lipid bilayer.

It is unlikely that any difference between the hydrophobic thicknesses of the lipid bilayer and the membrane protein will lead to exposure of either the fatty acyl chains or of hydrophobic amino acids to water, because the cost of exposing hydrophobic groups to water is high. It is much more likely that either the lipid bilayer or the protein, or both, distort so that the hydrophobic thickness of the protein matches that of the surrounding lipid bilayer. The fluorescence emission spectrum for KcsA reconstituted into bilayers of phosphatidylcholines shifts by no more than 3 nm on changing the lipid from di(C10:0)PC to di(C24:1)PC (Fig. 3; Table 1). The wavelength of maximum emission (λ_{\max}) for Trp can vary over a range of ~ 40 nm, depending on environment (Lakowicz, 1999). A Trp residue located within the hydrophobic core of a lipid bilayer has a λ_{\max} of ~ 320 to 330 nm (Webb et al., 1998), compared with 358 nm for Trp in buffer (Fig. 3); λ_{\max} for a Trp residue in a peptide bound to the surface of a lipid bilayer is ~ 331 nm (Chung et al., 1992). For the porin OmpF, λ_{\max} for Trp-318 on the outside of the porin exposed to lipid and close to the lipid-water interface is 318 nm, compared with 306 nm for Trp-61 located at the trimer interface (O'Keefe et al., 2000). The value for λ_{\max} for KcsA is 324 to 327 nm, suggesting a hydrophobic environment for the Trp residues. The very small effect on λ_{\max} of more than doubling the

hydrophobic thickness of the lipid bilayer (Fig. 3, Table 1) suggests that the environment of the Trp residues in KcsA changes little with changing bilayer thickness. Thus, hydrophobic matching between KcsA and the bilayer is very efficient.

Ladokhin et al. (2000) have shown that a single Trp shielded from water with λ_{max} value of 325 nm would be expected to have a spectral width of ~ 47 nm. The width of the Trp emission spectrum for KcsA is ~ 50 nm, independent of phospholipid chain length (Fig. 3). The fact that the observed spectral width corresponds to that expected for a single class of Trp residues suggests that both the lipid-exposed and the protein-buried Trp residues in KcsA are in similar hydrophobic environments; the presence of more than one class of Trp residues with different values of λ_{max} would have resulted in a broader emission spectrum. The observation that the spectral width does not change with bilayer thickness suggests that hydrophobic matching maintains a constant environment for both groups of Trp residues in KcsA.

Possible changes in the locations of the Trp residues in KcsA with changing bilayer thickness were also investigated by observing the quenching of Trp fluorescence by I^- . The fraction of the Trp fluorescence for KcsA in di(C18:1)PC quenchable by KI is 0.55 ± 0.02 (Fig. 5). Of the five Trp residues in KcsA, three are exposed to the lipid and two are located within the protein; molecular modeling shows that the Trp residues not exposed to lipid are partly exposed to the aqueous medium. From these experiments it is not possible to assign the fraction of Trp fluorescence quenchable by I^- to particular groups of Trp residues in KcsA. The observation that the fraction of quenchable fluorescence does not change significantly with changing phospholipid chain length from C10 to C24 (Fig. 5, Table 5) suggests that there are no major changes in the conformation of KcsA over this range of chain lengths. However, the observation that the Stern-Volmer quenching constant is significantly higher in di(C10:0)PC than in the other lipids, approaching the value of Trp free in buffer (Table 5), suggests that the Trp residues quenchable by I^- are more exposed to the aqueous medium in di(C10:0)PC than in thicker lipid bilayers.

Energetics of lipid-KcsA interactions

Most theories of hydrophobic mismatch assume that the lipid chains distort to match the protein with the protein remaining unchanged (Mouritsen and Bloom, 1984, 1993; Fattal and Ben-Shaul, 1993; Nielsen et al., 1998). If the hydrophobic thickness of the lipid bilayer is less than that of the protein then the fatty acyl chains will stretch to provide matching. If, on the other hand, the hydrophobic thickness of the bilayer is greater than that of the protein then the fatty acyl chains will compress to provide matching. Stretching or compressing the fatty acyl chains of a lipid requires work,

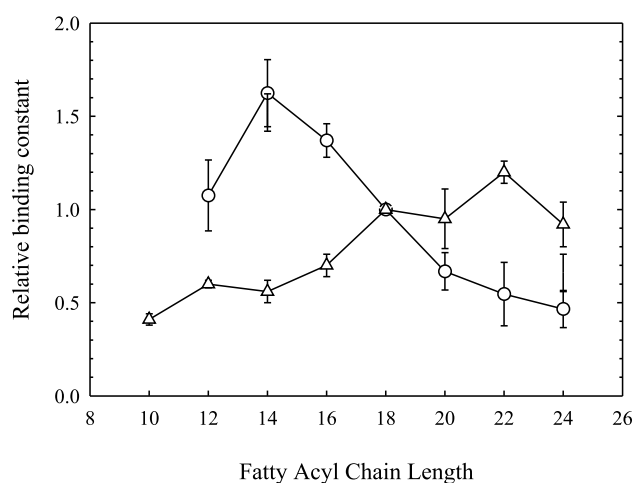


FIGURE 8 Relative lipid binding constants for KcsA. Binding constants for KcsA for phosphatidylcholines relative to that for di(C18:1)PC are plotted as a function of acyl chain length (Δ). Data are compared with relative lipid binding constants for OmpF (\circ).

and thus a lipid that has to change its hydrophobic thickness to bind to KcsA would be expected to bind less strongly than a lipid where no stretching/compressing was required. Binding constants for phosphatidylcholines relative to di(C18:1)PC have been determined (Table 7) and are plotted in Fig. 8 as a function of chain length. There is a gradual increase in relative binding constant with increasing chain length from C10 to C22 with a small decrease from C22 to C24. The changes in binding constant with chain length are, however, small compared with those seen with the β -barrel protein OmpF. For OmpF the changes in lipid binding constant between di(C14:1)PC and di(C18:1)PC are comparable with the changes expected if the lipid fatty acyl chains have to compress to match the hydrophobic thickness of a rigid protein, although changes in binding constant with further increases in chain length are less than expected from lipid compression, suggesting that hydrophobic matching to the thicker bilayers requires distortion of OmpF as well as distortion of the lipid bilayer (O'Keeffe et al., 2000). The very small changes in lipid binding constant with chain length for KcsA (Fig. 8) suggest that over the whole chain length range KcsA distorts to match the lipid rather than the lipid distorting to match the protein. Strongest binding is seen with di(C22:1)PC (Fig. 8). This gives a bilayer with a hydrophobic thickness of ~ 37 Å (Table 7). As shown in Fig. 1, a bilayer of this thickness would locate the Trp side chains totally within the hydrocarbon core of the bilayer. This is consistent with the recently published x-ray structure of KcsA (Zhou et al., 2001), which locates a lipid molecule modeled as a diacylglycerol close to Trp-87 with the side chain of the Trp located below the glycerol backbone of the lipid molecule (Fig. 1).

The change in relative free energy of binding per fatty acyl chain C atom is 0.1 kJ mol^{-1} (Fig. 9). The chain length

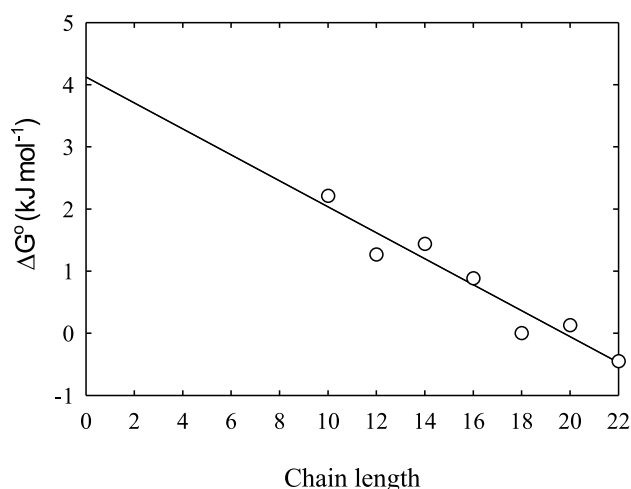


FIGURE 9 Relative free energy of lipid binding to KcsA. Free energy changes ΔG° were calculated from the lipid binding constants relative to di(C18:1)PC plotted in Fig. 7.

dependence of relative free energy of binding extrapolates to 4.1 kJ mol^{-1} at zero chain length. If this is equated with the loss of interaction energy of the fatty acyl chains with KcsA then the fatty acyl chain contribution to the di(C18:1)PC-KcsA interaction is more favorable than the fatty acyl chain contribution to the di(C18:1)PC-di(C18:1)PC interaction by $\sim 4.1 \text{ kJ mol}^{-1}$. This figure can be compared with the free energy cost of creating a void equivalent to a methyl group in the hydrophobic core of a soluble protein, which is $\sim 6.7 \text{ kJ mol}^{-1}$ (White and Wimley, 1999). Thus, a slightly larger fatty acyl chain contribution to the lipid-KcsA interaction than to the lipid-lipid interaction, with a gradual decrease in the free-energy of the lipid-KcsA interaction with decreasing chain length, would explain the results. The decreasing relative contribution of the chains to the lipid-KcsA interaction could represent the cost of distorting KcsA to achieve hydrophobic matching.

Distortion of KcsA required to achieve hydrophobic matching

The maximum thickness that can be achieved by a lipid bilayer is that in the gel phase with all-*trans* fatty acyl chains. The C-C separation in an all-*trans* chain measured along the long axis of the chain is 1.27 \AA , so that the thickness D_G of the hydrocarbon core of a gel phase bilayer is given by

$$D_G = 2.54(N_c - 1) \quad (8)$$

Thus, the maximum thickness for the hydrocarbon core of a bilayer of di(C10:0)PC is 23 \AA . The separation between the two layers of Trp residues in KcsA is $\sim 30 \text{ \AA}$ (Fig. 1) so that even with all-*trans* chains, the thickness of the hydrocarbon core of a bilayer of di(C10:0)PC would be insufficient to

cover all the Trp residues in an undistorted KcsA structure. Because the Trp residues in KcsA remain in a hydrophobic environment in di(C10:0)PC, KcsA must distort to match the thickness of the lipid bilayer.

It has been suggested that single transmembrane α -helices tilt in a lipid bilayer to match their hydrophobic lengths to the hydrophobic thickness of a lipid bilayer (Webb et al., 1998; Mall et al., 2000; Ren et al., 1997; Killian, 1998). It has also been suggested that aromatic residues at the ends of transmembrane α -helices could rotate relative to the long axis of the α -helix to adjust the effective hydrophobic length of the α -helix (Mall et al., 2000). We have characterized the changes in KcsA from the magnitude of the quenching of Trp fluorescence for KcsA reconstituted into brominated phospholipids. Quenching of Trp fluorescence by dibrominated quenchers fits to a Forster mechanism (Bolen and Holloway, 1990; Mall et al., 2001). The levels of fluorescence quenching observed with 0.83 M KI for KcsA reconstituted in di(C18:0)PC and di(Br₂C18:0)PC are very similar (Table 6). This shows that the 38% quenching of Trp fluorescence observed in di(Br₂C18:0)PC corresponds to a roughly equal degree of quenching of all Trp residues, consistent with a Forster energy transfer-type mechanism for quenching.

We have analyzed the quenching data for KcsA using the approaches of Dewey and Hammes (1980) and Koppel et al. (1979). As shown in Table 4, the separation distances between the planes of Trp and dibromo groups calculated using these two approaches differ by only $\sim 1 \text{ \AA}$. The measured values can be compared with estimates of the distance between the dibromogroups and the surface of the hydrocarbon core of the bilayer. The hydrophobic thickness of the bilayer D (\AA) is given by:

$$D = 1.75(N_c - 1) \quad (9)$$

in which N_c is the number of carbon atoms in the fatty acyl chains (Lewis and Engelman, 1983; Sperotto and Mouritsen, 1988). This equation gives a thickness for the hydrocarbon core of a bilayer of di(C18:1)PC of 30 \AA , close to the experimentally determined value of 32 \AA (Wiener and White, 1992). The position of the double bond in di(C18:1)PC has been shown to be 8.1 \AA from the surface of the hydrocarbon core of the bilayer (Wiener and White, 1992) and the position of the dibromo moiety in 1-oleoyl-2-dibromostearoylphosphatidylcholine has been shown to be the same as that of the double bond (Wiener et al., 1991). The distance d from the double bond to the surface of the hydrocarbon core of the bilayer can be estimated from a modified form of Eq. 9:

$$d = 0.875(n_c - 1) \quad (10)$$

in which n_c is the number of carbons between the double bond and the carbonyl group. For di(C18:1)PC Eq. 10 gives

a value for d of 7 Å (Table 2), 1.1 Å less than the experimentally determined value.

The separation h between the planes of the Trp and dibromo groups calculated using the fluorescence quenching method in di(Br₂C18:0)PC is ~5 Å greater than the separation between the dibromo groups and the surface of the hydrocarbon core (Table 4). The theories of Dewey and Hammes (1980) and Koppel et al. (1979) both treat the fluorescence acceptors as being distributed at random in a plane. However, KcsA will exclude lipids from a volume of the membrane corresponding to the volume occupied by the protein itself, and so will effectively “cast a shadow” on to the plane of the membrane, in which there will be no brominated lipid to take part in fluorescence energy transfer. The result will be to reduce the observed level of energy transfer, giving too large an estimate for the distance of separation h . Thus, the real distance of separation between the Trp residues and the dibromo groups is likely to be less than the estimated values. The sizes of the Trp and dibromo groups also become significant at the short distances being measured here; it is not obvious to what points in the Trp and dibromo groups the distance measurements correspond.

More useful than the absolute estimates of h are the calculated changes in h with changing chain length (Table 4). For the phospholipids of chain lengths C14 to C18, the dibromogroups are located at the same positions in the chains, at a constant distance from the surface of the hydrocarbon core. In contrast, the experimentally determined separation between the Trp residues and the dibromo groups increases by 2 to 3 Å from C14 to C18. This increase in separation is the opposite effect to that expected if the chains had to adapt their thickness to the hydrophobic thickness of KcsA; in that case, the C14 chain would have to be stretched or the C18 chain compressed, in which case the Trp-dibromo group separation would decrease with increasing chain length. The observed increase in separation from C14 to C18 suggests a small movement of the Trp residues away from the middle of the bilayer as the tilt of the helices decreases to match the thicker C18 bilayer.

The shift in the position of the double bond from the 9 position in di(C18:1)PC to the 15 position in di(C24:1)PC results in a 5-Å increase in the expected separation from the surface of the hydrocarbon core of the bilayer (Table 4). In this chain length range, the Trp-dibromo group separation only increases by ~2 Å (Table 4). Thus, it is possible that in this chain length range the Trp residues become more deeply buried within the hydrocarbon core of the bilayer as the bilayer thickens.

The transmembrane α -helices TM1 and TM2 in KcsA are organized as a pair of antiparallel coils in which each TM1 only contacts TM2 from its own subunit and the TM2 helices participate in subunit-subunit interactions (Doyle et al., 1998). The TM1/TM2 interface residues show a 3–4 heptad repeat, typical of a coiled-coil structure. The TM2 helices cross at an angle of approximately -40° . The rela-

tively steep packing angle shown by the TM2 helices means that the contact interface between the helices is localized to a fairly narrow region, making helix-helix rearrangements relatively easy. Indeed, it has been suggested that opening of the KcsA channel involves movement of the TM2 helices relative to the plane of the bilayer (Liu et al., 2001). The C-terminal ends of KcsA have been suggested to form a helical bundle on the intracellular side of KcsA (Cortes et al., 2001) that could restrict movement of the TM2 helices. However, the N-terminal region of KcsA has been suggested to be an interfacial α -helix pointing away from the core of the protein (Cortes et al., 2001) so that there will be relatively few constraints against movement of the TM1 helices. The level of quenching observed in brominated phospholipids is very similar at pH 4.0 and 7.2 (Table 4). Because the KcsA channel is in an open state at pH 4.0 (Liu et al., 2001), this shows that channel opening does not result in a significant movement of the Trp residues relative to the core of the lipid bilayer. Further, the observation that relative lipid binding constants are very similar at pH 4.0 and 7.2 (Table 7) also suggests that channel opening has only a small effect on the energetics of helix-helix and helix-lipid interactions in KcsA.

A simple geometrical calculation shows the required magnitude of tilting. The helices in the crystal structure of KcsA are tilted at $\sim 25^\circ$ with respect to the normal to the membrane. If it is assumed that the crystal structure corresponds to the structure in di(C22:1)PC then the length of the transmembrane α -helix required to span the bilayer of thickness 36.8 Å is 40.6 Å ($36.8/\sin 65^\circ$). The angle of tilt of the helix with respect to the bilayer normal will have to increase to 43° , 50° , and 56° to match the thicknesses of bilayers of di(C18:1)PC, di(C16:1)PC, and di(C14:1)PC, respectively. If the Trp residues are oriented perpendicular to the long axis of the helix (see Fig. 1) then, with a length for the Trp residue of ~ 10 Å, a rigid body tilt of an α -helix from 43° in di(C18:1)PC to 50° and 56° in di(C16:1)PC and di(C14:1)PC, respectively, will move the end of the Trp residue 0.7 and 1.5 Å closer to the bilayer center in di(C16:1)PC and di(C14:1)PC, respectively. These changes in distance can be compared with the changes observed experimentally (1.2 to 1.5 Å in di(C16:1)PC and 2.1 to 2.8 Å in di(C14:1)PC; Table 4). Thus, much of the change in position of the Trp residues as a function of chain length can be accounted for by rigid body tilting of the transmembrane α -helices.

Movement of its transmembrane α -helices might be expected to lead to a change in function for a membrane protein. The activities of a number of membrane proteins have indeed been shown to be dependent on the chain lengths of the surrounding phospholipids with highest activity being seen at a chain length of approximately C18 with lower activities for either shorter or longer chains (Pilot et al., 2001; Lee, 1998; Dumas et al., 2000). An optimal chain length of C22 for matching to KcsA is unexpected. Most biological membranes contain lipids with an

average chain length about C18. The fatty acyl chains of *Streptomyces* are unusual in being mostly branched-chain saturated C14, C15, and C16 iso-acids and C15 anteiso-acids (Verma and Khuller, 1983). The thicknesses of bilayers of branched-chain lipids appear not to have been determined and may be different to those of the normal unsaturated phospholipids. Alternatively, if the thickness of the lipid bilayer in *Streptomyces* is comparable with that in other organisms, then the tilt of the transmembrane α -helices for KcsA in the membrane may be different to those in the crystal structure.

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