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The αM1 transmembrane segment of the nicotinic acetylcholine receptor interacts strongly with model membranes

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The transmembrane domain of the nicotinic acetylcholine receptor (nAChR) plays a role in the regulation of the activity of this important ligand-gated ion channel. The lipid composition of the host membrane affects conformational equilibria of the nAChR and several classes of inhibitors, most notably anaesthetics, interact directly or indirectly with the four transmembrane M-segments, M1–M4, of the nAChR subunits. It has proven difficult to gain insight into structure–function relationships of the M-segments in the context of the entire receptor and the biomembrane environment. However, model membrane systems are well suited to obtain detailed information about protein–lipid interactions. In this solid-state NMR study, we characterized interactions between a synthetic αM1 segment of the *T. californica* nAChR and model membranes of different phosphatidylcholine (PC) lipids. The results indicate that αM1 interacts strongly with PC bilayers: the peptide orders the lipid acyl chains and induces the formation of small vesicles, possibly through modification of the lateral pressure profile in the bilayer. The multilamellar vesicle morphology was stabilized by the presence of cholesterol, implying that either the rigidity or the bilayer thickness is a relevant parameter for αM1–membrane interactions, which also has been suggested for the entire nAChR. Our results suggest that the model systems are to a certain extent sensitive to peptide–bilayer hydrophobic matching requirements, but that the lipid response to hydrophobic mismatch alone is not the explanation. The effect of αM1 on different PC bilayers may indicate that the peptide is conformationally flexible, which in turn would support a membrane-mediated modulation of the conformation of transmembrane segments of the nAChR.

KEYWORDS: NMR; solid-state NMR; ^{31}P NMR; ^{2}H NMR; nicotinic acetylcholine receptor; αM1 segment; peptide–lipid interactions; phosphatidylcholine model membranes; hydrophobic matching

INTRODUCTION

A prominent superfamily of membrane proteins is formed by the ligand-gated ion channels that act as neurotransmitter receptors, a prime example of which is the nicotinic acetylcholine receptor (nAChR).[1,2] The nAChR from the electric organ of the electric ray *Torpedo* is a large pentameric integral membrane protein that consists of four different subunits of about 470 amino acids each. One β, γ and δ and two α subunits are arranged around a central axis perpendicular to the postsynaptic membrane, forming a cation-selective ion channel which is essential for nerve impulse transduction over synaptic clefts.[3,4] Each subunit traverses the membrane with four polypeptide segments, M1–M4, and significant sequence homology exists between analogous M-segments of the α, β, γ and δ chains. The five M2 segments line the aqueous channel, whereas M1, M3 and M4 are exposed to the lipids that make up the host membrane.[1,4]

The transmembrane domains of the nAChR are important for the regulation of its activity. For example, conformational equilibria of the receptor are modulated by the lipid composition of the membrane,[5,6] and effectors such as local anaesthetics inhibit ion channel activity by targeting the transmembrane segments, either by direct binding or indirectly by altering membrane properties.[7,8] Despite their functional relevance, little is known with certainty about the structure of the transmembrane M-segments and about the mechanisms by which membrane constituents and anaesthetics exert their influence. In the case of a large integral membrane protein such as the nAChR, it is especially complicated to obtain structural information and

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insight into structure–function relationships in the complex environment of a biomembrane.

However, detailed information about protein–lipid interactions can be derived from well-defined model membrane systems, and several groups have studied synthetic M-segments in membrane-like environments. Opella and coworkers demonstrated by solid-state NMR methods that δM2 is present as an undistorted α-helix in bilayers of phosphatidylcholine lipids, and that multimers of M2, but not of the other M-segments, form functional cation-selective channel pores in these model membranes.[9] The conformation of an M4 segment of the nAChR in a bicelle environment is also being studied by solid-state NMR techniques.[10] Additionally, it has been shown by solution NMR that in micelles or in organic solvent, δM2, αM2 and αM3 adopt an α-helical conformation.[9,11,12] However, photoaffinity label studies on the entire nAChR indicate that the transmembrane domain is not entirely helical, and that the non-helical regions probably reside in M1,[13] which is the only M-segment that contains helix-destabilizing Pro residues.

If the M1 segment indeed contains non-helical structural elements, it can be expected to exhibit a degree of conformational flexibility, and consequently M1 could play a role in transmitting a membrane-induced conformational change to the lumen of the channel pore.[7,8,14] Therefore, we synthesized a 35-residue peptide corresponding to the M1 sequence of the α subunit of the *T. californica* nAChR for solid-state NMR characterization in model membranes. In this work, as a prelude to a conformational study on the peptide itself, we report the effects of αM1 incorporation on the order and morphology of a variety of phosphatidylcholine bilayers. We found that αM1 interacts strongly with phosphatidylcholine model membranes, a result which could point to a role of protein–lipid interactions in the regulation of nAChR activity.

EXPERIMENTAL

Peptide synthesis

The peptide sequence, H-Ile-Nle-Gln(Trt)-Arg(Pbf)-Ile-Pro-Leu-Tyr(tBu)-Phe-Val-Val-Asn(Trt)-Val-Ile-Ile-Pro-Cys(Trt)-Leu-Leu-Phe-Ser(tBu)-Phe-Leu-Thr(tBu)-Gly-Leu-Val-Phe-Tyr(tBu)-Leu-Pro-Thr(tBu)-Asp(OtBu)-Ser(tBu)-Gly-O-Wang-Argogel, representing the αM1-segment amino acid sequence 230–264 of the nicotinic acetylcholine receptor of *Torpedo californica*,[15] was synthesized automatically on an Applied Biosystems 433A Peptide Synthesizer using the FastMoc protocol[16] on 0.25 mmol scale. N^{α}-9-fluorenylmethoxycarbonyl (Fmoc)-Gly-OH was coupled to the Wang resin[17] using the method of Sieber;[18] Wang resin was used to obtain the C-terminal peptide acid. To facilitate the synthesis, the methionine residue at position 231 was substituted by norleucine (Nle), a common isosteric replacement. Protecting groups are abbreviated as trityl (Trt), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) and *tert*-butyl (tBu). Coupling of N^{α}-Fmoc-amino acids (1 mmol, 4 equiv.) was performed with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate–*N*-hydroxybenzotriazole[19] in the presence of 8 equiv. of *N*,*N*-diisopropylethylamine in *N*-methylpyrrolidone (NMP) for 45 min. Fmoc removal was carried out with 20% piperidine in NMP for 70 s. Any remaining amino groups after incomplete coupling were acetylated with acetic anhydride–*N*,*N*-diisopropylethylamine–*N*-hydroxybenzotriazole in NMP. After coupling of the last amino acid, Fmoc-Ile-OH, the Fmoc was removed and the resin was thoroughly washed with NMP and dichloromethane, after which the peptide–resin was dried in a vacuum desiccator. The peptide was cleaved from the resin and deprotected by treatment with trifluoroacetic acid (TFA)–H_2O–1,2-ethanedithiol–triisopropylsilane (85 : 8.5 : 4.5 : 2, v/v/v/v) for 3 h at room temperature. The peptide was precipitated at −20 °C with methyl *tert*-butyl ether–hexane (1 : 1, v/v). The precipitates were decanted and subsequently washed three times with cold methyl *tert*-butyl ether–hexane (1 : 1, v/v) and finally lyophilized from *tert*-butanol–H_2O (1 : 1, v/v).

Peptide purification and characterization

The peptide was purified by size-exclusion chromatography on a Sephadex LH20 column (130 × 1.5 cm i.d.) with dichloromethane–methanol (1 : 1, v/v) as eluent. Peptide purity was analysed by analytical HPLC on a Merck LiChroSpher CN column (250 × 4.6 mm i.d., 5 μm particle size, 100 Å pore size), at a flow-rate of 0.75 ml min^{-1} and using a 60 min linear gradient from 0.1% TFA in H_2O–acetonitrile (8 : 2, v/v) to 0.1% TFA in acetonitrile–2-propanol–H_2O (50 : 45 : 5, v/v/v). Purity was determined to be 90% or higher. The peptide was characterized by electrospray ionization mass spectrometry in the positive ionization mode. Mass spectrometric analysis was also carried out on a Kratos Axima CFR MALDI-TOF instrument. The calculated average mass of the peptide is $[M + H]^+$ 3973.85, and a value of 3973.23 was found.

Sample preparation

An amount of αM1 peptide appropriate to achieve a given peptide/lipid molar ratio was dissolved in 20 μl of TFA per mg of peptide. Subsequently, the excess of solvent was evaporated by a stream of nitrogen and the resulting peptide film was dissolved in 0.5 ml of trifluoroethanol. This peptide solution was added to 0.5 ml of a 50 mM phospholipid solution in trifluoroethanol [trifluoroethanol–ethanol (2.5 : 1, v/v) in the case of phospholipid–cholesterol mixtures], followed by addition of 15 ml of distilled water and overnight lyophilization to remove the organic solvents. This method ensures that peptides and lipids are mixed on a molecular level and that multilamellar vesicles are formed prior to lyophilization.[20] Lyophilized powders were packed into 5 mm silicon nitride rotors (Doty Scientific, Columbia, SC, USA); during this procedure some samples form a gel-like paste owing to the absorption of atmospheric water. Alternatively, the lyophilized multilamellar vesicles were rehydrated with 1 ml of distilled water, followed by centrifugation of the vesicle suspension for 5 min at 22 000 *g*. The pelleted vesicles in excess water, with a total volume of ~75 μl, were transferred to a 5 mm silicon nitride rotor. All phospholipids were obtained from Avanti Polar Lipids (Birmingham, AL, USA).

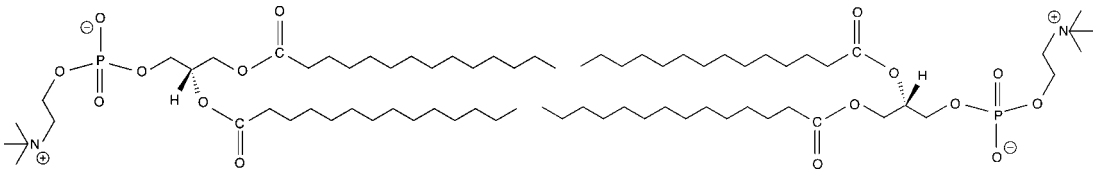

Figure 1. Sequence of the αM1 peptide and schematic depiction of a bilayer of DMPC lipids. Underlined residues are expected to prefer contact with the lipid acyl chains.

NMR measurements

Solid-state NMR measurements were performed on an Inova 300 instrument (Varian, Palo Alto, CA, USA) equipped with a 5 mm diameter Doty Scientific magic angle spinning (MAS) probe. The operating frequencies were 121.46 MHz for ^{31}P and 46.06 MHz for ^{2}H, while ^{1}H decoupling was applied at 300.04 MHz. Broadline ^{1}H-decoupled ^{31}P NMR spectra were recorded (3.5 µs $\pi/2$ pulse, 2 s recycle delay, 50 kHz sweep width, ~70 kHz ^{1}H decoupling) to characterize the macroscopic lipid organization. Typically 4096 scans were acquired and 4096 real data points were used, with zero filling to 8192 points and an exponential multiplication resulting in a 100 Hz line broadening prior to Fourier transformation. All spectra were scaled to the same height and chemical shift was referenced to an aqueous solution of H_3PO_4 (85%). To assess the lipid acyl chain order, broadline ^{2}H NMR measurements were performed using a quadrupolar echo sequence[21] (8.0 µs $\pi/2$ pulse, 30 and 20 µs pulse separations, 0.5 s recycle delay, 100 kHz sweep width, 8192 data points). Approximately 25 000 scans were accumulated. The free induction decays were left-shifted to begin at the top of the echo and multiplied with an exponential window function equivalent to a line broadening of 50 Hz. The ^{2}H chemical shift was referenced to D_2O.

RESULTS AND DISCUSSION

The sequence of the αM1 peptide of *T. californica* nAChR is shown in Fig. 1, together with a schematical depiction of two opposing DMPC lipids, resembling a cross-section of a DMPC model membrane. A description of the different phosphatidylcholine (PC) lipids employed in this study is given in Table 1. In a lipid bilayer, the lipid acyl chains form the hydrophobic membrane core, whereas the water-permeated polar headgroups constitute an interface between this core and the aqueous phase. Sequence analysis identifies the underlined residues of αM1 (Fig. 1) as the transmembrane stretch, and these hydrophobic residues can be expected to traverse the hydrophobic membrane core. To enable the αM1 peptide also to interact with the lipid headgroups, we extended the N- and C-termini with the adjacent, more polar, residues encountered in the *T. californica* nAChR sequence.[15]

Effects of αM1 incorporation on DMPC bilayers

The macroscopic organization of lipid assemblies can be conveniently monitored by wide-line ^{31}P NMR spectroscopy.[23,24] The shape of the ^{31}P NMR spectrum reflects the configuration and dynamics of the phosphorus-containing lipid headgroups and, because the rotation of multilamellar lipid vesicles is slow on the ^{31}P NMR time-scale, also the vesicle morphology. The spectrum of a dispersion of pure DMPC in excess water, as shown in Fig. 2(A), is characterized by a low-field shoulder at about 28 ppm and a high field peak at −15 ppm, typical for the preferred planar bilayer organization of this lipid in the biologically relevant liquid-crystalline (L_α) phase.[23,24] When αM1 is associated with the DMPC lipids at a 1 : 150 peptide/lipid molar ratio, a similar ^{31}P NMR spectrum was observed [Fig. 2(B)], although the spectral width, or residual chemical shift anisotropy (CSA), is about 6 ppm smaller for the peptide-containing sample. This small decrease in CSA is characteristic of peptide incorporation[20] and could reflect a partial disruption of the

Table 1. Description of the phosphatidylcholine lipids used in this study, together with their hydrophobic thickness in the liquid-crystalline phase

Abbreviation	Name	Acyl chain composition[a]	Hydrophobic thickness (Å)[b]
DMPC	1,2-Dimyristoyl-*sn*-glycero-3-phosphatidylcholine	14 : 0/14 : 0	23.0[c]
DPPC	1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine	16 : 0/16 : 0	26.0[c]
POPC	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine	16 : 0/18 : 1_c	26.5[d]
DOPC	1,2-Dioleoyl-*sn*-glycero-3-phosphatidylcholine	18 : 1_c/18 : 1_c	27.0[c]
DSPC	1,2-Distearoyl-*sn*-glycero-3-phosphatidylcholine	18 : 0/18 : 0	30.0[c]

[a] E.g., 16 : 0/18 : 1_c refers to a saturated acyl chain of 16 carbons (palmitoyl) at the 1-position and an acyl chain of 18 carbons with one unsaturated bond (oleoyl) at the 2-position.

[b] Hydrophobic thickness is defined as the distance between the acyl chain C-2 atoms of two opposing lipids in a bilayer.

[c] Based on Ref. 22.

[d] Estimated by taking the average value of both chains.

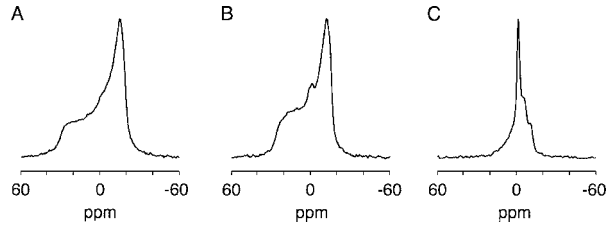

Figure 2. ^{31}P NMR spectra of dispersions of (A) pure DMPC, (B) αM1–DMPC-d_{54} (1 : 150 molar ratio) and (C) αM1–DMPC-d_{54} (1 : 30 molar ratio) in excess water at 34 °C.

hydrogen-bonding network between the lipid headgroups.[25] Superimposed on the L_α spectrum, a small spectral component is observed near −1.5 ppm, the resonance position of phospholipids that experience isotropic motion on the ^{31}P NMR time-scale. Since rapid reorientation of the lipids is unlikely to occur within large multilamellar vesicles, the isotropic signal most likely indicates that a minor fraction of the lipids in the αM1–DMPC (1 : 150) sample are present in undefined aggregates or in well-defined assemblies with a highly curved surface such as a cubic lipid phase or small (unilamellar) vesicles.

This effect on lipid organization is clearly induced by αM1 because the contribution of the isotropic signal was dramatically enhanced at a higher peptide concentration. As can be seen in Fig. 2(C), the spectrum of an αM1–DMPC (1 : 30) sample is dominated by an isotropic resonance at −1.5 ppm. The L_α component of this spectrum is not only reduced in relative intensity, but with a low-field shoulder around 16 ppm and a high-field peak at −11 ppm, its CSA is only 27 ppm. The occurrence of a second high-field peak at −6 ppm appears to originate from another L_α component with an even smaller CSA value. These significantly reduced CSA values correspond to vesicles that are considerably smaller than the large multilamellar vesicles which are formed in the absence of αM1, suggesting that the dominant isotropic component originates from even smaller vesicles, with a radius of 150 nm or less.[26] It should be noted that the spectra shown here were recorded shortly after hydration, and that in time the contribution of the isotropic component increased for both peptide samples. This growth in isotropic signal with increase in hydration was an irreversible effect that occurred only for peptide-containing lipid samples and under both static and MAS NMR conditions. However, the effect was enhanced by MAS, possibly because the resulting centrifugal force influences the kinetics of membrane rearrangement. After 24 h under static conditions, the αM1–DMPC (1 : 30) sample gave rise to an exclusively isotropic signal, whereas the αM1–DMPC (1 : 150) sample yielded a spectrum similar to that depicted in Fig. 2(C) (data not shown). Apparently, even at the lower peptide concentration, αM1 has a pronounced influence on the morphology of DMPC model membranes.

The molecular organization of the αM1–DMPC systems was further characterized by wide-line ^{2}H NMR measurements, which give information about the average order of the perdeuterated lipid acyl chains.[27,28] The ^{2}H NMR spectrum of a dispersion of DMPC-d_{54} in the absence of peptide is presented in Fig. 3(A). The shape of the spectrum is characteristic of a lipid bilayer, and consists mostly of overlapping doublet resonances that result from the different CD_2 segments of the acyl chain, while the central high-intensity doublet corresponds to the terminal CD_3 moiety. The distance between the two peaks of each doublet is the quadrupolar splitting, which is larger for chain segments that are more ordered. The spectral width of the entire spectrum is related to the fluidity of the lipid bilayer, and the range observed in the pure DMPC-d_{54} spectrum is typical for acyl chains in an L_α phase.[29] ^{2}H NMR spectra were also recorded for DMPC in the presence of αM1, using the samples that were first employed to obtain the ^{31}P NMR spectra depicted in Fig. 2. For the αM1–DMPC(-d_{54}) (1 : 150) sample, the ^{2}H NMR spectrum (not shown) was almost identical with the spectrum in the absence of peptide, which can be expected at relatively low peptide concentrations because the spectrum reflects the *average* acyl chain order, and only a small fraction of the lipids would be in direct contact with the peptide. However, as shown in Fig. 3(B), at the higher αM1 : DMPC molar ratio of 1 : 30 a spectrum was observed with significantly larger quadrupolar splittings and with less fine structure than the spectrum of pure DMPC-d_{54}. Note that no significant isotropic lipid signal is apparent in the ^{2}H NMR spectrum, in contrast to the situation for the longer ^{31}P NMR time-scale [Fig. 2(C)], thus confirming that the isotropic ^{31}P NMR signal corresponds to smaller, rapidly tumbling, vesicles and not to non-vesicular structures.

The αM1-induced increase in the quadrupolar splittings implies that the presence of the peptide significantly orders the acyl chains in the DMPC bilayer, concomitantly increasing the hydrophobic bilayer thickness. Interestingly, a similar increase of ∼3.5 kHz in quadrupolar splitting of the outer methylenes is observed for the 21-residue YALP21 and 23-residue WALP23 peptides at a 1 : 30 peptide : lipid molar ratio in DMPC bilayers.[30,31] These totally hydrophobic peptides, consisting of a stretch of alternating Leu and Ala

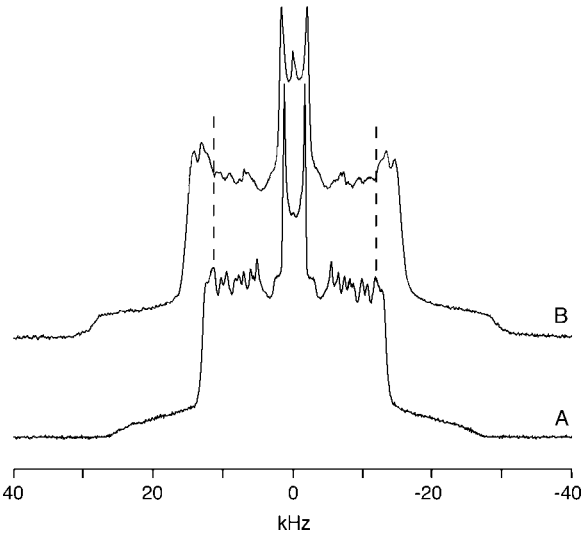

Figure 3. ^{2}H NMR spectra of dispersions of (A) pure DMPC-d_{54} and (B) αM1–DMPC-d_{54} (1 : 30 molar ratio) in excess water at 34 °C.

residues that is flanked by Tyr or Trp residues, were shown to be effectively too long to fit in a DMPC bilayer. Since the central hydrophobic stretch of αM1 (Fig. 1) consists of 24 hydrophobic residues, this peptide also could experience such a hydrophobic mismatch in a DMPC bilayer, which may underlie the strong effects on lipid organization as observed by ^{31}P NMR.

Effects of αM1 incorporation on thicker bilayers

To investigate whether the apparent αM1-induced conversion of multilamellar vesicles into smaller (unilamellar) vesicles is indeed related to the extent of peptide–bilayer hydrophobic matching, model membranes of lipids with longer acyl chains were also investigated. Since molecular motion can decrease the strength of the heteronuclear dipolar interaction,[32] for peptide-directed NMR it can be favourable to measure at lower water content because the decrease in mobility facilitates signal enhancement by cross-polarization from ^{1}H to ^{13}C or ^{15}N isotopic labels. Therefore, we compared the NMR spectra of samples with different levels of hydration. In Fig. 4(A), the ^{31}P NMR spectrum is depicted of POPC (see Table 1) in the presence of αM1 at a 1 : 30 peptide : lipid molar ratio. Although the lyophilized αM1–POPC powder was not hydrated as described in the Experimental section, the sample had absorbed some water from the ambient atmosphere. The αM1–POPC spectrum consists of an L_α component with a reduced CSA of ~25 ppm, on which a substantial isotropic resonance is superimposed. Under similar conditions, αM1–DMPC samples resulted in a comparable spectrum (not shown), but with a greater contribution from the isotropic signal, suggesting that the thicker bilayer formed by POPC is less prone to adopt the morphology that is represented by the isotropic resonance. Figure 4(B) shows the spectrum of a lyophilized powder of αM1–DSPC, the lineshape and large CSA of which are characteristic of multilamellar vesicles in the gel (L_β) phase,[26] a consequence of measuring below the main-transition temperature of ~55 °C of the DSPC lipids.[33] No spectral components are visible that can be related to relatively small vesicles, suggesting that a gel-state DSPC bilayer is a suitable matrix for the αM1 peptide.

However, in excess water and at a temperature above the main-transition temperature, the αM1–DSPC system gave rise to a ^{31}P NMR spectrum that consists exclusively of a broad isotropic resonance [Fig. 4C]. Within a few hours after hydration, samples with DMPC, DPPC, POPC and DOPC also yield spectra (not shown) that are characterized by a single isotropic signal, regardless of the hydrophobic thickness of these bilayers (Table 1). Why are multilamellar vesicles of DSPC more stable in the L_β than in the L_α phase? The gel-state DSPC bilayer is thicker than all the fluid-state bilayers studied, but αM1–DSPC was also the sample that as judged from its consistency, absorbed the smallest amount of atmospheric water, which is related to the high main-transition temperature of DSPC. Since the stretch of 24 hydrophobic residues in the αM1 peptide would have a length of 36 Å when in an ideal α-helical conformation, and the hydrophobic thickness of a gel-state DSPC bilayer is at least 36 Å,[34] a peptide–bilayer hydrophobic mismatch will not occur, which could explain why the apparent multilamellar morphology of the DSPC vesicles is retained in the gel phase. Alternatively, it can be envisaged that below a threshold water concentration the redistribution of the DSPC lipids into smaller vesicles is not possible, or that small vesicles have been formed but vesicle tumbling and/or lipid diffusion is slow. The partial retention of the L_α signal for αM1–POPC at reduced hydration [Fig. 4(A)], seems primarily related to the water content and not to hydrophobic matching, because the thickness of the already liquid-crystalline POPC bilayer is not expected to alter significantly upon full hydration, and in excess water only the isotropic ^{31}P NMR spectral component was observed.

Stabilization of vesicle morphology

We found that in excess water multilamellar vesicles in the presence of αM1 can be stabilized by the addition of 33% cholesterol to the phospholipid bilayer. As can be seen in Fig. 5(A), no isotropic component was present in the ^{31}P NMR spectrum of αM1 in a POPC–cholesterol mixture (2 : 1 molar ratio). Instead, the spectrum is characteristic of multilamellar vesicles of lipids in an L_α phase, and the influence of αM1 is only manifested in the reduced CSA value of ~30 ppm [cf. Fig. 2(A)]. A very similar spectrum was obtained for an αM1–DMPC–cholesterol sample, although the CSA value of ~37 ppm is somewhat larger and a minor isotropic signal is also visible [Fig. 5(B)]. It is noteworthy in this respect that functional properties of the entire nAChR are related to the lipid composition of the host membrane.[35] This could imply that receptor conformational equilibria are modulated by the physical properties of the lipid matrix,[5,6] a hypothesis that has been tested by reconstitution of purified nAChR in model membranes of defined composition. Recent spectroscopic and chemical labelling data indicate that the nAChR in

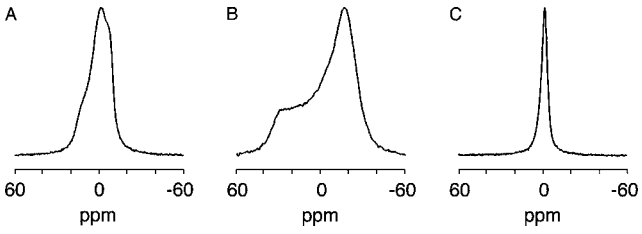

Figure 4. ^{31}P NMR spectra of (A) lyophilized powder of αM1–POPC at 34 °C, (B) lyophilized powder of αM1–DSPC at 34 °C and (C) dispersion of αM1–DSPC in excess water at 65 °C. The peptide : lipid molar ratio is 1 : 30.

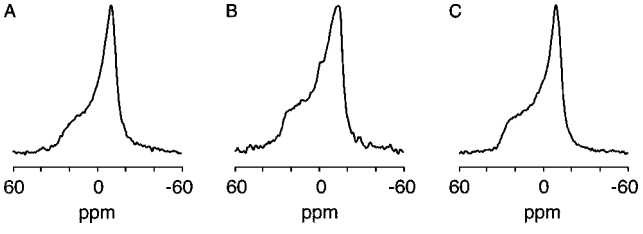

Figure 5. ^{31}P NMR spectra of dispersions in excess water of (A) αM1–POPC–cholesterol at 26 °C, (B) αM1–DMPC–cholesterol at 34 °C and (C) αM1–DTPC at 34 °C. The peptide : lipid molar ratio is 1 : 30 and the PC : cholesterol molar ratio is 2 : 1.

bilayers of PC species alone is present in a desensitized-like non-functional state, whereas the addition of either phosphatidic acid (PA) lipids or cholesterol, both present in neuronal membranes, enables a resting-like state that is capable of undergoing agonist-induced conformational change.[36,37] In the presence of the nAChR, POPC bilayers containing PA and/or cholesterol appear to experience a lesser degree of water penetration around the lipid carbonyls and consequently a higher density of headgroup packing, suggesting that the nAChR induces an ordering of the lipid bilayer. In addition, the nAChR can undergo agonist-induced conformational transitions in gel-state bilayers.[37] These results suggest that relatively ordered membranes, but not relatively fluid membranes, stabilize the receptor in a functional state.

Interestingly, the αM1 peptide mimics several aspects of the effect of the entire nAChR on model membranes. Not only does αM1 order DMPC acyl chains (Fig. 3), but it also appears to interact more favourably with cholesterol-containing PC bilayers [Fig. 5(A) and (B)], and perhaps with gel-state bilayers [Fig. 4(B)], as indicated by the fact that these systems retain the multilamellar vesicle morphology of peptide-free bilayers. Baenziger and Blanton and co-workers[37] have suggested that the ability of the nAChR to modulate the physical properties of model membranes could originate from hydrophobic mismatch between the transmembrane segments of the nAChR and the lipid bilayer. As outlined above, the hydrophobic length of αM1 might indeed correspond to the hydrophobic thickness of a gel-state DSPC bilayer, whereas fluid-state bilayers of all the phospholipids employed in this study could well be too thin to accommodate the peptide without lipid rearrangements. However, the addition of 30% cholesterol to a fluid-state POPC bilayer increases the hydrophobic bilayer thickness only by ~4 Å,[38] resulting in a thickness that is comparable to that of a fluid-state bilayer of DSPC (see Table 1). Since αM1 affects the morphology of fluid-state DSPC but not of POPC–cholesterol vesicles in excess water, it therefore seems unlikely that hydrophobic matching considerations are a dominant factor in αM1–PC interactions. Instead, either the relative rigidity of PC–cholesterol bilayers or specific peptide–cholesterol interactions seem likely to provide αM1 with a more favourable membrane environment.

However, these considerations fail to rationalize the observation that in excess water αM1 did not affect the multilamellar vesicle morphology of bilayers of 1,2-ditetradecyl-*sn*-glycero-3-phosphatidylcholine (DTPC), as evident from the lineshape and CSA of the corresponding ^{31}P NMR spectrum presented in Fig. 5(C). DTPC lipids are almost identical with DMPC, the only difference being that the 14-carbon acyl chains are connected to the glycerol backbone with an ether instead of an ester linkage (Fig. 1), which can be advantageous for ^{13}C=O labelled peptide-directed ^{13}C NMR studies.[39] The DTPC model membranes were not in the L_β phase, did not contain cholesterol and were of comparable hydration, fluidity and hydrophobic thickness to the DMPC vesicles that were readily deformed by αM1. If the resistance of the DTPC vesicles to αM1-induced adaptation of vesicle morphology is indicative of favourable αM1–DTPC interactions, bilayer rigidity as well as peptide–lipid hydrophobic matching alone may not constitute relevant parameters for αM1–membrane interactions. However, it should be noted that in determining the extent of hydrophobic mismatch, the aromatic residues that typically flank protein transmembrane segments play a crucial role because these residues prefer localization at the polar/apolar interface, probably through interaction with the lipid ester carbonyl moieties.[40] Since the hydrophobic stretch of the αM1 segment is flanked on both sides by aromatic residues, namely Tyr and Phe (Fig. 1), the minimal effect of αM1 on the macroscopic organization of the ether-linked DTPC systems might correlate with the potential insensitivity of DTPC bilayers to hydrophobic mismatch. In turn this would imply that the pronounced effects of the peptide on DMPC vesicles are at least to a certain extent related to αM1–DMPC hydrophobic mismatch.

Finally, the possibility exists that αM1 does not adopt the same conformation in the different model membranes. Conceivably, for example, the peptide has a tendency to kink around the central Pro residue, and could be in a conformational equilibrium between a kinked and a straight α-helix. It has been argued that depending on the lateral pressure profile in the bilayer, which is different for relatively rigid and relatively fluid membranes, a particular conformation of the transmembrane segment is favoured.[14] Since the effective hydrophobic length of αM1 will depend on its specific conformation, mismatch-driven membrane interactions cannot be identified by looking solely at the lipid components of model systems in the case of conformation-flexible peptides. We speculate that in cholesterol-free systems in the fluid phase, αM1 is in a conformation which induces a lateral pressure profile that is more compatible with the high curvature of small vesicles than with the small curvature of large multilamellar vesicle assemblies. Conversely, the conformation of αM1 in cholesterol-rich membranes would appear to be more compatible with larger vesicles or less highly curved membranes.

CONCLUSION

Solid-state NMR studies have revealed that the αM1 segment of the nAChR has a considerable influence on the organization of PC model membranes. Although the results are clearly indicative of strong peptide–lipid interactions, the underlying mechanism appears to be complex as neither hydrophobic matching nor bilayer rigidity considerations are sufficient to explain the combined observations of this study. Interestingly, the αM1 peptide mimics several aspects of the behaviour of reconstituted nAChR, as both the peptide and the entire protein order the lipid bilayer and are sensitive to the presence of cholesterol. The observed membrane interactions of the nAChR and of the isolated transmembrane segment, αM1, thus support the concept that physical properties of the membrane environment modulate the conformational equilibrium and consequently the function and regulation of the nAChR. To investigate the conformation of the αM1 segment in different model membranes, we are studying a specifically labelled αM1 analogue by ^{13}C and ^{15}N MAS NMR.

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