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Metal ion binding to phospholipid bilayers evaluated by microaffinity chromatography

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ABSTRACT

Group I and II ion binding to phospholipid membranes was evaluated by affinity chromatography utilizing a new stationary phase system based on lipid bilayers supported within large-pore particles composed of Stöber silica spheres. Using an inductively coupled plasma mass spectrometer for detection, robust determination of binding selectivity within group II ions is achieved with capillary columns containing nanomole quantities of lipid and using picomoles of metal analyte. Columns with a unique lipid formulation can be prepared within three hours using a solvent-casting assembly method. The observable thermotropic phase behavior of dipalmitoylphosphatidylcholine has a significant effect on alkaline metal binding and demonstrates the dynamic nature of the supported bilayers. Of the group I ions, only lithium exhibits retention with neutral phosphatidylcholine bilayer stationary phases. A comparison of Stöber-based supports with two commercially available large-pore silicas reveals the effect that particle structure has on analyte accessibility to the bilayer surface as evaluated by retention per supported lipid mass.

1. Introduction

Metal ion interactions with lipid components of biomembranes are low affinity in nature. Nevertheless, the interaction plays a role in vesicle fusion [1–3], phospholipase activity [4], and physical properties of the lipid bilayer such as stiffness [5], phase transitions [6], lipid clustering [7–9], and surface charge [10]. The low binding affinity and its sensitivity to membrane electrostatics that are influenced by solution composition complicate the determination of association binding constants (K_{assoc}) of ions to lipid membranes. Indirect measurements of affinity must accurately model competing interactions or non-binding processes that can contribute to a measured parameter change. This may factor into the variability in K_{assoc} values determined by different techniques. A recent work by Szekely et al. cataloged many of the reported K_{assoc} values for cations with zwitterionic lipids which are major components of many biomembranes [11]. The values for calcium ion binding to dipalmitoylphosphatidylcholine, for example, vary from 1 to 190 in different studies.

The interactions between inorganic ions and zwitterionic surfactants have been studied by electrostatic ion chromatography, also called zwitterionic ion chromatography (ZIC) [12–17]. Most commonly, single-tail surfactants with zwitterionic headgroups are adsorbed onto alkylated porous silica to form a surfactant monolayer. The work of several research groups [15,18–21] has led to an understanding of the mechanisms that give rise to the unique ion selectivity on different zwitterionic phases. While the development of ZIC has largely been for analytical separations, cation studies with monolayer films bearing phosphocholine headgroups [14,18,21] yield insight into interactions occurring at biological membranes. The biophysical relevance of the technique has been established through the characterization of anion binding to natural phosphatidylcholine lipids as reported by Hu et al. [19,20]. To date however, no K_{assoc} values between metal ions and phosphatidylcholine lipids have been reported by ZIC studies which would be useful for a technical comparison between chromatographic and indirect techniques.

Although the single-tail surfactants used in several ZIC studies with cations bear resemblance to phosphatidylcholine lipids, there are important differences between them. Dual-tail membrane lipids contain phosphodiester linkages that contribute to membrane potential [22] and may have a secondary role in some metal cation interactions [23]. They also exhibit crystalline phase transitions that are known to be affected by metal binding [6]. This transition is associated with changes in, among other things, bilayer density, order within the lipid tails, and the rate of lateral diffusion, and it is affected by the presence of solid supports [24].

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Although lipid monolayers on alkylated surfaces exhibit increased stability relative to lipid bilayers formed on hydrophilic ones [25], they have been shown to impact the phase transition temperature of adsorbed phospholipids in unexpected ways [26,27]. For this reason, the present work is focused on the use of lipid bilayers to measure metal cation binding to phospholipids.

The structure of lipid films is sensitive to surface features of solid supports. Synthetic silica particles that contain a distribution of pores sizes and geometries have nanometer scale features [28–30]. Such features create defects and high curvature in lipid films that affect bilayer integrity [31], conformational order [32], and phase properties [33–35]. Stöber silica spheres are non-porous and dense [36], and lipid bilayers supported on them [37–40] are similar to those supported on smooth, planar glass surfaces. The latter is a venerable bilayer system widely used for studies of membranes [41]. Stöber agglomerated particles (SAPs) were utilized in high performance size exclusion chromatography in the 1970s [42]. The smooth surface of Stöber spheres and the large, fully connected pores (>75 nm with 500 nm spheres) in SAPs suggest they are well-suited for hosting supported lipid bilayer stationary phases. In the present work, group I and II metal binding to natural zwitterionic lipids is evaluated using bilayers adsorbed on Stöber spheres. A capillary column format is described that requires several orders of magnitude less lipid than conventional scale HPLC columns.

2. Experimental methods

A more detailed account of procedures, instrument schematics, and additional supporting data is in the Supplementary document.

2.1. Reagents

Angstromsphere silica spheres (Stober spheres) with a diameter of 4 μm and 500 nm were purchased from Fiber Optic Center, Inc. (New Bedford, MA). The lipids 1,2-dipalmitoyl-*sn*-*glycero*-3-phosphocholine (DPPC) and L-α-phosphatidylcholine from chicken egg (Egg PC) were from Avanti Polar Lipids (Alabaster, AL). The emulsifying surfactant Kemelix 7475X was kindly donated by Croda Inc. (USA Headquarters, Edison, NJ). Commercial particles were obtained from two sources, a Viva silica column (5 μm, 300 Å) from Restek Corp. (Bellefonte, PA) and two Nucleosil particles, 1000 and 4000 Å (10 μm spherical) from GFS Chemicals (Powell, OH). IAM.PC silica (10 μm spherical, 300 Å) was from Regis Technologies (Morton Grove, IL). Deionized water (18.2 MΩ, Siemens Water Technologies, Munich, Germany) was used exclusively. A full list of chemicals is in Supplementary Section 1.1.

2.2. Formation of Stöber agglomerated particles (SAPs)

A water-in-oil emulsion procedure based on the work of Kim et al. [43] was used to prepare Stöber agglomerated particles (SAPs). The process is detailed in Supplementary Section 1.2. A scanning electron microscopy image of an SAP is shown in Fig. 1. SAPs composed of 500 nm Stöber spheres (SAP_{500}) had a mean diameter of 11.5 ± 3.3 μm as estimated from >100 spheres from three different SEM images.

2.3. Lipid bilayer formation

Lipid bilayers were formed within SAPs by the rehydration of dried films deposited by solvent-casting with TRIS buffer. This procedure has been used to assemble bilayers on Stöber spheres [44], and is principally similar to the process that results in sphere-supported bilayers within colloidal crystals which are laterally mobile and accessible to soluble proteins [45]. The idealized structure of lipid-SAP particles is depicted in Fig. 1. Stöber spheres have a surface area similar to smooth featureless spheres of the same diameter, measured by nitrogen adsorption to be 18–24 m^2/g for 270 nm particles [46,47]. The mass of lipid solvent-cast onto SAPs was based on the surface area of an equal mass of non-porous spheres. The process and calculation are detailed in Supplementary Section 1.3.

Vesicle fusion experiments used dispersions of small unilamellar vesicles (2.5 or 5.0 mg/mL) prepared by probe tip sonication (Fisher Scientific, model FB-120 with tip model Cl-18) to fuse to silica particles within capillary columns. Turbid lipid suspensions were sonicated to clarity using 2 s on, 2 s off pulse cycles at 30% power for 10 min. Supplementary Section 1.3 contains distribution plots for the vesicles determined by dynamic light scattering (Corvus DLS01 instrument and software, Spokane, WA). The z-average hydrodynamic diameter of the vesicles was 80 nm. The mass of lipid adsorbed onto columns was evaluated by frontal analysis using the phosphorus-31 signal to detect the breakthrough volume of the vesicles.

2.4. Capillary column preparation, lipid-SAP and column characterization

SAPs were packed into 150 μm i.d. fused silica capillaries (Polymicro Technologies, Phoenix, AZ) from aqueous slurries using a PC77 pressure injection cell (Next Advance, Averill Park, NY) and helium (100–500 psi). Frits were prepared from small plugs, nominally 1 mm long, of 4 μm Stöber spheres that were stabilized by exposure to silicon tetrachloride vapor (caution: silicon tetrachloride reacts violently with water).

The lipid:silica ratio of the SAPs was determined by thermal gravimetric analysis (TGA, Perkin Elmer TGA-7, Waltham MA) with approximately 15 mg of the slurry, air-dried overnight at 85 °C. Post use, column void was determined by weighing it before and after drying to a constant mass at 85 °C (microbalance, Cahn Instruments Inc, Cerritos, CA), which was then used with the t_m to determine volumetric flow rate. The empty capillary mass (periodically reevaluated, typical value = 1.712 ± 0.011 mg/cm) was then used to determine the silica mass on the column to calculate the phase ratio. A Perkin-Elmer DSC-7 instrument was used to determine the phase transition temperatures of DPPC multilamellar vesicles and bilayers on SAP_{500} particles.

2.5. Chromatography instrumentation

Capillary columns were coupled to an Agilent 7700x ICP-MS (Agilent Technologies, Inc. Tokyo, Japan). A schematic of the system is in Supplementary Fig. S3. The sub-microliter flow from the columns was coupled to flow from the ICP peristaltic pump for effective nebulization (Micromist nebulizer). Post-elution dead time was under three seconds. Metal ions, bromide, or phosphorous ions were simultaneously monitored in time-resolved analysis mode. Standard autotune-obtained parameters for no collision gas (group II ions) and H_2 collision gas (4.5 mL/min for group I ions) conditions were utilized. Supplementary Section 1.5 contains values for ICP-MS parameters.

A Series 1500 Lab Alliance pump (Scientific Systems, Inc. State College, PA) was used with two manual injection 6-port valves (360 μm fittings, Vici Valco Instrument Co. Inc. Houston, TX) linked in series. The first valve had a large volume injection loop (35 μL, approximately 30 min of flow) used for online changes to the mobile phase composition. All fittings (unions, reducers, tees) were from Vici Valco Instruments Co. Inc. (Houston, TX). A splitting tee with a 25 cm restriction capillary (50 μm i.d.) was used to reduce mobile phase flow rates from the pump. A 12 cm column heater