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Emulsification of Cholesterol in Bile Salt Micelles:relevance For Cholesterol Absorption

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A high level of cholesterol in the blood is associated with predisposition for cardiac diseases and is one of the major human health problems[1]. Some cholesterol is synthesized in the liver and a significant amount is also absorbed from dietary cholesterol at the small intestinal brush border membranes. Dietary cholesterol is first emulsified in mixed micelles of bile salts (BS) and fatty acids (FA) and the currently accepted mechanism suggests that absorption involves the interaction between the mixed micelles and the apical membrane of brush border cells where passive mechanisms play a significant role[2]. However, the detailed mechanism and the dependence on the dietary mixture of lipids are far from being completely understood. In most studies, mixtures of BS and FA have been used and the distribution of cholesterol between different phases and/or the in vitro cholesterol intake has been measured[3]. The complexity of the systems studied precludes the interpretation of the effect of each component in the process. Here we present the study of the solubilization of a cholesterol analogue, Deydroergosterol, in micelles of glycocholic acid and glycochenodeoxycholic acid (most abundant BS in the upper intestine[3]) followed by fluorescence. We develop a kinetic model to describe the rate of sterol emulsification and its maximum solubility in the BS micelles. The study was also performed with cholesterol labeled with C13 in carbon 4 in the sterol ring and followed by C13 NMR Spectroscopy. From preliminary results we can identify and quantify the emulsified cholesterol in the BSM at a chemical shift of 41.6 ppm that is well separated from the C13 NMR resonances of BS[4]. From the data obtained we obtain the cholesterol saturation index and the kinetic profile for the solubilization of cholesterol in the BS micelles.

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Partition of Amphiphilic Molecules To Lipid Bilayers By ITC

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The partition of the amphiphile Sodium Dodecyl Sulfate between an aqueous solution and a POPC bilayer was followed by ITC as a function of the total concentration of SDS. It was found that the obtained partition coefficient is strongly affected by the ligand concentration, even after correction for the charge imposed by SDS to the bilayer due to its partition. The partition coefficient decreased as the concentration of SDS increased and this was accompanied by an increase in the molar enthalpy. This behavior is due to saturation of the lipid bilayer leading to non-ideal behavior. Some rules are proposed to enable the retrieval of the parameters that describe the interaction of ligands with unperturbed lipid bilayers.

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Cholesterol Orientation and Tilt Modulus in DMPC Bilayers

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We performed molecular dynamics (MD) simulations of hydrated bilayers containing mixtures of dimyristoylphosphatidylcholine (DMPC) and Cholesterol at various ratios, to study the effect of cholesterol composition on its orientation, and to study the link between cholesterol tilt and overall phospholipid membrane organization. The simulations show a substantial probability for cholesterol molecules to transiently orient perpendicular to the bilayer normal. Our results further suggest that cholesterol tilt may be an important factor capable of inducing membrane ordering. In particular, we find that, as cholesterol concentration increases, the average cholesterol orientation changes in a manner strongly (anti)-correlated with the variation in membrane thickness. This correlation persists within the broad range of lipid/cholesterol ratios (1%-40% cholesterol) that we have studied. However, cholesterol orientation is found to be strongly determined by the aligning force induced by other cholesterol molecules.

To discuss this aligning field quantitatively, we analyzed cholesterol orientation using, to our knowledge, the first estimates from MD simulations of the cholesterol tilt modulus. Our calculations suggest that the tilt modulus (hence the aligning field) is indeed strongly dependent on sterol composition. Beyond providing valuable energetic insights pertaining to cholesterol orientation in phospholipid membranes, this empirical parameter should become a useful quantitative measure to describing cholesterol interaction with lipid bilayer, particularly in various coarse-grained force fields. The results discussed in this work should aid in understanding how cholesterol may induce a ''nematic'' aligning field on membrane proteins and thus shift their preferred conformational state.

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Combined Use of Steady-State Fluorescence Emission and Anisotropy of Merocyanine 540 To Distinguish Crystalline, Gel, Ripple, and Liquid Crystalline Phases in Dipalmitoylphosphatidylcholine Bilayers

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This study investigated the effects of lipid phase on monomer and dimer fluorescence of merocyanine 540. Emission and anisotropy spectra were assessed at multiple temperatures covering all four lamellar phases of pure dipalmitoylphosphatidylcholine. The probe segregates in the bilayer into two populations: monomers (emission maximum ~585 nm) and dimers (emission maximum ~621 nm). Induction of the crystalline (L_C) phase by extended pre-incubation at 4 °C produced a strong wavelength dependence of anisotropy values (0.33 at 580 nm, 0.14 at 625 nm). Wavelength dependence was strong at 15 and 25 °C, weak at 38 °C and absent above the main phase transition (>41.4 °C) or after returning the temperature from 46 to 25 °C. Average anisotropy values across the complete temperature range revealed both the sub-and main phase transitions. The temperature dependence of total fluorescence intensity likewise displayed both transitions. In contrast, changes in the shape of the emission spectrum were sensitive to the sub- and pre-transitions but not the main transition. These changes were quantified by calculating the ratio of intensities at the two peaks in the emission spectrum (585 and 621 nm). These results indicate that dimer fluorescence mostly vanishes at the pre-transition because the spectrum shape was unchanged above 35 °C. Thus, the absence of wavelength dependence of anisotropy values at higher temperatures was largely due to loss of fluorescence from probe dimers. These observations are consistent with a model in which merocyanine dimers are localized to the region between membrane leaflets where their motion is greater than that of the monomers, which reside among the packed lipid head groups. Moreover, dimer fluorescence intensity is enhanced by constraints on its movement imposed by highly-ordered lipids.

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Characterizing Vesicle Leakage By Fluorescence Lifetime Measurements

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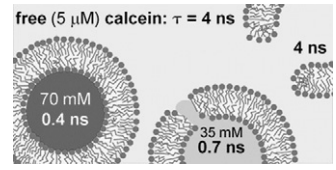
The membrane leakage assay based on vesicles loaded with self quenching dyes has been widely used for quantifying the activity of antibiotic peptides and other compounds that induce membrane pores or leaks. Here we show that this assay can be substantially improved if it is based on time-resolved decay curves rather than steady-state intensities only. A bi- or triexponential fit of calcein fluorescence decays allows for a parallel quantification of the free and (one or two) entrapped dye fractions and their effective local concentrations (governing the lifetime). The advantages of this technique are that it (i) allows the distinguishing of all-or-none from graded leakage for each sample, (ii) reveals the heterogeneity of graded leakage, (iii) truly quantifies the released dye rather than providing an empiric de-quenching value, and (iv) is independent of many errors that may affect the intensity. In addition to the example C12EO8 shown in Patel et al. (2009) Soft Matter 5:2849, we will present data obtained for a series of detergents and peptides, comprising all-or-none as well as graded leakage.



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The Effect of Lidocaine·HCL on the Physical Properties of Liposomes of Total Lipid and Phospholipids Extracted From Neuronal Membranes

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Fluorescent probe techniques were used to evaluate effect of lidocaine·HCl on physical properties (asymmetric lateral and rotational mobilities, membrane thickness) of liposomes of total lipid(SPMVTL) and phospholipids(SPMVPL) extracted from synaptosomal plasma membrane vesicles(SPMV). An experimental procedure was used based on selective quenching of 1,3-di(1-pyrenyl)-propane(Py-3-Py), 1,6-diphenyl-1,3,5-hexatriene(DPH) by trinitrophenyl