A Single-Residue Deletion Alters the Lipid Selectivity of a K^+ Channel-Associated Peptide in the β-Conformation: Spin Label Electron Spin Resonance Studies

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ABSTRACT Lipid-peptide interactions with the 27-residue peptide of sequence KLEALYILMVLGFFGFFTLGIMLSYIR reconstituted as β-sheet assemblies in dimyristoylphosphatidylcholine bilayers have been studied by electron spin resonance (ESR) spectroscopy with spin-labeled lipids. The peptide corresponds to residues 42–68 of the IsK voltage-gated K^+ channel protein and contains the single putative transmembrane span of this protein. Lipid-peptide interactions give rise to a second component in the ESR spectra of lipids spin-labeled on the 14C atom of the chain that corresponds to restriction of the lipid mobility by direct interaction with the peptide assemblies. From the dependence on the lipid/peptide ratio, the stoichiometry of lipid interaction is found to be about two phospholipids/peptide monomer. The sequence of selectivity for lipid association with the peptide assemblies is in the order phosphatidic acid > stearic acid = phosphatidylserine > phosphatidylglycerol = phosphatidylcholine. Comparison with previous data for a corresponding 26-residue mutant peptide with a single deletion of the apolar residue Leu^2 (Horváth et al., 1995. Biochemistry 34:3893–3898), indicates a very similar mode of membrane incorporation for native and mutant peptides, but a strongly modified pattern and degree of specificity for the interaction with negatively charged lipids. The latter is interpreted in terms of the relative orientations of the charged amino acid side chains in the β-sheet assemblies of the native and deletion-mutant peptides.

INTRODUCTION

In contrast to the complex, polytopic structures usually found for voltage-gated ion channels, the IsK protein, the expression of which induces delayed rectifier K^+ channels, contains only a single putative transmembrane segment (Takumi et al., 1988; Murai et al., 1989; Honoré et al., 1991). It therefore has been suggested that this 130-residue protein may act as an activator, rather than constituting the channel itself, because heterologous expression at high levels in oocytes also induces voltage-regulated Cl^- channels (Attali et al., 1993). However, such expression has been found to be a general property shared with a variety of quite unrelated proteins (Tzounopoulos et al., 1995; Shibo et al., 1995). Much of the available evidence, including mutagenesis studies (Takumi et al., 1991; Goldstein and Miller, 1991; Tsai and Goldstein, 1997), is therefore in favour of IsK being a true channel protein. Because of the relatively simple structure of the IsK protein, this has proved an attractive candidate for the study of synthetic peptides related to the putative transmembrane sequence that are reconstituted in lipid bilayers (Ben-Efraim et al., 1993, 1994; Horváth et al., 1995; Aggeli et al., 1996).

Previously we have studied the assembly in phospholipid bilayers of a 26-residue peptide (peptide of sequence KEALYILMVLGFFGFFTLGIMLSYIR [i.e., K27(ΔL2)]; K26) that is a deletion mutant but contains the putative transmembrane sequence of the IsK protein (Horváth et al., 1995). On dialysis from the helix-promoting solvent 2-chloroethanol, the K26 peptide at high concentration was found to be incorporated into lipid bilayers in a β-sheet form. In view of the known secondary structural polymorphism of membrane-bound peptides such as the M13 bacteriophage coat protein (Spruijt et al., 1989; Spruijt and Hemminga, 1991), this does not necessarily imply a β-sheet configuration for the IsK channel pore. Nonetheless, the observation is most interesting in light of the β-barrel structures proposed for the pore regions of voltage-gated sodium and potassium channels (Guy and Conti, 1990; Durrell and Guy, 1992; Bogusz et al., 1992). In addition, recent experiments on the reversible block by Cd^{2+} of the IsK channel with site-directed cysteine mutants of the IsK protein suggest that the transmembrane section in fact has an extended conformation (Tsai and Goldstein, 1997).

In the present work we have studied the lipid interactions with a 27-residue peptide (peptide of sequence KLEALYILMVLGFFGFFTLGIMLSYIR; K27) that corresponds to residues 42–68 of the native IsK protein and contains its single putative transmembrane segment. The native K27 peptide was reconstituted in lipid bilayers of dimyristoyl phosphatidylcholine, again in the β-sheet form. The previously studied deletion-bearing K26 peptide differs from the native K27 peptide only by omission of a single amino acid residue, Leu^2, in the sequence. This hydrophobic residue is situated between the charged residues, Lys^1 and Glu^3, at the

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N-terminus of the 23-residue apolar domain of the native K27 peptide, in a region that is likely to interact with the lipid polar groups in the membrane. This is therefore the region of the peptide that could be expected to affect the selectivity of interaction with different lipid species. It is found that, although the mode of membrane integration of the native K27 peptide is similar to that of the related K26 deletion-mutant peptide, the selectivity of the lipid-peptide interaction is modified strongly. To establish this comparison it was necessary to perform extensive studies over a range of lipid/peptide ratios with different spin-labeled lipids. This was done to differentiate precisely between differences in stoichiometry and differences in selectivity of lipid interaction with the native and deletion-mutant peptides. Lipid/peptide interaction stoichiometries provide an important control, because they are sensitive indicators of the state of peptide assembly (Marsh, 1993, 1997a; Cornea et al., 1997). The results that are presented here therefore have important implications not only for the local peptide structural features affecting the specificity of lipid-protein interactions in membranes, but also for determining the transmembrane topology of integral proteins by the study of lipid-protein interactions.

MATERIALS AND METHODS

Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was from Avanti Polar Lipids (Alabaster, AL). Spin-labeled phospholipids 14-PCSL, -PGSL, -PSSL and -PASL were synthesized from spin-labeled stearic acid (14-SASL) as described in Marsh and Watts (1982) (14-SASL, 14-(4,4-dimethyloxazolidine-*N*-oxyl)stearic acid; 14-PCSL, -PGSL, -PSSL, and -PASL, 1-acyl-2-[14-(4,4-dimethyloxazolidine-*N*-oxyl)]stearoyl-*sn*-glycero-3-phosphocholine, -phosphoglycerol, -phosphoserine, and -phosphoric acid). Spin-labeled 3-maleimido-2,2,5,5-tetramethyl-pyrrolidine-*N*-oxyl (5-MSL) was obtained from Aldrich (Milwaukee, WI). The K27 peptide was synthesized by FMOC chemistry (BOP chemistry for Ser and Thr) on an automated solid-phase Milligen-Biosearch 9050 synthesizer. Coupling efficiency was maintained above 99.5% by incorporating recoupling steps for residues showing poor attachment. The peptide was cleaved from the resin by using reagent K (King et al., 1990) for 2.5 h, excluding water from the mixture. After precipitation and washing thoroughly in diethyl ether, the peptide was dried in vacuo, dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), diluted with water to 1% (v/v) HFIP, and lyophilized. Amino acid sequence analysis indicated >90% purity of the K27 peptide, with respect to the presence of deletion peptides arising from incomplete coupling. Calculations show that deletion peptides at this low level are unlikely to appreciably affect the lipid selectivity assayed by spin label electron spin resonance (ESR).

Preparation of lipid/peptide complexes

The required amount of peptide and 2 mg of DMPC were dissolved together in freshly distilled 2-chloroethanol (4 ml) and dialyzed against 2 liters of 0.1 M NaCl, 2 mM HEPES, 1 mM EDTA (pH 7.4) for 24 h with three changes of the buffer. The lipid/peptide complexes were separated from free lipid by centrifuging in buffer containing 1 M NaCl. The resulting pellets were found to sediment to a single band on continuous sucrose density gradient centrifugation (10–55% sucrose), with no visible free lipid remaining at the top of the gradient. There was no evidence for any surface-bound peptide resolved from the complexes on density gradient centrifugation. Dialysis from 2-chloroethanol has been shown to produce large unilamellar or paucilamellar protein-lipid vesicles (Brophy, 1977). The lipid-peptide complexes containing ~2 mg 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, 1 mM EDTA (pH 8.3). Unincorporated spin label was then resolved by centrifugation and washing. The K27(L19C) cysteine-containing peptide was covalently spin-labeled with a maleimide nitroxide (5-MSL) by aqueous reaction with a threefold molar excess of 5-MSL at 20°C, in the above buffer, overnight. Unreacted spin label was removed by centrifugation and exhaustive washing. Lipid/peptide ratios were determined after the ESR measurements by phosphate (Eibl and Lands, 1969) and protein (Lowry et al., 1951) assays.

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 ID capillaries by low-speed centrifugation. 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. Spectral subtractions with the two-component ESR spectra were performed as described by Marsh (1982). Saturation transfer ESR spectra were recorded in the second-harmonic 90° out-of-phase mode with 50-kHz Zeeman field modulation, a mean microwave field intensity at the sample of $\langle H_1^2 \rangle^{1/2}$ = 0.25 G, and a Zeeman modulation amplitude of H_m = 5.0 G. Calibrations and standardized sample protocol for the saturation transfer ESR measurements were as described previously (Horváth et al., 1995).

Fourier transform infrared spectroscopy

Infrared spectra were recorded on a Bruker IFS 25 Fourier transform infrared (FTIR) spectrometer with a DTGS detector, at a resolution of 4 cm^{-1}. 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 thermostatted cuvette with CaF_2 windows and a 50-μm teflon spacer for recording the spectra.

RESULTS AND DISCUSSION

The K27 peptide is very hydrophobic, with a mean hydrophobicity of $\langle H \rangle$ = 0.56 and low hydrophobic moment of $\langle \mu_\beta \rangle$ = 0.12 and $\langle \mu_\alpha \rangle$ = 0.07 for a β-sheet and α-helical conformation, respectively (see Eisenberg et al., 1984; Marsh, 1996). For the 23-residue apolar domain of K27, $\langle H \rangle$ = 0.82, $\langle \mu_\beta \rangle$ = 0.11 and $\langle \mu_\alpha \rangle$ = 0.07. Therefore the peptide might be expected to be incorporated into the membrane as an integral transmembrane protein. The K27/DMPC lipid-peptide complexes of different lipid/peptide ratios were formed by dialysis from 2-chloroethanol solutions and, after collecting by centrifugation through buffer containing 1 M KCl, were found to be of homogeneous composition, as demonstrated by density gradient centrifugation. FTIR spectra of the reconstituted complexes in D_2O buffer revealed a single, narrow band in the amide I region. This band was centered at a frequency of 1626 cm^{-1} (width at half-height ~19 cm^{-1}), which is characteristic of a β-sheet structure (Byler and Susi, 1986). These features are shared by reconstituted DMPC complexes of the mutant K26 peptide, which differs from the native K27 peptide by a single-residue deletion at position 2 in the N-terminal

region (Horváth et al., 1995). Detailed characterization of the β-sheet structure of the K27 peptide in DMPC bilayers by polarized ATR-FTIR spectroscopy has been presented previously (Aggeli et al., 1996). Saturation transfer ESR of a maleimide spin-labeled cysteine-substitution mutant L19C of the K27 peptide revealed a high degree of rotational restriction, comparable to that found for the corresponding maleimide spin-labeled K26(L18C) deletion mutant in DMPC (cf. Horváth et al., 1995). The effective rotational correlation times for the spin-labeled peptides were close and were in the region of tens of microseconds in fluid membranes in both cases. These results indicate that the sizes of the peptide aggregates are similar for the β-sheet forms of the native and mutant peptides in fluid DMPC membranes. The high degrees of rotational restriction, which are sensitive to the DMPC lipid phase transition, are also consistent with the peptides being incorporated into the high-viscosity interior of the lipid bilayer membranes.

DMPC was chosen for the reconstitution because it is a lipid, not subject to peroxidation, that has previously been found to be a suitable host for reconstituting integral membrane proteins. For example, bovine rhodopsin is monomeric in fluid DMPC membranes, as it is in the retinal rod outer segment disc membrane (Kusumi and Hyde, 1982; Ryba and Marsh, 1992). Reconstitution in DMPC is found to support enzymatic activity of cytochrome *c* oxidase (Abramovitch et al., 1990) and formation of the meta-II photointermediate of rhodopsin (Ryba et al., 1993). It is also found that the bilayer thickness of DMPC in the fluid phase is comparable to or greater than that of the longer-chain *cis*-unsaturated dioleoyl phosphatidylcholine (see Marsh, 1990).

The ESR spectra of the 14-SASL stearic acid spin label in complexes of the K27 peptide with DMPC at various lipid/peptide ratios are given in Fig. 1. The spectra all consist of two components at temperatures in the fluid phase of the wild-type K27/DMPC complexes and qualitatively resemble those of the related deletion-mutant K26/DMPC complexes (cf. Horváth et al., 1995). This is a characteristic feature of the interactions of such spin-labeled lipids with integral transmembrane proteins, whereby the motionally restricted spin label population corresponds to those lipid chains that directly contact the intramembranous section of the protein (cf. Marsh, 1985). Correspondingly, the proportion of the motionally restricted population increases systematically with increasing peptide/lipid ratio. Significantly, it is found that the ESR spectra of the lipid spin labels in K27/DMPC and K26/DMPC complexes resemble more closely those of DMPC complexes of the M13 bacteriophage coat protein in its β-sheet form than those of its α-helical form (cf. Peelen et al., 1992; Wolfs et al., 1989). For the M13 coat protein, it was found that the lipid spin-label ESR spectra were sharper and better resolved in complexes with the β-sheet form than in those with the α-helical form. This was attributed to a slower rate of lipid exchange at the interface with the β-sheet protein. It is in this sense that the ESR spectra from K27/DMPC and K26/DMPC complexes resemble those from β-sheet lipid complexes of the M13 coat protein.

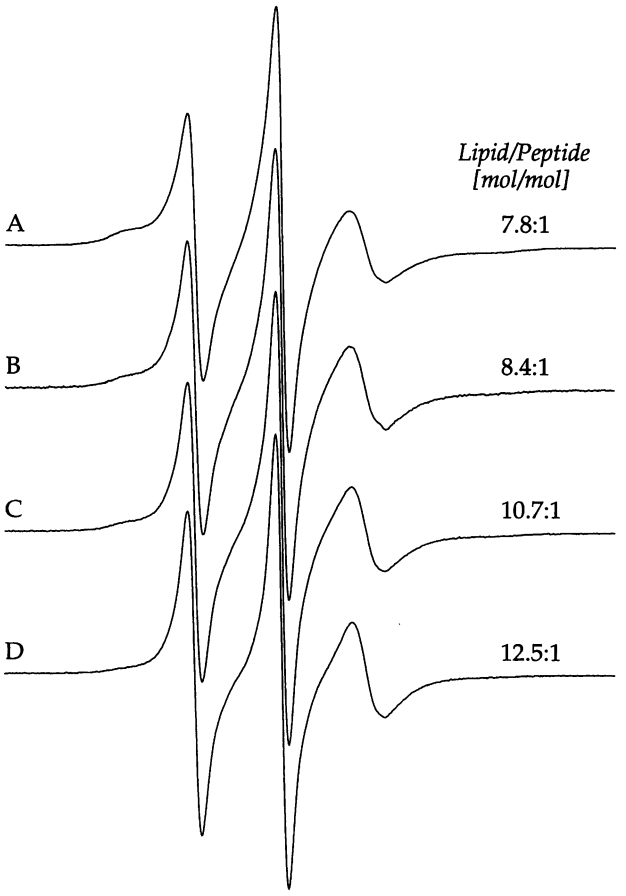

FIGURE 1 ESR spectra of the 14-SASL stearic acid spin label in complexes of the K27 peptide with dimyristoyl phosphatidylcholine at lipid/peptide ratios of (*A*) 7.8 mol/mol, (*B*) 8.4 mol/mol, (*C*) 10.7 mol/mol, and (*D*) 12.5 mol/mol, as indicated. T = 30°C; total scan width = 100 G.

ESR spectra of the various spin-labeled lipid species (14-PCSL, 14-PGSL, 14-PSSL, 14-SASL, and 14-PASL) were obtained from K27/DMPC complexes of given peptide contents (see Fig. 2). At a fixed lipid/peptide ratio, the spectra from the different spin-labeled lipids contain significantly different proportions of the two spectral components, as was demonstrated quantitatively by spectral subtraction. The greatest selectivity for interaction with the peptide is found with spin-labeled phosphatidic acid, 14-PASL (~45% motionally restricted component). The negatively charged lipids, stearic acid and phosphatidylserine, 14-SASL and 14-PSSL, respectively, also display a limited selectivity of interaction (~33% motionally restricted component) relative to spin-labeled phosphatidylcholine (~22% motionally restricted component).

The relative proportions of the fluid and motionally restricted spin-labeled lipids were determined by spectral subtraction and double integration of the component first-derivative ESR spectra. The dependence of the integrated intensity of the fluid component relative to that of the

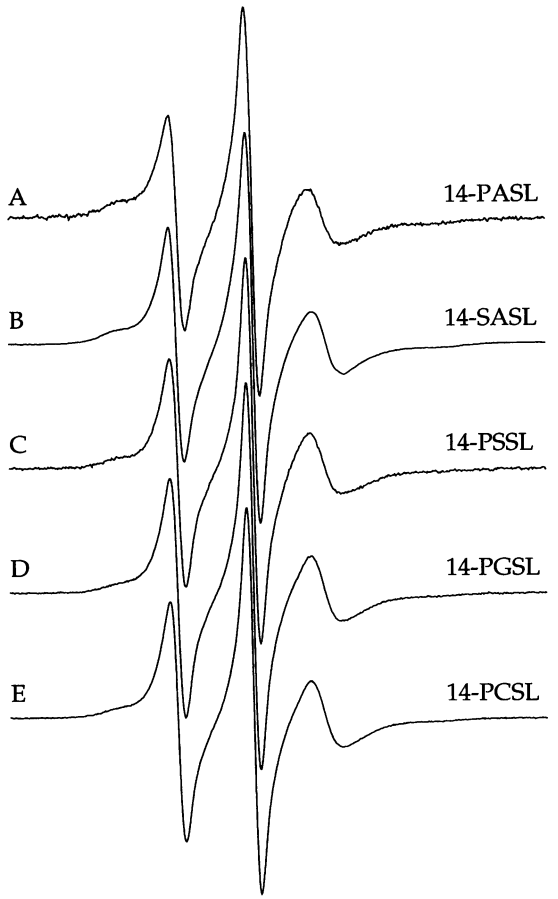

FIGURE 2 ESR spectra of the (*A*) phosphatidic acid (14-PASL), (*B*) stearic acid (14-SASL), (*C*) phosphatidylserine (14-PSSL), (*D*) phosphatidylglycerol (14-PGSL), and (*E*) phosphatidylcholine (14-PCSL) spin labels in complexes of the K27 peptide with dimyristoyl phosphatidylcholine at a lipid/peptide ratio of 10.7:1 mol/mol. T = 30°C; total scan width = 100 G.

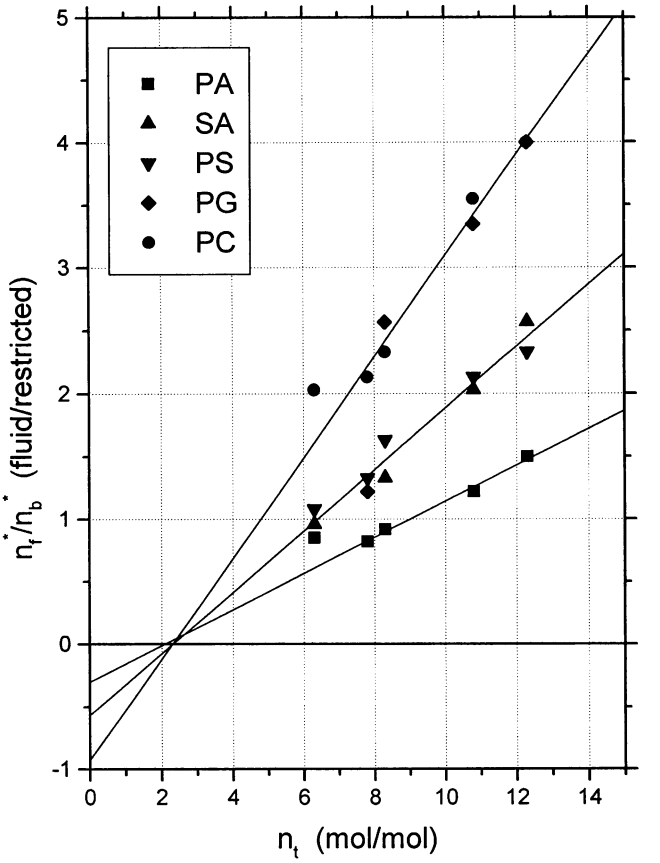

FIGURE 3 Dependence on the lipid/peptide ratio, n_t, of the interaction of spin-labeled lipids with the K27 peptide in complexes with dimyristoyl phosphatidylcholine. The ordinate is the ratio, n_f^*/n_b^*, of the double-integrated intensities of the fluid and motionally restricted components in the ESR spectra of the spin-labeled lipids. ●, 14-PCSL; ◆, 14-PGSL; ▼, 14-PSSL; ▲, 14-SASL; ■, 14-PASL. The solid lines are linear regressions to the data from 14-PCSL plus 14-PGSL, from 14-PSSL plus 14-SASL, and from 14-PASL.

motionally restricted component, n_f^*/n_b^*, is given as a function of the total lipid/peptide ratio, n_t, in the complex in Fig. 3. The data for each particular spin-labeled lipid display a linear dependence on the lipid/peptide ratio that conforms to the equation for equilibrium association with the protein (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 monomer and K_r is the association constant of the spin-labeled lipid relative to the unlabeled background host lipid (i.e., DMPC). Both of these latter quantities are therefore found to be constant for a given spin-labeled lipid species, as is expected. The lipid site stoichiometry, $N_b \approx 2.2$ lipids/K27 monomer, is the same for the different spin-labeled lipids, in agreement with the equilibrium association model, i.e., the same number of sites is available to each lipid species and the relative occupancies are determined solely by different relative affinities for these sites. This value of N_b is also similar to that for the stoichiometry of lipid association with the β-sheet form of the K26 peptide (Horváth et al., 1995), consistent with the mode of intramembranous assembly of the deletion-mutant peptide being very similar to that of the native K27 peptide in DMPC complexes (cf. Marsh, 1993, 1997a). The implications of this stoichiometry per monomer, in terms of β-sheet strands tilted relative to the membrane normal, have been discussed previously (Horváth et al., 1995). A 60° tilt or a hairpin structure is required to match the apolar stretch of the peptide to the hydrophobic span of the DMPC bilayer. A highly tilted β-strand structure has been found experimentally for the K27 peptide by polarized FTIR measurements (Aggeli et al., 1996; Marsh, 1997b). A 60° tilted structure or a β-hairpin structure is able to accommodate approximately two lipids/monomer on one side of an enclosed or otherwise sandwiched bilayer assembly.

Certain of the spin-labeled lipids display an enhanced selectivity for association with the K27 peptide assemblies, as has been already noted (cf. Fig. 2). This is characterized by the different slopes of the titration with respect to the

lipid/peptide ratio in Fig. 3. The relative association constants, K_r, obtained from linear regressions of the data to Eq. 1 are given in Table 1. For the purpose of these fits, the data for 14-PCSL and 14-PGSL are grouped together, as are those for 14-SASL and 14-PSSL, because the dependences on n_t are indistinguishable to within experimental precision. The 14-PCSL phosphatidylcholine spin label does not show an appreciable selectivity for association with the K27 peptide assemblies, relative to the unlabeled parent host lipid dimyristoyl phosphatidylcholine, i.e., $K_r \approx 1$ for 14-PCSL. A similar result was found previously for complexes of the deletion-mutant K26 peptide with DMPC, as well as for a range of integral membrane proteins (Horváth et al., 1995; Marsh, 1985). With the exception of 14-PGSL, the different negatively charged lipids, 14-PSSL, 14-PASL, and 14-SASL, display a selectivity relative to phosphatidylcholine for interaction with the K27 peptide assemblies. The pattern of lipid selectivity for the native K27 peptide differs, however, from that found previously for the K26 deletion-mutant peptide in complexes with DMPC (Horváth et al., 1995). The relative association constants for interaction of the spin-labeled lipids with the deletion-mutant peptide are given for comparison with the present data for the native K27 peptide in Table 1. It should be noted that the composition and pH of the buffer are identical in all cases.

In general, the relative association constants for the native K27 peptide are smaller than those for association of the corresponding negatively charged lipids with the K26 deletion mutant peptide in complexes with DMPC (Table 1). Particularly notable is the difference in the relative association constant of stearic acid. This is similar to that for phosphatidic acid, the phospholipid of highest specificity, in association with the K26 peptide, but is similar to that for phosphatidylserine, the phospholipid of intermediate specificity, in association with the K27 peptide. The corresponding values of $\Delta\Delta G$ caused by the deletion mutation are -2.2, -0.9, and -0.8 kJ/mol for the peptide interaction with stearic acid, phosphatidic acid, and phosphatidylserine, respectively (relative to phosphatidylcholine). The enhanced effect on the selectivity for stearic acid, relative to that for phosphatidic acid and phosphatidylserine, probably arises from the influence of the different electrostatic environments (see below) on the local pH. This could change the protonation state of peptide-associated stearic acid, which is known to affect its specificity of interaction, whereas phosphatidylserine and phosphatidic acid are far less affected in this pH range (Horváth et al., 1988; Esmann and Marsh, 1985).

TABLE 1 Relative association constants, K_r, for the interaction of spin-labeled lipids with the native K27 peptide and the ΔL2 deletion mutant (i.e., K26) peptide in complexes with DMPC, deduced from lipid-peptide titrations of the ESR spectra

	14-PASL	14-SASL	14-PSSL	14-PGSL	14-PCSL
K27	3.3	2.0	2.0	1.1	1.1
K26 [K27(ΔL2)]*	4.3	4.3	2.5	1.0	1.0

T = 30°C. Buffer: 2 mM HEPES, 1 mM EDTA (pH 8.3).
*Data from Horváth et al. (1995).

The deletion of a single nonpolar amino acid, leucine at position 2, from the sequence of the K27 peptide therefore has a rather pronounced effect on the selectivity of interaction of the negatively charged lipid polar headgroups with the peptide assemblies in DMPC bilayers. This finding further supports the conclusion that the observed specificity for negatively charged lipids locates the charged residues of the K27 peptide at the polar surface of the lipid membrane. Correspondingly, this (in addition to the lipid stoichiometries) again implies that the β-strands of the K27 and K26 peptides either are strongly tilted with respect to the membrane normal or contain a reverse turn, to accommodate the 7.7-nm-long apolar stretch of the peptide within the lipid bilayer (cf. Horváth et al., 1995; Aggeli et al., 1996).

The origin of the difference in lipid selectivity between the K26 deletion-mutant peptide and the native K27 peptide most probably lies not in a direct interaction of the lipid polar groups with the apolar leucine residue, but rather in the secondary structure of the peptide in the reconstituted complexes. For a β-sheet structure, the amino acid side chains of adjacent residues in the sequence alternate in their orientation between opposite sides of the sheet (see Fig. 4). In the K26 deletion-mutant peptide, the oppositely charged side chains of residues Lys^1 and Glu^2 at the N-terminus are therefore located on different sides of the β-sheet. This segment of the K26 peptide has a low net *hydrophobic* moment of $\langle\mu_\beta\rangle = 0.05$ (cf. Eisenberg et al., 1984; Marsh, 1996), resulting from the separation of charges. An optimal interaction of the negatively charged lipids with the positively charged lysine side chains may thus be the origin for

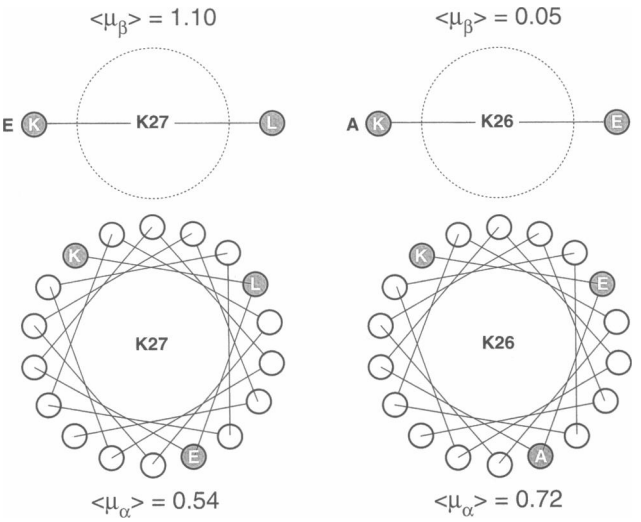

FIGURE 4 Helical wheel projections for the N-terminal sequences of the native K27 (*left*) and the ΔL2 deletion-mutant, K26 (*right*) peptides in a β-strand (*top*; pitch = 2 residues) and an α-helical (*bottom*; pitch = 3.6 residues) conformation. The *hydrophobic* moments of the N-terminal sequences are indicated on the figure.

the enhanced selectivity of interaction with the K26 peptide. Correspondingly, the local pH in the vicinity of the positively charged lysine will be higher, increasing the proportion of ionized stearic acid, which has a higher selectivity of interaction. In the native K27 peptide, on the other hand, the oppositely charged residues, Lys^1 and Glu^3, are located on the same side of the β-sheet. This segment of the K27 peptide has a high hydrophobic moment, $\langle\mu_\beta\rangle = 1.10$, resulting from the accumulation of (opposite) charges on one side of the sheet. Therefore, the favorable electrostatic interaction with the positively charged lysine will be partially neutralized, resulting in a lower selectivity of lipid interaction for the native K27 peptide than for the K26 deletion-mutant peptide. In the case of the K27 peptide, the local pH will be less enhanced, resulting in a higher proportion of the protonated stearic acid, which has a lower selectivity. Hence the relative orientations of the peptide side chains could also explain the preferential effect that deletion of Leu^2 has on the selectivity for stearic acid, compared with that for phosphatidic acid and phosphatidylserine. The considerations above refer to only one of the two sides of the β-sheet. From the stoichiometries of lipid association, it was argued that only one side of the β-sheet was accessible to the lipid (see previously and Horváth et al., 1995). However, it is not necessarily expected that one particular face of the sheet is preferentially oriented toward the lipid in DMPC bilayers, because the hydrophobic moment of the apolar section, $\langle\mu_\beta\rangle = 0.11$, is not particularly high (cf. Eisenberg et al., 1984). A random orientation seems more likely and, in this case, it must be assumed that it is the interaction with the Lys^1-containing face that determines the differences in lipid specificity.

Whatever the exact mechanism, it is clear that deletion of a single nonpolar residue in a region of the native peptide expected to be situated close to the phospholipid headgroups can have a rather marked effect on the lipid specificity. Conversely, the study of lipid-protein interactions by spin label methods can provide a useful means of probing the local structure of integral proteins and, via mutagenesis, of identifying those residues in the protein sequence that are located in the proximity of the lipid polar groups.

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