Experimental report

03/04/2017

Proposal: 8-02-758 **Council:** 4/2016

Title: The interaction between amyloid beta peptides and model membrane containing cholesterol and/or melatonin

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DOPC
DOPC + 3 mol% Amyloid beta
DOPC + 29 mol% Cholesterol + 3 mol% Amyloid beta
DOPC + 29 mol% Melatonin + 3 mol% Amyloid beta
DOPC + 29 mol% Cholesterol+Melatonin + 3 mol% Amyloid beta

Instrument	Requested days	Allocated days	From	To
D16	8	7	26/09/2016	03/10/2016

Abstract:

Alzheimer`s disease (AD) is a devastating neurodegenerative disease caused by the formation of senile plaques, primarily consisting of amyloid-beta (Ab) peptides. The crucial role in this process is imparted by peptide-membrane interactions, changing the structural properties of membrane. These changes are known to be modulated also by membrane composition. In particular, cholesterol increases the order of lipid hydrocarbon chains and increases the stiffness of membrane. On the other hand, melatonin increases the fluidity of membrane. Our previous experiments [Drolle et al., BBA 2013] revealed the counteracting effect of melatonin to that of cholesterol. We propose to investigate model membranes loaded with transmembrane Ab peptide with the addition of cholesterol, melatonin, and cholesterol and melatonin. We expect the bilayer structure to reflect the elevated amounts of cholesterol by its thickening, while the fluidizing effect of melatonin should evoke the membrane thinning. Results of this experiment may shed more light on the melatonin's potential role in preventing the development of AD.

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Experimental Report

Experiment title The interaction between amyloid-β peptides and model membrane containing cholesterol and/or melatonin

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Abstract

Alzheimer's disease (AD) is a devastating neurodegenerative disease caused by the formation of senile plaques, primarily consisting of amyloid-beta (Ab) peptides. The crucial role in this process is imparted by peptide-membrane interactions, changing the structural properties of membrane. These changes are known to be modulated also by membrane composition. In particular, cholesterol increases the order of lipid hydrocarbon chains and increases the stiffness of membrane. On the other hand, melatonin increases the fluidity of membrane. Our previous experiments [Drolle et al., BBA 2013] revealed the counteracting effect of melatonin to that of cholesterol in neat lipid membranes. We have extended our investigations recently by including transmembrane Ab peptide in these model membranes.Small angle neutron diffraction measured at four different contrast conditions was utilized for an unambiguous determination of structure in transversal direction. The obtained bilayer structure reflected the elevated amounts of cholesterol by its thickening, while the fluidizing effect of melatonin evoked the membrane thinning. Results of our experiments possibly confirm the melatonin's potential role in preventing the development of AD.

Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized by dementia and memory loss for which no cure or effective prevention is currently available. One of the hallmarks of AD is the formation of senile plaques, primarily consisting of amyloid-β (Aβ) peptides. The crucial role in this process is thought to be imparted by peptide-membrane interactions, modulated by membrane composition. In particular, cholesterol was found to segregate in the immiscible plaques when present in elevated concentrations, and even further intensification was observed in the anionic membranes by the presence of Aβ peptides.[1]

In contrary to the effect of cholesterol, melatonin has been shown to increase the fluidity of membrane and counteracted the effect of cholesterol.[2]It is intriguing to note the correlations between both mentioned membrane additives and the probability of AD. The decreasing levels of melatonin in the brain tissue with the aging was linked to the melatonin's potential role in preventing the development of AD whose probability also increases with age.[3] The mechanism of its action may be – similarly but in opposite way to the cholesterol that increases the order of lipid hydrocarbon chains and increases the stiffness of membrane – in affecting the properties of membrane. Small molecules such as cholesterol and melatonin may conceivably alter the physical properties of lipid membrane and thus affect amyloid fibril formation and toxicity.

While our previous results showed clearly the effect of the addition of cholesterol and/or melatonin on the structural properties of underlying phospholipid membrane,[4] it did not provide any information about the interactions with Aβ peptide itself. Based on the previous results, we have therefore extended our studies by scrutinizing the system with the addition of 3 mol% of transmembrane Aβ segment A$\beta_{25\text{-}35}$. The previously investigated system of multilayered stacks prepared of dioleoyl-phosphatidylcholine (DOPC) bilayers was selected to provide a liquid-crystal matrix, to which Aβ peptide was incorporated. Melatonin and/or cholesterol were also added to the system at the previously utilized concentration of 29 mol %. In total, 5 samples including 2 standards were measured. It is interesting to note, the set of samples prepared ahead of time in our home laboratory and transported to ILL showed a high mosaicity and was therefore excluded from the measurements. The fresh samples prepared immediately before the experiment on the other hand revealed a high-quality alignment.

The experimental technique employed in the investigation continued to be the neutron diffraction due to its ability to provide important characterization of model membranes.[5] The advantage of D16 instrument with 2D detector resulted additionally in more effective measurements.[6] The instrument configuration allowed to measure both diffraction curve (i.e., along the membrane transversal direction) and rocking curve (i.e., along the constant size of scattering vector) at the same time. While the former curve is used to calculate the membrane's neutron scattering length density profile, the latter was used to assess the alignment of lipid stacks. Fig. 1 shows the rocking curves measured for various samples. The low-quality sample is revealed by small central peak, which on the other hand raises up from general background (see the graph showing an extended PSI range) by several orders in the case of high-quality samples. The width of central peak for all the samples was less than 0.06 degree (Gaussian sigma) confirming the high level of membrane alignement.[7]

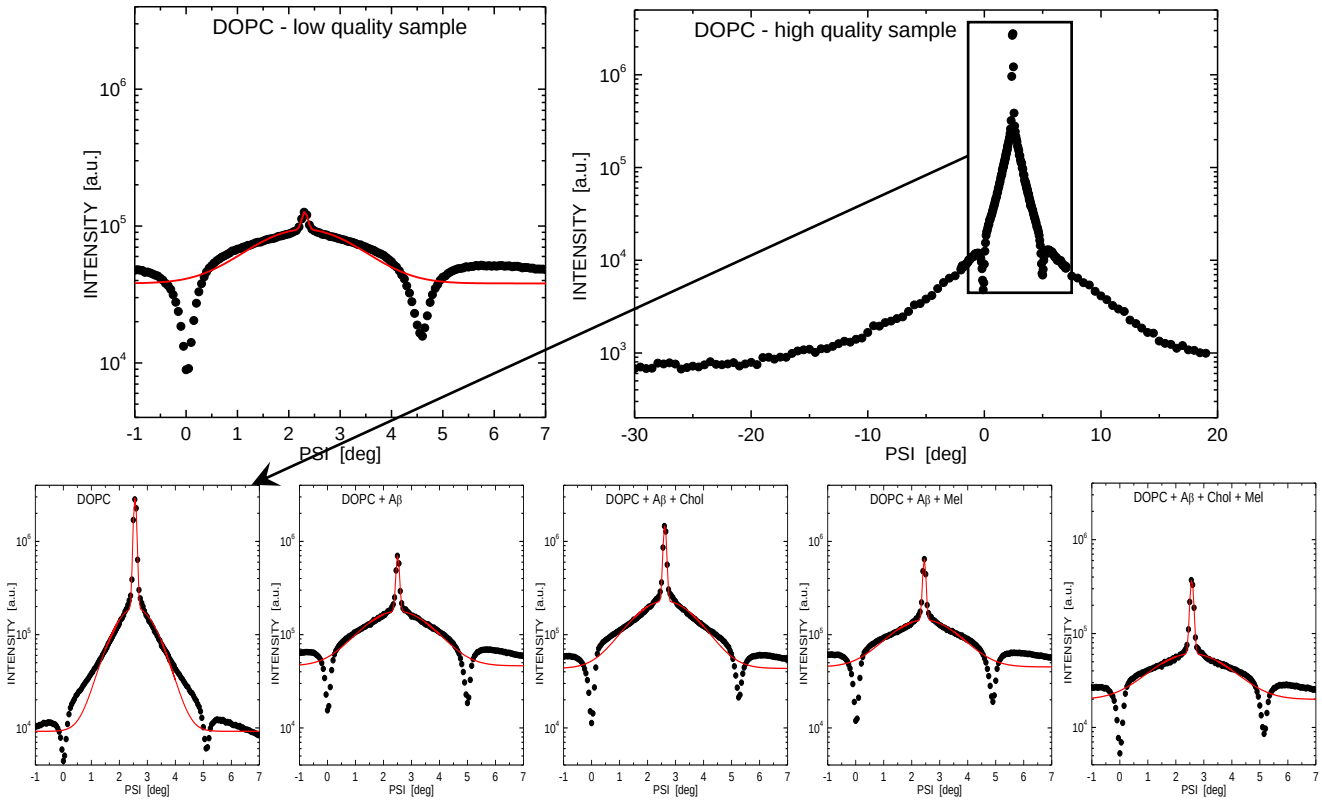

Figure 1: Uncorrected rocking curves measured for various samples investigated in the experiment. The diffraction intensity is collected as a function of the sample angle PSI, while the detector active area is fixed at the position of the first diffraction order.[8]

The samples were held during the measurements vertically at 25°C in an air-tight hydration chamber provided by ILL. The chamber's bottom was filled with a saturated K_2SO_4 (97% RH)[9] solution of different D_2O/H_2O mixtures. The well-controlled hydration conditions slightly below the full hydration assure the sample stability throughout the course of the experiment. Under these conditions, the amount of inter-bilayer hydration water is sufficient to avoid structural changes due to steric constraints.[5] Consequently, the interlamellar repeat d-spacing represents a good measure of bilayer steric thickness. We have evaluated this parameter first.

The repeat d-spacing relates directly to an inverse peak position ($2\pi/q$). We improve the reliability of its calculation by utilizing the entire set of measured diffraction orders (thus $d=2\pi n/q_n$). A typical number of orders detected in our measurements is 5, while the 6th order has been detected for some samples. The evaluated d-spacings for various samples are visualized in Fig. 2 and compared to the results obtained previously for samples without Aβ peptide incorporated.

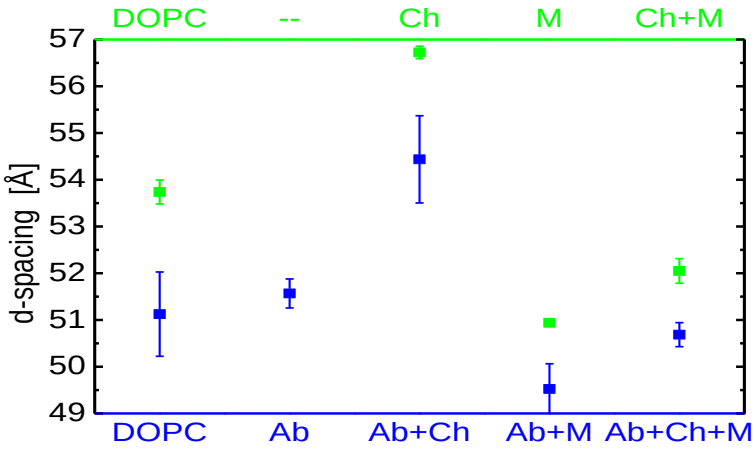

Figure 2: A transversal lamellar spacing in the multilayered model membranes composed of DOPC bilayers. The addition of cholesterol increases, while that of melatonin decreases the thickness of both the neat DOPC (green points) as well as Aβ-loaded DOPC(blue points) bilayers.

It is obvious from our analysis that all the membrane additives investigated affect the bilayer thickness parameter. The addition of Aβ peptide shows a small effect as its concentration was kept low to avoid peptide aggregation while preserving model conditions for studying lipid-peptide interactions. The addition of both cholesterol and melatonin, on the other hand, resulted in significant thickness changes. Agreeably to the previous results, cholesterol increases bilayer thickness – most likely through increasing the bilayer stiffness – and melatonin decreases the thickness. The latter effect

has been postulated to be a result of the melatonin's location in lipid head group region that in turn fluidizes the membrane while increasing the disorder of lipid chains.[2] Finally, the concurrent addition of cholesterol and melatonin at equimolar concentrations brings the bilayer thickness close to its neat value.[4]

Our results thus confirm the effect that both cholesterol and melatonin have on the model mimicking the pre-AD membrane. We suggest to continue using this model as a platform for studying the modulation of Aβ peptide-lipid interactions due to the structural and dynamical changes of lipid matrix. It is of our further interest to reveal further structural details based on the neutron scattering length density profiles that can be calculated from our current data.

Sample preparation

Dioleoyl-phosphatidylcholine (DOPC) was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Melatonin and cholesterol were purchased from Sigma Aldrich (St. Louis, MO), and transmembrane amyloid-beta peptide segment 25-35 ($A\beta_{25-35}$) was obtained from Abbiotec (San Diego, CA).The weighted amounts of DOPC, cholesterol, melatonin, and Aβ were dissolved in chloroform:trifluoroethanol solvent (volume ratio 1:1).The Aβ peptide was in addition subjected to a pre-treatment, in which it was first dissolved in 0.5 mL trifluoroacetic acid (TFA), and sonicated for 15 min. Much of TFA was then evaporated under the vacuum followed by the addition of solvent. A residual amount of TFA was allowed in solution to prevent the aggregation of peptide before its interactions with membrane lipids.[10,11]

The calculated amounts of DOPC with the addition of 3 mol% of Aβ were mixed with and without 29 mol% cholesterol, 29 mol% melatonin, and29 mol% cholesterol and 29 mol% melatonin in separated glass vials. Approximately 10 mg total was deposited onto a 25 x 60 mm^2 silicon wafer forming about 15 μm thick layer by the 'rock and roll' method.[12] The remaining traces of solvent were removed by placing the samples under vacuum overnight. Five high-quality oriented samples were confirmed by observing 5-6 orders of diffraction peaks in diffraction curves and narrow central peaks in rocking curves (Fig. 1).

Small-Angle Neutron Diffraction

Neutron diffraction data were collected at the Institut Laue-Langevin (ILL) in Grenoble, France on D16 small momentum transfer diffractometer with variable vertical focusing. Neutrons of 4.55Åwavelengthwere selected by the (002) reflection of a pyrolytic graphite (PG) monochromator. Incoming beam was formed by the set of slits (S_1=150x6 mm^2 and S_2=25x6 mm^2) and sample-to-detector distance was 0.95 m. All samples were measured at two detector positions with ^{3}He position sensitive detector. Γ_1=12° was utilized for the detection of up to 4th order diffraction peak, and Γ_2=27° for the detection of higher order peaks. The intense first order peak, which appeared for most of the samples, was measured with the 5 mm attenuator reducing the intensity by a factor of 10. The data of 2D area detector were visualized and reduced by the Lamp software provided by ILL.[13]

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