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Electrostatic peptide–lipid interactions of amyloid-β peptide and pentalysine with membrane surfaces monitored by ^{31}P MAS NMR

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High-resolution ^{31}P magic angle spinning (MAS) NMR spectroscopy is presented as a direct and non-perturbing method for measuring changes in surface charge density occurring in mixed phospholipid membranes upon binding of charged surface-active peptides. ^{31}P MAS NMR was used to investigate mixed lipid membranes of neutral phosphatidylcholine and negatively charged phosphatidylglycerol where the molar fraction of the charged lipid was varied from 0 to 1. The chemical shifts of the individual membrane lipids showed a simple variation in response to changes in the fraction of the negatively charged component phosphatidylglycerol. Addition of the positively charged amyloid-β_{1-40} peptide, a key substance in Alzheimer's disease, resulted in changes in the isotropic chemical shifts of the membrane lipid phosphates in a way consistent with reduction in the negative surface charge of the mixed lipid bilayers. Binding of different amounts of the positively charged peptide pentalysine to L-α-dioleoylphosphatidylcholine/L-α-dioleoylphosphatidylglycerol (DOPC/DOPG) vesicles (2 : 1 molar ratio) also showed a systematic variation of both chemical shift values. These changes were described by a simple two-site model and indicate purely electrostatic binding of pentalysine.

Introduction

The interaction of peripheral proteins with lipid membranes plays a key role in many cellular processes. The initial binding of surface-active proteins and peptides to lipid membranes is usually driven by electrostatic interactions between protein domains rich in positive (basic) amino acid residues and negatively charged (acidic) lipid membranes.[1–11] It is common for membrane-perturbing toxins and antimicrobial peptides to use positively charged amphipatic segments for initial non-specific binding to negatively charged target lipid membranes prior to inducing membrane poration and lysis.[4,9] Other proteins, where positively charged domains are used for electrostatic binding to lipid membranes include apocytochrome *c*,[5] myosin,[10] HIV matrix protein[11] and myristoylated alanine-rich C-kinase substrate (MARCKS).[7]

Traditionally, the effects of surface-active molecules and ions on lipid bilayers are investigated by monitoring the changes in membrane surface charge density using wideline deuterium NMR.[1–3,12–21] This approach relies on the specific introduction of deuterium labels into the headgroup of one membrane lipid constituent, typically phosphatidylcholine (PC). In cases where the headgroup choline α- and β-methylene deuterons can be resolved by their deuterium quadrupole splittings a counterdirectional change in those splittings upon introduction of membrane inclusions is used as an indicator of changes in membrane surface charge density, a concept described as "molecular voltmeter".[3,12–17] Changes of opposite sign have been observed in the presence of positively or negatively charged effector molecules, or during increase or reduction in the lateral order in the membrane headgroup region. This methodology has been used extensively to describe membrane surface charge effects in the presence of a wide range of biologically important compounds including surface ligands, divalent cations, peptides, proteins, local anaesthetics, charged lipids, or changes through hydrostatic pressure.[3,12–21] Drawbacks of the described approach include the required use of specifically headgroup deuterated lipids and their often not straightforward synthesis. Also, spectroscopic observation is limited to one deuterated lipid species in each experiment, and introduction of deuterated compounds into native lipid membranes is often not possible. In some cases, such as amyloid-β peptide (Aβ) or pentalysine, the method has failed to respond to surface charge changes outside the lipid headgroup region.[6,16]

The 100% natural abundance phosphorus-31, present in all phospholipid constituents of the lipid membranes, has been used for wideline NMR investigation of the phase behaviour of phospholipid dispersions and possible changes thereof, induced upon addition of peptides, proteins or ions.[22–27] Multicomponent phospholipid systems, however, produce NMR spectra consisting of overlapping intensity distributions of the same type and of similar effective chemical shift anisotropy values (CSA), where the individual lipid components cannot be resolved.[20,22–24] These individual membrane phospholipid constituents can be observed simultaneously by high-resolution natural abundance ^{31}P MAS NMR where sample rotation is used to average in part or completely the effective CSA of the lipid phosphates. With this method specific interactions have been identified between cytochrome *c* and the individual components in mixed lipid bilayers of cardiolipin/PC/phosphatidylethanolamine (PE), where by ^{31}P MAS NMR all membrane phospholipids have been resolved according to their ^{31}P isotropic chemical shifts (CS).[24] The method has been used to show segregation of phosphatidylglycerol (PG) in PC/PG mixtures in the presence of cardiotoxin II,[8] and antibiotic nisin,[9] as well as to demonstrate vesicle fusion and quantify proteolipid association induced by bacterial toxin pneumolysin.[27]

In the present study the use of high-resolution ^{31}P MAS

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NMR as a selective and non-perturbing method has been extended to investigations of the changes in phospholipid headgroup electrostatics occurring during membrane association of amyloid-β peptide and pentalysine. Systematic changes in the isotropic chemical shifts of mixed lipid bilayers of PC and PG have been observed for both phosphates during variation of bilayer composition from pure PC (neutral) to pure PG (negatively charged) and have been interpreted as resulting from variation of the bilayer surface charge density. It has been possible to offset these changes by addition of amyloid-β peptide (positively charged). Binding of positively charged peptide pentalysine caused changes in ^{31}P chemical shifts in both PC and PG, opposite to that observed during PG titration.

Fibril formation of amyloid-β peptide has been closely associated with neuronal membranes, composed mainly of phospholipids, cholesterol and various glycolipids. Because these membranes have invariably a net negative membrane surface, association of Aβ peptide with these charged systems has been proposed to be the dominant mechanism behind the accelerated conversion of the precursor Aβ-peptide into neurotoxic aggregates during Alzheimer's disease.[6,28,29] Here, high-resolution ^{31}P MAS NMR results from Aβ peptide, bound to lipid bilayers of variable negative surface charges are presented and a mixed electrostatic/hydrophobic character of the peptide association with charged membranes is proposed.

The basic peptide pentalysine, which corresponds to the first 5 residues of the MARCKS peptide,[7] the effector region of the myristoylated alanine-rich C kinase substrate, is responsible for initial binding of MARCKS to negatively charged lipid membranes. The purely electrostatic nature of the interaction between pentalysine and acidic lipid bilayers has been proposed recently.[7,30] In this study the binding of pentalysine at different peptide/lipid ratios to mixed lipid bilayers of PC/PG has been investigated through changes in the ^{31}P chemical shifts of the two phospholipid species using high-resolution ^{31}P MAS NMR. Isotropic CS changes of opposite sign to those, induced by negative charges, support the proposed electrostatic origin of the interaction.

Materials and methods

Materials

Lipids were obtained from Sigma (UK). Amyloid-$\beta_{1\text{-}40}$ peptide was synthesized by standard solid-phase FMOC chemistry, subsequently purified by high-performance liquid chromatography (HPLC) and quality checked by MALDI-TOF mass spectroscopy. Pentalysine, KKKKK (Multiple Peptides Systems, San Diego, CA) and was >95% pure as shown by mass spectrography and analytical HPLC.

Sample preparation

Multilamellar vesicles (MLV) mixed lipid suspensions were prepared for ^{31}P NMR experiments by co-solubilizing the desired lipid ratios in $CHCl_3/CH_3OH$ (2 : 1 v/v) followed by removal of the solvent under high vacuum for 5 h. The dried lipid films were subsequently hydrated by vigorous agitation in excess of buffer A (low ionic strength: 10 mM Tris, 10 mM KCl, 0.5 mM EDTA, pH 7.8) for L-α-dimyristoylphosphatidylcholine/L-α-dimyristoylphosphatidylglycerol (DMPC/DMPG) systems and Aβ-experiments or buffer B (1 mM MOPS, 1 mM EDTA, 10 mM KCl, pH 7.2) for preparation of DOPC/DOPG vesicles in pentalysine binding studies.

Aβ peptide was dissolved in 500 µl TFA (trifluoroacetic acid) to obtain a monomeric form. After removal of TFA under nitrogen gas flow the peptide film was resuspended in TFE (trifluoroethanol). The solvents were removed by rotary evaporation followed by 5 h exposure to high vacuum. The peptide film was resuspended in an appropriate amount of buffer A under vigorous agitation for 10 min and then added to the DMPC/DMPG suspension at 30 : 1 L/P molar ratio (100 µM Aβ, total volume 7.35 ml per sample). The final suspensions were incubated for 30 min at 37 °C and subjected to three freeze–thaw cycles. The MLV suspensions were concentrated in a benchtop centrifuge, and the pellets kept frozen prior to the NMR studies.

Binding of pentalysine to the DOPC/DOPG vesicles was performed as described previously.[30] The desired amount of peptide was dissolved in buffer B, which was used subsequently for hydration of the dried lipid films. The resulting MLVs were incubated for 30 min at 37 °C under vigorous agitation and subjected to three freeze–thaw cycles. The final suspensions were concentrated in a benchtop centrifuge (30 min at 50 000*g*) and the pellets were loaded into the MAS NMR rotors.

Solid state NMR measurements

^{31}P MAS NMR experiments were carried out on Infinity spectrometers (Chemagnetics, USA) at proton frequencies of 200 and 100 MHz and an MSL spectrometer (Bruker, Germany) at a proton frequency of 400 MHz. Double resonance 4 and 7 mm MAS probes (Bruker, Germany) were used to allow for ^{31}P observation under proton decoupling (60 kHz). A single phosphorus-31 $\pi/2$ pulse with 5 µs was used for excitation. For static ^{31}P NMR measurements a Hahn echo pulse sequence was applied with an interpulse delay of 50 µs. Between 200 and 4000 transients were collected for each spectrum, with a repetition period of 3 s. All ^{31}P NMR spectra were referenced externally to 0 ppm for 10% H_3PO_4. High speed MAS lineshape analysis was performed by fitting simulated Lorentzian lineshapes to the experimental spectra using Felix (MSI, Cambridge, UK).

Analysis

As previously shown by ^{2}H NMR[1,10] it is possible to obtain information about the exchange rate of lipids between two coexisting bilayer environments in the presence of membrane proteins. In the case of fast exchange of lipid molecules between a protein-free part of the bilayer and a protein-associated part, the isotropic ^{31}P chemical shift σ_t can be expressed in a similar way as a weighted average of the chemical shift values in each of the two environments:

$$n_t = n_c + n_f$$
$$\sigma_t = n_c(\sigma_c - \sigma_f)/n_t + \sigma_f \quad (1)$$

Here n_c and n_f denote the molar fractions of protein-associated (in proteolipid complexes) and protein-free bilayer lipid, respectively, of total bilayer lipid n_t. Similarly, σ_t denotes the observed average ^{31}P chemical shift and σ_c and σ_f are the chemical shifts from protein-associated and protein-free bilayer lipid environments. Plotting σ_t *vs.* $1/n_t$ can be used to assess the rate of lipid exchange between the two environments, where in the case of rapid exchange (on the ^{31}P MAS NMR timescale) a straight line with slope of $n_c(\sigma_c - \sigma_f)$ is expected.

Results

^{31}P MAS NMR

Wideline ^{31}P NMR was used to monitor any potentially occurring changes in the phase behaviour of the phospholipid systems upon addition of peptides. A typical static ^{31}P NMR spectrum from a DOPC/DOPG mixture (2 : 1 molar ratio) is shown in Fig. 1a. It is dominated by the chemical shift anisotropy of the phosphates from both PC and PG, partially

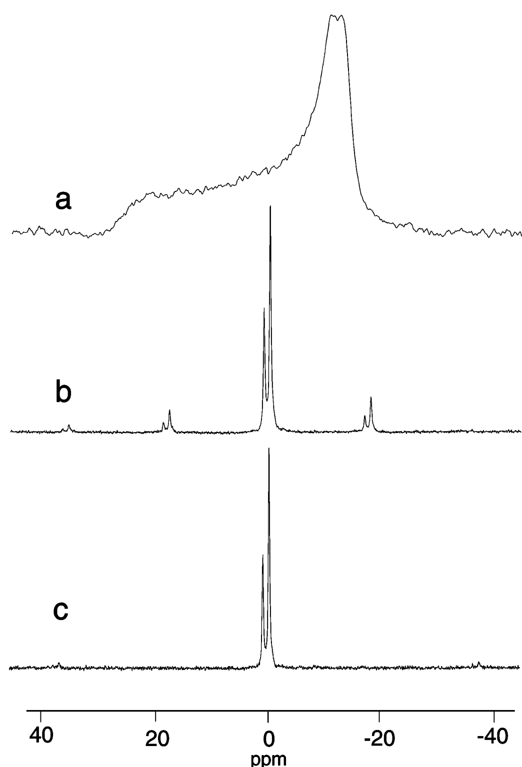

Fig. 1 ^{31}P NMR spectra at 400 MHz proton frequency of DOPC/DOPG MLV suspensions (2 : 1 molar ratio) in buffer B at 308 K: (a) static wideline spectrum; (b) MAS spectrum at 2.9 kHz spinning speed; (c) MAS spectrum at 6.0 kHz spinning speed.

averaged by fast axial rotation of the lipid molecules.[22–24] The spectrum itself is a superposition of two powder subspectra originating from the phosphorus nuclei of DOPG and DOPC. Because of the different headgroup structure for the two lipids, each powder spectrum is slightly shifted ($\sim$2 ppm) with respect to each other, as observed at the intense high field spectrum edge with two maxima at -11 and -13 ppm. Since the individual spectral contributions from the two lipids cannot be resolved due to the similarity in their effective CSA, an overall effective CS value of the order of 37 ppm is estimated, in good agreement with earlier studies.[22,24]

Mechanical rotation of the MLV suspensions about the magic angle (54.7°) with respect to the static magnetic field during NMR experiments facilitates averaging of the effective CSA and results in high-resolution-like spectra where the phosphates from the individual membrane lipids are well resolved according to their isotropic chemical shift values. At 2.9 kHz sample rotation (ω_r), the NMR spectrum obtained (see Fig. 1b) is composed of a pair of central resonances at 0.3 and -0.79 ppm, and accompanied by a series of spinning sidebands separated by multiples of the spinning rate, ω_r. At higher spinning rates ($\omega_r = 6.0$ kHz) these sidebands disappear (Fig. 1c), while the central resonances remain unchanged in position. At this speed a quantitative analysis can be carried out by integration of the indivual resonances with an intensity ratio of 2 : 1 reflecting the chemical composition of the bilayer. Since our studies focus on the isotropic chemical shift variations of each lipid component as a function of membrane surface charge, experiments were carried out at higher spinning speeds (depending on the ^{31}P Larmor-frequency).

^{31}P MAS NMR as a monitor of surface charge changes

Multilamellar suspensions of lipid mixtures containing different fractions of neutral DMPC and negatively charged DMPG, were studied by high-resolution ^{31}P MAS NMR. Spectra from DMPC/DMPG mixtures where the bilayer DMPG fraction was varied between 0 and 100 mol% in 20% steps are shown in Fig. 2 (left panel). Resonances from the individual bilayer components are well resolved with chemical shift values varying between -0.91 ppm for pure DMPC and 0.48 ppm for pure DMPG. The relative intensity of the individual spectral components reflects the chemical composition of the bilayer. Values of the FWHH between 7 and 14 Hz agree with earlier measurements[24,31] and reflect long transverse relaxation times T_2 and high lipid mobility in the liquid crystalline bilayers. Particularly important is the observation that the isotropic chemical shifts of both lipid resonances move downfield as the fraction of negative lipid is increased (see Fig. 3), changing from 0.27 ppm (20% DMPG) to 0.48 ppm for pure DMPG, and from -0.91 ppm for pure DMPC to -0.68 ppm (80% DMPG). The same sign of chemical shift

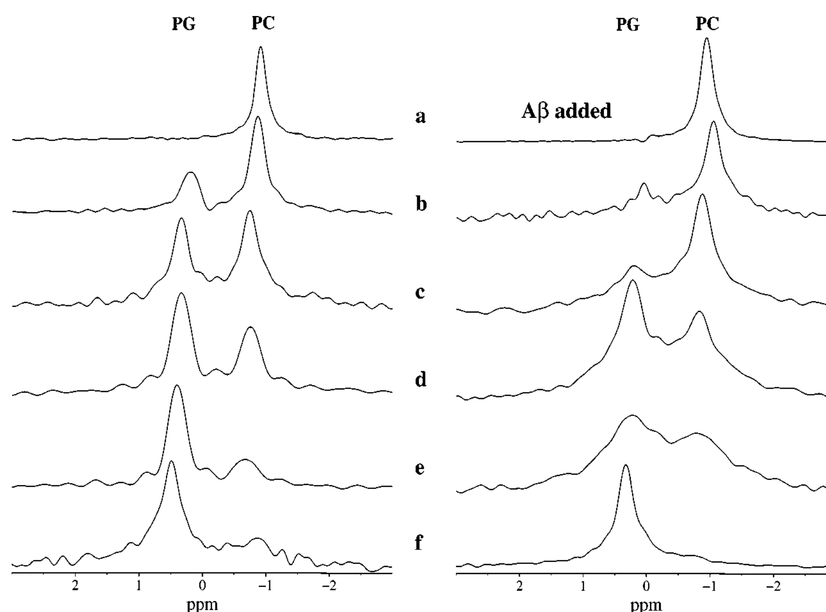

Fig. 2 Expanded ^{31}P MAS NMR spectra at 100 MHz proton frequency from DMPC/DMPG MLV suspensions before (left panel) and after addition of $A\beta_{1-40}$ peptide at 30 : 1 lipid to peptide molar ratio (right panel). The DMPC/DMPG molar ratio was (a) ∞; (b) 4 : 1; (c) 3 : 2; (d) 2 : 3; (e) 1 : 4; (f) 0. The MAS speed was maintained at 2.5 kHz and the temperature at 308 K.

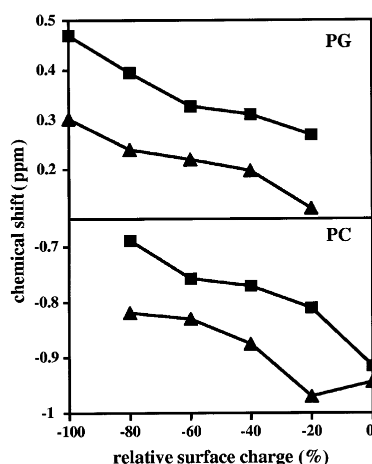

Fig. 3 Isotropic ^{31}P chemical shift values at 308 K for DMPG (top) and DMPC (bottom) as a function of the DMPC/DMPG lipid molar ratios (expressed as relative surface charge before binding) of the MLV before (squares) and after addition of $A\beta_{1\text{-}40}$ peptide (triangles) at 30 : 1 lipid to peptide molar ratio.

changes for both phosphates strongly indicates a common response to a change in the membrane surface charge.

Association of $A\beta_{1\text{-}40}$ peptide with charged membrane surfaces

The interaction between Aβ peptide and lipid bilayers, which has been implicated in peptide aggregation into neurotoxic plaques,[6,28,29,32] and its electrostatic nature were investigated by ^{31}P wideline and MAS NMR. For this purpose Aβ peptide was added to DMPC/DMPG vesicles (30 : 1 L/P molar ratio) and the molar fraction of the charged component, DMPG, was varied between 0 and 100% as in the pure lipid case. The wideline spectra from all mixtures in the presence of peptide showed no deviation from the powder distribution (spectra not shown), and confirm that the lipid system remains in the liquid crystalline lamellar phase upon addition of Aβ peptide.[6,22] Under fast MAS conditions the individual ^{31}P NMR resonances from each membrane lipid (Fig. 2, right panel) do not reveal any dramatic changes, *e.g.* no indication of a specific interaction between the peptide and a lipid component, but show a systematic variation in their chemical shift, akin to the observed variation in the absence of peptide. Only for neutral DMPC bilayers is no change observed, in agreement with earlier binding studies.[6] The relative intensity of the DMPG peak at a fixed PC/PG ratio had undergone a slight reduction compared to the pure lipid case, which has been interpreted previously as a preference of the peptide for the charged lipid species.[9] A slight increase in the FWHH of both lines, compared to the pure lipid case, suggests that Aβ peptide imposes slight restrictions on the motions of both phospholipid species. At a fixed lipid ratio an overall upfield change in the chemical shifts of both PG and PC after addition of the positively charged peptide is also observed, where, for example, the DMPG resonance at 80% charged lipid contents moves from 0.4 to 0.24 ppm, and the DMPC resonance, from −0.69 to −0.82 ppm. The chemical shift values for both lines are plotted in Fig. 3 alongside the corresponding values from the pure lipid system and reveal two clear features. First, the chemical shift values for both lipid components change in the same way. Second, upon binding, Aβ induces a general upfield shift for all lipid resonances in a way identical to that observed when the amount of negatively charged lipid is lowered. This effect reflects a compensation of the negative surface charge by the positive charged peptide residues upon binding,[32] except for neutral DMPC bilayers.[6] The chemical shift changes, induced upon increasing the acidic lipid fraction, appear to be offset by addition of cationic peptide.

Changes in the membrane surface charge induced by pentalysine

Results from ^{2}H NMR[16] and computer modelling[30] have suggested that the interaction of the highly basic (5 positive charges) peptide pentalysine with lipid bilayers is purely electrostatic. Wideline ^{31}P NMR showed, as for Aβ peptide, no changes in the lipid phase state upon addition of pentalysine, as expected from earlier studies.[16] High speed ^{31}P MAS NMR was used here to investigate how the addition of pentalysine at between 30 : 1 and 6 : 1 lipid to peptide molar ratio affects negatively charged lipid bilayers of DOPC/DOPG at 2 : 1 molar ratio. The NMR spectra are shown in Fig. 4, where the DOPG resonances are expanded in the left panel and the DOPC resonances, in the right panel. The overall linewidth

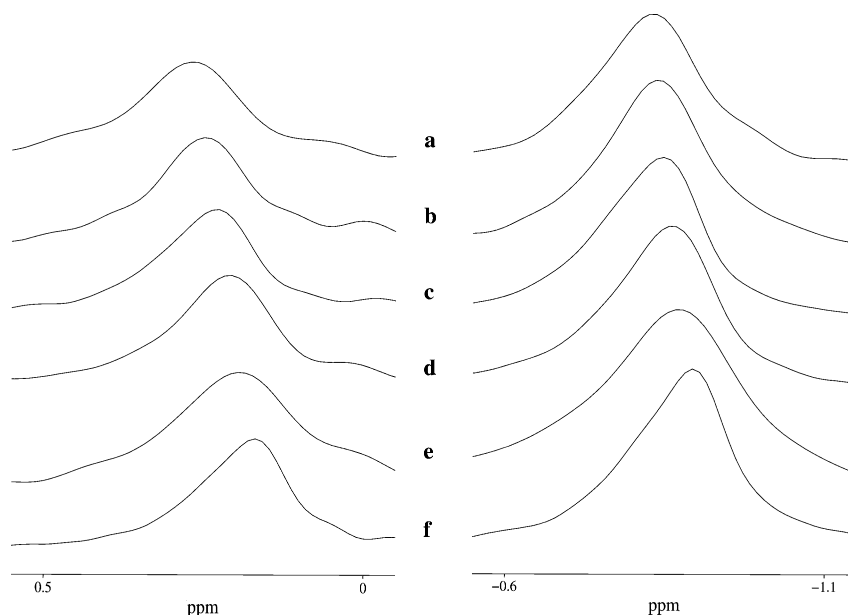

Fig. 4 Expanded ^{31}P MAS NMR spectra at 200 MHz proton frequency from DOPC/DOPG MLV before (a) and after addition of pentalysine at lipid to peptide molar ratios of: (b) 30 : 1; (c) 15 : 1; (d) 12 : 1; (e) 9 : 1; (f) 6 : 1. Left panel: DOPG; right panel: DOPC. The MAS speed was maintained at 2.6 kHz and the temperature at 308 K.

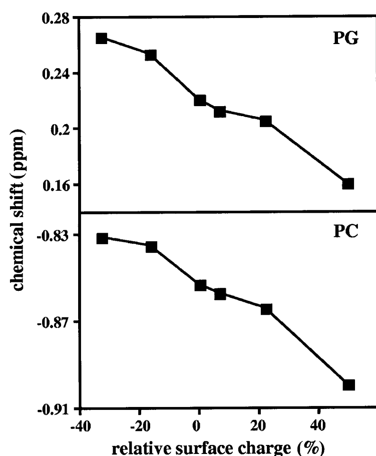

Fig. 5 Isotropic ^{31}P chemical shift values at 308 K for DOPG (top) and DOPC (bottom) as a function of the lipid to pentalysine molar ratio (expressed as relative surface charges for complete electrostatic binding).

appears to be unaffected by addition of the pentalysine. A slight deviation from pure Lorentzian lineshape is observed in the presence of pentalysine, similar to that reported for ^{31}P MAS NMR spectra from phospholipids in the presence of cytochrome *c*.[24] The asymmetry increases in a concentration-dependent way and most probably reflects distortions in the surface charge distribution in the immediate vicinity of the peptide.[30,33] There seems to be no change in the relative intensity of the PG *vs.* PC line, which suggests that there is no preferential association of pentalysine with any particular lipid species, in agreement with earlier experiments.[16] As plotted in Fig. 5, the isotropic chemical shifts of both DOPC and DOPG lines undergo an upfield change, akin to that induced by addition of positively charged Aβ peptide. Addition of pentalysine at a 6 : 1 lipid/peptide ratio induced chemical shift changes from 0.27 to 0.16 ppm for the DOPG line and from −0.83 to −0.90 ppm for the DOPC line, in comparison to pure lipids. The phospholipid chemical shift dependence on pentalysine concentration was analysed in terms of eqn. (1) and linear fits with slopes of −0.6 for PG and −0.4 for PC are shown in Fig. 6 alongside the experimental data. A good agreement between the experimental data and the model suggests that a simple fast two-site exchange mechanism between the bulk lipid and short-lived peptide-associated lipid domains is responsible for the observed changes in phospholipid chemical shift values.

Discussion

One common way in which peripheral proteins and peptides reach their biologically active membrane-bound state, involves initial electrostatic binding *via* positively charged domains to negatively charged lipid bilayers followed by membrane insertion of hydrophobic domains. Examples include apocytochrome *c*, myosin, cardiotoxin, nisin and the antibacterial peptide PGLa.[4,5,8–10] Proteins such as Src, K-RAS, MARCKS and HIV-I include clusters of basic residues (*e.g.* pentalysine in MARCKS), which establish electrostatic anchoring of these proteins to acidic lipid membranes.[7,30,33] Electrostatic interactions between Aβ peptide and acidic lipid bilayers have been implicated in the accelerated conversion of non-toxic peptide into neurotoxic aggregates.[6,28,29] The ability to describe quantitatively the electrostatic phenomena occurring in pure lipid membranes and the changes thereof resulting from membrane association of charged proteins or peptides is, therefore, of paramount importance.

For a long time the use of wideline ^{2}H NMR has been a successful approach for the investigation of changes in lipid headgroup conformation and dynamics in response to variations in the bilayer surface charge density.[1–3,12–20] This approach relies on detection of the changes in deuterium quadrupole splittings from specifically headgroup labelled phospholipid reporters induced by electrostatically driven reorientation of their headgroups. The method is sensitive to charge density variations due to a favourable orientation of the reporter groups in the most commonly used phospholipid marker, deuterated DMPC and offers a simple spectral interpretation in the liquid crystalline phase. However, it suffers from a few essential shortcomings, amongst which are the need for introduction of a synthetic labelled reporter lipid into the model membranes, a difficulty in resolving electrostatic effects from effects due to lipid dynamics, a complex spectral interpretation in cases of close values of the quadrupole splittings from the deuterium reporter groups and extremely complex spectral interpretation for protein or peptide-associated lipid fractions (domains) where the lipid motions are not axially symmetric.[9] In addition, the synthesis of deuterated lipid reporters can be difficult and, when possible, obtaining information from different bilayer components requires separate experiments to be performed on individually prepared samples containing the desired deuterated lipid.

High-resolution ^{31}P MAS NMR as shown here, can be used for the investigation of the modulation in the surface charge densities of lipid membranes. Systematic downfield changes in the isotropic chemical shifts of the membrane lipid phosphates have been correlated with an increase in the bilayer negative surface charge density. These changes have been quantified simultaneously for both lipid species in a two-component model membrane from natural abundance ^{31}P. The phosphates of most phospholipid species encountered in natural membranes produce spectroscopically well resolved lines at characteristic chemical shifts,[8,9,24] which allows for simultaneous and independent observation of the phospholipid constituents of multicomponent model membranes, as well as of phospholipids in native membranes.[34] Changes in membrane electrostatics are observed as changes in the isotropic chemical shift of all reporter species, while dynamic effects are reflected in the individual spectral linewidths and are, therefore, clearly distinguishable from each other. In cases where protein or peptide–lipid interactions result in new phases, high-resolution ^{31}P MAS NMR provides additional quantitative information on the phospholipid partitioning between the different phases.[9,27]

A direct comparison between ^{2}H wideline and ^{31}P MAS NMR as methods sensitive to membrane surface charge changes, is possible. The results presented here from DMPC/DMPG mixtures of different composition follow a change in effective negative charge per phospholipid molecule from 1 for 100% DMPG to 0 for 100% DMPC. If 60 Å^2 is taken as the average area per phospholipid molecule and is assumed to be the same for PC and PG, the investigated surface charge

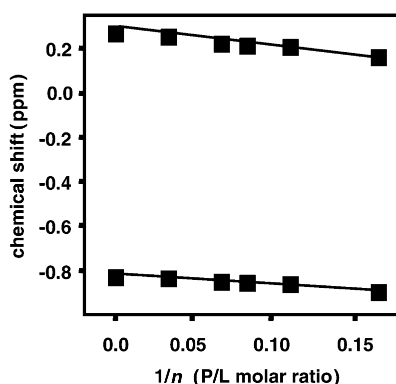

Fig. 6 Variation of the isotropic chemical shift values (DOPG top; DOPC bottom) as a function of the reciprocal of the lipid to pentalysine molar ratio ($1/n_t$) according to eqn. (1).

range is between -1.66 and 0 e nm^2 in steps of 0.33 e nm^{-2}. The chemical shifts of both lipid species respond similarly with DMPG showing a slightly stronger response than DMPC. A ^{2}H NMR study of the same system,[15] where headgroup deuterated lipids have been introduced in separate experiments, has also reported a monotonic effect of the change in effective negative charge per lipid molecule, approximately four times stronger from deuterated DMPC than from deuterated DMPG. Therefore, the two approaches complement each other and can be used independently for surface charge investigation.

Earlier studies have reported observations of electrostatic effects on ^{31}P isotropic chemical shifts of the same nature as discussed here, for example during binding of nisin or cardiotoxin to lipid membranes, without discussing in detail the underlying phenomena.[8,9] Analysis of ^{2}H NMR data from headgroup labelled DMPC has pointed to a surface charge-induced lipid headgroup reorientation, where changes in the electric field normal to the membrane surface affect the orientation of a molecular dipole formed by the positively charged quaternary nitrogen and the negatively charged phosphate.[3,35] Surface charges affect only the normal component of the electric field since rapid molecular rotation[31] and lipid diffusion average the in-plane components.[20] In contrast to deuterium quadrupole splittings, which respond directly to changes in chemical bond orientation, isotropic chemical shifts are orientationally independent and reflect only the distribution of electron density in the immediate vicinity of the observed nucleus.[36] The observed changes in chemical shift, therefore, arise most probably from a redistribution of electron density in direct response to changes in the electric field normal to the lipid bilayer (induced atomic polarisation).

The ^{31}P MAS NMR data presented here support the proposed electrostatic model for interaction between pentalysine and negatively charged lipid bilayers as seen in the good agreement between the charge dependence of the phosphorus-31 chemical shifts and the proposed fast exchange model. On the other hand the interaction of Aβ peptide with lipid bilayers includes not only an electrostatic but also a hydrophobic component. This can be inferred from the observed larger changes in the chemical shifts of both PC and PG upon addition of Aβ peptide than predicted for an increase in the molecular charge by only two electronic charges per molecule. Indeed, a close examination of the chemical shift values obtained during titration of PG shows that addition of Aβ peptide at 30 : 1 lipid/peptide molar ratio to bilayers of 80% PG for example, results in chemical shift values observed from the 20% PG mixture. Such a strong reversal of the charge effect cannot be explained by the addition of 3% positive charge. Also, the chemical shift dependence analysed in terms of eqn. (1) provides not a linear but a complex relationship. One possible explanation is that the observed apparent decrease in negative surface charge may result from a reduction in the surface density of the acidic lipid when the surface area of the bulky hydrophobic domain of the Aβ peptide and the possible surface insertion of its polar domain are taken into account. The balance between hydrophobic and electrostatic components in the Aβ peptide–lipid interaction ensures successful initial binding followed potentially by proper peptide incorporation into the membrane, a process most recently described by Kremer *et al.* who showed that peptide upon aggregation can penetrate bilayers but not in its initial monomeric form.[37] The ^{31}P MAS NMR results also confirm previous findings, based on circular dichroism (CD) measurements, that electrostatic binding of Aβ to charged membranes is essential to peptide aggregation.[6,28,29,32] Aggregation of the membrane inserted Aβ peptide is most likely triggered by hydrophobic interactions in a second step, akin to the observed folding of apocytochrome *c* on charged lipid membranes.[5]

One final remark on the applicability of ^{31}P MAS NMR is warranted. This concerns the sensitivity of the method to electric fields due to the presence of charges outside the lipid headgroup region. Both pentalysine and Aβ peptide belong to this category. Electrostatic adsorption of these peptides to negatively charged lipid membranes produces easily measurable effects, while no effect has been observed using wideline ^{2}H NMR from deuterated lipids.[6,16] In both cases the lack of response from the deuterated PC headgroups has been explained by the inability of a bilayer to accommodate a measurable headgroup tilt, most likely due to steric hindrance.[20] In contrast, the changes observed by ^{31}P MAS NMR reflect directly the magnitude of the electric field at the site of the reporter nucleus and are, therefore, insensitive to molecular rearrangements in the headgroup region.

Conclusions

The application of high-resolution ^{31}P MAS NMR as a method for monitoring changes in membrane surface charge density has been proposed. The method has been demonstrated on a model lipid system of variable surface charge and then has been applied to study the binding of positively charged surface active peptides to acidic lipid membranes. The interaction of pentalysine, a basic domain mediating MARCKS binding acidic membranes, with negatively charged bilayers has been shown to be purely electrostatic. The association of the amyloid-β peptide involved in Alzheimers disease, with acidic lipid bilayers has been investigated. An electrostatic component, implicated in the initial binding, has been identified in the interaction alongside a hydrophobic interaction, which has been suggested to facilitate Aβ peptide aggregation into neurotoxic plaques.

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