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Short communication

The critical micelle concentrations of lysophosphatidic acid and sphingosylphosphorylcholine

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Abstract

The critical micelle concentrations (CMC) of lysophosphatidic acid (LPA) and sphingosylphosphorylcholine (SPC) were measured by isothermal titration calorimetry. The CMC of LPA decreases with salt concentration and acyl chain length. In water at 25 °C, the CMC values of 1-acyl-2-lyso-*sn*-glycero-3-phosphatidic acid are 1.850, 0.540, 0.082, and 0.346 mM, respectively, when the acyl group is myristoyl, palmitoyl, stearoyl, and oleoyl. The CMC of SPC in 10 mM sodium phosphate buffer, pH 7.4, at 25 °C was 0.158 mM, and did not change with an increase in salt concentration.
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1. Introduction

Lysophosphatidic acid (1-acyl-2-lyso-*sn*-glycero-3-phosphatidic acid, LPA) and sphingosylphosphorylcholine (SPC) are mediators of a myriad of physiological events, including Ca^{2+} flux, neural development, and cell growth/death. LPAs exert their activities through several G protein-coupled receptors (GPCR) as well as through non-GPCR proteins (Tigyi and Parrill, 2003). Several proteins present in biological fluids, such as albumin, fatty acid binding proteins, gelsolin, and apolipoproteins, have been implicated in LPA transport and activity. Although the specific receptors to which SPC binds with high affinity have not yet been characterized (Bektas et al., 2003), SPC has important cell signaling functions (Meyer zu Heringdorf et al., 2002). LPA and SPC also have potential pathophysiological roles in angiogenesis and metastasis (English et al., 2001) and ischemia (Karliner, 2002). Therefore, a great deal of current interest is directed toward developing specific antagonists for the various lysophospholipid receptors (Tigyi et al., 2000). The binding of LPA and SPC to their putative protein receptors or to proteins that enhance their cellular activity has been studied over a broad range of ligand concentrations (from low nanomolar to hundreds of micromolar). As no detailed examination of critical micelle concentration (CMC) values has yet been reported for these lysophospholipids, it is not known whether the ligands exist as monomers or

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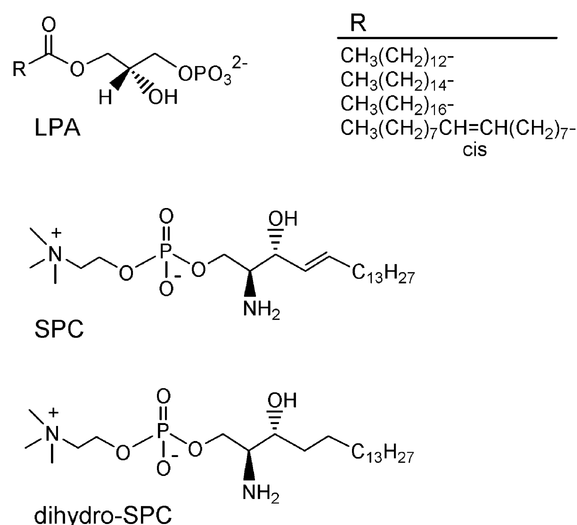

Fig. 1. Structures of the lysophospholipids that were used for the CMC measurements.

micellar aggregates under the conditions of the binding experiment. In the present study, we have estimated the CMC values of several synthetic LPAs, SPC, and dihydro-SPC (for structures, see Fig. 1) in water and in aqueous buffer at various ionic strengths at 25 °C.[1]

2. Materials and methods

2.1. Materials

LPAs and SPC were purchased from Avanti Polar Lipids (Alabaster, AL, USA). 4,5-Dihydro-SPC was prepared by catalytic hydrogenation of SPC (PtO_2, dry EtOH, overnight); purification was by silica gel column chromatography. Octyl-β-thioglucoside (OTG) was from Sigma–Aldrich.

2.2. Isothermal titration calorimetry

Microcalorimetry was performed on an MCS ITC microcalorimeter (Microcal, Northampton, MA, USA). Five- or ten-microliter aliquots of lipid suspension in pure water or buffer were injected into a sample cell containing ~1.35 ml of water or buffer at 25 °C, with stirring at 400 rpm. The heat flow accompanying the dilution was measured, and the data were analyzed with the Origin software provided by Microcal.

3. Results and discussion

3.1. Estimation of CMC values by ITC

A wide variety of physical methods have been applied to estimate the CMC of surfactants (Patist, 2002). A particularly attractive method to measure the process of micellization/demicellization of surfactants is isothermal titration calorimetry (ITC), which provides CMC as well as thermodynamic data for micelle formation without the use of an exogenous probe (Heerklotz and Seelig, 2000). In the ITC method, microliter volumes of an aqueous micellar solution of the surfactant are injected serially (up to a total of ~250 μl) into a cell containing >1 ml of water or buffer at a constant temperature. The dissociation of the surfactant molecules from the injected micelles on this ~100–200-fold dilution in the cell is monitored as the heat of demicellization. The heat is measured at each titrant concentration, from far below the CMC to concentrations at which the CMC is approached, at which point the injection of additional micelles does not result in dissociation into the individual surfactant monomer molecules. The CMC value is the maximal value of the first derivative of the exothermic heat of titration or the minimum value of the endothermic heat of titration (Paula et al., 1995).

To confirm that we could measure the CMC of the surfactant OTG by analysis of the ITC profiles we carried out a demicellization experiment in which OTG micelles were injected into water. The CMC value we calculated at 25 °C was 11.23 ± 0.02 mM (data not shown), which is consistent with a previous estimation of the CMC of OTG (15.94 mM at 60 °C) (Lasch and Hildebrand, 2002); at a higher temperature, the CMC value is expected to increase.

The demicellization of 1-oleoyl-LPA in pure water results in an endothermic heat of reaction (Fig. 2A).

[1] Attempts to measure the CMC of the important lysosphingolipid sphingosine 1-phosphate in water or phosphate buffer failed because this lipid could not be solubilized under the conditions used to disperse LPA and SPC.

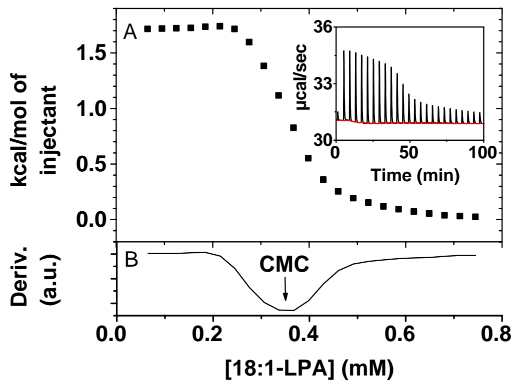

Fig. 2. ITC demicellization of C18:1 Δ^9*cis*-LPA in water (no salt) at 25 °C. (A) Heat observed on each injection vs. final C18:1-LPA concentration in the calorimeter cell. A 7.96-mM micellar dispersion of C18:1-LPA in water was titrated into pure water. *Inset*: Measured heat power vs. time elapsed during the titration. (B) The first derivative of the curve in A (in arbitrary units). The minimum is taken as the CMC.

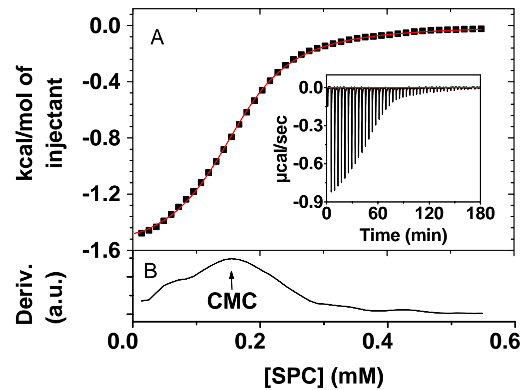

Fig. 3. ITC demicellization of SPC in 10 mM Na_2HPO_4 buffer (0 mM NaCl), pH 7.4, at 25 °C. (A) Heat observed on each injection vs. final SPC concentration in the calorimeter cell. A 3.1-mM micellar SPC dispersion in buffer (10 mM sodium phosphate, pH 7.4) was titrated into buffer. *Inset*: Measured heat power vs. time elapsed during the serial injections of 5-μl aliquots. (B) The first derivative of the curve in A (in arbitrary units). The maximum is taken as the CMC.

The CMC value calculated from the data presented in Fig. 2B is 0.346 mM.

The ITC profiles for the demicellization of SPC in 10 mM phosphate buffer without any added salt are presented in Fig. 3A. A CMC value of 0.158 mM was calculated from the exothermic heat of “reaction” shown in Fig. 3B.[2]

3.2. Effect of salt on the CMC of LPA and SPC

Fig. 4 compares the dependence of the CMC values of 1-oleoyl-LPA and SPC on NaCl concentration. As the NaCl concentration is increased, the CMC of SPC is invariant; however, the CMC of 1-oleoyl-LPA decreases markedly, reflecting the ability of the added salt to shield the negative charges of the neighboring phosphate groups in the LPA micelles. In contrast, the zwitterionic nature of the phosphocholine headgroup of SPC and its 2-amino group contribute to the lack of a salt effect on its CMC. The effect of [NaCl] (especially between 0 and ∼30 mM) on the CMC of LPA is more marked than that observed with SDS and sodium cholate (Chattopadhyay and London, 1984) and with CHAPS (Chattopadhyay and Harikumar, 1996). A plot of log (CMC—the limiting CMC at high salt concentration) versus [NaCl] is linear (correlation coefficient 0.98, plot not shown), and fits the empirical equation $\log \Delta\mathrm{CMC} = \mathrm{constant} - k_s C_s$, where k_s is the salt effect constant and C_s is the concentration of sodium chloride (Mukerjee, 1965).

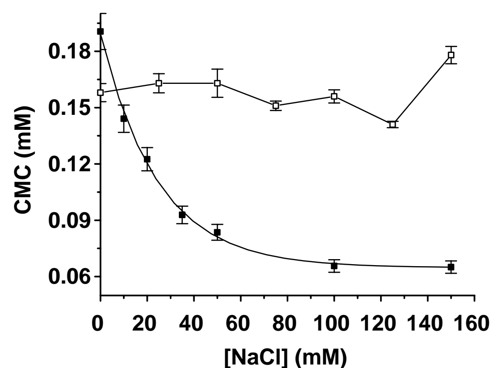

Fig. 4. Salt dependence of the CMC of C18:1 Δ^9*cis*-LPA and SPC at 25 °C: (■) C18:1 Δ^9*cis*-LPA; (□) SPC.

[2] Previous studies of demicellization of various lipids have revealed both exothermic and endothermic heats of reaction. For example, endotherms were observed for injections of sodium dodecylbenzenesulfonate (Hait et al., 2003) and lysophosphatidylcholine (Heerklotz and Epand, 2001) into water, whereas exotherms were observed for the demicellization of surfactin (Heerklotz and Seelig, 2000), $C_{12}EO_8$ (Heerklotz and Seelig, 2000), and OTG (the work described herein).

Table 1
CMCs of various LPAs in water

1-Acyl-LPA	CMC (mM)[a]
Myristoyl	1.850
Palmitoyl	0.540
Oleoyl	0.346
Stearoyl	0.082

[a] Data are averages of duplicate runs. Errors are typically ±10%.

3.3. *Effect of acyl-chain structure on the CMC of LPA*

The CMC values of synthetic 1-acyl-LPAs in water (no salt) at 25 °C are summarized in Table 1. The methylene group contribution to the change in enthalpy of micelle formation at 25 °C for the C14:0-, C16:0-, and C18:0-LPAs was obtained from the slope of a plot of ΔH of micellization versus the number of methylene groups in the acyl chain. The value thus obtained was −1.14 kcal/mol, which is higher than that obtained for liquid hydrocarbons and lysophosphatidylcholines (LPCs) (Heerklotz and Epand, 2001). On introduction of a *cis* double bond into the 18-carbon acyl chain, the CMC increased from 0.082 to 0.346 mM, reflecting the looser packing in micelles containing an oleoyl chain than a stearoyl chain. Similarly, marked reductions in the melting temperature T_m and ΔH of the gel-to-liquid-crystalline phase transition have been found in bilayers on introduction of *cis* monounsaturation into phospholipids (Huang and Li, 1999). Catalytic hydrogenation of the trans double bond of SPC, producing dihydro-SPC, resulted in a marked decrease in the CMC, i.e., 0.056 mM for dihydro-SPC versus 0.158 mM for SPC in pH 7.4 buffer at 25 °C.

3.4. *Previous estimates of CMC values of lysophospholipids*

The CMC values of LPCs were estimated by ITC to be 6.8, 0.71, 0.045, and 0.005 mM for C10:0-, C12:0-, C14:0-, and C16:0-LPC at 25 °C in pure water; moreover, the identical CMC values were found for these LPCs in pH 7.4 buffer containing 150 mM NaCl (Heerklotz and Epand, 2001). We found that the CMC values of C14:0- and C16:0-LPAs are much higher than those for C14:0- and C16:0-LPCs in pure water (Table 1). In addition, as noted above, the CMC of LPA is strongly dependent on the salt concentration (Fig. 4).

4. Summary

In summary, although it is recognized that the binding of lysophospholipids such as LPA and SPC to receptors mediates their bioactivities, the CMC values of these important lipids have not yet been reported. The ITC studies reported here provide the CMC values of two important bioactive lysophospholipids, LPA and SPC. LPAs have much higher CMC values than the corresponding LPCs in the absence of added salt. On increasing the NaCl concentration, the CMC of LPA decreases whereas the CMC of SPC does not. The presence of unsaturation in LPA and SPC markedly raises the CMC.

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