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Structural transition in aqueous lipid/bile salt [DPPC/NaDC] supramolecular aggregates: SANS and DLS study

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ABSTRACT

Small angle neutron scattering (SANS) and dynamic light scattering (DLS) were used to study different aggregation states in sodium deoxycholate (NaDC)-phosphatidylcholine systems at $T = 60\ ^{\circ}C$. Size and shape of the aggregates investigated as a function of the NaDC bile salt concentration (at the constant DPPC concentration of 6 mM) indicate a strong dependence of the size and morphology of the generated aggregates on the relative amount of NaDC bile salt. More specifically large occupied area of the bile salt induces a steric interaction which promotes the transition toward a variety of supramolecular structures ranging from ellipsoidal vesicles, ribbon-like structures, up to final spherical mixed micelles at the large amount of bile salt of 10 mM NaDC. The findings of the obtained results give important insight for understanding the formation of different topologies in aqueous lipid–bile salt mixtures as well as stimulate new routes for liposome reconstitution–solubilisation processes suitable for technological applications.

1. Introduction

The study of the self-assembly processes in a water environment is an important problem for the sake of understanding the physical and chemical bases of the existence of lipid bilayers. It is also interesting for the creation of new materials on the basis of the principle of the self-assembly of supramolecular bioaggregates and for the design of new drug delivery systems such as liposomes and mixed micelles [1,2]. Water structure, as well as the interfacial water behavior around hydrated bio-systems, play an important role in determining the favorite environment where biological structures and functions can be preserved [3–6]. In many aspects of bio-physiscs, structural processes involved in water solution of supramolecular aggregates stimulated in recent years new effective routes to control the main characteristic of the microstructure and the macroscopic phase transition for the design of new drug delivery systems. More specifically the study of the phase transitions in water solution of phospholipid vesicles are very important in many different fields of science and technology [7–9]. In basic research they serve as models for the investigation of cell membranes and membrane proteins that can be reconstituted in vesicles. They also serve as delivery agents for drugs, genetic material and enzymes through living cell membranes and other hydrophobic barriers in pharmacology, medicine, genetic engineering, cosmetic, and food industry [10–12]. Vesicles size appears to be a major factor in its permeation through tumour microvessels and its local residence in tumour tissue. A vesicle size of 1000 Å may be a pivotal size of vesicles for tumour targeting and long vesicle circulation in blood [13].

Bile salts are physiological detergents in humans and play an important role in intestinal digestion and absorption of dietary lipids and cholesterol as well as in the formation of cholesterol-gallstones [14,15]. Sodium deoxycholate (NaDC) is a secondary bile salt, which is formed from the primary sodium cholate by a bacterial enzyme in the upper small intestine. The bile salts solubilise the lipid vesicles under formation of mixed micelles. In contrast to classical detergents, where the hydrophilic headgroup and the lipophilic flexible aliphatic chains are clearly separated, bile salt molecules have a lipophilic surface, the convex side of the rigid steroid backbone, and a hydrophilic surface, the polyhydroxylated concave side of the molecule. Due to their structure and rigidity the aggregation properties are completely different compared to 'normal' detergents [16,17].

The mixed lipid/detergent systems are currently of great interest because of their wide use in membrane studies and in particular for the solubilization of biomembranes and the reconstitution of membrane proteins and lipids. Despite the successful reconstitution of a great number of membrane functions, molecular mechanisms, and thermodynamics of the membrane assembly on reconstitution are still debated [18–19]. Bio-membranes, in fact,

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are essentially 2D systems with a heterogeneous lipid compositions. Recently bio-membranes has been observed to form raft-like (liquid-ordered) nanodomains, which are floating inside a liquid-disordered lipid matrix at particular compositions and temperature range of multi-component mixtures [20].

The vesicle to micelle transition, represents an essential stage in the transformation of closed bilayer vesicles into solubilized micellar aggregates [20–23]. Various methods were applied to study the aggregation behavior of mixed lipid/detergent systems and the vesicle to micelle transition [24,25] including time-resolved fluorescence [20], transmission electron microscopy [24,26], static and dynamic light scattering [23,27,28], gel exclusion chromatography [22], differential scanning calorimetry [27], isothermal titration calorimetry [29,30] and NMR [31]. Among the methods used, the scattering techniques such as small angle scattering of X-rays (SAXS) and neutrons (SANS) are probably the most important and widely utilized experimental approach employed for the structural investigation of bio-membranes [32–34], and in special way for what concern the mixed lipid/bile salt systems investigations [35–39].

Herein, we describe the different aggregation states in sodium deoxycholate (NaDC)-phosphatidylcholine (DPPC) systems at the constant temperature of $T = 60$ °C (i.e. above the main phase transition temperature $T = 41$ °C of DPPC). The formation of intermediate structures with different topologies, evidenced by SANS and DLS experiments, indicate a strong dependence of the size and morphology of the supramolecular aggregates on the amount of NaDC bile salt at the constant DPPC concentration of 6 mM. The micelle-to-vesicle transition investigated in this study play an important role in the self-assembly processes involved in liposome reconstitution and have implications for the physiology of lipid solubilization in bile as well as intestinal absorption of dietary lipids.

2. Experimental section

2.1. Materials

The phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) and the bile salt sodium deoxycholate (NaDC) were purchased from Sigma (Deisenhofen, Germany). Water-d_2 (D_2O) was obtained from Aldrich (Milwaukee, MI, USA). Sodium chloride (NaCl) of p.A. grade was purchased from Merck (Darmstadt, Germany). The phospholipid was pure as checked by thin-layer chromatography. The purity of the bile salt was tested by mass spectrometry (Finnigan LCQ; Thermoquest, CA, USA). All substances were used without further purification.

2.2. Sample preparation

Mixed DPPC/NaDC were prepared by dissolving a certain amount of both substances in D_2O with 0.1 M NaCl, followed by ultrasonication for 10 min at 60 °C. All samples were stirred continuously for 5 h at 50 °C in a water bath and then filtered though a membrane filter of 0.2 μm pore size into dust-cleaned sample-cells. The sample cells are made of quartz glass and have a path length of 2 mm. All mixtures had a constant phospholipid concentration of 6 mM and a different bile salt concentration of 1.5, 3.5, 5.5, 7.5, and 10 mM, respectively.

2.3. Method

The SANS measurements were carried out at the small-angle neutron spectrometer in BENSC (Berlin). Incoherent background was subtracted from the experimentally measured and normalized SANS curve to receive the macroscopic cross-section of the coherent neutron scattering. Incoherent background is calculated as value of $6.178 \cdot 10^{-24}\,\mathrm{n}\,(\mathrm{cm}^{-1})$, where n is the concentration of hydrogen atoms in a sample. All measurements were carried out at the temperature of $T = 60$ °C.

The dynamic light scattering measurements were carried out on an ALV-5000 light scattering apparatus equipped with a 200 mW frequency doubled Nd:YAG laser with a wavelength of 5320 Å and a multiple tau digital correlator with a sampling time of 12.5 ns.

2.4. Structural models

Although SANS techniques have been widely used over the years in the field of biophysics and materials science, due to the intrinsic disorder of bio-systems, the achievement of the average structural information is not always a straightforward task. Analysis of the recent literature indicate that lipid bilayers systems are mainly composed of fluid phase with a heterogeneous lipid composition, while the relevant structures are best described by broad statistical distributions.

2.5. Guinier approximation

Guinier approximation of the coherent small-angle macroscopic scattering cross-section [32] was used as one of the methods to interpret the neutron small-angle scattering spectra. In the case of particles with commensurable sizes in each dimension (with radius of gyration, R_g, volume V, and scattering length density relative to the solvent $\Delta\rho$) the expression for the macroscopic differential cross-section in the so called Guinier region (i.e. for $q < 1/R_g$) is:

$$\frac{d\Sigma}{d\Omega} = n \cdot (\Delta\rho)^2 \exp\left[-\frac{q^2 R_g^2}{3}\right] \tag{1}$$

where q is the scattering wavevector, and n is the particles concentration. The radius of gyration R_g is simply related to the particle dimensions. For *spherical particles* with a homogeneous distribution of scattering length density and radius R, one gets $R = R_g\sqrt{5/3}$, while for ellipsoidal particles with semi-axes a, b, and c, one has $R_g^2 = \frac{1}{5}(a^2 + b^2 + c^2)$. For *cylindrical particles* with a cylinder length L much larger than its radius R, the expression for scattering macroscopic cross-section in the Guinier approximation is:

$$\frac{d\Sigma}{d\Omega} = n \cdot \frac{\pi \cdot L \cdot (\Delta\rho \cdot S_c)^2}{q} \exp\left[-\frac{q^2 R_c^2}{2}\right] \tag{2}$$

This equation, which is valid in the wavevector range $2\pi/L < q < 1/R_c$, describes also flexible cylinders with persistence length L_p much larger than the radius of the cross-section. For an homogeneous distribution of the scattering length density, in the case of circular cross-section the cylinder radius R is connect with the corresponding gyration radius R_c via expression $R = R_c\sqrt{2}$, while for ellipsoidal cross-section of rod-like micelles we have $R_g^2 = \frac{1}{5}(a^2 + b^2 + c^2)$, where a and b are the semi-axes of the ellipse.

For *extended unilamellar structures* (sheets or vesicles) with a surface area S, at the conditions when thickness d_l of the layer (or radius of gyration R_t) is much smaller than the radii of curvature of the surface (or the lateral dimensions of the layer), the approximation valid for scattering vectors q in the domain $2\pi/\sqrt{S} < q < 1/R_t$ is given by equation:

$$\frac{d\Sigma}{d\Omega} = n \cdot \frac{2\pi \cdot S \cdot (\Delta\rho \cdot d_l)^2}{q^2} \exp[-q^2 R_t^2] \tag{3}$$