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Effects of Lipid Structure on the State of Aggregation of Potassium Channel KcsA

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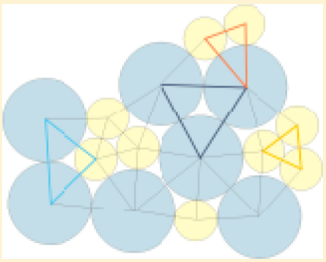
ABSTRACT: The state of aggregation of potassium channel KcsA was determined as a function of lipid:protein molar ratio in bilayer membranes of the zwitterionic lipid phosphatidylcholine (PC) and of the anionic lipid phosphatidylglycerol (PG). EPR (electron paramagnetic resonance) with spin-labeled phospholipids was used to determine the number of motionally restricted lipids per KcsA tetramer. Unexpectedly, this number decreased with a decreasing lipid:KcsA tetramer molar ratio in the range of 88:1 to 30:1, consistent with sharing of annular lipid shells and KcsA−KcsA contact at high mole fractions of protein. Fluorescence quenching experiments with brominated phospholipids showed a decrease in fluorescence quenching at low lipid:KcsA tetramer mole ratios, also consistent with KcsA−KcsA contact at high mole fractions of protein. The effects of low mole ratios of lipid seen in EPR and fluorescence quenching experiments were more marked in bilayers of PC than in bilayers of PG, suggesting stronger association of PG than PC with KcsA. This was confirmed by direct measurement of lipid association constants using spin-labeled phospholipids, showing higher association constants for all anionic lipids than for PC. The results show that the probability of contacts between KcsA tetramers will be very low at lipid:protein molar ratios that are typical of native biological membranes.



The biological membrane is a very crowded environment. Dupuy and Engelman have estimated that ∼23% of the area in the middle of the membrane of a red blood cell is occupied by protein.[1] How membrane proteins interact in this crowded environment will obviously depend on their shape. Proteins with large domains extending beyond the lipid bilayer will interact with other proteins in the membrane through these extrabilayer domains, with little contact between their membrane-spanning domains. In contrast, proteins with small extrabilayer domains may well be able to interact through their membrane-spanning domains. In some cases, such interactions could be important for the proper function of a membrane protein, allowing the formation of homo-oligomers or of multiprotein complexes. However, interaction within the membrane between the membrane-spanning domains of unrelated proteins could be detrimental to function. The question about the likelihood of such interactions at the concentrations of membrane proteins found in a typical biological membrane, therefore, arises.

As well as the dependence on overall protein shape, the likelihood of membrane-spanning domains interacting will depend on the concentration of proteins in the membrane, on how well the surfaces of the membrane-spanning domains pack together, and on the strength of lipid interactions with the membrane-spanning surfaces, because interactions between membrane-spanning domains will necessarily reduce the extent of the protein surface available to interact with the lipid bilayer. The free energy of association of two membrane-spanning domains, ΔG_A, can be written as[2]

$$\Delta G_A = \Delta G_{PP} + (n/2)\Delta G_{LL} - n\Delta G_{PL} \quad (1)$$

where ΔG_{PP}, ΔG_{LL}, and ΔG_{PL} are the free energies of protein−protein, lipid−lipid, and protein−lipid interactions, respectively, and it is assumed that formation of a protein−protein pair results in displacement of n lipid molecules from around the two proteins.

Protein−protein interaction could be driven by a favorable value of ΔG_{PP} resulting, for example, from good packing at the protein−protein interface. An unfavorable value of ΔG_{PL} would also favor protein−protein interaction and could arise from poor packing of the lipid fatty acyl chains with the rough surface of a membrane protein or weak interactions of the polar headgroups of the lipid with the protein.

Here we use EPR and fluorescence quenching methods to study aggregation of potassium channel KcsA in lipid bilayers as a function of lipid:protein molar ratio and as a function of lipid headgroup. The functional unit of KcsA is a tetramer, with each monomer contributing two transmembrane α-helices.[3] The membrane-spanning domain is roughly cylindrical, with a small extracellular domain and a long, narrow C-terminal domain on the intracellular side that extends away from the membrane[4] and would not interfere with packing of the membrane-spanning domain. The short N-terminal domain has not been resolved in crystal structures but is believed to be flexible and located close to the lipid−water interface.[5]

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EXPERIMENTAL PROCEDURES

Dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylglycerol (DOPG), and lissamine rhodamine B-labeled dioleoylphosphatidylethanolamine (rPE) were obtained from Avanti Polar Lipids (Alabaster, AL). DOPC and DOPG were brominated as described by East and Lee[6] to give di(9,10-dibromostearoyl)phosphatidylcholine (BrPC) and di(9,10-dibromostearoyl)phosphatidylglycerol (BrPG), respectively. The spin-labeled phospholipids 1-acyl-2-[14-(4,4-dimethyloxazolidinyl-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphocholine (14-PCSL), 1-acyl-2-[14-(4,4-dimethyloxazolidinyl-*N*-oxyl)-stearoyl]-*sn*-glycero-3-phosphoglycerol (14-PGSL), 1-acyl-2-[14-(4,4-dimethyloxazolidinyl-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphoserine (14-PSSL), and 1-acyl-2-[14-(4,4-dimethyloxazolidinyl-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphoric acid (14-PASL) were synthesized as described by Marsh.[7]

KcsA was purified as described by Marius et al.[8] For fluorescence experiments, we reconstituted KcsA into lipid bilayers by mixing lipid and KcsA in cholate followed by dilution into buffer [20 mM Hepes and 100 mM KCl (pH 7.2)] to decrease the concentration of cholate below its critical micelle concentration and re-form membranes.[8] For EPR measurements, samples were reconstituted in a similar way, followed by dialysis to remove detergent and pelleting in a benchtop centrifuge.

Gradient Centrifugation. Discontinuous sucrose gradient centrifugation was used to characterize the reconstituted KcsA samples. KcsA was reconstituted with DOPC at DOPC:KcsA tetramer molar ratios of 30:1 and 100:1, the lipid containing rPE at concentrations of 3 and 1.5 mol % respectively. Samples (1.5 mL) were loaded onto sucrose gradients containing the following solutions of sucrose in 20 mM Hepes and 100 mM KCl (pH 7.2): 70, 60, 50, 40, 30, and 2.5 (w/w). Samples were spun at 104000*g* for 18 h at 4 °C, and then 1.5 mL fractions were collected from the gradients, diluted into 1% SDS to avoid light scattering, and then analyzed for lipid and protein by the absorbance at 573 and 280 nm, respectively; the absorbance at 280 nm was corrected for the absorbance of rPE at 280 nm using an OD_{573}/OD_{280} ratio of 5.54 for rPE.

EPR and Fluorescence Measurements. EPR spectra were recorded on a 9 GHz Varian Century-line EPR spectrometer. Spin-labeled phospholipids were incorporated into reconstituted KcsA samples at approximately 1 mol % with respect to total lipid by addition of a concentrated solution of spin-labeled lipid in ethanol to the sample. Following centrifugation to separate any unincorporated spin-label, samples were transferred to 50 μL glass capillaries and placed in a standard 4 mm quartz sample tube. EPR spectra were analyzed by spectral addition or subtraction, as described previously.[7,9] Reference libraries for the fluid and motionally restricted components of the composite EPR spectra were obtained from 14-PCSL in DOPC and in sonicated vesicles of dimyristoylphosphatidylcholine, respectively, recorded over the temperature range of 0−37 °C.

Fluorescence was recorded on a model 8000C fluorimeter (SLM, Urbana, IL) with excitation at 290 nm, at 25 °C. Fluorescence emission intensities were measured at 333 nm and corrected for light scatter by subtracting a blank consisting of lipid alone in buffer. The reported fluorescence intensities represent averages of triplicate measurements from two or three separate reconstitutions.

RESULTS

Homogeneity of Samples. Sucrose gradient centrifugation was used to confirm homogeneous mixing of lipid and protein in the KcsA reconstitutions. As shown in Figure 1,

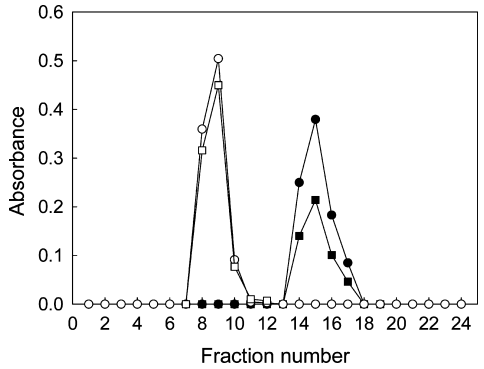

Figure 1. Sucrose gradient analysis of reconstituted KcsA samples. Samples of KcsA reconstituted with DOPC, doped with rPE, at a lipid:KcsA tetramer molar ratio of 100:1 (○ and □) or 30:1 (● and ■) were separated on discontinuous sucrose gradients from 2.5 to 70% sucrose; 1.5 mL fractions were taken and absorbances measured for rPE at 573 nm (□ and ■) and KcsA at 280 nm (○ and ●). Absorbances at 280 nm were corrected for the absorbance of rPE at 280 nm as described in the text.

when KcsA was reconstituted with DOPC at a lipid:KcsA tetramer molar ratio of 100:1 or 30:1, KcsA and lipid colocalize toward the center of the gradients, at the positions expected from their calculated densities. The lipid:KcsA tetramer molar ratios for the reconstituted samples, calculated from the measured absorbances, were 92 ± 2.7 and 26.5 ± 0.6 for the 100:1 and 30:1 samples, respectively.

Effect of KcsA Content on EPR Spectra. Figure 2 shows the EPR spectra of spin-labeled phosphatidylcholine, 14-PCSL, in bilayers of DOPC containing KcsA at lipid:KcsA tetramer molar ratios between 100:1 and 30:1. The spectra all consist of two components, a broad component that is characteristic of lipids motionally restricted at the membrane-spanning surface of the KcsA channel and a sharper component characteristic of lipids in fluid bilayer regions of the membrane.[7] Spectral subtractions and additions were used to confirm that the spectra were indeed composed of two components and to determine the relative proportions of the two spectral components.[7,9] From the fraction f of motionally restricted lipid and the molar ratio of total lipid per KcsA tetramer, N_t, the number of lipid molecules directly associated with each tetramer, N_b, can be calculated as[7]

$$N_b = fN_t \quad (2)$$

because spin-labeled phosphatidylcholine shows no selectivity relative to unlabeled phosphatidylcholine.[10−14]

Figure 3 shows a plot of N_b against the DOPC:KcsA tetramer molar ratio. The numbers of DOPC molecules contacting the protein, 32 ± 4, were the same at DOPC:KcsA tetramer molar ratios of 88:1 and 100:1, but unexpectedly, this number decreased with DOPC:KcsA tetramer molar ratios of <88:1 (Figure 3). For a very wide range of other membrane proteins studied using EPR, the number of protein-interacting lipid molecules has been found to remain constant over a wide range of lipid:protein molar ratios.[7,15−18]

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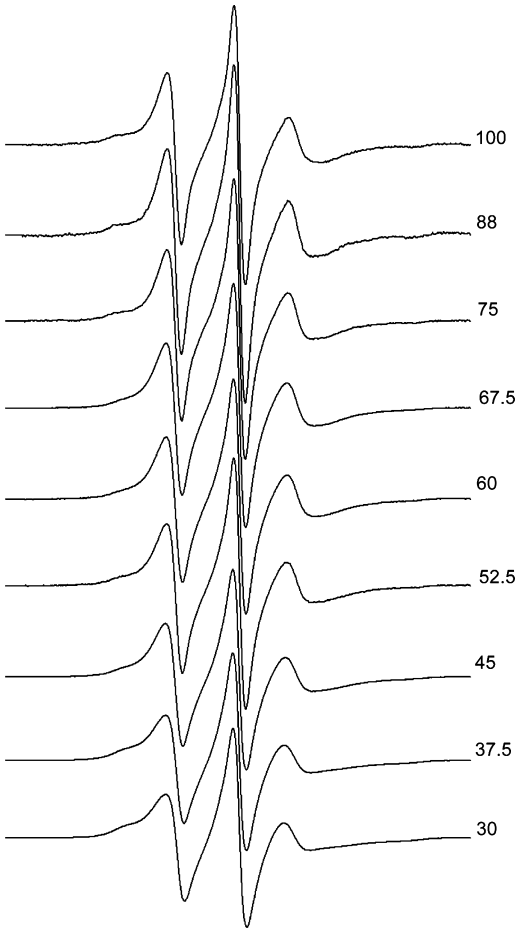

Figure 2. EPR spectra of 14-PCSL in DOPC membranes containing KcsA, at the indicated DOPC:KcsA tetramer molar ratios. Spectra were recorded at 25 °C and normalized to equal double-integrated areas. The total scan width was 100 G.

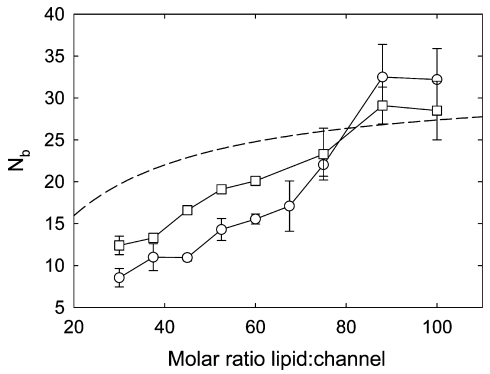

Figure 3. Dependence of the number of protein-associated lipid molecules per KcsA tetramer, N_b, according to eq 2, on the lipid:KcsA tetramer molar ratio, in DOPC (○) and DOPG (□). The dashed line is the prediction for random protein−protein contacts based on eq 5, with an N_1 of 32 mol/mol.

A similar behavior was observed for KcsA in DOPG (Figure 4). Again, the lipid stoichiometry was approximately the same at DOPG:KcsA tetramer molar ratios of 100:1 and 88:1 but then decreased with a decreasing DOPG:KcsA tetramer molar ratio (Figure 3). For DOPG, the decrease in the number of motionally restricted lipids with a decreasing molar ratio of lipid was less steep than that observed in bilayers of DOPC,

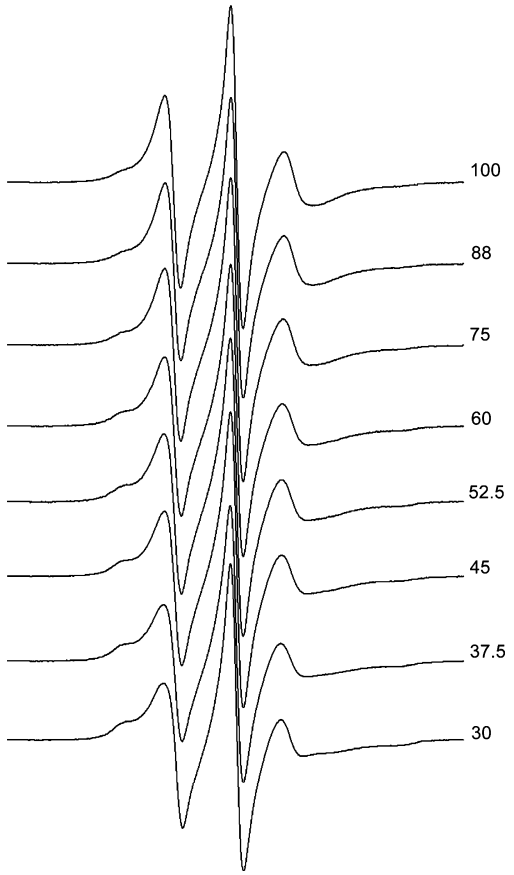

Figure 4. EPR spectra of 14-PGSL in DOPG membranes containing KcsA, at the indicated DOPG:KcsA tetramer molar ratios. Spectra were recorded at 25 °C and normalized to equal double-integrated areas. The total scan width was 100 G.

suggesting that DOPG interacts with KcsA more strongly than does DOPC (Figure 3).

Relative Association Constants for Anionic Lipids. Relative lipid association constants for a membrane protein can be obtained by analysis of EPR spectra from probe amounts of a spin-labeled lipid of one species in bilayers of another species of lipid that contain the membrane protein of interest.[7] EPR spectra of spin-labeled anionic lipids with DOPC as the host lipid at a lipid:KcsA tetramer molar ratio of 60:1 are shown in Figure 5. The fractional populations, f, of the motionally restricted lipid determined from these spectra are listed in Table 1. Comparing the values for f with the fraction f_o of motionally restricted lipid for spin-labeled PC in DOPC gives the relative association constant, K_r, for the anionic lipids, relative to that for the spin-labeled PC, K_r^{PC}:[7,19]

$$\frac{K_r}{K_r^{PC}} = \frac{f(1-f_o)}{f_o(1-f)} \quad (3)$$

Because spin-labeled PC shows no selectivity relative to the unlabeled host PC lipid (see above), i.e., $K_r^{PC} \approx 1$, eq 3 directly gives the association constant for the anionic lipid, K_r, relative to the host DOPC lipid. Note that eq 3 holds quite generally, independent of the particular lipid:protein ratio and lipid stoichiometry.[19] The relative association constant that is determined from the EPR spectra of spin-labeled lipid probes, at low concentration in the host lipid, is an average over all N_b accessible lipid sites on the protein:[20]

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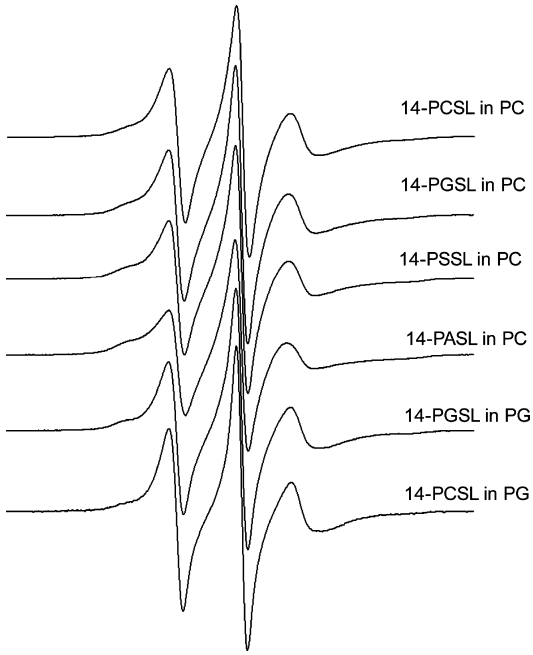

Figure 5. EPR spectra of 14-PCSL or the given spin-labeled anionic lipid in DOPC membranes containing KcsA (top four spectra) and of 14-PGSL or 14-PCSL in DOPG membranes containing KcsA (bottom two spectra). The lipid:KcsA tetramer molar ratio was 60:1. Spectra were recorded at 30 °C and normalized to equal double-integrated areas. The total scan width was 100 G.

Table 1. Relative Lipid Association Constants[a]

spin-labeled lipid	fraction of motionally restricted lipid (f)	ratio of relative association constants (K_r/K_r^{PC} or K_r/K_r^{PG})	relative association constant from fluorescence[b] (K_r)
DOPC as the host lipid			
14-PCSL	0.29 ± 0.07	1	1
14-PGSL	0.42 ± 0.03	1.76 ± 0.24	1.66 ± 0.20
14-PSSL	0.52 ± 0.01	2.69 ± 0.14	2.16 ± 0.17
14-PASL	0.60 ± 0.02	3.65 ± 0.41	1.95 ± 0.22
DOPG as the host lipid			
14-PGSL	0.36 ± 0.07	1	
14-PCSL	0.26 ± 0.07	0.62 ± 0.26	

[a]The fractional populations of the motionally restricted lipid were determined from the EPR spectra shown in Figure 5, and relative association constants were calculated using eq 3, for DOPC as the host lipid, or using the equivalent of eq 3 for 14-PGSL, for DOPG as the host lipid. [b]Association constants of brominated lipids, relative to DOPC, obtained using fluorescence quenching, taken from ref 36.

$$K_r = \frac{1}{N_b}\sum_i n_i K_i \quad (4)$$

where n_i is the number of lipid sites with relative association constant K_i and the summation is over groups of sites with the same affinity for lipids.

In all cases, the fractions of the motionally restricted anionic phospholipid are greater than that found with spin-labeled PC, showing that the relative binding constants for all the anionic lipids were greater than 1 (Table 1). A similar experiment was performed with DOPG as the host lipid (Figure 5), and as shown in Table 1, the association constant determined for 14-PCSL relative to 14-PGSL with DOPG as the host lipid (0.62 ± 0.26) is close to the reciprocal of the value determined for 14-PGSL relative to 14-PCSL with DOPC as the host lipid (1/1.76 = 0.57), as expected for simple competitive association of PC and PG at the lipid sites on KcsA.

Fluorescence Quenching Studies of Interactions between KcsA Tetramers. Fluorescence quenching by phospholipids containing brominated fatty acyl chains has been used to probe lipid–protein interactions in a number of systems.[6,21−23] Quenching of Trp fluorescence by brominated phospholipids is short-range so that only a brominated phospholipid bound close to a Trp residue in a membrane protein can quench its fluorescence.[23] Because the time for two lipid molecules to exchange between the bulk lipid phase and the annular shell of lipids around a membrane protein is greater than the fluorescence lifetime,[9,24] the level of fluorescence quenching observed in a bilayer of a brominated lipid will depend on the extent of coverage of the membrane-spanning surface of the protein by lipid molecules. Interaction between KcsA tetramers, by reducing the surface area available for lipid binding, would be expected to result in a decrease in the level of quenching by brominated lipids. Fluorescence intensities for KcsA as a function of lipid:protein ratio in brominated and nonbrominated lipid bilayers are shown in Figure 6. In bilayers of DOPC or DOPG, the fluorescence intensity decreases with decreasing lipid:KcsA tetramer molar ratios of <100:1. This could reflect a change in the Trp environment as a result of aggregation or could simply be an example of the concentration quenching observed with many fluorescent molecules including indoles (the fluorophore in tryptophan) that arises from the transfer of energy to nonfluorescent dimers.[25,26] In contrast to the results with nonbrominated lipids, with BrPC and BrPG fluorescence is low at high lipid:protein molar ratios but then increases at low molar ratios, as expected if the number of brominated lipids on the protein surface decreases as a result of protein–protein interaction (Figure 6). The decrease in the level of fluorescence quenching is observed in bilayers of BrPC below a BrPC:KcsA tetramer molar ratio of ∼100:1, whereas the decrease in the level of fluorescence quenching in bilayers of BrPG occurs only below a BrPG:KcsA tetramer molar ratio of ∼50:1, again consistent with stronger binding of BrPG than BrPC to KcsA.

■ DISCUSSION

This paper addresses questions about the probability of contact between the transmembrane domains of proteins in the crowded environment of a biological membrane. Rough estimates can be made for the mole fraction of membrane protein in a typical biological membrane. The red blood cell membrane contains approximately equal amounts by weight of lipid and protein.[1] If we assume average molecular weights of 750 and 50000 for lipids and membrane proteins, respectively, this corresponds to an average lipid:protein molar ratio of 66:1. In skeletal muscle sarcoplasmic reticulum, a membrane containing largely one membrane protein, the Ca^{2+}-ATPase, the lipid:protein molar ratio is ∼100:1.[27] The important question, therefore, is what is the probability of contacts between proteins in a membrane with these kinds of lipid:protein molar ratios.

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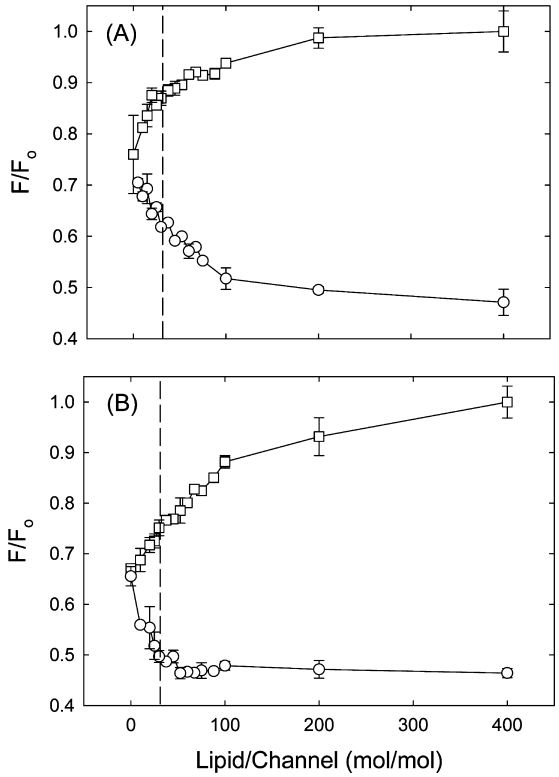

Figure 6. Effect of the lipid:KcsA tetramer molar ratio on Trp fluorescence intensity. Fluorescence intensities F/F_o are plotted as a function of the phospholipid:KcsA tetramer molar ratio: (A) in DOPC (□) or BrPC (○) and (B) in DOPG (□) or BrPG (○). F_o is the fluorescence intensity measured at a lipid:KcsA tetramer molar ratio of 400:1 in DOPC or DOPG, and F is the fluorescence intensity measured at the given lipid:KcsA tetramer molar ratio. The dashed vertical line corresponds to a lipid:KcsA tetramer molar ratio of 33:1.

Contact between membrane-spanning domains will not be possible for proteins with large domains extending out of the lipid bilayer that occupy surface areas greater than those of the transmembrane cross section; the presence of a large extrabilayer domain will ensure that the lipid-exposed surface of a membrane protein is covered by a full shell of lipid molecules, even at low lipid:protein molar ratios. This has been observed by EPR spectroscopy with spin-labeled lipids for a wide range of membrane proteins, where the number of motionally restricted lipids remains constant until very low lipid:protein molar ratios are reached,[28−35] but the results obtained with KcsA are very different (Figure 3). At high lipid:protein molar ratios, the average number of motionally restricted lipid molecules is 30.5 ± 2.0 per KcsA tetramer in DOPC or DOPG, consistent with the crystal structure for the KcsA tetramer that shows a hydrophobic surface that would accommodate ∼33 lipid molecules.[15] However, at lower molar ratios, the number of motionally restricted lipid molecules decreases markedly with a decreasing lipid:protein ratio. The decrease in the number of motionally restricted lipids could follow from contact between the transmembrane domains of neighboring KcsA tetramers at high concentrations of KcsA in the membrane, and from sharing of annular shells of lipid molecules between adjacent KcsA tetramers. However, sharing of annular lipid shells could reduce the number of motionally restricted lipids to only ∼15 per KcsA tetramer, but the number of motionally restricted lipid molecules falls below this, showing that direct protein−protein contacts must also be present.

These conclusions are consistent with the results of fluorescence quenching studies with brominated phospholipids (Figure 6). A reduction in the number of brominated lipid molecules on the protein surface will result in an increase in Trp fluorescence intensity, as is observed. In this case, it is likely that sharing of annular shells of lipid molecules will have relatively little effect on the level of fluorescence quenching, so that the observed recovery of fluorescence intensity is more likely to indicate protein−protein contact.

As described by eq 1, the probability of protein−protein contact will depend on the relative strengths of lipid−protein and protein−protein interactions and so could vary with lipid structure. We determined binding constants for a series of anionic phospholipids from EPR spectra of spin-labeled lipids in the presence of KcsA (Table 1). Association constants for phosphatidylglycerol and phosphatidylserine agree well with those obtained previously by fluorescence quenching methods,[36] but the value obtained for phosphatidic acid by EPR analysis is significantly greater than that obtained by fluorescence methods (Table 1). Fluorescence quenching studies with native KcsA give an association constant averaged over all the lipid binding sites on KcsA close enough to the five Trp residues per KcsA monomer to result in quenching when the sites are occupied by brominated lipid molecules. In contrast, an association constant determined from EPR studies, where the mole fraction of spin-labeled lipid in the membrane is low, will be weighted toward any available site of high affinity (see eq 4). Fluorescence quenching studies with Trp mutants of KcsA suggested the presence of a site of high affinity for phosphatidic acid (binding constant relative to DOPC of 2.93 ± 0.77) on the intracellular side of the membrane,[37] and the average K_r that is determined by spin-label EPR should definitely include any such site; the EPR studies could also be detecting association with the lipid-binding site present at protein−protein interfaces in the KcsA tetramer.[8,38] However, the important observation is that DOPG and other anionic lipids associate more strongly with KcsA than does DOPC, explaining the observation that aggregation of KcsA is first seen at lower mole fractions of DOPG than of DOPC.

These observations can be compared with the expectations for simple random mixing of lipids and proteins in a membrane. Lipids and proteins are treated as disks with a ratio of protein to lipid radii of 4:1, corresponding to a maximal number of annular lipids around each protein of ∼32, as expected for the KcsA tetramer. It has been shown that this problem can be treated as a simple mixture of triangles of four types, LLL, PLL, PPL, and PPP, where L and P represent lipid and protein, respectively.[9] Calculations show that the probability of protein−protein contacts, given by the frequencies of LPP and PPP triangles, is very low at lipid:protein molar ratios of greater than ∼30:1 simply because the much larger number of lipid molecules than of protein molecules makes the probability of lipid−protein contacts much higher than the probability of protein−protein contacts.[9] Comparing the results expected for random mixing with the fluorescence quenching results (Figure 6) suggests that mixing of KcsA and DOPG is close to random but that in DOPC protein−protein contacts are more frequent than expected for random mixing because of weaker association of DOPC than DOPG with KcsA.

Estimates for the effect of random protein−protein contacts on the stoichiometry of lipid−protein interaction that is

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determined from spin-label EPR can be made using a simple lattice theory:[39,40]

$$N_b = N_t\left[1 - \exp\left(-\frac{N_1}{N_t}\right)\right] \quad (5)$$

where N_1 is the value of N_b for the first lipid shell, in the absence of protein−protein contacts. The predictions of eq 5 for an N_1 value of 32 lipids/tetramer are given by the dashed line in Figure 3. It is seen that measurements for KcsA in DOPG correspond more closely to the predictions for random contacts than do those in DOPC. Even in DOPG, the experimental values decrease more rapidly than does the random prediction, particularly at low lipid:protein ratio. The latter may be attributed in part to limitations of the lattice theory.[39]

It is not possible to draw any detailed conclusions from the results presented here about the structures of any aggregates adopted by KcsA tetramers at high protein concentrations in a membrane. The observed decrease in Trp fluorescence intensity with an increasing protein content (Figure 6) could simply be the result of the concentration quenching observed at high concentrations of indoles and many other fluorescent molecules.[25,26] It could, however, also reflect a relocation of Trp residues on KcsA relative to the lipid−water interface or some more major change in the structure of the KcsA tetramer. A further complication is that the reconstitution method used here probably results in a random orientation of the KcsA tetramers within the membrane, and at high protein concentrations, interactions could then occur between tetramers oriented with the N-terminus facing out and tetramers oriented with the N-terminus facing in. Finally, coarse-grained molecular dynamics simulations have suggested that protein−protein interactions within a membrane are likely to result in aggregates with a distinct shape, such as an elongated linear cluster.[41] However, the most significant result from both spin-label and fluorescence measurements is that, even in bilayers of DOPC, the probability of protein−protein contact is very low at lipid:KcsA molar ratios of ∼100:1 (Figures 3 and 6), typical of the molar ratios found in biological membranes.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

DOPC, dioleoylphosphatidylcholine; DOPG, dioleoylphosphatidylglycerol; BrPC, di(9,10-dibromostearoyl)-phosphatidylcholine; BrPG, di(9,10-dibromostearoyl)-phosphatidylglycerol; 14-PCSL, 1-acyl-2-[14-(4,4-dimethyloxazolidinyl-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphocholine; 14-PGSL, 1-acyl-2-[14-(4,4-dimethyloxazolidinyl-*N*-oxyl)-stearoyl]-*sn*-glycero-3-phosphoglycerol; 14-PSSL, 1-acyl-2-[14-(4,4-dimethyloxazolidinyl-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphoserine; 14-PASL, 1-acyl-2-[14-(4,4-dimethyloxazolidinyl-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphoric acid; rPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl); EPR, electron paramagnetic resonance.

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