Role of peptide structure in lipid-peptide interactions: nuclear magnetic resonance study of the interaction of pentagastrin and [Arg^4]pentagastrin with dimyristoylphosphatidylcholine

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Complexes formed between dimyristoylphosphatidylcholine (DMPC) and the peptide pentagastrin or [Arg^4]pentagastrin were examined by ^31^P- and ^2^H-NMR. The cationic [Arg^4]pentagastrin produces larger changes in the lipid NMR spectra than does the anionic pentagastrin. ^31^P-NMR spectra of DMPC with [Arg^4]pentagastrin below the phase transition exhibits two components one of which is motionally restricted compared with the pure lipid. The exchange between these two lipid domains is slow on the millisecond time scale. The interactions between this peptide and phospholipid are diminished above the melting temperature of the complex. The ^2^H-NMR spectra of DMPC which had been labelled in a choline methylene group is also altered more by the [Arg^4]pentagastrin than by pentagastrin. In the presence of [Arg^4]pentagastin, even above the lipid phase transition, an additional doublet with a smaller quadrupole splitting is observed. These results clearly demonstrate the importance of peptide charge in determining the effects of peptides on lipid bilayers.

Keywords: pentagastrin; peptide-lipid interaction; deuterium NMR; dimyristoylphosphatidylcholine; phosphorous NMR.

Introduction

There has been increasing interest in the molecular interactions between small peptides and phospholipids [1–3]. In addition to providing an understanding of the forces which stabilize membrane and lipoprotein structures, these studies may also aid in understanding some of the factors which contribute to the potency of biologically active peptides. We have previously shown, using fluorescence spectroscopy [4] as well as differential scanning calorimetry and electron spin resonance [5], that a cationic analog of pentagastrin, \(^N-t\)-Boc-\(\beta\)-Ala-Trp-Met-Arg-Phe-NH\(_2\), interacts stronger and in a somewhat different manner with DMPC than does pentagastrin (\(^N-t\)-Boc-\(\beta\)-Ala-Trp-Met-Asp-Phe-NH\(_2\)), although the molecular basis for these differences is not fully understood. In this work, we show by nuclear magnetic resonance spectroscopy that the cationic analog of pentagastrin has a more pronounced effect on the DMPC headgroup than does pentagastrin. Signal sequences of membrane and secreted proteins are cationic [6, 7] as are numerous peptide hormones and toxins such as calcitonin and mellitin. The ability of these positively-charged peptides to partition into membranes may in part be a result of their tendency to interact with phospholipids.
Experimental procedures

Materials

DMPC and deuterium-labelled DMPC (protons of the CH\textsubscript{2}N\textsuperscript{+} methylene groups of choline substituted by deuterium) were purchased from Avanti Polar Lipids, Birmingham, AL. The deuterium-labelled lipid will be referred to as (β-d\textsubscript{2}) DMPC. The peptides were synthesized by Peninsula Labs, Torrance, CA. The purity of these substances was ascertained [4, 5]. Deuterium-depleted water was purchased from Aldrich Chem. Co. The position of deuteration of DMPC was confirmed by \textsuperscript{1}H-NMR using previously published assignments [8]. TLC analysis of this lipid showed one spot. Differential scanning calorimetry showed that the deuterated lipid was pure and had transitions similar to the fully protonated compound at 11.2 and 23.2°C. The latter transition had a van't Hoff enthalpy of 1200 kcal/mol.

Sample preparation

Lipid and peptide (6:1, mol ratio) were dissolved in chloroform/methanol (1:1). After evaporation of the solvent, PIPES buffer (20 mM PIPES, 150 mM NaCl, pH 7.4) in deuterium-depleted water was added to yield a final lipid concentration of 3 mM. The mixture was vortexed for approximately 5 min during which time the sample is warmed and cooled repeatedly through the transition temperature.

NMR spectroscopy

\textsuperscript{31}P-NMR spectra were obtained at 109 MHz with a broad band probe on a JEOL FX270 multinuclear Fourier transform NMR spectrometer. Spectra were obtained with a fully phase cycled Hahn echo sequence [9] and the transform was calculated from the top of the echo. The first pulse of the sequence was 10 μs. A spectral width of 50 kHz was used. Ten-millimeter tubes were used and 2048 data points were collected. Temperature was controlled at the indicated value (±1°C).

\textsuperscript{2}H-NMR spectra were obtained at 41 MHz using a solid echo on the same instrument under the same conditions as the \textsuperscript{31}P-NMR spectra. A spectral width of 50 kHz was used and 2048 data points were collected. Samples were contained in 10 mM tubes.

Results

\textsuperscript{31}P-NMR spectra were obtained of DMPC in the presence and absence of the peptides at 15°C (Fig. 1). The pure phospholipid shows the axially symmetric powder pattern typical of phospholipid bilayers [10, 11]. An axially symmetric powder pattern is the result of axial diffusion of the anisotropic \textsuperscript{31}P chemical shift tensor of the phospholipid phosphate. Axial diffusion occurs about an axis or director approximately perpendicular to the surface of the phospholipid bilayer. The \textsuperscript{31}P-NMR powder patterns produced by the phospholipid are therefore indicative of the motion experienced by the phospholipid headgroup.

The influence of the two peptides used in this study on the motional properties of the DMPC headgroup was examined using \textsuperscript{31}P-NMR powder patterns of the phospholipid. The \textsuperscript{31}P-NMR pow-

![Fig. 1. 109 MHz \textsuperscript{31}P-NMR spectra of DMPC in the presence and absence of peptides at 15°C. Bottom is pure DMPC; middle is DMPC plus pentagastrin; top is DMPC plus Arg peptide. The latter two samples have a 6:1 lipid to peptide molar ratio.](image-url)
der pattern for the DMPC in the presence of pentagastrin is axially symmetric identical to that observed for the pure phospholipid, within the signal-to-noise limitations of the experiment. Also shown in Fig. 1 is the effect of the Arg peptide on the $^{31}$P-NMR powder pattern of the DMPC. The axially symmetric powder pattern of the normal bilayer DMPC can be detected in this spectrum. However, that axially symmetric powder pattern does not fully account for the spectrum observed. Additional resonance intensity is observed on the upfield side of the main resonance.

To understand this interesting spectral shape, the following operation was performed. It appears that a normal axially symmetric powder pattern may be part of the resonance. Therefore, the spectrum obtained from the pure DMPC was subtracted from the spectrum obtained of the phospholipid in the presence of the Arg peptide. The magnitude of the "pure bilayer" spectrum that should be subtracted was determined from the shape of the resulting difference spectrum. Increasing magnitudes of the bilayer spectrum were subtracted, in real time computations, until the difference spectrum showed significant negative deflections. Then the magnitude of the bilayer spectrum subtracted was decreased just to the point of the disappearance of the negative deflections. This was taken as representative of a maximum contribution of the "pure bilayer" spectrum to the overall resonance shape. The result of this subtraction (Fig. 2) suggests that there may be two powder patterns contributing to the spectrum observed in Fig. 1 for the DMPC in the presence of the Arg peptide. One powder pattern is that obtained from pure phospholipid bilayers. The other is that represented in Fig. 2. The powder pattern represented in Fig. 2 is indicative of considerable motional restriction. In particular, it can be representative of a slow axial diffusion, much slower than that observed for pure phospholipids in a fully hydrated bilayer. The observation of two separable powder patterns for the DMPC in the presence of the Arg peptide indicates that the exchange of phospholipids between the two domains represented by these powder patterns is slow on the millisecond time scale.

The temperature dependence of this phenomenon was then measured. Previous calorimetric and fluorescence studies suggested that interactions between the Arg peptide and DMPC was enhanced in the gel state, that a phospholipid domain with a higher melting temperature than pure DMPC was created by the Arg peptide, and that the interactions between the Arg peptide and the DMPC are diminished above that melting temperature [4, 5]. Therefore, it is of interest to know whether the presence of the additional powder pattern in the $^{31}$P-NMR spectra is in any way altered by raising the temperature.

Figure 3 shows the results of a temperature
study. The $^{31}$P-NMR spectra obtained between 15°C and 40°C are shown. The resonance intensity protruding on the upfield side of the axially symmetric powder pattern is an indicator of the presence of the powder pattern presented in Fig. 2. Such a feature is not observed in the $^{31}$P-NMR spectra of pure DMPC [12]. The contribution of the non-axially symmetric powder pattern was estimated by fitting the observed spectrum to a sum of the spectrum of the pure lipid, plus that of the broadened component shown in Fig. 2. The contribution of this latter component is reduced at elevated temperature. It is approximately 50, 40, 30 and 5% of the total spectrum at 15, 20, 30 and 40°C, respectively.

$^{31}$P-NMR studies suggest that the Arg peptide produces a domain of phospholipids whose headgroups undergo different motional behavior than found for phospholipids in bilayers. $^2$H-NMR can provide additional information on phospholipid headgroup motional behavior. Therefore, DMPC labelled with deuterium in the choline methylene groups was used in a study of the interactions of these peptides with the DMPC. For these studies, only measurements above the gel-to-liquid crystalline phase transition temperature of the DMPC are shown. This is because below the phase transition temperature, the spectra are too broad to adequately obtain this spectra with the instrumentation available to this study.

$^2$H-NMR spectra of the deuterium-labelled ($\beta$-d$_2$) DMPC were obtained for the pure phospholipid. The spectrum at 30°C appears in Fig. 4. One doublet is observed. Galley et al. [13] obtained a doublet with a similar quadrupole splitting for ($\beta$-d$_2$) DPPC. A similar, although slightly broadened, spectrum is obtained in the presence of pentagastrin. The $^2$H-NMR spectrum of the deuterium-labelled DMPC in the presence of the Arg peptide also appears in Fig. 4. A distinctly different spectrum is obtained. Two doublets are observed. One corresponds to the quadrupole splitting of pure DMPC (outer peaks). The other doublet represents a smaller quadrupole splitting. Thus there are two spectral components in the presence of the Arg peptide.

Discussion

Our present results demonstrate that N-t-Boc-$\beta$-Ala-Trp-Met-Arg-Phe-NH$_2$ has a more marked effect on the DMPC headgroup order and motion than does N-t-Boc-$\beta$-Ala-Trp-Met-Asp-Phe-NH$_2$. The relatively greater effects of the arginine-containing peptide is in accord with a number of other observations [4, 5]. However, the differences between the $^2$H and $^{31}$P-NMR spectra for the mixtures of the two peptides with DMPC is more pronounced (Figs. 1 and 4) than the differences observed by other techniques. The apparent dissociation constant derived from fluorescence titration studies for lipid binding of the Asp and Arg peptides, respectively, is 0.81 and 0.50 mM at 8°C and 2.08 and 1.18 mM at 40°C [4]. The Stokes shift for tryptophan fluorescence is 17.0 and 18.5 nm at 8°C and 10.9 and 15.0 nm at 40°C for the Asp and Arg peptides, respectively [4]. Thus both the binding affinity and depth of penetration of N-t-Boc-$\beta$-Ala-Trp-Met-Asp-Phe-NH$_2$ and N-t-Boc-$\beta$-Ala-Trp-Met-Arg-Phe-NH$_2$ with DMPC are not dramatically...
different, although the interaction with the Arg peptide is slightly greater. Also, the ability for these two peptides to alter the properties of DMPC are qualitatively similar. For example, if we compare the phase transition properties of peptide-DMPC mixtures at a peptide mole fraction similar to that used in this work (compare Figs. 4b and 6a in Ref. 5), both transition curves indicate the presence of two lipid populations; one melting slightly below and one above the transition temperature of pure lipid. Again, the extent of the temperature shift for the higher melting lipid component is greater for the Arg-containing peptide (see Fig. 5, Ref. 5). It should be also noted that for the peptide/lipid ratio used in the present work, NMR spectra taken at 20°C or below represent gel state lipid, while 30°C or above is liquid crystalline state lipid. Thus the difference in the NMR properties of the two lipid-peptide systems does not arise from a difference in their phase behaviour as measured by differential scanning calorimetry [5]. The difference in motional properties of DMPC, particularly in a gel phase, with either of the two peptides were also clearly demonstrated by electron spin resonance using a 5-deoxystearic acid spin-label [13]. In addition to the large apparent shift of the transition temperature, the Arg-peptide causes a greater degree of immobilization of the probe at temperatures beyond the phase transition region. However, besides the usual caveats about using a bulky nitroxide probe [15, 16], the use of an anionic fatty acid probe to study the effects of a cationic peptide may be particularly prone to artifact as a result of sequestering of the probe around the Arg-peptide.

The effects of the Arg-peptide on the motional properties of the lipid have some similarities to the effects of some peptides and integral membrane proteins on lipid motion. Certain hydrophobic peptides [17] and membrane proteins [18] as well as the Arg-peptide, narrow the quadrupolar splittings of deuterium-labelled phospholipids. The effects of the proteins are generally somewhat greater and extend to positions of the lipid molecule deeper in the membrane. However, using d$_{27}$-DMPC (results not shown) narrowing of the $^2$H quadrupole splitting from acyl chain positions in the presence of the pentagastrin peptides was also observed. A separate component in the $^{31}$P-NMR spectra for protein-bound lipid is also observed with integral membrane proteins [19, 20]. However, the shape of the powder pattern is somewhat different for the two cases. Both the $^{31}$P- and $^2$H-NMR results indicate the presence of two components in the mixtures of DMPC with the Arg-peptide. One of the components is similar to pure DMPC while the other lipid must be more strongly influenced by the presence of the peptide. The ability to observe NMR spectra with two components indicates that the peptide-bound and bulk lipid components are exchanging slowly on the ms time scale.

Previous fluorescence measurements indicated that these peptides have a stronger interaction with DMPC in the gel state than they do with this lipid in the liquid crystalline state [4]. This observation is reinforced by the temperature dependence of the perturbed lipid component using $^{31}$P-NMR (Fig. 3). The lipid whose $^{31}$P-NMR powder pattern is altered by the presence of peptide is still present above the lipid phase transition but is a smaller fraction of the total lipid. There are several examples of peptides which exhibit preferential interaction with gel state lipid [14].

In summary, the present NMR results demonstrate the marked influence of charge on the effects of peptides on lipid properties. The observation that a cationic peptide has a much stronger influence on the zwitterionic DMPC headgroup than does the anionic peptide suggests that the lipid is acting like a negatively-charged substance. It has been noted that the phase transition behaviour of phosphatidylcholines resemble more closely the behaviour of repulsive anionic lipid than they do neutral lipids [21]. The reason for this is not apparent but it does explain both the phase transition behaviour of DMPC as well as its stronger interaction with a cationic peptide.
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References