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The vesicle-to-micelle transformation of phospholipid–cholate mixed aggregates: A state of the art analysis including membrane curvature effects

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ABSTRACT

We revisited the vesicle-to-micelle transformation in phosphatidylcholine–cholate mixtures paying special attention to the lipid bilayer curvature effects. For this purpose, we prepared unilamellar vesicles with different starting sizes ($2r_v$ = 45–120 nm). We then studied mixtures of the unilamellar vesicles (1–8 mmol kg^{-1}) and sodium cholate (0–11.75 mmol kg^{-1}) by static and dynamic light scattering. The transformation generally comprises at least two, largely parallel phenomena; one increases and the other decreases the average mixed aggregate size. In our view, cholate first induces bilayer fluctuations that lead to vesicle asphericity, and then to lipid bilayer poration followed by sealing/reformation (or fusion). The cholate-containing mixed bilayers, whether in vesicular or open form, project thread-like protrusions with surfactant enriched ends even before complete bilayer solubilisation. Increasing cholate concentration promotes detachment of such protrusions (i.e. mixed micelles formation), in parallel to further softening/destabilising of mixed amphipat bilayers over a broad range of concentrations. Vesicles ultimately fragment into mixed thread-like micelles. Higher cholate relative concentrations yield shorter thread-like mixed micelles. Most noteworthy, the cholate-induced bilayer fluctuations, the propensity for large aggregate formation, the transformation kinetics, and the cholate concentration ensuring complete lipid solubilisation all depend on the starting mean vesicle size. The smallest tested vesicles ($2r_v$ = 45 nm), with the highest bilayer curvature, require ~30% less cholate for complete solubilisation than the largest tested vesicles ($2r_v$ = 120 nm).

1. Introduction

Bile salts are a special group of physiological detergents, or biosurfactants, with a major role in lipid absorption in the intestine. The chemical structure of bile salts differs from that of typical head–tail surfactants (e.g. alkyl glucosides, alkyl sulphates, fatty acid polysorbates), which consist of a polar "head" with one or several hydrophilic groups attached to a separate hydrophobic chain/s, or a "tail". In contrast, each bile salt molecule has a rigid steroid core with one lipophilic, convex surface and one hydrophilic, polyhydroxylated, concave surface [1] (Fig. 1). The ring system is central in bile salt interactions with other molecules. The self- and hetero (with various lipids)-aggregation of bile salts and head–tail surfactants therefore differ in detail.

Bile salt–lipid interactions are of physiological and fundamental interest. They are a key to understanding intestinal fat digestion and absorption [2] and the basis for various applications. In biochemistry, for example, bile salts are used to isolate membrane proteins and to reconstitute such proteins into lipid bilayers [3–6]. In pharmaceutics, bile salts are included into some drug delivery systems, such as mixed micelles [7] (e.g. Konakion® MM, Valium® MM) or ultradeformable mixed lipid vesicles [8–16]. The self-assembly, i.e. the homo-aggregation, of bile salts into micelles and their hetero-aggregation with other lipids that tend to form bilayers, such as the ubiquitous phosphatidylcholines, are of physicochemical significance as well.

Phospholipid–bile salt mixtures were extensively investigated for decades, with a special early focus on lipid/cholesterol solubilisation and a later focus on vesicle-to-micelle transformation [1–3,5,17–31]. A large body of experimental data was thus collected over the years, but some important questions remain open. To our knowledge, the mixed aggregates detailed morphology and molecular composition, and especially their interrelationship in the transition region, are not yet fully elucidated. Trying to avoid repetition, we tackle herein these questions only to the extent necessary to understand the whole picture. In contrast, we pay full attention to the previously neglected curvature effects on bilayer solubilisation and on to the comparability of vesicle-to-micelle with micelle-to-vesicle transformation. Unlike previous researchers in the field we also ensure complete cholate ionisation during the process, as cholate like other ionic surfactants is (maximally) active only in its ionised form.

In this report we address the complete sequence of structural and morphological changes occurring during the vesicle-to-micelle

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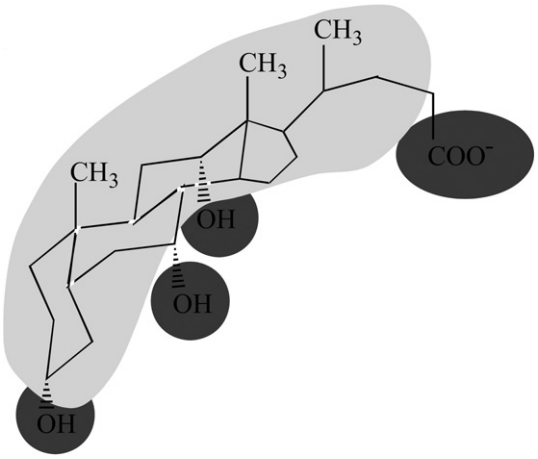

Fig. 1. The structural and chemical formula of a cholate molecule, showing the hydrophobic surface (light grey) and the hydrophilic groups (dark grey) on the other molecular side (modified from [1]).

transformation in phosphatidylcholine–sodium cholate mixtures under consideration of (local) bilayer curvature effects and with special focus on the presolubilisation region. For this purpose, we studied the initial vesicle size effects on natural phosphatidylcholine vesicle transformation into the cholate dominated mixed micelles, relying on static and dynamic light scattering data. We analysed the results with several theoretical models to explain experimental observations more quantitatively, and paid some attention to the studied process kinetics as well. The emerging picture of vesicle-to-micelle transformation is richer but also more complex than previously known, which raises some experimental caveats. To list but the most important ones: the very popular optically measured "bilayer-saturating concentration" is an ill-defined "descriptor" with an assay-, preparation history-, and size-dependent value; the "three-step solubilisation" model is pedagogically useful but never strictly applicable to reality, where continuous solubilisation prevails; the bilayer solubilising concentration is the only robust descriptor of lipid solubilisation in the tested mixtures, but it also depends on the starting aggregate size and is potentially burdened with the slow transition kinetics; the results measured with only partially ionised surfactant molecules are questionable due to interfacial effects on molecular charge, and vice versa. Arguably, these caveats apply to most, if not all, lipid–surfactant mixtures.

2. Materials and methods

2.1. Materials

We obtained soybean phosphatidylcholine (SPC, Lipoid S 100, purity = 97.8%, the assumed average molecular weight ~800 g/mol) from Lipoid GmbH (Ludwigshafen, Germany). Sodium cholate hydrate (purity ≥ 97%) was from Sigma-Aldrich (Steinheim, Germany). All the other chemicals and reagents were of analytical grade. Polycarbonate membranes were from GE Water & Process Technologies (Trevose, PA, U.S.A.).

2.2. Preparation of lipid vesicles

In brief, we dissolved the necessary amount of phosphatidylcholine in a sufficient amount of chloroform in a 500 mL round-bottom flask. We evaporated the solvent under vacuum at 50 °C in a rotary evaporator. This yielded a thin lipid film, which we hydrated at the same temperature with bidistilled water. The ensuing suspension of multilamellar vesicles (MLV) had a total phospholipid concentration of 120 mg g^{-1} (~150 mmol kg^{-1}). We then produced large unilamellar vesicles (LUV) from such MLV by extruding the original "crude" suspension 10 times through a set of polycarbonate membranes with 80 nm pores under 1.75 MPa (254 psi) nitrogen gas pressure. To gain intermediate-size unilamellar vesicles (IUV), we further extruded the LUV suspension eight times through a set of polycarbonate membranes with 30 nm pores under nitrogen gas pressure of 2.50 MPa (= 363 psi). To obtain the smallest achievable unilamellar vesicles (SUV), we sonicated the IUV suspension on ice with a Sonopuls HD 3100 ultrasonic probe homogenizer (Bandelin electronic, Berlin, Germany) until the suspension became opalescent (using an MS 73 tip and a sonication power of 20 W, this took approximately 150 min for a 25 g sample). Finally, we filtered the sonicated SUV suspensions through a polycarbonate membrane with 80 nm pores under 1.75 MPa nitrogen gas pressure to remove the titanium particles originating from the ultrasound transducing tip. We measured the final phospholipid concentration with HPLC to confirm that no lipid material was lost during preparation. For this purpose, we used an in-house modification of the method described by Nasner and Kraus [32], with refractive index detection [33].

2.3. Preparation of samples

We prepared vesicle suspensions with different lipid concentrations in an aqueous carbonate buffer (50 mM, pH = 10.25) adjusted with NaCl to a final ionic strength of 150 mM. For this purpose, we diluted the original LUV, IUV, or SUV suspensions, prepared in distilled water, with appropriate volumes of the buffer. We always prepared fresh samples immediately before starting an experiment to minimise lipid degradation/hydrolysis at the high chosen pH. We also prepared a series of sodium cholate solutions/suspensions with different concentrations in a similar buffer, and adjusted each preparation to 150 mM ionic strength with NaCl. We then mixed an aliquot of the tested vesicle suspension with an equal volume of the appropriate sodium cholate solution/suspension by stirring the blend thoroughly. For the steady state experiments, we left each mixture to equilibrate at room temperature (~25 °C), until its optical density became constant. We then recorded the static and the dynamic light scattering data of each separately prepared mixture. To conduct the time-resolved measurements, we mixed the individual components in a jacketed (25 °C) glass reservoir connected to a flow-through quartz cuvette with peristaltic pump tubing. An eight-channel Gilson (Villiers le Bel, France) Minipuls-3 peristaltic pump maintained a steady suspension flow through the cuvette, in which we recorded the optical density continuously until reaching a constant value. To assess phosphatidylcholine hydrolysis during experiments, we checked phosphatidylcholine and lysophosphatidylcholine concentrations in representative samples with the described HPLC method.

2.4. The static light scattering (turbidimetry)

We measured the static light scattering (optical density) with a Shimadzu UV-1601 double-beam UV–VIS spectrophotometer equipped with a 6 position, automated sample changer and the Shimadzu UVProbe version 2.0 software (Shimadzu Corporation, Kyoto, Japan). We first confirmed that the light absorbed by SPC in the employed concentration range is negligible between 400 nm and 500 nm. We then read the optical density of the tested LUV and IUV mixtures at 500 nm. For the SUV mixtures we recorded the optical density at 400 nm to increase sensitivity and then properly allowed for the difference in the final data analysis.

2.5. The dynamic light scattering (photon correlation spectroscopy)

We used an ALV-NIBS/HPPS particle sizer (ALV-Laser *Vertriebsgesellschaft mbH*, Langen, Germany) for the dynamic light scattering measurements. We characterised each sample at 25 °C in three