Experimental report

14/10/2015

Proposal: 8-03-828 **Council:** 4/2014

Title: Interaction of Hyaluronan and Phospholipids

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: hyaluronan
1,2-dihexadecanoyl-sn-glycero-3-phosphocholine
1,2-dipalmitoyl-d62-sn-glycero-3-phosphocholine
1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine

Instrument	Requested days	Allocated days	From	To
D33	2	0		
D22	2	0		
D11	2	1	06/11/2014	07/11/2014

Abstract:

We propose an experiment to study the interaction of hyaluronan (HA) with the two phospholipids DPPC and DPPE. A deeper understanding of the interaction between lipids and HA will improve the knowledge about the reasons for the outstanding lubrication in joints. For our experiment, we will use unilamellar vesicles as a model system which mimics the bilayer self-assembly. Interesting is the question if HA influences vesicle properties like the bilayer structure and phase behaviour and how the structure of HA is influenced by the lipids. To obtain this information the possibilities of contrast variation will be needed.
This experiment will be performed in the frame of the Röntgen-Angstrom-Cluster BMBF “Verbundprojekt 05K2012: JOINT”.

Experimental Report, ILL, Beamline D11

Interaction of Hyaluronan and Phospholipids

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The motion of joints works almost frictionless (friction coefficients between 0.001-0.01), even under various conditions such as slow and fast motion and high and low shear and pressure. [1, 2] One of the origins of the low friction is thought to be the interplay of the cartilage surface and the components of the synovial fluid, which is a highly effective lubricant. However up to now, it is poorly understood how the different components interact in order to enable the exceptional good lubrication properties. Two of the most important constituents, which already have proven to reduce friction very efficiently, are hyaluronan (HA) and lipids. [2]

It was observed that phospholipids form multilayers on the cartilage surfaces. [3, 4] Phospholipids might interact with other components of the synovial fluid, which results in the formation of supramolecular structures which might have a huge influence on the tribological properties. Favorable lubrication properties of DPPC-HA composite layers have already been reported. [5]

The aim of the proposed experiment was to explore the interaction of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) with HA of different molecular weight. For this purpose we planned to study the structural parameters (radius of gyration, persistence length) of HA (Mw = 10kDa, Mw = 1500kDa) in the presence of vesicles as well as the size and shape of the vesicles. To get access to these parameters we matched the contrasts of the solvent and the lipids, so that scattering predominantly from HA occurs.

Since we only got half of the proposed beam time we focused on the interaction of DPPC and HA and could not investigate DPPE and HA.

Measurements:

From calculations of the scattering length densities of our samples we decided to measure DPPC with and without HA in different H_2O/D_2O mixtures: 100% D_2O (full contrast), 88% D_20 and 80% D_2O. We also performed measurements with deuterated DPPC (d-DPPC) with and without HA in 88% D_2O (contrast match with d-DPPC) and 0% D_2O (full contrast).

A very low sample concentrations was of 2 mg/mL for DPPC and HA and 2.17 mg/mL for d-DPPC was chosen. To obtain data over a large q-range, measurements were performed at three different sample – detector distances: 1.2 m, 8 m and 39 m. All measurements were performed at 55 °C to be in physiological relevant fluid phase of the DPPC lipids.

Results:

The scattering curves of DPPC and DPPC with HA in 100%, 88% and 80% D2O are shown in figures 1-3. From these curves it can be seen, that the scattering from HA is very weakly. The scattering is dominated by the DPPC vesicles and the curves of DPPC and DPPC/HA only differ in the low q region. Sole HA shows even at maximum contrast (HA in 100% D2O) almost no scattering, which can be seen in Figure 4. Therefore an evaluation of the data of sole HA is not possible and no information about the conformation of HA and its possible change in the presence of DPPC can be drawn from the data. Results from SAXS experiments will be used to evaluate the structure of HA.

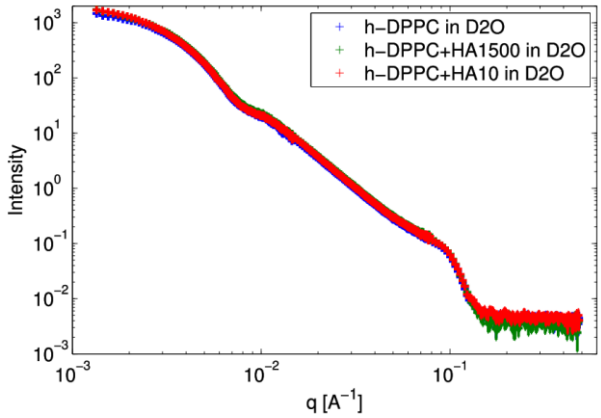

Figure 1 *Scattering curves of DPPC, DPPC/HA1500 and DPPC/HA10 in D_2O.*

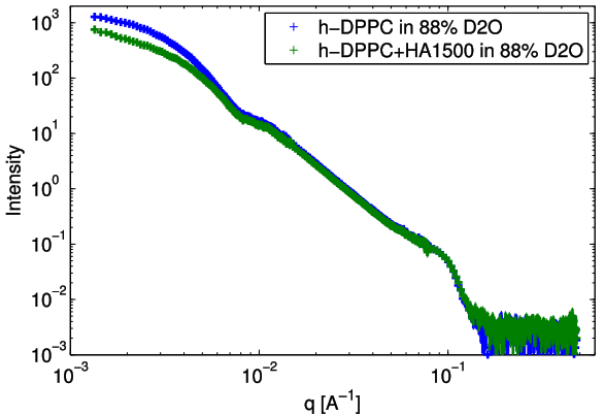

Figure 2 *Scattering curves of DPPC and DPPC/HA1500 in 88% D_2O.*

As a result of the insufficient scattering of HA only information about structural changes of the DPPC vesicles due to an adsorption of HA can be obtained. The curves in the figures 1-3 indicate that the bilayer structure of the vesicles do not change in the presence of HA as now change in the slope of the scattering curve can be observed for intermediate and high q values. However, a deviation in the low q regime for sample with and without HA can be seen. An evaluation of the radii of gyration revealed that vesicles increase in size in the presence of HA, which hints at an adsorption of HA to the vesicle.

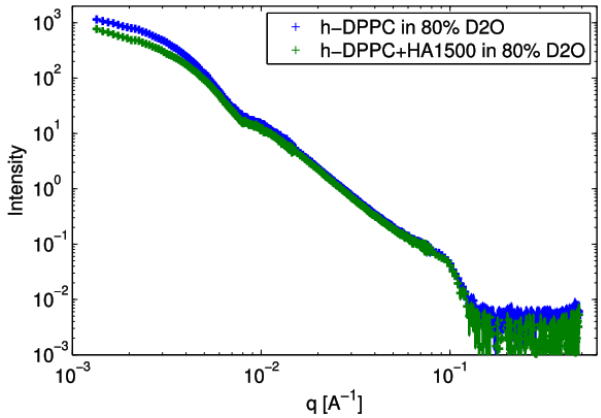

Figure 3 *Scattering curves of DPPC and DPPC/HA1500 in 80% D_2O.*

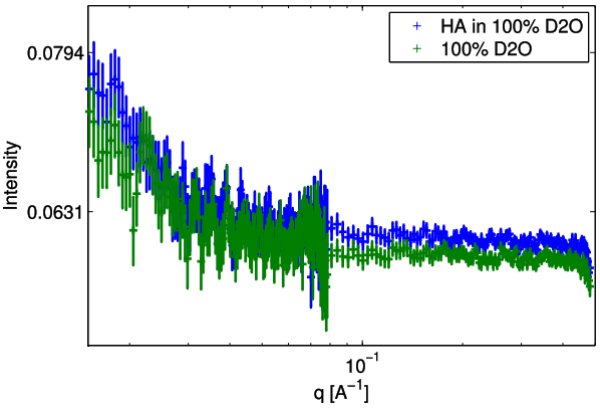

Figure 4 *Scattering curve of HA in 100%D2O.*

The first results of our experiment showed that DPPC and HA interact weakly with each other. It seems likely that the bilayer structure of the vesicles is not changed by HA. However

an increase of the vesicle size due to HA can detected, which might be due to adsorbed HA itself or due to an agglomeration of vesicles mediated by HA.

[1] Hills, B. A. (2002) Intern. Med. J. 32, 242-251 [2] Klein, J. (2006) Proc. IMechE, Part J: J. Engineering Tribology 220, 691-710 [3] Hills, B.A. (1989) J. Rheumatol. 16(1), 82-91 [4] Trunfio-Sfarghiu, A.-M., Berthier, Y., Meurisse, M.-H., and Rieu, J.-P. (2008) Langmuir 24, 8765-8771 [5] Liu, C., Wang, M., An, J., Thormann, E., Dedinaite, A. (2012) Soft Matter 8, 10241