Integration of a K^+ Channel-Associated Peptide in a Lipid Bilayer: Conformation, Lipid−Protein Interactions, and Rotational Diffusion

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ABSTRACT: The 26-residue peptide of sequence KEALYILMVLGFFGFFTLGIMLSYIR, which contains the single putative transmembrane domain of a small protein that is associated with slow voltage-gated K^+ channels, has been incorporated in bilayers of dimyristoylphosphatidylcholine by dialysis from 2-chloroethanol to form complexes of homogeneous lipid/peptide ratio. Fourier transform infrared spectroscopy indicates that the peptide is integrated in the lipid bilayer wholly in a β-sheet conformation. The electron spin resonance spectra of spin-labeled lipids in the lipid/peptide complexes contain a component corresponding to lipids whose chains are motionally restricted in a manner similar to those of lipids at the hydrophobic surface of integral transmembrane proteins. From the dependence of the lipid spin label spectra on the lipid/peptide ratio of the complexes, it is found that ca. 2.5 lipids per peptide monomer, independent of the species of spin-labeled lipid, are motionally restricted by direct interaction with the peptide in the bilayer. This value would be consistent with, e.g., a β-barrel structure for the peptide in which the β-strands either are strongly tilted or have a reverse turn at their center. A preferential selectivity of interaction with the peptide is observed for the negatively charged spin-labeled lipids phosphatidic acid, stearic acid, and phosphatidylserine, which indicates close proximity of the positively charged residues at the peptide termini to the lipid headgroups. The saturation-transfer electron spin resonance spectra of the peptide spin-labeled at a cysteine residue replacing Leu18 evidence rather slow rotational diffusion in the lipid complexes. This indicates that the presumably enclosed β-sheet units of the peptide are aggregated in oligomeric assemblies in the lipid bilayer. The results suggest a way in which one type of channel unit may be integrated in the membrane.

Ion channels are generally large, often multimeric, proteins containing many transmembrane segments (Stephenson, 1991). In spite of the molecular complexity, it is likely that the channel itself is lined with a limited range of, possibly homologous, transmembrane segments. Correlating with this, it is found that certain relatively short synthetic peptides, of sequences resembling those of putative pore-lining segments, are able to sustain channel activity when incorporated in lipid bilayers (Montal, 1990; Ben-Efraim et al., 1993). Such synthetic peptides are therefore suitable models for studying particular aspects of channel structure and assembly by using biophysical or biochemical techniques. The direct relevance to native channels depends, of course, on the correct identification of the pore-lining transmembrane segments.

In addition to the complex channel proteins mentioned above, the DNA clone encoding a small protein associated with a slowly activating voltage-gated potassium channel has been identified in rat kidney (Takumi et al., 1988). This protein, I_{sK}, contains only 130 amino acids with a single putative 23-residue transmembrane domain. A clone encoding a similar I_{sK} protein has also been identified in human, and the amino acid sequence was found to have a high degree of homology with that of the rat protein, particularly in the conserved transmembrane domain (Murai et al., 1989). The I_{sK} protein from neonatal mouse heart is >90% identical with the rat protein and has been shown to be associated with a delayed rectifier K^+ channel with characteristics suggesting it to be involved in control of the cardiac action potential and beating rate (Honoré et al., 1991). In view of the relatively simple composition of the I_{sK} protein, it is of considerable interest to study the behavior of the single transmembrane sequence incorporated in lipid bilayers.

In the present work, a 26-residue peptide (K26)[1] with sequence KEALYILMVLGFFGFFTLGIMLSYIR, which is a direct analogue of the conserved putative transmembrane domain of I_{sK}, and a variant with the single residue

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[1] Abbreviations: K26, peptide of sequence KEALYILMVLGFFGFFTLGIMLSYIR; K26(L18C), peptide of sequence KEALYILMVLGFFGFFTCGIMLSYIR; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; 14-SASL, 14-(*N*-oxy-4,4-dimethyloxazolidin-2-yl)stearic acid; 14-PCSL, -PGSL, -PSSL, and -PASL, 1-acyl-2-[14-(*N*-oxy-4,4-dimethyloxazolidin-2-yl)stearoyl]-*sn*-glycero-3-phosphocholine, -phosphoglycerol, -phosphoserine, and -phosphoric acid; 6-MSL, 4-maleimido-2,2,6,6-tetramethylpiperidineoxyl; 5-InVSL, 2-[(1-oxy-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)methenyl]indan-1,3-dione; EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; ESR, electron spin resonance; STESR, saturation-transfer ESR; V_1, first harmonic ESR absorption signal detected in-phase with respect to the field modulation; V_2', second harmonic absorption ESR signal detected 90° out-of-phase with respect to the field modulation; FTIR, Fourier transform infrared.

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replacement L18C have been synthesized and incorporated in dimyristoylphosphatidylcholine bilayers by dialysis from 2-chloroethanol. The conformation, lipid interactions, and rotational diffusion of the peptide in lipid membranes have been studied by Fourier transform infrared (FTIR) spectroscopy and by electron spin resonance spectroscopy of both spin-labeled lipids and spin labels covalently attached to the peptide.

The characterization of the secondary structure is important, because molecular models that have been suggested for other potassium channels contain pore-lining segments in β-sheet conformations, rather than the more familiar transmembrane α-helices proposed for many membrane proteins (Guy & Conti, 1990; Bogusz et al., 1992). Study of the lipid−peptide interactions by conventional spin label ESR spectroscopy gives an estimate of the number of lipids in direct contact with the peptide assembly, which in turn is determined both by the conformation of the peptide and by its mode of incorporation in the bilayer (Marsh, 1993). Additionally, the selectivity of interaction of the peptide with different lipid species can give information on the proximity of charged residues in the peptide to the lipid polar headgroups (Marsh, 1985). Finally, determination of the rotational diffusion rates of the spin-labeled peptide by saturation-transfer ESR spectroscopy allows estimation of the size of the peptide assembly in the bilayer (Marsh & Horváth, 1989). Together, these measurements are therefore able to delineate several important features of the integration of the channel-associated peptide in a lipid membrane.

MATERIALS AND METHODS

Materials. Dimyristoylphosphatidylcholine (DMPC) was obtained from Fluka (Buchs, Switzerland). Spin-labeled stearic acid (14-SASL) was synthesized according to Hubbell and McConnell (1971). The spin-labeled phospholipids 14-PCSL, -PGSL, -PSSL, and -PASL were synthesized from 14-SASL as described in Marsh and Watts (1982). The indandione vinyl nitroxide derivative, 5-InVSL, used for covalent spin labeling of the K26(L18C) peptide was synthesized according to Hankovszky et al. (1989). The maleimide spin label, 6-MSL, was obtained from the Institut Jozef Stefan (Ljubljana, Slovenia).

Peptide Synthesis. Peptides K26 and K26(L18C) were synthesized on an automated Milligen/Biosearch 9050 solid-phase synthesizer. Fmoc chemistry was used for amino acid coupling. Peptides were cleaved from the resin for 12 h using a cleavage mixture of 95% trifluoroacetic acid, 2.5% phenol, and 2.5% ethanedithiol. After precipitation and washing thoroughly in diethyl ether, the peptides were dried under vacuum, dissolved in 100% trifluoroethanol (TFA), diluted in water to 10% (v/v) TFA, and lyophilized. Further purification of the peptides by reverse-phase HPLC was not possible, because of their extreme hydrophobicity. Amino acid sequence analysis of the peptides indicated >80% purity (in relation to deletion peptides).

Preparation of Lipid/Peptide Complexes. The required amounts of peptide and DMPC were codissolved in freshly distilled 2-chloroethanol (4 mL) and dialyzed against 2 L of 0.1 M NaCl, 2 mM Hepes, and 1 mM EDTA, pH 7.4, over a period of 24 h with three changes of the buffer. The lipid complexes were resolved from free lipid by centrifuging in buffer containing 1 M NaCl and discarding the supernatant. The lipid/peptide complexes recovered as the pellets were found to sediment to a tight, single band on continuous sucrose density gradient (10−55% sucrose) centrifugation, with very little free lipid remaining. Lipid/peptide ratios of the complexes were determined by phosphate (Eibl & Lands, 1969) and protein (Lowry et al., 1951) assays.

Spin Labeling. The dialyzed lipid/peptide complexes containing ca. 2 mg of DMPC were doped with spin-labeled lipid by adding 5 μg of spin label in concentrated ethanol solution to dispersions of the complexes in 2 mM Hepes and 1 mM EDTA, pH 8.3. Unincorporated spin label was then resolved by centrifugation and washing. The K26-(L18C) peptide was labeled with the 5-InVSL spin label (Esmann et al., 1990), or with the 6-MSL spin label, either in 2-chloroethanol or in lipid complexes in buffer, by reacting 500 μg of peptide with up to 1:1 mole ratio of spin label for 45 min at room temperature. For the peptide spin-labeled in 2-chloroethanol, lipid complexes of the peptide with DMPC were then prepared as described above. All spin-labeled lipid/peptide complexes were suspended in 2 mM Hepes and 1 mM EDTA, pH 8.3, for ESR measurement.

ESR Spectroscopy. ESR spectra were recorded on a Varian Century Line Series 9-GHz spectrometer equipped with a nitrogen gas flow temperature regulation system. Spin-labeled lipid/peptide complexes were packed in 1-mm i.d. capillaries to a height of 5 mm to minimize the effects of microwave and modulation field inhomogeneities (Fajer & Marsh, 1982; Hemminga et al., 1984). The sample capillaries were accommodated within standard 4-mm quartz ESR tubes containing light silicone oil for thermal stability. Temperature was measured by a fine-wire thermocouple located at the top of the microwave cavity within the silicone oil. Conventional, in-phase ESR spectra (V_1 display) were recorded at a modulation amplitude of 1.6 G p-p and a modulation frequency of 100 kHz. Saturation-transfer, quadrature-phase ESR spectra (V_2' display) were recorded at a modulation amplitude of 5 G p-p and a modulation frequency of 50 kHz with second-harmonic detection. The microwave power was adjusted so as to give an average microwave field at the sample of $\langle H_1^2\rangle^{1/2} = 0.25$ G for recording the STESR spectra (Fajer & Marsh, 1982; Hemminga et al., 1984). Calibrations of the effective rotational correlation times in terms of the STESR spectra of spin-labeled hemoglobin in isotropic solution were taken from Horváth and Marsh (1988). Spectral subtractions with the conventional ESR spectra were performed as described in Marsh (1982).

FTIR Spectroscopy. Infrared spectra were recorded on a Bruker IFS 25 FTIR spectrometer with DTGS detector, at a resolution of 4 cm^{-1}, apodized with a triangular function and Fourier transformed after one level of zero filling. Samples prepared as for ESR spectroscopy were freeze-dried and resuspended in a volume of D_2O equal to that of the original buffer. The sample was contained in a thermostated cuvette with CaF_2 windows and a 50-μm Teflon spacer for recording the spectra. Peak positions in the amide I band were resolved by Fourier self-deconvolution (Kauppinen et al., 1981) and quantitated by band fitting of the deconvoluted spectra, using programs kindly provided by Dr. H. H. Mantsch.

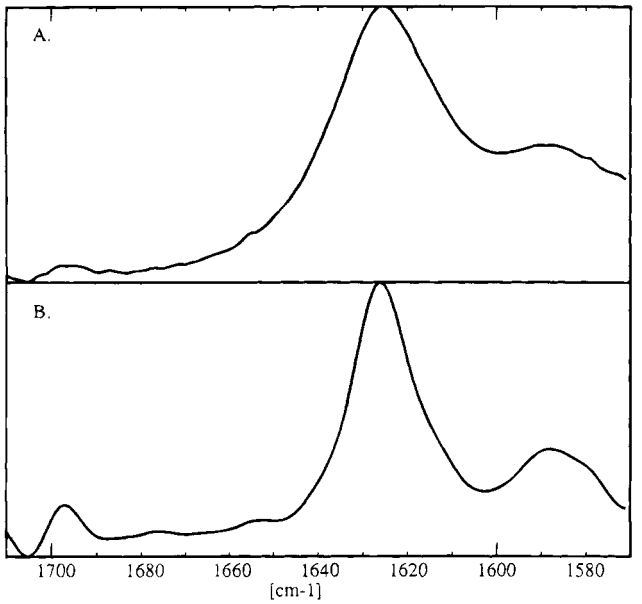

FIGURE 1: FTIR spectra of the K26 peptide in DMPC at a lipid/peptide ratio of 7.5 mol/mol dispersed in D_2O buffer at 30 °C: (A) the original spectrum and (B) the spectrum after Fourier self-deconvolution with a band-narrowing factor of $K = 1.8$ and Lorentzian line width of 17 cm^{-1}.

RESULTS

FTIR Spectroscopy. The amide I region of the FTIR spectrum of a complex of the K26 peptide with DMPC is given in Figure 1. The FTIR spectrum in the amide I region of the peptide consists essentially of a single band at 1626 cm^{-1}, which is characteristic of a β-sheet or extended chain structure (Byler & Susi, 1986). As revealed by Fourier self-deconvolution, virtually no intensity is present in the regions of the spectrum expected for α-helix, β-turns, or random structures. Essentially identical spectra are obtained in this region also for samples of higher lipid/protein ratios.

Conventional ESR of Spin-Labeled Lipids. The conventional ESR spectra of spin-labeled stearic acid, 14-SASL, in complexes of the K26 peptide with DMPC of different lipid/peptide ratios are given in Figure 2. The spectra are recorded at a temperature of 30 °C, which is above the chain-melting phase transition of DMPC bilayers. As found for reconstituted lipid complexes with integral proteins [cf. Marsh (1985)], the spectra consist of two components: one corresponding to the fluid bilayer regions of the lipid complexes and the other, with larger hyperfine anisotropy (visible in the outer wings of the spectra), corresponding to the motionally restricted lipid environment at the intramembranous surface of the incorporated peptide. The direct association of the motionally restricted component with the peptide is seen from the dependence on lipid/peptide ratio: with increasing lipid content in the complex the sharp three-line spectrum characteristic of a fluid lipid bilayer environment grows at the expense of the broader motionally restricted spectral component.

The conventional ESR spectra of different spin-labeled lipids in complexes of the K26 peptide with DMPC at the same lipid/peptide ratio are shown in Figure 3. In spite of the constant lipid/peptide ratio, considerable differences are seen in the ratio of the intensities of the fluid and motionally restricted components in these spectra. This is a clear indication of selectivity between the different lipids for

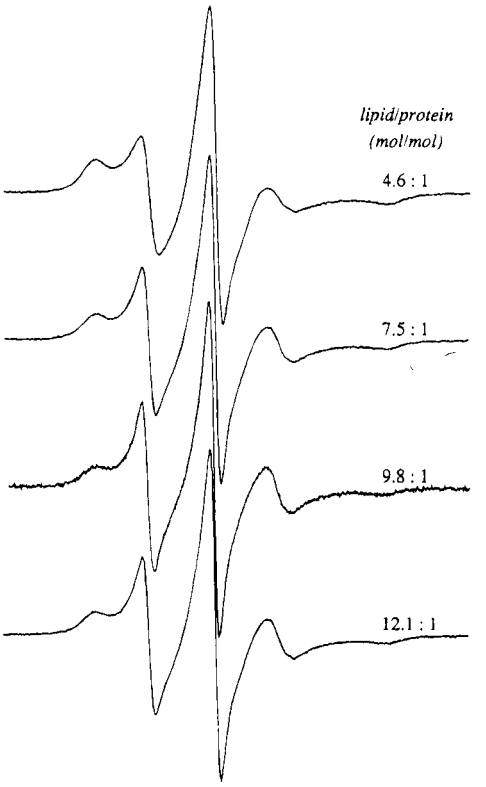

FIGURE 2: Conventional ESR spectra (V_1 display) of the 14-SASL stearic acid spin label in complexes of the K26 peptide with DMPC at lipid/peptide ratios of 4.6, 7.5, 9.8, and 12.1 mol/mol, as indicated. $T = 30$ °C; total scan width = 100 G.

interaction with the intramembranous surface of the peptide incorporated in the complex, a feature that is also observed with many integral proteins [cf. Marsh (1985)]. Clearly, the negatively charged phospholipids phosphatidic acid (14-PASL) and stearic acid (14-SASL), for instance, display a considerably higher selectivity for interaction with the peptide than does the zwitterionic phosphatidylcholine (14-PCSL).

The relative proportions of the fluid and motionally restricted components in the ESR spectra of the spin-labeled lipids were determined by spectral subtraction and integration as described in Marsh (1982). The dependence of the ratio of the intensity of the fluid component to that of the motionally restricted component (n_f^*/n_b^*) is given as a function of the lipid/peptide ratio of the complexes (n_t) for the different spin-labeled lipids in Figure 4. The data are displayed in terms of the equation for equilibrium lipid–peptide association (Brotherus et al., 1981; Marsh, 1985):

$$n_f^*/n_b^* = (n_t/N_b - 1)/K_r \quad (1)$$

where N_b is the number of lipid association sites per peptide, and K_r is the association constant of the spin-labeled lipid with the peptide, relative to that for the unlabeled host lipid (DMPC). Although there is some scatter in the data, particularly at the higher lipid/peptide ratios for the spin labels showing least selectivity and hence the lowest values of n_b^*, for a given spin label they do correspond reasonably well to the dependence depicted by eq 1. The linear dependences cross the x-axis at a common value, $N_b \approx 2.5$ lipids/peptide monomer, as would be expected on structural grounds. The intercepts on the y-axis ($-1/K_r$) differ for the different spin-labeled lipids, reflecting the lipid selectivities for interaction with the peptide, which are characterized by

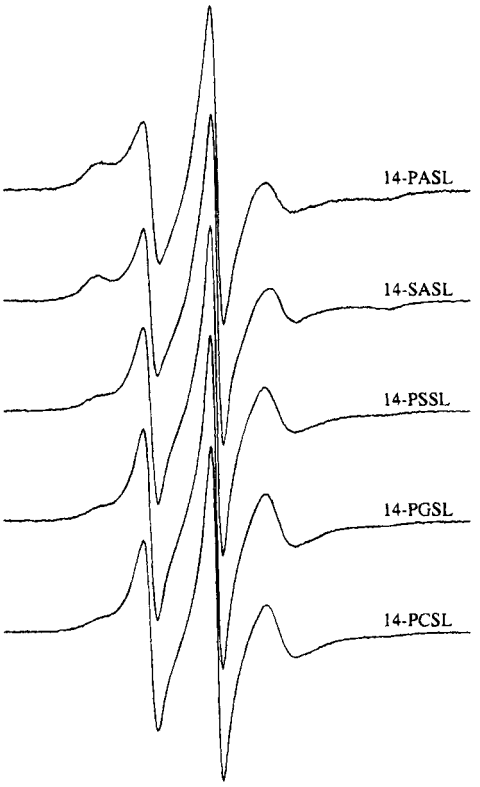

FIGURE 3: Conventional ESR spectra (from upper to lower) of the 14-PASL phosphatidic acid, 14-SASL stearic acid, 14-PSSL phosphatidylserine, 14-PGSL phosphatidylglycerol, and 14-PCSL phosphatidylcholine spin labels in complexes of the K26 peptide with DMPC at a lipid/peptide ratio of 7.5 mol/mol. T = 30 °C; total scan width = 100 G.

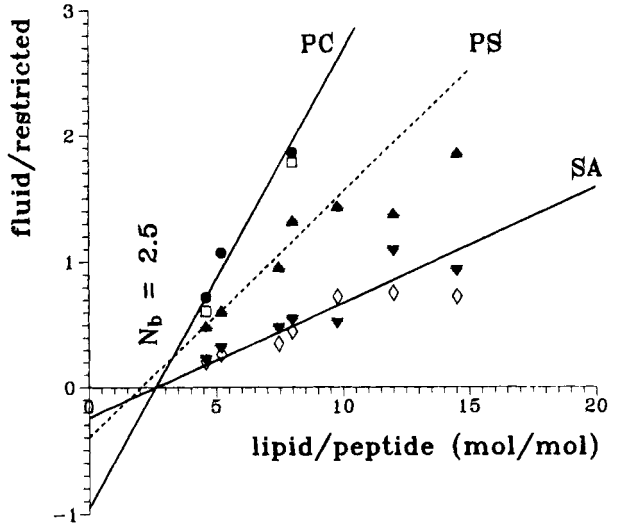

FIGURE 4: Lipid/peptide titration of the K26 peptide complexes with DMPC obtained from ESR difference spectra of the 14-PASL phosphatidic acid (◊), 14-SASL stearic acid (▼), 14-PSSL phosphatidylserine (▲), 14-PGSL phosphatidylglycerol (□), and 14-PCSL phosphatidylcholine (●) spin labels. The ordinate is the ratio, n_f^*/n_b^*, of the double-integrated intensity of the fluid and motionally restricted components in the ESR spectra of the complexes, and the abscissa is the total lipid/peptide ratio, n_t. Solid lines represent linear regressions to the data for the 14-PCSL and 14-SASL spin labels, and the dashed line represents that for the 14-PSSL spin label (for 14-PCSL only three points at lower values of n_t are included in the regression).

relative association constants of K_r = 4.3 for 14-PASL and 14-SASL, 2.5 for 14-PSSL, and ≈1 for 14-PGSL and 14-PCSL. In particular, 14-PCSL has a relative association

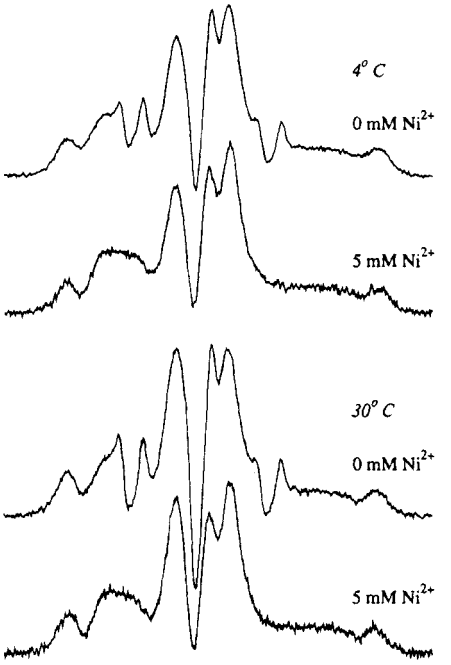

FIGURE 5: Second harmonic, 90° out-of-phase, absorption saturation-transfer ESR spectra (V_2' display) of the 5-InVSL spin label covalently bound to the K26(L18C) peptide in a complex with DMPC of lipid/peptide ratio ca. 12:1 mol/mol. Spectra were recorded at 4 °C (upper pair) and at 30 °C (lower pair). The upper spectrum in each pair is in the absence, and the lower spectrum in the presence, of 5 mM Ni^{2+}. Total scan width = 100 G.

constant close to unity, indicating that the spin label on phosphatidylcholine does not affect appreciably the thermodynamics of interaction with the peptide, relative to the parent unlabeled DMPC. This is in common with the situation for a range of reconstituted integral proteins (Marsh, 1985; Marsh & Watts, 1982).

STESR of the Spin-Labeled K26(L18C) Peptide. The second harmonic, 90° out-of-phase absorption STESR spectra of the K26(L18C) peptide labeled with 5-InVSL in a complex with DMPC are given in Figure 5. The spectra contain a sharp component, most probably arising from spin labels that are released slowly from the peptide, which can be quenched by addition of 5 mM Ni^{2+} in the aqueous phase to reveal the undistorted line shape of the spin label bound to the peptide. The intensity of the latter is reduced somewhat as a result of paramagnetic relaxation by the Ni^{2+}. From calibrations with model systems (Páli et al., 1992), the extent of this quenching indicates that the spin label attached to residue 18 is situated in the hydrophobic region of the lipid bilayer.

At low temperatures, corresponding to the gel phase of DMPC, the spectra of the 5-InVSL label have a large intensity in the intermediate regions at low and high field, corresponding to a rather slow rotational motion of the peptide assembly in the lipid complex [cf. Thomas et al. (1976)]. Using calibrations for isotropic rotational diffusion (Horváth and Marsh, 1988), the effective rotational correlation times deduced from the diagnostic line-height ratios in these regions are of the order of $\tau_R^{eff} \approx 100-150$ μs. Corresponding values deduced from samples with the maleimide spin label, 6-MSL, which is known to display additional segmental motion when attached to some integral proteins (Esmann et al., 1989; Horváth et al., 1990), are in the region of 40−80 μs. As the temperature is increased,

the relative spectral intensity in the diagnostic regions of the STESR spectra decreases only slightly, indicating that the rotational mobility of the peptide labeled with 5-InVSL remains rather low. At 30 °C, which would correspond to the fluid phase of DMPC bilayers, the effective rotational correlation times deduced from the STESR spectra of the 5-InVSL spin label are in the region of 100−120 μs, and $\tau_R^{eff} \approx 20-40$ μs for the peptide labeled with 6-MSL.

DISCUSSION

The K26 peptide and its variant K26(L18C) in which Leu18 is replaced by cysteine can be incorporated into dimyristoylphosphatidylcholine bilayers by dialysis from 2-chloroethanol to produce complexes that are of homogeneous lipid/peptide ratio, as judged by continuous sucrose density gradient centrifugation. The incorporation of the peptide in the lipid bilayer is further evidenced by the ESR spectra of spin-labeled lipids, which display the motionally restricted spin label component and lipid selectivity that is characteristic of integral transmembrane proteins [see, e.g., Marsh (1985)]. In the following, the mode of incorporation of the K26 peptide in the lipid bilayer will be discussed in terms of its conformation, lipid−peptide interactions, and rotational diffusion.

Secondary Structure of the Lipid-Bound Peptide. The results of FTIR spectroscopy indicate that the peptide incorporated in DMPC is wholly in a β-sheet conformation. Examination of the peptide sequence reveals a continuous, mainly hydrophobic stretch of 22 residues with charged residues at both ends. In the extended β-conformation, this hydrophobic stretch would have a length of 77 Å, which is much greater than the entire thickness of a DMPC bilayer, which is approximately 37 Å in the fluid phase (Tardieu, 1972). If the β-strands are tilted by an angle of 60° relative to the bilayer normal, which is the maximum tilt found in the β-sheet structure of porin (Weiss et al., 1991), the hydrophobic stretch would be reduced to 38.5 Å. This value approaches the thickness of a fluid DMPC bilayer, which suggests that the K26 peptide might be incorporated in the lipid complexes in a highly tilted β-sheet structure. An alternative structure which could accommodate the peptide within the DMPC bilayer is a double β-strand with a reverse turn of two to four residues at the center of the apolar sequence. Such a conformation would also reduce the hydrophobic stretch of the peptide by a factor of 2 and therefore is also a possible candidate for the mode of integration in the lipid bilayer. The FTIR spectra provide no evidence for such a hairpin structure, although this cannot be excluded entirely, since the number of residues involved in the putative reverse turn is small. It is interesting to note, in this connection, that a β-barrel structure composed of strand pairs connected by hairpin turns has been proposed for the pore-lining region of a different voltage-activated potassium channel (Bogusz et al., 1992). Also, consensus target sites for two protein kinases which regulate I_{sK}-associated activity intracellularly have been identified on opposite sides of the putative transmembrane sequence (Honoré et al., 1991). Further discussion of the general points relating to membrane-spanning β-structures is given in Marsh (1993).

Lipid−Peptide Interactions. The ESR spectra of the spin-labeled lipids indicate that the K26 peptide assemblies present a well-defined hydrophobic surface in the bilayer at which the lipid chains are restricted in their rotational mobility. It has been observed previously that the M13 phage coat protein incorporated in lipid bilayers in a polymeric β-sheet conformation gives rise to lipid spin label ESR spectra in which the motionally restricted component is much better resolved than in those arising from the protein incorporated in a mainly α-helical conformation (Peelen et al., 1992; Wolfs et al., 1989). The reason for this spectral difference lies partly in the exchange rate of the lipids at the protein interface, which is rather slow in the case of the polymeric β-sheet structure. One possible reason advanced for the slow exchange rate was that the extended nature of the β-sheet conformation causes the lipid chains at the hydrophobic protein surface to adopt a more extended configuration, thereby reducing the chain flexibility [see also Marsh (1993)]. It is likely that a similar effect is operative for the K26 peptide, since the hydrophobic stretch of even a strongly tilted β-sheet or a β-hairpin structure is somewhat larger than that of the DMPC bilayer in the fluid phase.

The lipid/peptide titration of the ESR spectra from spin-labeled lipids indicates that approximately two lipids per monomer are associated with the hydrophobic surface of the peptide in the bilayer. This value is considerably smaller than the number of lipids that could be accommodated around the hydrophobic region of the peptide monomer in an α-helical conformation, which is approximately 10 [see Marsh (1993)]. The FTIR spectra indicate that the peptide is in a β-sheet conformation, for which one lipid per strand could be accommodated on one side of the sheet, if this is in an enclosed structure with strands that are not tilted (Peelen et al., 1992; Marsh, 1993). If the strands in such a structure are tilted by 60°, this value is increased to two lipids per monomer. This is close to that determined experimentally and, as noted above, gives a structure that is reasonably compatible with the thickness of the DMPC bilayer. Again, the double-strand structure with a reverse turn would also fulfill these requirements, being able to accommodate two lipids per monomer at one surface, assuming that the peptide hairpins are assembled into an enclosed structure. Further discussion of lipid/peptide stoichiometries with respect to secondary structure and assembly may be found in Marsh (1993).

The relative association constants of the different spin-labeled lipids with the membrane-bound peptide indicate a clear selectivity of interaction with certain of the anionic lipids. This result suggests that the K26 peptide must be positioned in the lipid in such a way that one or both of the positively charged lysine and arginine residues at the N- and C-termini, respectively, of the peptide must be located in a region close to the negatively charged lipid headgroups. The incorporated peptide therefore does not extend far into the aqueous phase beyond the surface of the lipid bilayer. This conclusion is again in accord with the K26 peptide being integrated in the bilayer as a strongly tilted β-sheet structure or as an assembly of β-hairpin conformations.

Peptide Rotational Diffusion. The STESR spectra of the K26(L18C) peptide spin-labeled at the single cysteine residue indicate that the peptide incorporated in the DMPC lipid undergoes only slow rotational diffusion. This implies that the peptide must be integrated in the bilayer in a polymeric β-sheet form consisting of many more strands than a single peptide monomer [cf. Marsh and Horváth (1989) and

Knowles and Marsh (1991)]. Such a structure, e.g., a β-barrel, would completely satisfy the H-bonding capacity of the peptide backbone, as is required on energetic grounds for a peptide embedded in the hydrophobic region of a membrane. Even the shortest effective rotational correlation time, recorded with the maleimide spin label (6-MSL) at 30 °C, corresponds to an effective intramembranous radius for the peptide aggregate of ca. 32−36 Å [cf. Fajer et al. (1989)]. An aggregate in a β-barrel conformation of this size would consist of 20−22 individual peptide monomer strands, but such a structure would have an extremely large internal pore. A β-barrel with only 8 strands would be more compatible with forming a K^+ channel (Bogusz et al., 1992). The high-resolution porin structure, which has a large internal lumen partially obstructed by a long irregular peptide loop, consists of 16 strands in a β-barrel, and three of these are assembled in a trimer unit (Weiss et al., 1991). Judging from the large values of the effective rotational times obtained from the STESR spectra of the peptide labeled with 5-InVSL, it seems likely, whatever the exact form of the enclosed β-sheet structures of the K26(L18C) peptide is, that these are also assembled in oligomeric aggregates of what are possibly pore structures. Consistency with the models proposed above for the stoichiometry of the motionally restricted lipid associated with the K26 peptide would require that some of the motionally restricted lipids be trapped within the oligomeric structures, a feature that is exhibited by the lipid-bound M13 phage coat protein in its β-sheet form (Wolfs et al., 1989).

Conclusions. The K26 peptide, which contains the single putative transmembrane sequence of a small protein that is associated with slow voltage-gated K^+ channels, is integrated in lipid bilayers in a β-sheet conformation, at the peptide/lipid ratios studied here. The incorporated peptide presumably spans the bilayer, with an intramembranous hydrophobic surface that restricts the lipid chain mobility in a manner similar to that of integral transmembrane proteins. The positively charged residues at the N- and/or C-termini of the peptide are located close to the surface of the bilayer and give rise to a selectivity of interaction with certain negatively charged lipids. The β-sheets are present possibly as enclosed pore-forming units, and these are assembled into larger aggregates which undergo slow uniaxial rotational diffusion within the membrane.

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