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Interaction of prostatic acid phosphatase fragments with a lipid bilayer as studied by NMR spectroscopy

Andrey Filippov,*[a,b] Aidar Khakimov,[a] Sergii Afonin[c] and Oleg N. Antzutkin[b,d]

[a] *Institute of Physics, Kazan (Volga Region) Federal University, 420008 Kazan, Russian Federation. Fax: +7 843 231 5189; e-mail: Andrey.Filippov@kpfu.ru*
[b] *Chemistry of Interfaces, Luleå University of Technology, SE-97187 Luleå, Sweden*
[c] *Karlsruhe Institute of Technology, 76021 Karlsruhe, Germany*
[d] *Department of Physics, Warwick University, CV4 7AL Coventry, UK*

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The effects of five fragments of prostatic acid phosphatase on dimyristoylphosphatidylcholine lipid multi-lamellar liposomes were studied by ^{2}H and ^{31}P NMR spectroscopy and those on planar supported multi-bilayers of the same lipid, by ^{1}H and ^{31}P NMR spectroscopy. It was found that hydrophobic interaction is a dominated factor of the peptide–membrane binding, while the short α-helical fragments PAP(262-270) and PAP(262-272) strongly interact with the membrane at the interface, generally following to the Gibbs free energy of water-to-interface insertion.

Prostatic acid phosphatase (PAP) is a protein present in human seminal fluid. PAP plays an important role in fertilization and infectivity by the HIV virus due to its interaction with cellular membranes.[1,2] A 39-amino-acid fragment within PAP, PAP(248–286), forms amyloid fibrils,[1] which can significantly increase the risk of HIV infection by promoting virus attachment to the host cell. These amyloid fibrils, known as semen-derived enhancer of viral infection (SEVI), are thought to act as polycationic bridges, neutralizing a negative charge on the membrane surface between the viral capsid and the host cell membrane.[3] Because of the functioning of PAP peptide implemented cellular membrane, it is of importance to study the interaction of the peptide and its fragments with lipid membranes. A literature survey reveals that this interaction has been studied earlier but mainly in SDS micellar solutions.[4–6] In this work, we applied NMR technique to study changes in the ordering and local dynamics of lipid molecules of zwitterionic dimyristoylphosphatidylcholine (DMPC) due to their interaction with five structurally different PAP fragments (Table 1).[†]

Figure 1 shows the ^{2}H and ^{31}P NMR spectra of vesicular peptide–DMPC systems. The presence of PAP fragments does not change the quadrupolar splitting of ^{2}H NMR spectra, which remain the same as that of pure DMPC,[10] and may be characterized by the C–D bond order parameter $S_{CD} \sim 0.20$. The ^{31}P NMR 'powder' spectra demonstrate that peptide–DMPC systems maintain in a lamellar phase typical of pure DMPC.[10] However, small changes occur in the ^{31}P NMR spectra in the presence of PAP fragments, which may be arranged in the order PAP(248-261) < < PAP(262-270) < PAP(262-272) < PAP(248-286) < PAP(274-284). Thus, the presence of PAP fragments did not affect the ordering of hydrocarbon chains; however, the orientation and/or mobility of the phosphate groups of lipid molecules changed.

It is known that the ^{1}H and ^{31}P NMR spectra can be more sensitive to changes produced by macromolecules in a macroscopically oriented lipid in comparison with the spectra of vesicles.[8,11,12] The ^{31}P NMR spectra of the oriented multi-bilayers are presented in Figure 2. For pure DMPC, the spectrum reveals a broad symmetric peak centred at 0 ppm, which corresponds to

[†] DMPC and DMPC with deuterated hydrocarbon chains (DMPC-d_{54}) were purchased from Avanti Polar Lipids (Alabaster, AL). The peptide synthesis was made using a 0.1 mmol automated fast Fmoc solid phase procedure and HBTU activation[7] on an ABI 433A peptide synthesizer (Applied Biosystems, Foster City, CA). Separation of the peptide substrate and the protecting groups was carried out in a mixture containing trifluoroacetic acid. The peptide was purified using a Series 200 Perkin–Elmer HPLC System instrument equipped with a Vydac C18 column (Grace, IL). Purity of the final products (>98%) was characterized using MALDI-TOF mass spectrometry. Five different PAP fragments were synthesized (Table 1) keeping in mind their different secondary structures in SDS micellar solutions.[4] The peptide/DMPC weight ratio was 0.025 in all of the test samples, which corresponds to ~7 amino acids per lipid molecule.

To prepare a vesicular sample, the lipid was dissolved in a sufficient amount of methanol. The methanol was then evaporated under a constant flow of dry nitrogen. Finally, the sample was vacuum-pumped overnight to remove solvent traces. The resulting lipid film was hydrated by thoroughly mixing with an aqueous peptide solution (lipid to solution, 1:1 by weight). Afterwards, five freeze–thaw cycles were applied using liquid nitrogen and warm (40 °C) water, which resulted in the formation of a homogeneous sample of multi-lamellar vesicles. Before the measurements, samples were incubated overnight in the dark at a temperature higher than the gel–liquid crystalline transition temperature (24 °C for DMPC) for equilibration.

Macroscopically glass-supported oriented multibilayers of DMPC were prepared in accordance with a published procedure.[8] DMPC dissolved in ethanol was mixed with an aqueous peptide solution and deposited onto glass plates. Then, the solvent was evaporated and the plates were placed in a high vacuum overnight to remove the traces of ethanol. Afterwards, around 40 plates were stacked on the top of each other and placed in a glass tube with a square cross-section. The sample tube was then sited into a humid atmosphere of saturated D_2O vapour and kept there at a temperature higher than the gel–liquid transition of DMPC for three to five days. During this time, hydrated and oriented multi-bilayers were formed. Hydration was controlled by weighing. During that time bilayers absorb water (up to ~50%), that is sufficient just to be saturated (35–50 wt%)[9] and not to change their properties at a higher water content. Finally, the tube was sealed and left for several hours for final equilibration.

A Chemagnetic InfinityPlus NMR spectrometer (Agilent) operating at a proton frequency of 359.2 MHz was used for oriented samples, while Bruker AVANCE III operating at a proton frequency of 400 MHz was used for suspensions. The ^{31}P NMR spectra were recorded at 35 °C using a single pulse excitation without proton decoupling. For measurements on oriented lipid multi-bilayers, a square sample tube containing the sample was placed in a specifically designed goniometer probe that enabled the bilayers to be oriented with the bilayer normal at any desirable angles with respect to the magnetic field of the spectrometer.

Table 1 Characteristics of the test peptides and lipid–peptide systems.

Peptide	Lipid–peptide system	Charge (pH 7)	Number of amino acids	Peptide molecular mass/Da	Peptide to lipid ratio[a]	% helix[4]	ΔG_{if}[15]/ kcal mol^{-1}
PAP(248-286)	GIHKQKEKSRLQGGVLVNEILNHMKRATQIPSYKKLIMY	6.2	39	4551	1:286	35	4.43
PAP(248-261)	acetyl-GIHKQKEKSRLQGG-amide	3.1	14	1606	1:95	0	3.87
PAP(262-270)	acetyl-VLVNEILNH-amide	–0.9	9	1091	1:64	100	–4.41
PAP(262-272)	VLVNEILNHMK	0.1	11	1350	1:80	81	–1.06
PAP(274-284)	acetyl-ATQIPSYKKLI-amide	2.0	11	1302	1:77	45	–3.2

[a]This work.

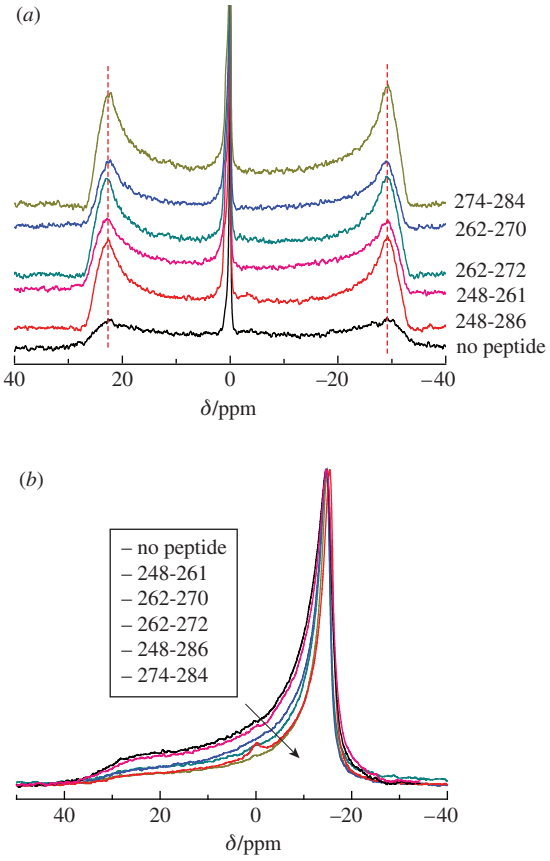

Figure 1 (*a*) ^{2}H NMR and (*b*) ^{31}P NMR spectra of DMPC multi-lamellar liposomes without and with PAP fragments. (DMPC-d_{54})/DMPC ratio, 1:3; peptide/DMPC weight ratio, 0.025. Water concentration, 50 wt%. An arrow shows the order of spectra listed.

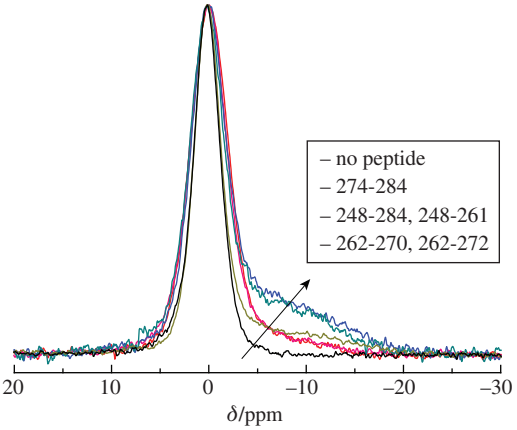

Figure 2 ^{31}P NMR spectra of DMPC planar supported multi-bilayers without and with PAP fragments. The angle of bilayer orientation is MA (54.7°). Peptide/DMPC weight ratio, 0.025. Water concentration, 50 wt%. An arrow shows the order of spectra listed.

the mean orientation of the phosphate groups at a magic angle (MA). In the presence of peptides, a wide shoulder appears similarly to that observed earlier in the presence of polyethylene oxide.[12] The effect is stronger, as compared to that observed in powder spectra [Figure 1(*b*)], and it increases in the order PAP(274-284) < PAP(248-286) ~ PAP(248-261) < PAP(262-270) ~ ~ PAP(262-272). A position of the shoulder centre is around –12 ppm, which corresponds to a maximum of the powder spectrum because the shoulder is related to headgroups randomly distributed over all possible angles.[12]

Figure 3 shows changes in the ^{1}H NMR spectrum of DMPC as a function of the orientation of bilayers near the MA, which was measured as reported previously.[8,11] Signals from chains and heads of the lipid in peptide–DMPC samples decay more slowly than that in pure DMPC [Figure 3(*b*)]. This validates the worsening orientation of DMPC molecules in the presence of peptides in the order PAP(274-284) < PAP(248-286) ~ PAP(248-261) < < PAP(262-270) < PAP(262-272). This order is similar to that observed in ^{31}P NMR measurements (Figure 2).

For the first sight, data for lipid chains in Figure 3(*b*) are inconsistent with ^{2}H NMR spectra [Figure 1(*a*)] because they demonstrate disordering of chains with a magnitude depending on the used peptide. However, note that some disordering in lipids is proper to the flat oriented lipid membranes. Alongside with the anisotropy of molecular rotation, this gives a contribution to the broadening of ^{31}P NMR spectra (Figure 2). Therefore, angular dependences in Figure 3(*b*) demonstrate worsening lipid molecules orientation in the presence of peptides. On the other hand, Figure 2 shows inhomogeneity in the orientation of phosphate groups of DMPC in a domain-like type. Only a part of phosphate groups keeps their orientation at the normal to the bilayer, while another part of the phosphate groups changes their orientation in the presence of peptides.

Changes in the ^{31}P NMR spectra of DMPC vesicles in the presence of PAP fragments (Figure 1) resemble those observed earlier for the same lipid with adsorbed Cytotoxin I.[13] To describe these alterations, a model of prolate phospholipid liposomes, which formed due to interaction of homogeneous diamagnetic vesicle surface with the constant magnetic field of magnet of NMR spectrometer was proposed. However, it is not the case of our PAP–DMPC inhomogeneous bilayers in dense suspensions of multi-lamellar vesicles. Evidently, PAP fragments destabilize the polar part of DMPC vesicles and worsen the order of lipid molecules in their glass-supported oriented multibilayers.

To characterize the binding of PAP fragments to the lipid membrane interface, we applied an approach used earlier[14] for short antimicrobial peptides. The Gibbs free energy of water-to-interface insertion ΔG_{if} was suggested as a sum of two terms, representing binding in an unfolded state and folding at the interface according to their α-helix content. ΔG_{if} of the peptide-to-membrane binding was calculated with the membrane protein explorer (MPEx[15]). The α-helix contents for different fragments of the PAP(248-286) peptide were published elsewhere.[4] The charges of Asp and Glu amino acid residues were taken negative,

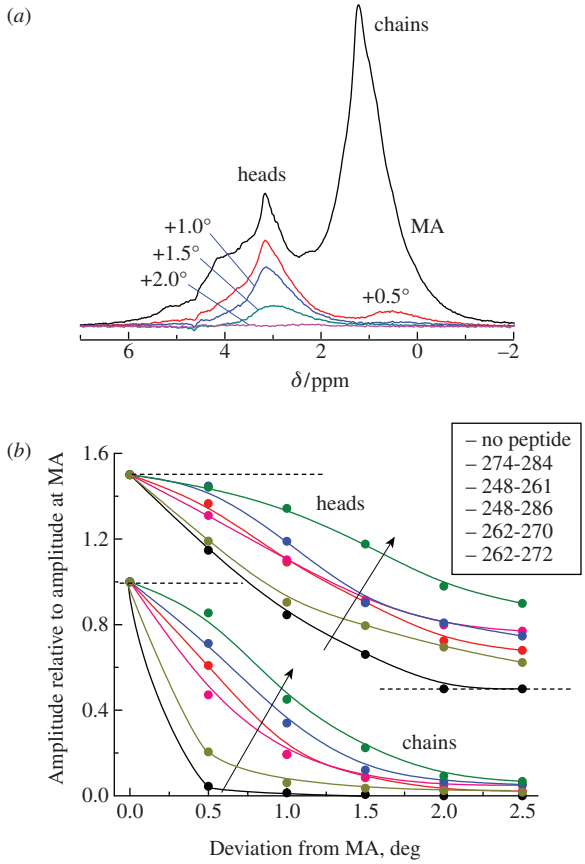

Figure 3 (*a*) ^{1}H NMR spectra obtained after the Fourier transformation of the descending half of stimulated echo of DMPC bilayers without peptides oriented at MA and at angles subsequently deviating from MA by 0.5°. (*b*) Dependences of heads and chain amplitudes as a function of the deviation of peptide–DMPC bilayers on the deviation from MA. Arrows indicate the order of curves corresponding to the PAP fragments listed.

while the charges of Lys and Arg were taken positive, and His was neutral at pH 7.4. The values of ΔG_{if} are listed in Table 1.

The ^{2}H and ^{31}P NMR data show that all of the studied PAP fragments bind to the interface of the DMPC membrane. It is evident (Table 1) that electrostatic interaction is not a dominating factor for the peptide–membrane interaction because strongly charged PAP(248-286) and PAP(248-261) are bound rather weakly. PAP(262-272) and PAP(262-270) have a minor charge. These two peptides bind almost equally; thus, the acetylation and amidation of PAP(262-270) has almost no influence on the binding. Previously, the major role of hydrophobic interactions in the PAP(266-272)–sodium dodecyl sulfate system was determined.[6] Based on NMR chemical shift data, the highly hydrophobic cluster Ile-Leu was identified as the peptide part mainly responsible for the binding.[6] Evidently, for the neutral membranes of DMPC, hydrophobic interaction should be effective even in a higher degree. Calculations with MPEx, which take into consideration the charges and hydrophobicity of amino acid residues, as far as the helicity of the peptide, demonstrated a correlation between ΔG_{if} (Table 1) and the effect of peptide on the membrane (Figures 2 and 3). However, this approach predicts different ΔG_{if} for PAP(262-272) and PAP(262-270), which really bind equally, and also provides low ΔG_{if} (strong binding) for PAP(274-284), while the last has only a minor effect on the membrane. Limitations of the MPEx program in the calculations of ΔG_{if} for δ-lysin and cecropin A were illustrated previously.[14]

Our results generally agree with previous observations,[4,5] demonstrating that PAP(248-286) peptide rather weakly interacts with a lipid membrane. At the same time, the short α-helical fragments of PAP(262-270) and PAP(262-272) strongly interact with the membrane interface. Hydrophobic interaction is a dominating factor for the test PAP fragments and DMPC membrane interaction.

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