



Experiment title: Investigation of ionization state of the Langmuir monolayer of novel cationic lipids for gene transfection

Experiment number:
SI-1390

Beamline: ID10B	Date of experiment: from: 30-NOV-2006 at 8:00 to:05-DEC-2006 at 8:00	Date of report: 26.06.2007 <i>Received at ESRF:</i>
Shifts: 12	Local contact(s): Dr. Oleg Konovalov	
Names and affiliations of applicants (* indicates experimentalists): Dr. Maria N. Antipina Dr. Gerald Brezesinski Dr. Vladimir L. Shapovalov		

Report:

In order to be used in versatile DNA delivery systems novel cationic lipids 2-tetradecylhexadecanoic acid-2-[(2-aminoethyl)amino]ethyl}amide (CI), 2-tetradecylhexadecanoic acid-2-[bis(2-aminoethyl)amino]ethylamide (CII), N-2-[[Bis-(2-aminoethyl)amino]ethyl]-2,N'-dihexadecylpropanidamide (CIII), 2-amino-3-hexadecyloxy-2-hexadecyloxymethyl-propan-1-ol (CIV) were synthesized. The cationic groups of the newly synthesized lipids are represented either by oligoamines (CI, CII, CIII) or by monoamine (CIV), which can be protonated and therefore positively charged or deprotonated/uncharged depending on the environmental pH value. Since their pK values are unknown, the protonation properties of these lipids have been studied in a wide pH range. In our experiments, the amphiphilic molecules were organized as a Langmuir monolayer at the air-water interface. Total reflection x-ray fluorescence (TRXF) was used to determine the 2D-concentration of bromide counterions bound to a positively charged (protonated) Langmuir monolayer. TRXF investigations were supplemented with results of film-balance measurements, grazing incidence x-ray diffraction (GIXD) and x-ray reflectivity (XR) data.

The monolayers of CI-CIV were formed on subphases with a constant concentration (2 mM) of Br⁻ anions and the x-ray fluorescence intensity of Br, which is proportional to the amount of Br⁻ anions coupled to the protonated lipid head groups, was measured in dependence on the bulk phase pH. Selected X-ray fluorescence spectra of CIII are presented in Figure 1. The Br K_α and Br K_β fluorescence peaks are well

observable. It could be clearly seen that their intensity decreases with increasing pH and is close to 0 at pH 11. The same tendency was observed for all other compounds. Plotting the integral intensity of fluorescence peaks versus pH one can obtain a titration curve for each investigated lipid (Figure 2).

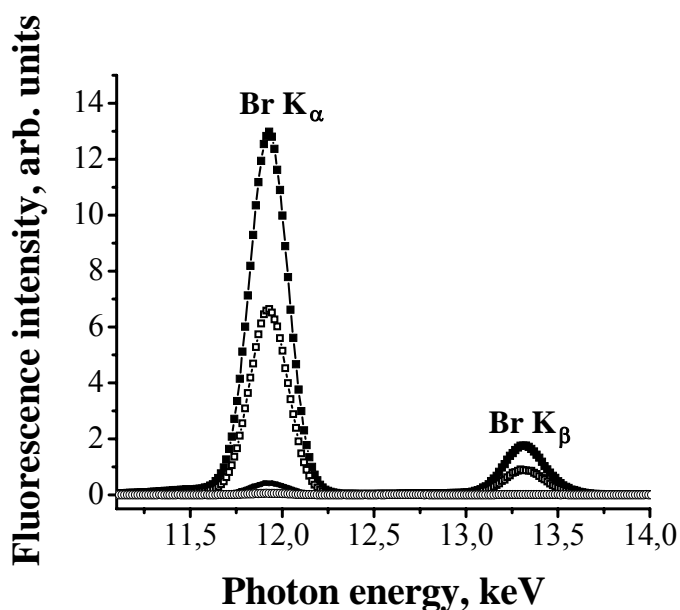


Figure 1. Selected X-ray fluorescence spectra of the CIII Langmuir monolayer at $40 \text{ \AA}^2 \cdot \text{molecule}^{-1}$ on Br^- containing subphases at pH 3 (■), pH 6 (□), pH 8 (●) and pH 11 (○).

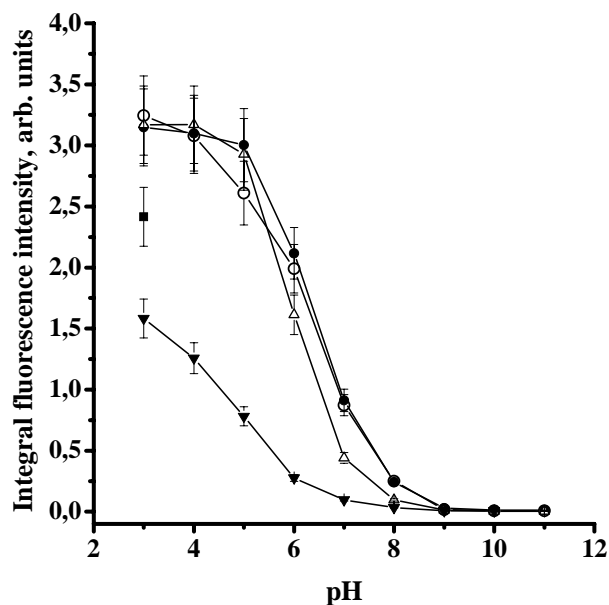


Figure 2. Integral X-ray fluorescence intensity from Br^- coupled to the Langmuir monolayers of DODAB at $50 \text{ \AA}^2 \cdot \text{molecule}^{-1}$ (■), CI at $64 \text{ \AA}^2 \cdot \text{molecule}^{-1}$ (○), CII at $60 \text{ \AA}^2 \cdot \text{molecule}^{-1}$ (●), CIII at $40 \text{ \AA}^2 \cdot \text{molecule}^{-1}$ (Δ) and CIV at $43 \text{ \AA}^2 \cdot \text{molecule}^{-1}$ (\blacktriangledown) in dependence on the subphase pH.

The protonation rate of the novel cationic lipids was estimated by comparing the fluorescence intensity with that of dioctadecyldimethylammonium bromide (DODAB) monolayers as a reference. It was shown that the molecules of all novel compounds are deprotonated at subphase pH values above 9. In contrast, at pH 3 all molecules in the monolayers of CI, CII and CIII have at least one protonated group. Furthermore appr. 40% of the CI and CII molecules have two positive charges per head group. 100 % protonation of molecular head groups does not occur in CIV monolayers even at pH 3. A rough estimation in comparison with DODAB gives a surface charge density of 1 positive charge per 72 \AA^2 for this compound. The X-ray observations support the conclusion which can be drawn from the chemical structure of the CIV. Due to the geometrical arrangement of the four groups attached at the tertiary carbon atom which are competing for the contact with the aqueous subphase, the protonation of the amine group is hindered. With the use of TRXF it was experimentally confirmed that the protonation rate depends not only on the chemical structure but also on the packing density in the monolayer. In fact, the protonation rate of the fluid monolayer of compound II is 1.4 whereas only 1 positive charge per molecule was found in the case of the condensed monolayer of compound III exhibiting the same head group. This experimental finding seems to be very reasonable because the higher surface density of positive charges leads to the decrease of the surface proton concentration according to the Boltzmann equation, what will result in the decrease of the monolayer protonation rate.