Experimental report

25/05/2020

Proposal: 9-13-875 **Council:** 4/2019

Title: Interactions of Surfactant with Model Lipid Membranes Studied by Neutron Diffraction

Research area: Chemistry

This proposal is a new proposal

Main proposer: Yao CHEN

Experimental team: ZI WANG
Yao CHEN
Peixun LI
Kun MA

Local contacts: Bruno DEME

Samples: Cholesterol
Behenic acid
C21H46BrNO2
C23H48BrNO2
C23H50BrNO2
CER EOS
Ceramide NS

Instrument	Requested days	Allocated days	From	To
D16	5	2	17/02/2020	21/02/2020

Abstract:

The skin is a barrier to protect our body from hazardous external environment. As the outermost layer of skin, Stratum Corneum (SC) of mammalian epidermis consists of keratin-filled dead corneocytes and extracellular multiple crystalline lamellar lipid, it forms the main barrier against diffusion of substances across the skin. The SC lipids assemble into two coexisting crystalline lamellar phase with repeat distance of ~6 and 13 nm, which refers to short periodicity phase (SPP) and long periodicity phase(LPP), respectively. Surfactant is widely used as drug carrier due to its amphiphilic property and self-assembly ability. However, how the surfactant interacts with model lipid membrane, especially with the LPP and SPP layers of model membrane, is still not clear yet.In this proposal, we will study the interaction of these surfactants with model SC lipid composed of an equimolar mixture of CERs, CHOL and FFAs using neutron diffraction.We try to study the interaction between surfactants and different SC model membranes, and understand these interactions from the architecture and fluidity of surfactant.

Interactions of Surfactant with Model Lipid Membranes Studied by Neutron Diffraction

(Proposal 9-13-875, PI, Yao Chen)

Sample preparation and neutron diffraction measurements. Five kinds of model lipid mixtures were constructed in this study, shown in Figure 1. Pure ceramide mixture (CER^{pure}) was made by an equimolar mixture of ceramide, cholesterol and fatty acids, among which fatty acids is a mixture of C22 acid and C24 acid (molar ratio1:1). To investigate the lipid-surfactant interaction, surfactant at a 1.0 % molar ratio of the total lipid was added to the lipid mixtures before sample preparation. Their name, composition, and molar ratios are also shown in Table 1. The sample preparation method and equilibration procedure was similar to references. For each model membrane, the appropriate amount of individual lipids and surfactants were dissolved in a methanol/chloroform (1:2 v/v) mixture with a total concentration of 10mg/mL. Subsequently, this mixture was sprayed on the silicon surface in an area of 2.0 × 2.1 cm^2 by the rock and roll method. The solvent was removed by a gentle flow of nitrogen. In total, ~5.0 mg of lipids and surfactants were sprayed on the silicon substrate. After removing the solvent under vacuum, the sample was equilibrated at ~70 °C for half an hour and then cooled down to room temperature. Afterwards, the sample was hydrated with D_2O/H_2O buffer under 90% relative humidity (RH) at 32 °C for ~ 6 h. For each sample, three D_2O/H_2O ratios, namely 8%, 50% and 100%, were used before neutron diffraction experiments. The hydration time for the same sample between two measurements was ~ 8 h.

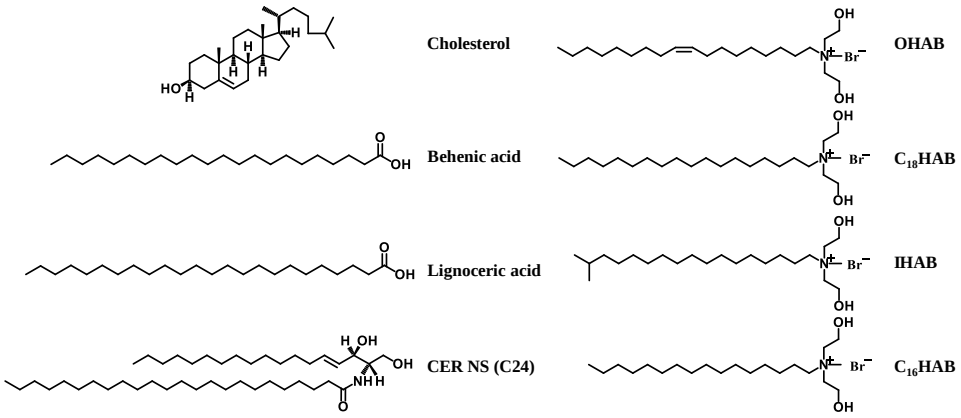

Figure 1. Chemical structures of ceramide, cholesterol, fatty acids and surfactants

Data analysis and results. In order to understand the interactions between surfactants and CER/CHOL/FFA model membranes, the neutron diffraction measurements were performed for both pure membrane and surfactant-CERs mixed membranes. The neutron diffraction results are analyzed from the following aspects: (a) one dimensional plot of intensity vs q for all the model membranes, (b) calculation of repeat distance of the unit cell, (c) structure factors calculated from the Gaussian fitting of the individual peaks, (d) determination of phase sign for each structure factor, and (e) SLD profiles obtained by Fourier reconstructions, indicating the water and surfactant distribution in the unit cell.

The repeat distance for the short periodicity phase is 53.4 ± 0.5 Å, and it keeps constant after adding surfactant. The phase sign for the structure factor was proved to be – + – + – for all the samples, a linear fitting of structure factor was also observed. The SLD profiles for the model membrane hydrated and measured at 8%, 50% and 100% are derived from Fourier reconstructions by using the structure factor and the corresponding phase sign. The distribution of water and surfactant is obtained by comparing selected contrasts. The addition of surfactants significantly increase the hydration level of model membrane, and more cholesterol is incorporated into the SPP structure. The above results are all understood from the molecular architecture of surfactants. Further measurements with deuterated surfactants is essential for understanding the configuration of surfactant in SPP structures.

Figures and Tables

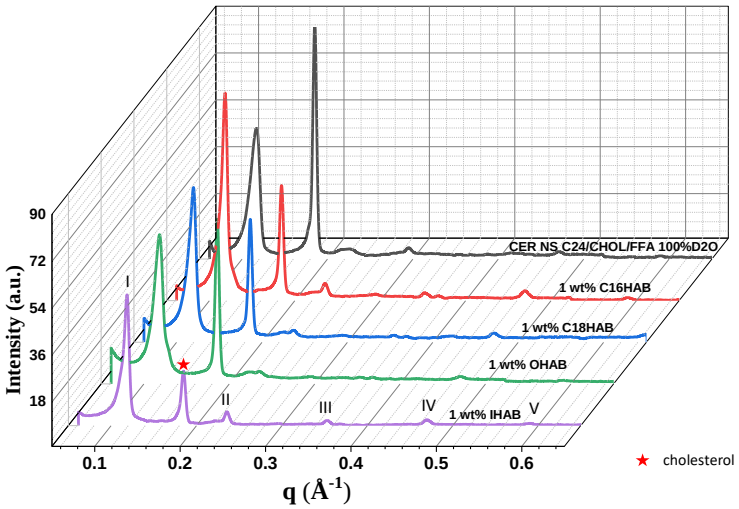

Figure 2. Neutron diffraction one dimensional plot of intensity vs q for the pure CER/Chol/FFA membrane and the surfactant/CER/Chol/FFA mixtures hydrated and measured at 100% D_2O/H_2O. See legends for detail. The higher diffraction orders of the SPP lamellae are indicated by the Arabic numbers and the CHOL peaks by means of a star.

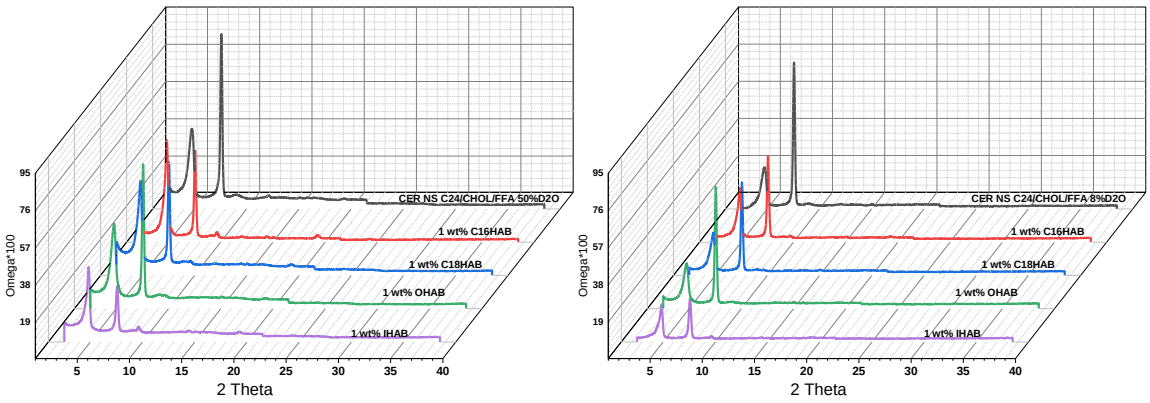

Figure 3. Neutron diffraction one dimensional plot of intensity vs q for the pure CER/Chol/FFA membrane and the surfactant/CER/Chol/FFA mixtures hydrated and measured at 50% D_2O/H_2O(left) and 8% D_2O/H_2O (right).

Table 1. The lipid classes and their corresponding molar ratios used to make the mixtures for neutron diffraction experiments Model lipid membrane densities and linear attenuation coefficients for different membranes at different D_2O% ratios.

Membrane type	Lipid composition	Molar ratio	Repeat distance (Å)	D_2O/H_2O (%)	Density(g/cm^3)	Attenuation coefficient (cm^{-1})
CER^{pure}	CER/CHOL/FFA	1:1:1	53.4 ± 0.5	8	0.92	5.628
				50	0.95	5.758
				100	1.03	6.183
CER^{C16HAB}	CER/CHOL/FFA/C_{16}HAB	1:1:1:0.03	53.2 ± 0.3	8	0.92	5.628
				50	0.95	5.758
				100	1.03	6.183
CER^{C18HAB}	CER/CHOL/FFA/C_{18}HAB	1:1:1:0.03	53.1 ± 0.3	8	0.92	5.628
				50	0.95	5.758
				100	1.03	6.183
CER^{OHAB}	CER/CHOL/FFA/OHAB	1:1:1:0.03	53.2 ± 0.3	8	0.92	5.628
				50	0.95	5.758
				100	1.03	6.183
CER^{IHAB}	CER/CHOL/FFA/IHAB	1:1:1:0.03	53.2 ± 0.3	8	0.92	5.628
				50	0.95	5.758
				100	1.03	6.183

Table 2. Structure factor with corresponding phase signs for the membranes.

Membrane type	D_2O/H_2O (%)	F1	F2	F3	F4	F5
CER^{pure}	8	-3.79	0.65	-0.57	1.11	-0.72
	50	-5.20	0.98	-0.83	1.85	-0.96
	100	-6.87	1.49	-1.26	2.40	-1.41
CER^{C16HAB}	8	-6.30	1.75	-1.02	1.61	-0.78
	50	-8.88	3.13	-2.18	2.88	-1.41
	100	-10.99	4.37	-3.21	3.83	-2.34
CER^{C18HAB}	8	-4.92	1.40	-0.64	1.20	-0.81
	50	-7.16	2.09	-1.33	2.05	-1.05
	100	-9.15	3.00	-2.00	2.74	-1.28
CER^{OHAB}	8	-4.24	1.28	-0.72	0.96	-0.64
	50	-5.54	1.85	-1.03	1.63	-0.77
	100	-7.41	2.66	-1.70	2.21	-1.18
CER^{IHAB}	8	-6.35	2.60	-1.47	1.68	-0.74
	50	-8.23	3.66	-2.37	2.56	-1.22
	100	-10.76	5.18	-3.47	3.77	-1.94

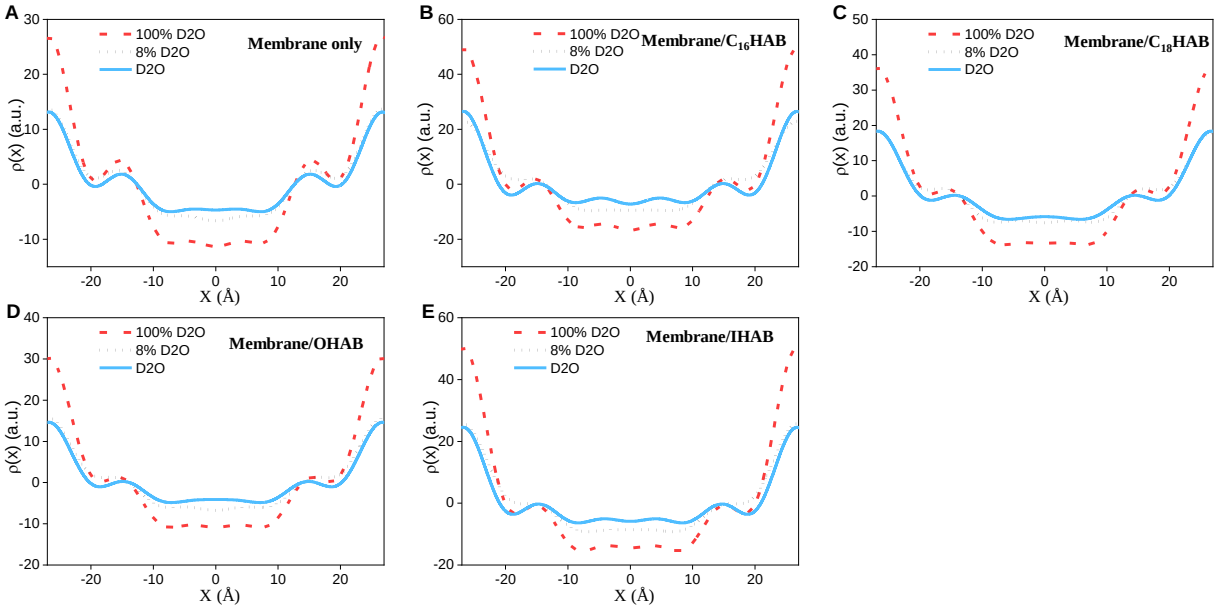

Figure 4. The relative SLD profiles of the pure CER/CHOL/FFA and the CER/CHOL/FFA/surfactant mixtures hydrated and measured at 8%D_2O (black dotted line), 100%D_2O (red dashed line). Difference profile (blue solid line) shows the water SLD profile. A) Pure membrane (CER^{pure}), B) C_{16}HAB/CER/Chol/FFA mixed membrane (CER^{C16HAB}), C) C_{18}HAB/CER/Chol/FFA mixed membrane (CER^{C18HAB}), D) OHAB/CER/Chol/FFA mixed membrane (CER^{OHAB}), E) IHAB/CER/Chol/FFA mixed membrane (CER^{IHAB}).