Experimental report

10/11/2022

Proposal: **9-13-1031**

Council: 4/2021

Title: Impact of Hyaluronic Acid and Polymeric Analogues on the Mechanics ofLipid Oligobilayers Studied by Off-Specular Neutron Scattering

Research area: Soft condensed matter

This proposal is a resubmission of 8-02-949

Main proposer: **Reiner DAHINT**

Experimental team:
Christian BUSCH
Wasim ABUILLAN
Reiner DAHINT

Local contacts: Bruno DEME

Samples:
DMPC
Hyaluronic acid
Poly(allylamine hydrochloride) (PAH)

Instrument	Requested days	Allocated days	From	To
D16	6	6	22/09/2021	28/09/2021

Abstract:

The main component of synovial fluid is negatively charged hyaluronic acid (HA). Together with oligolamellar lipid bilayers, which cover the cartilage in mammalian joints, it contributes to joint lubrication. Using specular neutron reflectometry and infrared spectroscopy we have already shown that contact with aqueous solutions of HA significantly improves the shear stability of the lipid layers. The same effect is observed upon exposure to aqueous solutions of positively charged poly(allylamine hydrochloride) (PAH) with the advantage of much shorter equilibration times. The degree of stability depends both on the molecular weight and type of polymer. Therefore, we now want to unravel the impact of HA and PAH on the mechanical properties of the lipid layers utilizing off-specular neutron scattering, and correlate the extracted vertical compression modulus and bending rigidity with the observed shear stability.

Experimental report 9-13-1031

Reiner Dahint,[1] Wasim Abuillan[2], Christian Busch,[1] and Bruno Demé[3]

[1] Applied Physical Chemistry, Institute for Physical Chemistry, Heidelberg University, Im Neuenheimer Feld 253, 69120 Heidelberg, Germany

[2]Physical Chemistry of Biosystems, Institute for Physical Chemistry, Heidelberg University, Im Neuenheimer Feld 253, 69120 Heidelberg, Germany

[3] Institut Laue-Langevin, 71 Avenue des Martyrs, CS 20156, 38042 Grenoble Cedex 9, France

1. Scientific Background

Oligolamellar lipid bilayers cover the cartilage in mammalian joints. Together with negatively charged hyaluronic acid (HA), the main component of the synovial fluid, they contribute to joint lubrication.[1] In case of osteoarthritis, clinical studies show a decrease in concentration and average molecular weight (MW) of HA in the synovial fluid.[2] Based on these findings, a new treatment called viscosupplementation has been established, which uses intraarticular injections of HA or HA/lipid mixtures to regain the functionality of the joint.[3] However, the effectiveness as well as the molecular mechanisms behind the healing process are still under debate.

2. Aim

With this in mind, the goal of our studies was to investigate the mechanisms of interaction between HA and oligolamellar lipid bilayers on a molecular scale. In particular we intended to clarify to what extent HA affects the mechanical properties of the lipid layers, as enhanced mechanical stability might improve protection of joint cartilage and reduce the risk of osteoarthritis. In previous investigations using specular neutron reflectometry (NR) and infrared (IR) spectroscopy we have already demonstrated improved shear stability of the lipid layers in contact with aqueous solutions of HA and poly(allylamine hydrochloride) (PAH).[4] Here, PAH served as a substitute for HA for time reasons as it essentially showed the same effects at much shorter equilibration times.[5,6] Shear stability depended both on the MW and type of polymer. Now we intended to directly study the impact of HA (or PAH serving as a substitute) on the mechanical properties (vertical compression modulus B and bending rigidity κ) of the lipid layers by off-specular neutron scattering, and search for potential correlations with shear stability.

3. Results

All samples used were prepared in the home laboratory at Heidelberg University. For this purpose, Si wafers (100 mm diameter, 0.65 mm thickness, p-doped) were cut into rectangular pieces of about 55 mm × 24 mm. For cleaning, they were rinsed with chloroform and then stored in ethanol for 1 h. Afterwards, they were again rinsed with ethanol and dried in a nitrogen stream. Next, the polished side of the rectangular Si substrates was covered with about 1 ml of a 0.5 mg/mL vesicle solution of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) in D_2O and stored in an oven at 70 °C until the liquid had completely been removed. In this way, a stack of about 100 DMPC bilayers was obtained. One coated and one non-coated Si substrate were assembled with the polished sides facing inward for each sample. Spacing between the two Si pieces was provided by rectangular fragments of microscope coverslips of about 0.2 mm thickness. Some samples were already incubated with the respective polymer solutions approximately two weeks prior to the start of the experiments to allow for long incubation times. PAH with a molecular weight of 15, 58, 900 kDa and HA with a molecular weight of 731 kDa were employed in the studies.

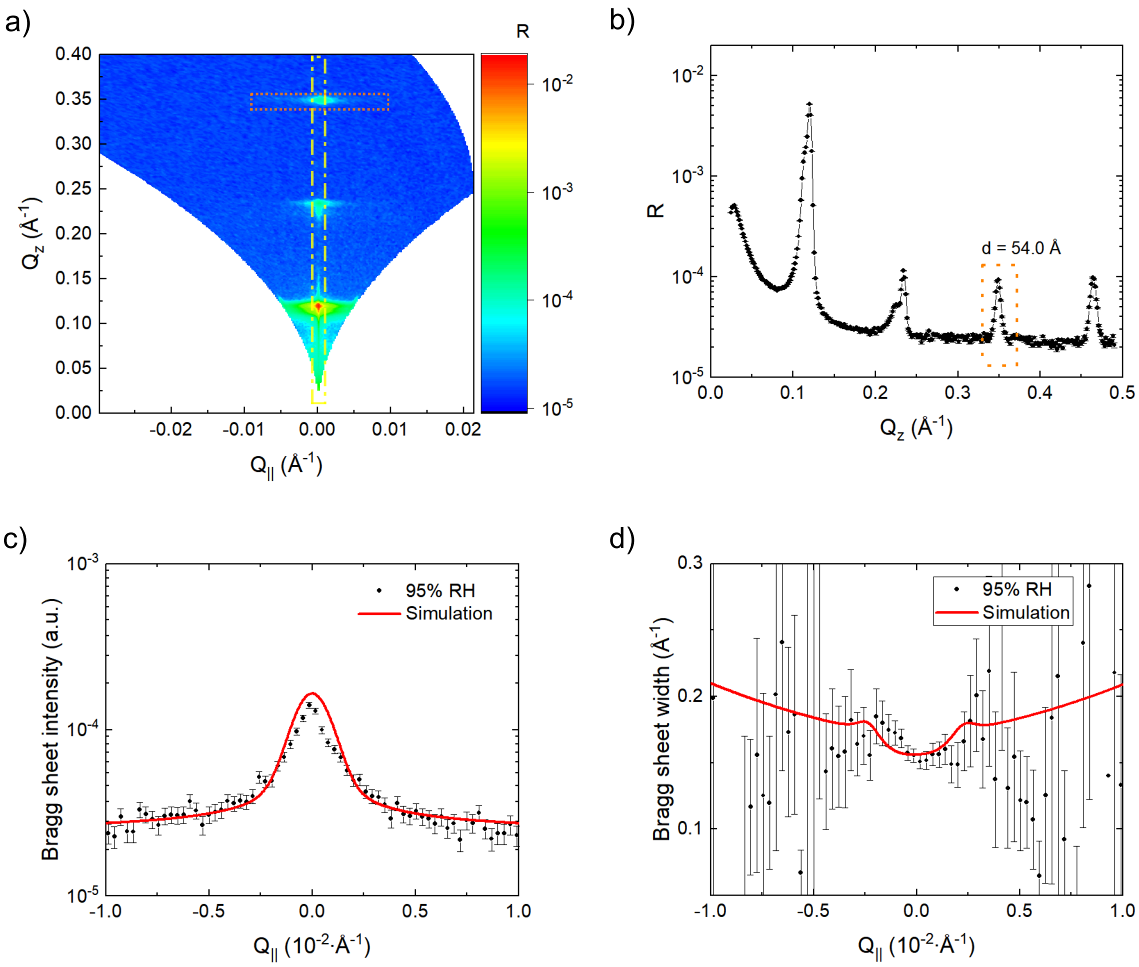

Figure 1: Experimental results for DMPC monolayers exposed to 95% relative humidity. (a) Plot of Q_Z versus $Q_{||}$. The specular reflectivity is marked with a yellow, the analyzed Bragg sheet with an orange box. (b) Specular reflectivity of the sample. (c) Measured Bragg sheet intensities and corresponding simulation. (d) Measured Bragg sheet widths and corresponding simulation.

The samples were mounted in a humidity chamber provided by ILL. To probe the impact of polymer size and type on the mechanical properties of the films, we measured the reflectivity of the samples at 50 and 95% relative humidity (RH) of D_2O, in bulk D_2O, and after esposure to a 3 mg/mL polymer solution in D_2O. Temperature was maintained constant at 20 °C below the main phase transition temperature of DMPC. From the measured neutron reflectivity as a function of wave vector transfer perpendicular and parallel to the sample plane, Q_Z and $Q_{||}$, we determined the de Gennes and the Caillé parameters, λ and η, in a fitting procedure, and used them to calculate B and κ of the films according to the relationship $\lambda = \sqrt{\frac{\kappa}{Bd}}$, $\eta = \frac{\pi k_B T}{2d^2\sqrt{\kappa B/d}}$. k_B denotes the Boltzmann constant, T the absolute temperature and d the lamellar lamellar spacing of the bilayer system. Details of the procedure have been decribed in literature.[7]

Table 1: Lamellar spacing *d*, compression modulus *B* and bending rigidity *κ* of DMPC multi-bilayers exposed to 50 and 95% RH of D_2O, bulk D_2O and a 3 mg/mL solution of PAH (58 kDa) in D_2O. PAH incubation time was 60 h.

	d (Å)	*B* (MPa)	*κ* (K_BT)
50% RH	54,1 ± 1,0	19.7	21.6
95% RH	53,9 ± 0,2	18.1	24.2
D_2O	62,6 ± 1,2	0.44	2.75
PAH (58 kDa)	193 ± 8	0.11	2.18

For most samples, only very weak second and third order Bragg sheets were observed, in particular after exposure to bulk liquid. The best results were obtained for PAH (58 kDa), and the corresponding data are shown in Figure 1 for 95% RH. Table 1 summarizes the mechanical parameters obtained in the experiment with PAH (58 kDa) for the different sample environments investigated. The most striking observation is the strong decrease in *B* and *κ* when changing the sample environment from 95% RH to D_2O. This reduction suggests a significant softening of the intermembrane potential and less resistance against bending deformation, respectively. In contrast, the values of *B* and *κ* for 50 and 95% RH are comparable.

Addition of PAH reduces rather than increases both mechanical parameters. Thus, in contrast to our original expectation, the significantly enhanced shear stability in aqueous PAH solutions compared to pure D_2O does not correlate with enhanced values of *B* and *κ*. The reasons for this behavior are still being investigated. The pronounced increase in the intermembrane separation upon addition of PAH indicates that the polyelectrolyte has a strong impact on the intermembrane interactions. It is caused by the adsorption of the charged polyelectrolyte molecules onto the lipid headgroups, resulting in enhanced Coulomb repulsion between the lipid bilayers.[8] However, there is no significant impact of the adsorbed PAH film on *κ*, most likely, because it is only weakly coupled to the lipid bilayers due to its high water content.[8] On the other hand, the slight decrease in *B* upon the addition of PAH reflects reduced membrane confinement due to larger bilayer separation.

References

[1] Hills, B. A. *Proc. Inst. Mech. Eng., Part H* **2000**, 214, 83 – 94.

[2] Dahl, L.; Dahl, I.; Engström-Laurent, A.; Granath, K. *Ann. Rheum. Dis.* **1985**, 44, 817 – 822.

[3] Bellamy, N.; Campbell, J.; Robinson, V.; Gee, T.; Bourne, R.; Wells, G. *Cochrane Database of Systematic Reviews* **2005**, 2, 1 – 656.

[4] Schwörer, F.; Trapp, M.; Ballauff, M.; Dahint, R.; Steitz, R. *Langmuir* **2015**, 31, 11539 – 11548.

[5] Kreuzer, M.; Strobl, M.; Reinhardt, M.; Hemmer, M.C.; Hauß, T.; Dahint, R.; Steitz, R. *Biochim. Biophys. Acta* **2012**, 1818, 2648 – 2659.

[6] Kreuzer, M., Trapp, M., Dahint, R. & Steitz, R. *Membranes* **2016**, 6, 2 – 16.

[7] Yamamoto, A.; Abuillan, W.; Burk, A. S.; Körner, A.; Ries, A; Werz, D. B.; Demé, B.; Tanaka, M. *J. Chem. Phys.* **2015**, 142, 49071 – 49078.

[8] Schwörer, F.; Trapp, M.; Xu, X.; Soltwedel, O.; Dzubiella, J.; Steitz, R; Dahint, R. *Langmuir* **2018**, 34, 1287 – 1299.