ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2013, **23**, 313–315

Mendeleev Communications

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*

DOI: 10.1016/j.mencom.2013.11.002

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} ~ 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.

© 2013 Mendeleev Communications. All rights reserved.