

Adsorption of DNA on lipid/polyelectrolyte covered support

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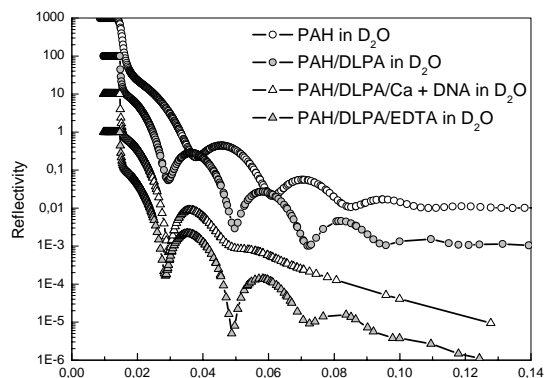
The interaction between polyelectrolytes (DNA) and lipids plays a crucial role in the construction of artificial viruses as gene delivery systems. As the interaction of positively charged lipids with DNA is so strong that it acts toxic for the target cell, negatively charged and zwitterionic phospholipids are of special interest. In this work two systems were studied by neutron reflectometry: the adsorption of DNA to the zwitterionic DMPC and to the negatively charged DLPA. The experiments were performed in presence and absence of calcium ions. Both experiments exhibited the formation of smooth bilayers on the particular polyelectrolyte and no adsorption of DNA in absence or presence of calcium.

Hollow polyelectrolyte (PE) capsules can be built, using the layer-by-layer technique. They can be used as model systems for artificial viruses, which are determined to deliver genetic material into target (cancer) cells. To guarantee an uptake of such capsules into the cell, the capsules are covered by a lipid bilayer. To understand the interaction between lipid and polyelectrolyte (e.g. PSS, PAH, DNA) its structural molecular basis has to be studied. Unique information about the interaction between the three components at their individual interfaces, i.e. PE/lipid, PE/aqueous phase or lipid/aqueous phase can be achieved by neutron reflectometry.

With cationic lipids it was shown that one can easily form stable and structured complexes with DNA^{1,2}. Unfortunately cationic lipids are frequently toxic for cells, which exclude their use in the construction of artificial viruses. Complexes composed of neutral or negatively charged lipids offer an alternative as they are completely non-toxic. The neutral lipids do not interact with DNA directly, but the interaction can be mediated by divalent cations^{3,4}. Recent studies on lipid monolayers at air/water interface using infrared reflection absorption spectroscopy (IRRAS) and grazing incidence X-ray diffraction (GIXD) showed that DNA can couple to zwitterionic DMPE (1,2-dimyristoyl-phosphatidylethanolamine) monolayers in presence of magnesium⁵.

In the present experiments the adsorption of 1 mM DNA + 10 mM NaCl on a lipid covered support was examined above the phase transition temperature of the lipid. NaCl was added to increase the stability of the DNA. The lipid dispersion adsorbed to the polyelectrolyte support had an initial concentration of 0,5 mg/mL and the size of the vesicles prepared by ultrasonication and followed extrusion was 50 nm. Neutron reflectometry experiments were carried out in a solid/liquid experimental cell in ToF mode at the reflectometer AMOR at SINQ, PSI. The reflectivity from the sample was scanned over the q-range up to 0.15 Å⁻¹ at three incident angles of the incoming beam. Two systems were studied: A) The zwitterionic phospholipid DMPC (1,2-dimyristoyl-phosphatidylcholine) was adsorbed onto a negatively charged PSS (poly(styrene sulfonate)) terminated polyelectrolyte cushion, resulting in the formation of a smooth bilayer with a thickness of 4.5 nm. In a following step DNA was injected into the experimental

cell and incubated for 1 h. Subsequently non-adsorbed DNA was washed out with D₂O. In the reflectivity curve no change was observed indicating no distinct adsorption of DNA to the DMPC bilayer. B) The negatively charged phospholipid DLPA (1,2-dilauroyl-phosphatidic acid) was adsorbed onto a positively charged PAH (poly(allyl amine hydrochloride)) terminated polyelectrolyte cushion. A smooth bilayer was built with a thickness of 5.0 nm. DNA was injected and after an hour, non-adsorbed DNA was washed out with D₂O. An adsorption of DNA could not be observed. Subsequently the influence of Ca²⁺ and DNA was studied. It was found that Ca²⁺+DNA doesn't change the thickness of the DLPA bilayer but increases the roughness. No adsorption of DNA to the lipid bilayer could be achieved. In an additional step, 50 mM EDTA was introduced causing a decrease of the roughness compared to the presence of Ca²⁺ and DNA. Also in this experiment no change of the layer thickness was observed (see Fig.).



- [1] P. L. Felgner, T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold and M. Danielsen, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 7413.
- [2] J. O. Rädler, I. Koltover, T. Salditt and C. R. Safinya, *Science*, 1997, **275**, 810
- [3] V. G. Budker, A. A. Godovikov, L. P. Naumova and I. A. Slepneva, *Nucleic Acids Res.*, 1980, **8**, 2499.
- [4] D. Huster, G. Paasche, U. Dietrich, O. Zschörnig, T. Gutberlet, K. Gawrisch and K. Arnold, *Biophys. J.*, 1999, **77**, 879.
- [5] S. Gromelski and G. Brezesinski, *PCCP*, 2004 (in press)

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