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Solubilization of sphingomyelin vesicles by addition of a bile salt

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Abstract

The interactions of the bile salt sodium taurocholate (TC) in 50 mM Trizma–HCl buffer and 150 mM NaCl (pH 9) at 37 °C with membranes composed of sphingomyelin (SM) were studied by dynamic light scattering, cryogenic transmission electron microscopy (cryo-TEM) and turbidity measurements. Small unilamellar SM vesicles were prepared by extrusion. Below the CMC of TC, taurocholate addition leads to vesicle growth due to incorporation of the taurocholate molecules into the vesicle bilayer. At around half the CMC of the bile salt, the SM vesicles are transformed into SM/TC mixed worm-like micelles, which are visualized by cryo-TEM for the first time. Further increase in the taurocholate concentration leads to the rupture of these structures into small spherical micelles. Interestingly, large non-spherical micelles were also identified for pure taurocholate solutions. Similar threadlike structures have been reported earlier for the bile salt sodium taurodeoxycholate [Rich, A., Blow, D., 1958. Nature 182, 1777; Blow, D.M., Rich, A., 1960. J. Am. Chem. Soc. 82, 3566–3571; Galantini, L., Giglio, E., La Mesa, C., Viorel-Pavel, N., Punzo, F., 2002. Langmuir 18, 2812] and for mixtures of taurocholate and phosphatidylcholate [Ulmius, J., Lindblom, G., Wennerström, H., Johansson, L.B.-Å., Fontel, K., Söderman, O., Ardvisson, G., 1982. Biochemistry 21, 1553; Hjelm, R.P., Thiyagarajan, P., Alkan-Onyuksel, H., 1992. J. Phys. Chem. 96, 8653] as determined by various scattering methods.

Keywords: DLS; Cryo-TEM; Bile salts; Taurocholate; Sphingomyelin; Lipids; Worm-like micelles

1. Introduction

Bile salts are one of the most important groups of natural occurring surfactants. They are produced by the hepatic metabolism of cholesterol and stored in the gall bladder after secretion by the liver. At low concentration, they form micellar aggregates (Marquez et al., 2000; Amenitsch et al., 2003) that act as solubilizers of lipophilic substances in the gastrointestinal tract. Therefore, bile salts play a vital role in lipid digestion and adsorption. Bile salts have a small aggregation number (and micellar size) compared to synthetic surfactants, with reported values ranging between 3 and 16 (Sugioka and Moroi, 1998; Matsuoka and Moroi, 2002; Mazer et al., 1979). The bile salt taurocholate (TC), for example, form micelles in water with a hydrodynamic radius of 1.4 nm (Nagadome et al., 1998). However, the aggregation number and micellar size increase considerably with concentration and ionic strength (Sugioka and Moroi, 1998; Matsuoka and Moroi, 2002; Mazer et al., 1979; Kratohvil et al., 1986). In some cases, helical arrangements of steroid units lead to the formation of fibre-like structures (Rich and Blow, 1958; Blow and Rich, 1960; Galantini et al., 2002).

Sphingomyelin (SM), together with cholesterol, are the main constituents of lipid rafts, which might play a determining role for the attachment of proteins involved during signal transduction in cell membranes (Simons and Ikonen, 1997). Additionally, SM is believed to protect RNA from hydrolysis within the cell nucleus and to favour DNA unwinding during DNA synthesis (see for example ref. Albi and Viola Magni, 2004). Indeed, SM represents about 40% of the cell nucleus lipids. Furthermore, SM plays a very important role in cell membrane formation and plasma lipoprotein metabolism (Ridway, 2000). SM rich diets have shown to significantly reduce the early stages in colon car-

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cinogenesis and tumour formation, which provides an insight into the association between diet and cancer (Schmelz, 2000). In another recent study sphingolipid-rich diets were shown to influence the plasma and tissue levels of other lipids (Imaizumi et al., 1992). Specifically, SM intake inhibits the intestinal absorption of cholesterol (Noh and Koo, 2004). Since SM is widely present in food, a deeper understanding of its solubilization by bile salts is required. Moreover, an increase of the SM content in the blood plasma have been correlated with the development of arteriosclerosis (Li et al., 2005). Therefore, controlling the digestion and uptake of dietary SM or inhibiting SM biosynthesis might have a therapeutic value for treatment of arteriosclerosis.

Bile salts can be incorporated into liquid crystalline phases in mixed lipid systems but to a limited extent (Almgren, 2000; Hildebrant et al., 2004). Instead, they are very effective solubilizing agents of phospholipid bilayers inducing the formation of mixed micelles (Andrieux et al., 2004; Walde et al., 1987). Indeed, glyceryl monooleate bilayers can only take up to 0.14 cholate molecules per lipid, whereas the phosphatidylcholine (PC) bilayers can accommodate up to 0.3 mol (Almgren, 2000). Above such molar ratio, bile salts and PC/monoolein mixtures form worm-like micelles (Ulmius et al., 1982; Hjelm et al., 1992). Upon increasing the bile salt concentration, various liquid crystalline phases are formed including a hexagonal phase for the phosphatidylcholine and a cubic phase for monoolein (Small et al., 1966a, 1966b). Interactions of bile salts with SM, however, have been poorly studied at so far. Eckhardt et al. (1999) have studied the interaction between PC/SM mixed vesicles (containing up to 40% SM) and the bile salt TC. They found that vesicles containing SM were considerably more sensitive to solubilization into micelles upon TC addition. Based on these results, we carry out a systematic study of pure SM vesicle solubilization by increasing the TC concentration using mainly dynamic light scattering (DLS) and cryo-TEM. The interactions have been followed at conditions that are physiologically relevant for the human small intestine where alkaline sphingomyelinase, the key enzyme for SM digestion (Duan, 2006), is present.

2. Experimental section

2.1. Materials

Bovine milk sphingomyelin was provided by Skånemejerier (Malmö, Sweden) and used without further purification. This sample has at least 95% of purity as determined by high performance liquid chromatography. The composition of sphingomyelin from bovine milk was determined earlier by Malmsten et al. (1994). It contains a large fraction of long acyl groups mainly of the saturated type (∼90%). The authors also determined the gel to liquid crystal transition temperature of the hydrated material to be ∼37 °C. Sodium chloride (Sigma) and sodium taurocholate (Sigma) were used without further purification. The CMC of taurocholate in 50 mM Tris buffer pH 9 containing 150 mM NaCl was found to be 2 mM by surface tension measurements at 25 °C. Trizma–HCl buffer, 1 M, pH 9 was purchased from Fluka biochemika. All water used was of ultra high quality (UHQ), processed in Elgastat UHQ II (Elga Ltd., High Wycombe, Bucks, England).

2.2. Methods

2.2.1. Vesicle preparation

Sphingomyelin was dissolved in chloroform. The solvent was evaporated under a nitrogen stream and left under vacuum overnight. Next, the thin lipid layer was dispersed in the final buffer solution (50 mM Tris buffer pH 9, 150 mM NaCl) that had been previously filtered through 0.2 μm filters (Sartorius). The lipid solution was then extruded 15 times through a 100 nm pore size polycarbonate filter using an avanti mini-extruder that was thermostated at 60 °C (McDonald et al., 1991). Then, aliquots of the lipid and taurocholate stock solution were mixed and taken to a total volume of 1 mL to give the desired sphingomyelin to taurocholate molar ratio (SM/TC m.r.). All samples (except otherwise indicated) were left to equilibrate overnight before used for further measurements.

2.2.2. Absorbance measurements

A Helios β UV Spectrophotometer was used to monitor the changes in absorption at the fixed wavelength of 532 nm with time for the sphingomyelin/taurocholate samples at the same total concentration and molar ratios used for the dynamic light scattering experiments. The absorbance measurements were performed at 37 °C.

2.2.3. Dynamic light scattering (DLS)

The experimental goniometer setup (ALV-GmbH, Langen, Germany) to perform DLS measurements has been described previously with the difference that *cis*-decahydronaphtalene is used instead as the refractive index matching liquid (Jansson et al., 2004). The light source is a 532 nm diode-pumped Nd:YAG solid-state Compass-DPSS laser (COHERENT, Inc., Santa Clara, CA). In this work, the temperature was controlled at 37 ± 0.1 °C and the scattering angle (θ) was varied from $\theta = 40°$ to 110°. Two multiple τ digital correlators (with a total of 320 exponentially spaced channels) were utilized to produce the time (auto and pseudo-cross) correlation function of the scattered intensity. The normalized intensity correlation function $g^{(2)}(t)$ is related to the normalized time correlation function of the electric field [$g^{(1)}(t)$], by Siegert's relation: $g^{(2)}(t) - 1 = \beta \mid g^{(1)}(t)|^2$, where t is the lag time and β (≤ 1) is a coherence factor which accounts for deviation from ideal correlation and the experimental geometry. For polydisperse particle sizes, $g^{(1)}(t)$ may be described by:

$$g^{(1)}(t) = \int_{-\infty}^{\infty} \tau A(\tau) \exp\left(\frac{-t}{\tau}\right) \mathrm{d}\ln\tau, \quad (1)$$

where τ is the relaxation time and $A(\tau)$ is the distribution of relaxation times.

The experimental intensity correlation functions ($g^{(2)}(t) - 1$) were analyzed by non-linear regularized inverse Laplace transformation to obtain the relaxation time distribution $A(\tau)$. The analysis uses the calculation algorithm REPES (Nicolai et al., 1990a), incorporated in the GENDIST analysis package