Structural Study on the Incorporation of Antitumor Agents into Lipid Multilayers

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The incorporation of the anti-cancer agent paclitaxel into lipid bilayers was studied. Drug/lipid liposome suspensions were reconstituted on glass slides, and oriented multilamellar stack of lipid bilayers were obtained, such as proven by previous X-ray scattering measurements from the same system. In the neutron scattering experiments, H_2O/D_2O contrast variation was applied in order to derive the density profile of the drug/lipid membranes to further detail. Aim is to insight into the influence of the paclitaxel on the membrane structure and to elucidate the molecular organization of the drug in the lipid matrix.

Liposomes play a significant role in medical and pharmaceutical sciences as drug delivery systems. For example, a lipophilic active compound can be encapsulated in the bilayer lipid membrane of the liposome and a hydrophilic one can be inserted into the aqueous compartment, in order to have it delivered to a desired site of action. Drug toxicity can be reduced and targeting of the liposome can be used for enhancing efficacy. Recently it was shown that liposomes made by cationic lipids have high affinity to angiogenic blood vessels around a solid tumor [1]. Therefore, they are useful for targeted delivery of a drug to the tumor vasculature. By neo-vascular targeting of paclitaxel-loaded cationic liposomes an improved retardation of tumor growth with respect to the conventional paclitaxel formulation was achieved [2].

In the experiments we investigate the molecular organization of paclitaxel in different bilayer membranes comprising cationic and zwitterionic lipids. We used the zwitterionic lipid DPPC and the cationic lipids DMTAP (1,2-Dimyristoyl-3-Trimehylammoniumpropan) and DOTAP (1,2-Dioleoyl-3-Trimehylammoniumpropan). Dispersions made by the lipids in H_2O were mixed with the drugs, deposited on a Si substrate and dried in a controlled humidity environment in order to form oriented multilamellar thin films. The measurements were performed using a closed sample chamber for neutron reflectivity, which allows the control of humidity during the experiments.

In previous X-ray reflectivity measurements at ID10B, ESRF, France, it was demonstrated, that well ordered multilamellar membranes can be obtained with these systems [3]. In the presence of the drugs, significant changes of the lamellar spacing in the lipid bilayers were found. The presence of Bragg peaks to several orders enabled to derive information on the internal electron density profile of the individual lamella.

The aim of the neutron scattering experiments was to get further information on the internal density profile. In the present pilot measurements at the AMOR instrument, PSI, Switzerland, due to the limited q-range of that instrument, higher orders of diffraction could not be measured. However contrast variation was successfully applied for the first time in our systems by means of a very simple procedure of sample preparation in a humid D2O environment. Fig 1 shows the reflectivity patterns in H_2O and D_2O atmosphere at 85% relative humidity, as adjusted by a saturated KCL solution. The different intensities of the Bragg peaks are due to the contrast change on H_2O and D_2O exchange indicating successful D/H exchange in the hydrophilic compartment of the bilayer. By additional measurements at different levels of relative humidity it was confirmed that the state of swelling was stable and reproducible. Quantitative data analyses are still under going and we are planning to continue the experiments with neutron techniques using the same protocol of sample preparation, but aiming to improve the geometry to be able to acquire higher diffraction orders to determine the electronic density profile of the membranes.

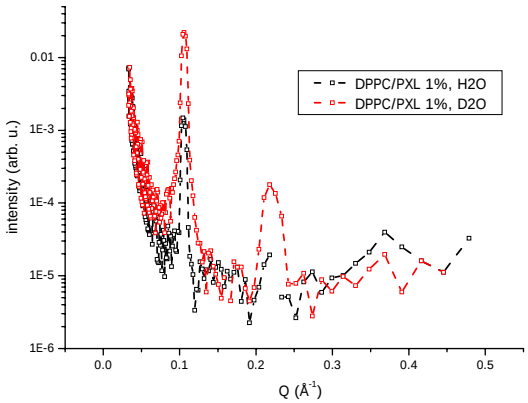

Figure 1: Neutron reflectivity measurements from a DPPC/PXL multilayer in H2O and D2O atmosphere.

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