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Membrane interactions in small fast-tumbling bicelles as studied by ^{31}P NMR

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ABSTRACT

Small fast-tumbling bicelles are ideal for studies of membrane interactions at molecular level; they allow analysis of lipid properties using solution-state NMR. In the present study we used ^{31}P NMR relaxation to obtain detailed information on lipid head-group dynamics. We explored the effect of two topologically different membrane-interacting peptides on bicelles containing either dimyristoylphosphocholine (DMPC), or a mixture of DMPC and dimyristoylphosphoglycerol (DMPG), and dihexanoylphosphocholine (DHPC). KALP21 is a model transmembrane peptide, designed to span a DMPC bilayer and dynorphin B is a membrane surface active neuropeptide. KALP21 causes significant increase in bicelle size, as evidenced by both dynamic light scattering and ^{31}P T_2 relaxation measurements. The effect of dynorphin B on bicelle size is more modest, although significant effects on T_2 relaxation are observed at higher temperatures. A comparison of ^{31}P T_1 values for the lipids with and without the peptides showed that dynorphin B has a greater effect on lipid head-group dynamics than KALP21, especially at elevated temperatures. From the field-dependence of T_1 relaxation data, a correlation time describing the overall lipid motion was derived. Results indicate that the positively charged dynorphin B decreases the mobility of the lipid molecules – in particular for the negatively charged DMPG – while KALP21 has a more modest influence. Our results demonstrate that while a transmembrane peptide has severe effects on overall bilayer properties, the surface bound peptide has a more dramatic effect in reducing lipid head-group mobility. These observations may be of general importance for understanding peptide–membrane interactions.

1. Introduction

The membrane bilayer is a highly dynamic system with many motional modes. Lipid molecules undergo lateral diffusion within the bilayer [1–4], they rotate around their molecular axis, and possess several degrees of internal motion [5,6]. All these motions affect the interactions between a lipid molecule and a peptide or protein. Consequently it is of great interest to understand how proteins and peptides affect lipid properties including their dynamics. There are a variety of biophysical methods available that are suitable for studying these motions and the influence of proteins and peptides on lipid order and dynamics. NMR spin relaxation provides one way to examine the reorientational motion of individual lipid molecules, a parameter that may be critically altered by introducing components such as peptides to a bilayer.

Studies of dynamics, however, inevitably require suitable membrane mimicking models that are both realistic enough to properly reflect on true lipid bilayer motions, and at the same time provide simple enough systems to allow for detailed studies on a molecular level. Accordingly, small ($q \leq 0.5$) isotropic bicelles [7–9] have been developed for the purpose of investigating the behavior of peptides and membrane proteins in a membrane-like environment [10–15]. Bicelles are ideal for combining structural and membrane interaction studies, since their tumbling is isotropic and the reorientational diffusion of the lipids is fast enough to give reasonable solution-state NMR spectra for both the peptides and the lipids. In addition to the classical ^{1}H, ^{15}N and ^{13}C measurements used for structural studies of the membrane inserted (or bound) peptides, ^{31}P NMR [16] as well as ^{2}H NMR [17,18] have for a long time been used to determine the phase properties of lipid mixtures, including magnetically aligned bicelles [9,12,19–22], and to characterize the effect of bioactive peptides on lipid bilayers [11,23–25]. Several studies have been reported by Killian and coworkers [26,27] on the insertion of model transmembrane peptides into phospholipid bilayers in which peptide-induced membrane effects, such as hydrophobic mismatch and effect of flanking residues have been investigated [28–37]. The acyl chain dynamics in fast-tumbling bicelles and their dependence on interactions with peptides and proteins have earlier been studied by ^{13}C NMR relaxation methods [38,39] and the dynamics of phosphate head-groups in lipids have previously been studied by ^{31}P NMR relaxation [40–42] or a combination of NMR methods and molecular

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dynamics simulations [43,44]. To date, however, no attempts have been made to correlate the location of peptides with the effects that they have on lipid mobility or on assembly properties. To address this subject we have explored the approach of using a combination of ^{31}P relaxation and dynamic laser light scattering to characterize the effect of two topologically different peptides on the head-group dynamics and lipid assembly properties in zwitterionic DMPC/DHPC (hereafter denoted PC), and partly negatively charged (DMPC + DMPG)/DHPC (hereafter denoted PC/PG) bicelles. We examined the influence of two membrane-active peptides: the surface-bound neuropeptide dynorphin B (DynB) and the model transmembrane peptide KALP21.

Dynorphins, including DynB, derive from prodynorphin [45] and are primarily endogenous ligands to the κ-opioid receptor, but due to direct membrane interactions, they have non-opioid functions as well [46–50]. It has been demonstrated that the basic 13 residue long DynB, with the sequence YGGFLRRDFKVVT, interacts with different bilayers and resides on the bilayer surface, but remains relatively unstructured in the membrane-bound form [50–53]. The KALP peptide series were designed to contain a stretch of alternating leucine and alanine residues, providing a model hydrophobic transmembrane segment, flanked by lysine residues [26,32]. The 21 residue long KALP21, with the sequence Ac-GKKLALALALALALALALKKA-NH_2, has been shown to insert in a transmembrane fashion in DMPC bilayers, with no or little mismatch [27,37,54]. Both peptides contain a large number of positively charged residues, mostly arginines, and both have positive net charges (+2 for DynB and +4 for KALP21). Therefore, it is also of interest to examine the effects of these membrane-active protein fragments not only on zwitterionic (PC), but also on partly negatively charged bilayers (PC/PG).

The very different peptides chosen in this study are representatives for two ways in which peptides can interact with membranes, either located at the surface or inserted into the bilayer in a transmembrane configuration. Results based on ^{31}P T_1 and T_2 relaxation time data and dynamic light scattering demonstrate that there are large differences in how they affect lipid properties, such as overall reorientational dynamics of the lipid molecules and bicelle size. The data clearly suggest a correlation between the location of a peptide with respect to the bilayer and the effect that it has on lipid properties. Moreover, this correlation demonstrates that ^{31}P relaxation of phospholipids can be used to discriminate between a transmembrane and a peripheral configuration of a membrane-interacting peptide.

2. Materials and methods

2.1. Materials

Synthetic peptides corresponding to either the KALP21 or DynB amino acid sequence were obtained from PolyPeptide Group (Strasbourg, France) and used without further purification. Phospholipids, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (DMPG) and 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) were purchased as powder from Avanti Polar Lipids (Alabaster, AL, USA). All the lipids were used without further purification.

2.2. Preparation of bicelles

Solutions containing fast-tumbling bicelles were produced by mixing the appropriate amount of DMPC, or DMPC/DMPG (8:2) carefully with H_2O by vortexing and centrifuging the sample repeatedly until a homogeneous slurry was formed, and then adding an aliquot of a 1 M DHPC stock solution to obtain the molar ratio [DMPC] / [DHPC] = 0.5 (for PC bicelles) or [DMPC(0.2) + DMPG(0.8)] / [DHPC] = 0.5 (for PG bicelles) as described earlier [15,55,56]. This mixture was subjected to several cycles of heating (up to 45 °C) and cooling (to room temperature) and to gentle vortexing, until a clear non-viscous solution was obtained. The total concentration of DMPC and DHPC, or DMPC + DMPG and DHPC, was 150 mM (c_L (w/v) ≈ 8%). The pH was in all cases adjusted to 6.5–6.7 with 50 mM HEPES buffer.

For the samples containing the peptide, the necessary amount of lyophilized peptide (powder) was measured by weight and mixed with the bicelle solution. This mixture was subjected to freezing and lyophilization, after which it was dissolved in distilled H_2O. DynB samples resulted in clear, transparent solutions under all experimental conditions, while KALP21 samples of higher than 1 mM concentration could not be prepared with the charged bicelles, due to a permanent formation of precipitate. According to the GRAVY (Grand average of hydropathicity) solubility index of proteins, DynB has a value of −0.108, while KALP21 has 1.371. The positive number indicates a requirement of a hydrophobic environment, and the negative value a hydrophilic environment. Therefore the highly hydrophobic KALP21 was difficult to solubilize in negatively charged (PC/PG) bicelles above a concentration of 1 mM. NMR samples had the typical volume of 550 μl containing 10% D_2O.

2.3. Dynamic laser light scattering measurements

The size of the bicelles was determined with dynamic light scattering over a temperature interval of 25–45 °C. Measurements were performed on an ALV/CGS-3 instrument equipped with a Light Scattering Electronics and Multiple Tau Digital correlator ALV/LSE-5004, using a 5 mm glass cuvette. Light scattering data were collected for 10–30 s and repeated 10–30 times and were exported as autocorrelation functions. The results are presented as hydrodynamic radii, R_h, by relating derived decay constants via the Stokes–Einstein relationship:

$$R_h = \frac{k_B T}{6\pi\eta D} \quad (1)$$

where k_B is the Boltzmann's constant, T is the absolute temperature, η is the viscosity of the sample and D is the diffusion coefficient of the particles. For monodisperse particles undergoing Brownian motion D is obtained from the decay rate, Γ, of the autocorrelation function from DLS, given by $\Gamma = Dq^2$, where q (not to be confused with q = [DMPC] / [DHPC]) is the magnitude of the scattering wave vector. $q = (4\pi n/\lambda)\sin(\theta/2)$ is determined by the wavelength of the light source, λ, the refractive index of the medium, n, and the scattering angle θ. Data were processed with the software provided by the manufacturer.

2.4. ^{31}P NMR measurements

Measurements were performed on a Bruker Avance spectrometer (9.4 T) operating at 162.0 MHz for ^{31}P, equipped with a 5-mm BBO probe-head and a 500 DRX spectrometer (11.7 T) operating at 202.5 MHz frequency for ^{31}P using a 5-mm BBI probe-head. ^{31}P chemical shifts were referenced to an external 85% H_3PO_4 standard and temperatures were checked with the ethylene glycol standard. Chemical shift values and integrated intensities were determined from one-dimensional inverse gated $^{31}P\{^1H\}$ spectra.

T_1 measurements were recorded using the standard inversion recovery pulse-sequence with power gated decoupling in pseudo-2D mode. A typical set of 14 spectra with delays varying between 1 ms–6 s was acquired, and at least 3 sets of experiments were performed. T_2 measurements were performed using the Carr–Purcell–Meiboom–Gill pulse-sequence with power gated decoupling in pseudo-2D mode. Typically 12 spectra were collected with delays between 0.03 s and 0.58 s, and at least 3 sets of experiments were acquired. The delay between scans was 8 s and 32 scans were recorded using a 20 ppm spectral window for both T_1 and T_2 measurements. All relaxation measurements were performed at three temperatures: 25 °C, 42 °C and 59 °C.

Data evaluation was done using the relaxation module of the TopSpin software. Intensity values were fitted to the $I(t) = I(0)[1-2A\exp(-t/T_1)]$ equation for T_1 evaluation, and integrated intensities were fitted to