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SPECIFIC PARTIAL AREA OF CHOLESTEROL IN MONOUNSATURATED DIACYLPHOSPHATIDYLCHOLINE BILAYERS

J. Gallová, gallova@fpharm.uniba.sk, Faculty of Pharmacy, Comenius University, 832 32 Bratislava, SR, D. Uhríková, uhrikova@fpharm.uniba.sk, Faculty of Pharmacy, Comenius University, 832 32 Bratislava, SR, N. Kučerka, Norbert.Kucerka@nrc.gc.ca, Canadian Neutron Beam Centre, National Research Council, Chalk River, Ontario K0J 1P0 Canada, J. Teixeira, jose.teixeira@cea.fr, Laboratoire Leon Brillouin (CEA-CNRS), CEA Saclay, Gif Sur Yvette Cedex, F-91191 France and P. Balgavý, balgavy@fpharm.uniba.sk, Faculty of Pharmacy, Comenius University, 832 32 Bratislava, SR

INTRODUCTION

Phospholipid bilayer is a basic structural element of cell membranes. Polar groups of phospholipids are located at the lipid - water interface, nonpolar acyl chains are in the interrior of bilayer to avoid contact with water. Different kinds of proteins are more or less embeded into the bilayer. The function of these proteins depends on physicochemical properties of the lipid bilayer. Cholesterol (CHOL), a ubiquitous component of mammalian cell membranes, plays an important role as a modulator of the structural and dynamical properties of the bilayer. If the phospholipid bilayer is in a liquid - crystalline state, CHOL (A) increases the conformational order of phospholipid acyl chains, thereby increasing bilayer thickness (ordering effect) and (B) decreases the surface area occupied by phosphatidycholine molecules on the lipid - water interface (condensing effect). These effects were verified by different experimental methods for bilayers consisting of saturated acyl chains but are less documented for acyl chains containing double bonds.

In this work, the effect of increasing concentration of CHOL on the bilayer thickness and area per molecule at water-lipid interface was studied by a small angle neutron scattering (SANS). Unilamellar liposomes made of monounsaturated diacylphosphatidylcholines (diCn–1PC, n=14, 18, 22) were used as an appropriate model of lipid part of biological membranes.

EXPERIMENT

diCn–1PC was co-solubilized in chloroform with an appropriate amount of CHOL. The chloroform was then evaporated under a stream of nitrogen gas followed by vacuum pumping. The lipid film was then dispersed in D_2O at a total lipid concentration 10 g/l. After homogenization, unilamellar liposomes were prepared by the dispersion extrusion through polycarbonate filter with pores of 50 nm diameter.

The SANS measurements were performed on the PAXE spectrometer located at the end of the G5 cold neutron guide on the Orphée reactor (Laboratoire Léon Brillouin, CEA Saclay, France). The experiments were performed with the sample to detector distance of 1.77 and 5.07 m and the neutron wavelength of λ=0.6 nm. The sample temperature was 30.0±0.1°C. The acquisition time for one sample was 30 min.

The normalized SANS intensity $I_{exp}(Q)$ in cm^{-1} units as a function of the scattering vector modulus $Q=4\pi\sin\theta/\lambda$, where 2θ is the scattering angle, was obtained as described in detail in [1]. An example of data is shown in Fig. 1, together with the best fits as obtained using an advanced evaluation model of lipid bilayer as a three-strip structure with a triangular shape of the probability distribution of polar head groups [2]. The volumetric data used in the analysis are the same as in [3]. The model's mathematical description [2] allows to evaluate the lateral area A_{UC} (surface per unit cell consisting of one diCn–1PC molecule and a particular fraction of CHOL) and the total thickness d_{TOT} while the polar region thickness is constrained to d_H=1 nm [2].

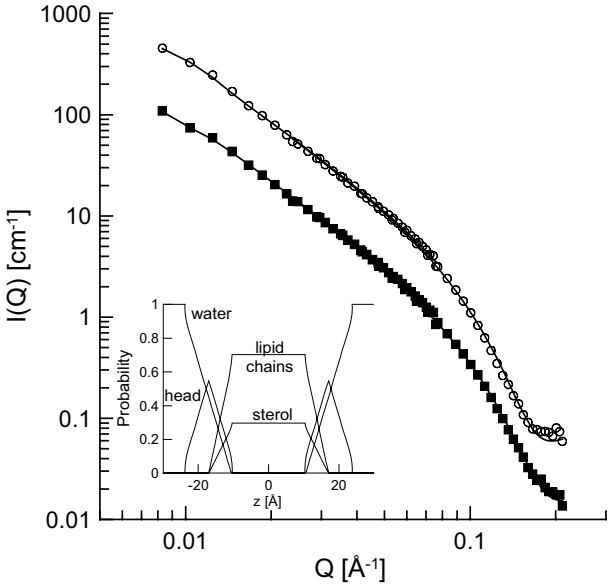

Fig. 1. Experimental SANS data obtained from unilamellar vesicles prepared from pure diC14–1PC bilayers (■) and those containing 33 mol% of CHOL ( ). Scattering curves are shifted vertically for clarity of presentation. Solid lines correspond to the best fits. The volume probability distributions is shown in the inset as a dependence on the distance z from the bilayer centre.

RESULTS

To estimate the magnitude of the condensing effect, one has to calculate independently the areas per diCn–1PC molecule, a_{PC}, and CHOL molecule, a_{CHOL}, using known values of A_{UC}. According to [4], specific partial areas for diCn–1PC and CHOL as a function of CHOL molar fraction $X=N_{CHOL}/(N_{CHOL}+N_{PC})$ were defined

$$a_{CHOL}(X)=\left(\frac{\partial A(X)}{\partial N_{CHOL}}\right)_{N_{PC}} \qquad a_{PC}(X)=\left(\frac{\partial A(X)}{\partial N_{PC}}\right)_{N_{CHOL}} \qquad (1)$$

where A is the whole surface of phospholipid bilayers,

N_{CHOL} and N_{PC} are the numbers of CHOL and diCn−1PC molecules in the sample. It was shown [4] that

$$\frac{A(X)}{N_{PC}} = A_{UC} = a_{PC}(X) + \frac{X}{1-X} a_{CHOL} \qquad (2)$$

The example of the concentration dependence of A_{UC} for diC14−1PC is in the Fig 2. A_{UC} depends linearly on the molar ratio CHOL : diCn−1PC for all phosphatidylcholines studied. As follows from this fact and Eq. 2, specific partial areas a_{PC} and a_{CHOL} are constant and do not depend on the concentration of CHOL in the diCn−1PC bilayer. They represent real areas of CHOL and diCn−1PC at lipid – water interface. Their values are listed in Tab. 1. Thereby we have shown that CHOL does not condense the area occupied by diCn−1PC molecule at lipid – water interface.

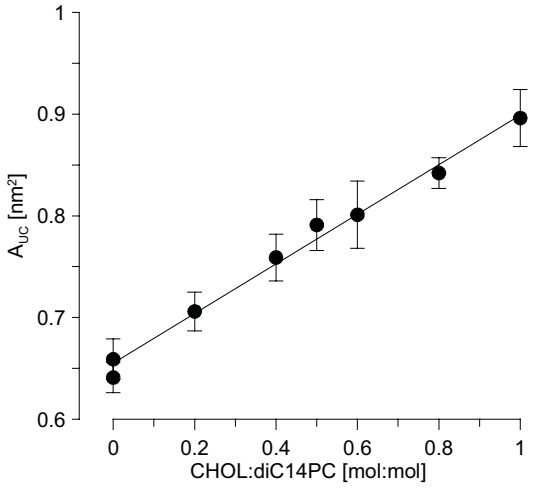

Fig. 2. Dependence of A_{UC} on the molar ratio of CHOL to diC14−1PC.

The area for diC18−1PC is in excellent agreement with [5]. Similarly as in [6], the area of diC18−1PC is larger than those for shorter and longer diCn−1PC.

TAB. 1. The partial-specific area of CHOL and diCn−1PC (n=14, 18, 22)

	a_{CHOL} [nm^2]	a_{PC} [nm^2]
diC14-1PC	0.246±0.010	0.652±0.005
diC18-1PC	0.234±0.007	0.673±0.003
diC22-1PC	0.259±0.014	0.634±0.008

Similar a_{CHOL} value (0.27 nm^2) was determined at high CHOL concentration as X approached 0.5 in diC16−0PC with saturated acyl chains [4]. Of course, for $X \leq 0.1$, a_{CHOL} was negative, what indicated a strong condensing effect of CHOL on diC16−0PC [4].

Different results regarding CHOL condensing effect were obtained by several other authors (see [7] for citations) who assume that the area per CHOL molecule in phospholipid bilayer does not depend on the lipid type and is the same as in CHOL monolayer (0.39 nm^2).

The ordering effect of CHOL on diCn−1PC acyl chains is manifested by the increase in the bilayer thickness (Fig. 3). These results are in agreement with [3].

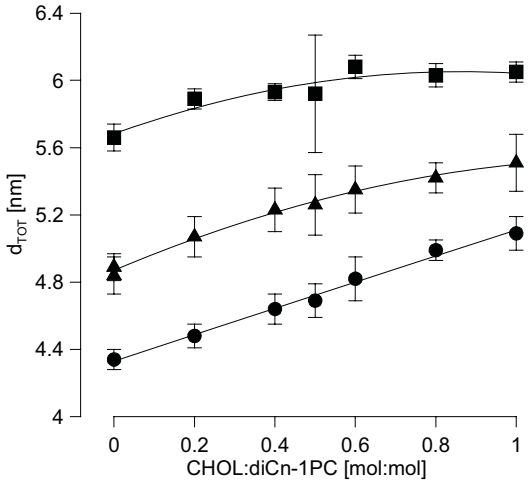

Fig 3. Dependence of A_{UC} on the molar ratio of CHOL. diC14−1PC (●), diC18−1PC (▲), diC22−1PC (■).

CONCLUSIONS

Cholesterol occupies cca 0.25 nm^2 at the interface of diCn−1PC bilayeyr and aqueous phase.

The condensing effect of CHOL on the surface of diCn−1PC at lipid - water interface is negligible.

The increase of diCn−1PC bilayer thicknes with increasing CHOL content proves the ordering effect of CHOL.

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