



Phospholipid containing mixed micelles Characterization of diheptanoyl phosphatidylcholine (DHPC) and sodium dodecyl sulfate and DHPC and dodecyl trimethylammonium bromide

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Abstract

Mixed micelles of 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (DHPC) with ionic detergents were prepared to develop well characterized substrates for the study of lipolytic enzymes. The aggregates that formed on mixing DHPC with the anionic surfactant sodium dodecyl sulfate (SDS) and with the positively charged dodecyl trimethylammonium bromide (DTAB) were investigated using time-resolved fluorescence quenching (TRFQ) to determine the aggregation numbers and bimolecular collision rates, and electron spin resonance (ESR) to measure the hydration index and microviscosity of the micelles at the micelle–water interface. Mixed micelles between the phospholipid and each of the detergents formed in all compositions, yielding interfaces with varying charge, hydration, and microviscosity. Both series of micelles were found to be globular up to 0.7 mole fraction of DHPC, while the aggregation numbers varied within the same concentration range of the components less than 15%. Addition of the zwitterionic phospholipid component increased the degree of counterion dissociation as measured by the quenching of the fluorescence of pyrene by the bromide ions bound to DHPC/DTAB micelles, showing that at 0.6 mole fraction of DHPC 80% of the bromide ions are dissociated from the micelles. The interface water concentration decreased significantly on addition of DHPC to each detergent. For combined phospholipid and detergent concentration of 50 mM the interface water concentration decreased, as measured by ESR of the spin-probes, from 38.5 M/L of interface volume in SDS alone to 9 M/L when the phospholipid was

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present at 0.7 mole fraction. Similar addition of DHPC to DTAB decreased the interfacial water concentration from 27 M/L to 11 M/L. Determination of the physicochemical parameters of the phospholipid containing mixed micelles here presented are likely to provide important insight into the design of assay systems for kinetic studies of phospholipid metabolizing enzymes. © 2005 Elsevier Ireland Ltd. All rights reserved.

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1. Introduction

Phosphatidylcholines with two short fatty acyl chains of four to eight carbons each readily form micelles in aqueous solutions (Tausk et al., 1974). They have been widely used as model substrates for the study of lipolytic enzymes (Roberts, 1991; El-Sayed and Roberts, 1985; El-Sayed et al., 1985; Boegeman et al., 2004) as well as for studies of model membranes, including the extraction and reconstitution of membrane-bound proteins (Kessi et al., 1994; Burns and Roberts, 1980). Phospholipid containing mixed micelles and vesicles have been widely used as substrates for measuring the activities of lipid metabolizing enzymes (Boegeman et al., 2004; Cajal et al., 2000; Edwards et al., 2002; Gadd and Biltonen, 2000). Usually, in these assay systems the phospholipid-to-surfactant ratio is varied empirically in order to obtain surface dilution of the phospholipid component, so as to achieve the necessary curve-fitting for interfacial kinetics (El-Sayed et al., 1985; Cajal et al., 2000), without being able to take into account the structure and properties of the micelle lipid–water interface. Specifically, phospholipases have been shown to exhibit several orders of magnitude higher activity toward aggregated substrates versus molecularly dispersed phospholipids in solution (Pierson et al., 1974; Brzozowski et al., 1991). While the observed catalytic rate enhancements have been attributed to favorable properties of the lipid–water interface (e.g. state of hydration, fluidity, interface charge, surface curvature, conformation of the lipid in the micelle and the micelle-bound enzyme (Tatulian, 2001), the properties of the interface remain to be elucidated. Thus, despite the fundamental importance and widespread application of micelle aggregates in biochemistry, relatively little information is available at the present on the structure and dynamics of these assemblies pertaining to phospholipase enzyme kinetics.

In the present article we report the physicochemical characterization of a series of short-chain phospholipid containing mixed micelles, including diheptanoyl phosphatidylcholine in conjunction with sodium dodecyl sulfate (SDS), and the same phospholipid in mixed micelles with dodecyl trimethylammonium bromide (DTAB), designed to determine the relationship between the properties and composition of these aggregates. The anionic surfactant (SDS) has been selected to provide a simple model for the more complicated, negatively charged bile-salt containing micellar aggregates of phospholipids that serve as substrates of secretory phospholipase A₂ enzymes (Roberts, 1991; El-Sayed and Roberts, 1985), while the cationic series including the DTAB-based mixed micelles are used as reference to obtain a more complete picture of the effect of the charge on the properties of the micellar aggregates.

The short chain lecithin micelles have been studied for their critical micelle concentrations (cmc), size, shape, and structure, by NMR (Burns and Roberts, 1980), Raman spectroscopy (Burns et al., 1982), quasi-elastic light scattering (Burns et al., 1983) and small angle neutron scattering (Lin et al., 1986; Lin et al., 1987). Mixed micelles of ionic/non-ionic or ionic/zwitterionic surfactants yield interfaces of varying charge, hydration, and microviscosity, depending on composition. When well-characterized, they can provide a controlled medium for bio-chemical assays and investigating interfacial phenomena. In the past few years we have applied the techniques of time-resolved fluorescence quenching (TRFQ), electron spin resonance (ESR), and small angle neutron scattering (SANS) to the characterization of micelles and the micelle/water interface (Griffiths et al., 2004; Bales et al., 2001; Ranganathan et al., 2001; Bales and Zana, 2002). This characterization has been framed in the core-shell model, essentially the classical model originally proposed by Hartley (1936). The claim was already made twenty years ago (Halle and Carlström, 1981) that “the overwhelming majority

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