



Short Communication

Lipid membranes supported on optically transparent nanosilicas: Synthesis and application in characterization of protein-membrane interactions

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ABSTRACT

We describe methods of preparation of lipid membranes supported on optically transparent nanosilicas (20 and 30 nm diameter) and an initial application of these materials to characterize membrane-protein binding using conventional circular dichroism and fluorescence spectroscopy.

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1. Introduction

Lipid membranes supported on solid surfaces are of fundamental interest as models of biological membranes [1,2] and as a prospective platform for bio-sensors [3]. Following pioneering work [4,5], several methods to prepare solid-supported lipid membranes have been reported; perhaps the most versatile one involves adsorption and fusion of lipid vesicles onto solids (e.g., Si wafers, glass, mica, metals, silica or polymer beads) [6]. To increase stability and decouple the membranes from the substrate, various methods of surface functionalization have been explored [7]. The use of self-assembled monolayers (silanes on silica [8] and thiols on gold [9]), hydrated polymer cushions [10,11], surface-grafted lipopolymer tethers and spacers [12,13], and structural protein anchors [14,15] have been reported.

However, while most work has focused on planar surfaces or relatively large silica beads ($\sim \mu\text{m}$), membranes supported on small “nanoparticles” extend many advantages for bio-analytical applications [3,16]. For example, there is considerable interest in how surface curvature is recognized by proteins involved in vesicle and viral envelope formation and transport; the introduction of a solid support should facilitate preparation of highly stable, homogeneously sized vesicles. Moreover, as the size of the beads increases, the weight fraction of phospholipid relative to silica becomes small limiting the concentrations of bilayer and associated proteins that can practically be achieved. Finally, light-scattering becomes problematic for supported bilayers with diameters greater than ~ 50 – 100 nm. Thus, supported

bilayers in the 10–50 nm size range would share numerous advantages of larger beads – including size-homogeneity, stability, ease of preparation, and facile centrifugal separation – but also enjoy advantages of reaching greater bilayer concentrations and optical transparency for bio-chemical and bio-analytical applications (Fig. 1).

2. Experimental

Two samples of fumed silicas were used: SiO₂-20 (CabOSil LM150, $d \sim 20$ nm, $S(\text{N}_2, \text{BET}) = 140 \text{ m}^2/\text{g}$) and SiO₂-30 (Aerosil A-90, $d \sim 30$ nm, $S(\text{N}_2, \text{BET}) = 90 \text{ m}^2/\text{g}$). For each silica, three types of surfaces were prepared: (1) hydroxylated bare silica (SiOH), (2) a series of monolayers of oligomeric ethylene glycol groups (CH₂OH, [EO]₃OH, and [EO]_{8–12}OH), and (3) monolayer of C₁₈H₃₇ groups. Small unilamellar vesicles (SUVs) were prepared from POPC-POPS (9:1 by mol plus ~ 1 mol% of the POPE-NBD as a fluorescent label) by sonication of vacuum-dried lipid films in PBS buffer (pH 7.1, 75 mM NaCl). The experimental details are provided as Supplement.

3. Results

The adsorption of SUVs on silicas was carried out under static conditions at room temperature for ~ 12 h. The equilibrium concentrations and the amounts of lipids adsorbed were determined by fluorescence of the NBD labels ($\lambda_{\text{ex}} = 480$ nm, $\lambda_{\text{fl}} = 530$ nm). For all the silica surfaces studied, the adsorption isotherms demonstrated a sharp increase of lipid adsorption followed by the plateau, Fig. 2. For the ease of comparison of the adsorption isotherms obtained for different silicas, the amount of lipid ad-

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Fig. 1. POPC/POPS bilayers supported on $\varnothing 20$ nm (left) and $\varnothing 100$ nm (right) silica particles. Both samples contain the same interfacial area (~ 2 m²/sample) and the same amount of adsorbed lipids (~ 7 mg/sample) respectively.

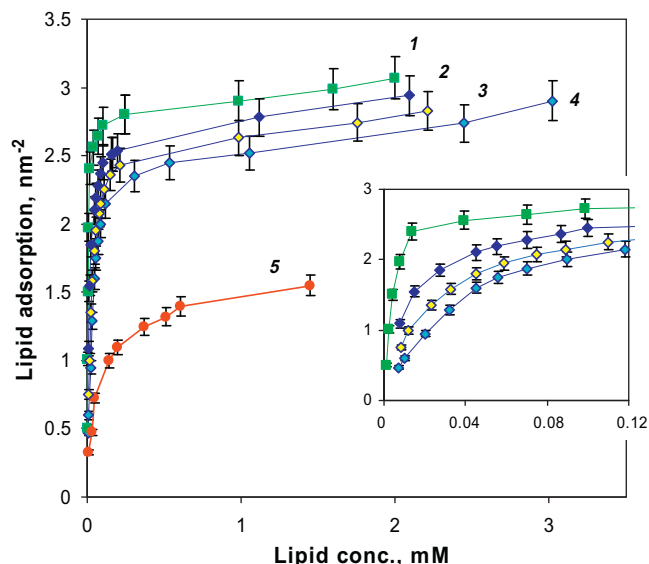


Fig. 2. The adsorption isotherms (298 K) of POPC-POPS SUVs on A-90 fumed silicas with different surface functionalities: 1 – SiOH, 2 – CH(OH)CH₂OH, 3 – [EO]₃OH, 4 – [EO]_{8–12}OH, 5 – C₁₈H₃₇ (see text for notations). The initial parts of the isotherms 1–4 are shown in the insert. Buffer: PBS, pH 7.1, 75 mM NaCl.

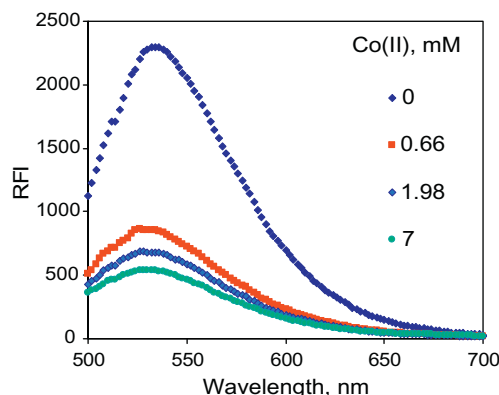
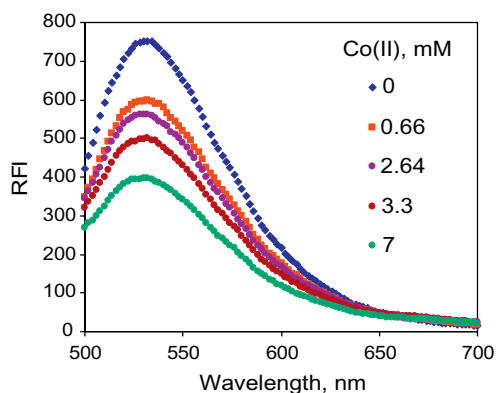


Fig. 3. Quenching of fluorescence of NBD-lipids in silica supported bilayers (left) and single layers (right) in presence of Co²⁺.

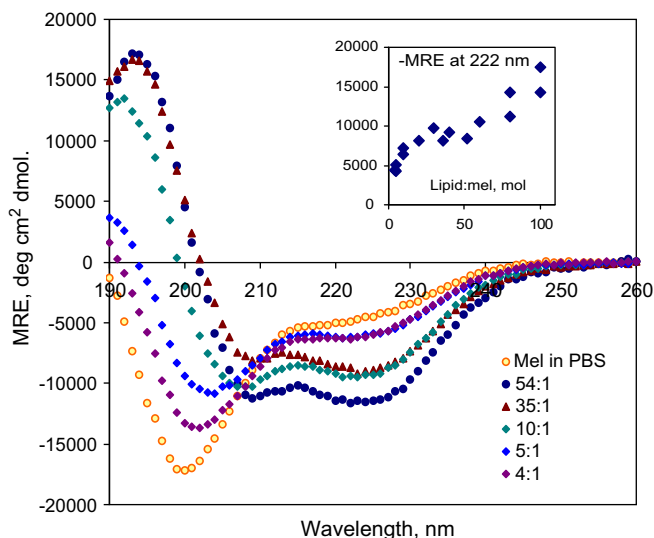


Fig. 4. The CD spectra of melittin in presence of lipid-coated silicas suspended in PBS for different lipid-melittin ratios (mol). In the insert: MRE at 222 nm.

sorbed was normalized per unit surface area and, thus given as number of lipid molecules adsorbed per nm² of the surface (1 lipid/nm² corresponds to 1.6 μ mol/m²). The adsorption on hydrophobic C₁₈H₃₇ silicas yielded plateau at ~ 1.5 lipids/nm² (67 Å² per lipid), in good agreement with the cross-sectional areas reported for the PC head group [17,18] indicating deposition of single lipid layers on hydrophobic surfaces [9]. For bare silicas and PEG-terminated silicas, the adsorption plateaus were $\sim$ twice as high (~ 2.5 – 2.8 lipids/nm²) with ~ 36 – 40 Å² per lipid, consistent with the formation of lipids bilayers [19]. The adsorption isotherms on the PEG-coated silicas were close to those on bare silicas, yet the adsorption plateaus were somewhat (5–10%) lower than for bare silicas. This was attributed to weakening of the adsorption interactions due to the presence of PEG groups. The effect of the size of the PEG group on the adsorption was illustrated with the differences in the initial regions of the isotherms (insert, Fig. 2). The slopes of the initial regions of the isotherms, hence the constants of the adsorption equilibrium decreased in the following range: SiOH > CH₂OH > [EO]₃OH > [EO]_{8–12}OH, which was consistent with the idea of the steric stabilization and reduction of the adsorption of biopolymers for PEG-surfaces [20]. Interestingly, no notable differences were found in the adsorbed amount of lipid (expressed per unit of surface area) for 20 and 30 nm silicas. We also noted that the value of the

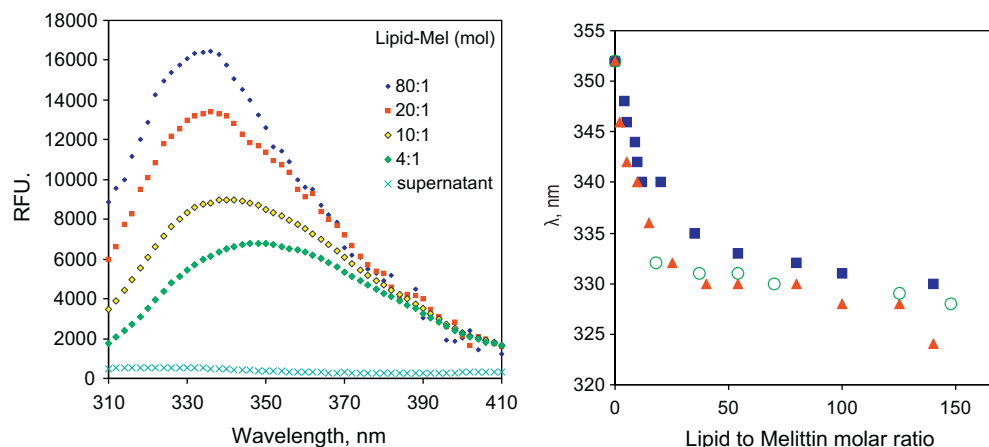


Fig. 5. On the left: fluorescence spectra of melittin in presence of lipid-coated 20 nm silicas suspended in PBS at different lipid–melittin ratios and the spectra of supernatant obtained after centrifugation of silica particles (molar ratio 20). On the right: The emission maximum at different lipid to melittin ratio (mol) for SiO₂-20 (■) and PEG-SiO₂-20 (▲), and SUVs (○).

adsorption plateau obtained in this work were in a close agreement with the ones reported [19] for ~50 nm and larger silicas demonstrating little effect of the substrate curvature (for the range of particles size investigated) on the lipid deposition [21].

The structural integrity of the membranes surrounding the silica particles was investigated by fluorescence quenching [22] of the NBD labels with Co²⁺. The typical fluorescence spectra of the NBD-lipids single- and bilayers adsorbed on silicas in presence of increasing amounts of Co²⁺ are shown in Fig. 3. For the membranes supported on bare and PEG-silicas, the fluorescence intensity decreased and leveled off at ~50–55% of the original intensity at ~10–30 mM of Co²⁺. Since NBD moiety was attached to the lipid polar ends, these results indicated that ~half of all the NBDs were present in the external layer of the membranes, i.e. in agreement with a single bilayer adsorbed on silica. For the membranes supported on C₁₈H₃₇ silicas, the fluorescence decreased by ~80–90%, i.e. close to 100% which was expected for single layers.

The silica-supported membranes prepared were tested in the interactions with melittin, 26 amino acids peptide. We used circular dichroism (CD) and fluorescence spectroscopies to determine the secondary structure and the local polarity of melittin upon its binding with the lipid-coated silicas. The spectra were taken using the suspensions of lipid-coated silicas in PBS (~0.1 mg of silica per mL) in presence of varying amounts of dissolved melittin so the lipid–melittin ratio varied from ~100:1 to 4:1 (mol). In pure PBS, melittin was present in a random coil conformation with a characteristic CD peak at 200 nm. As lipid to melittin ratio increased, the 200 nm peak disappeared and the 208 and 222 nm peaks appeared and grew indicating transition from a random coil to an alpha-helix conformation and suggesting incorporation of melittin into the membranes (Fig. 4). The interactions of melittin with lipid-coated silicas were also accompanied by substantial changes in the fluorescence of melittin (intrinsic fluorescence due to tryptophan). The fluorescence intensity increased and the emission maximum shifted from 353 nm for melittin in pure PBS to 330–335 nm for samples with the lipid–melittin ratios greater than ~40:1 (mol) indicating changes in the local environment from polar to non-polar respectively [23], which was consistent with the incorporation of melittin into the membranes (Fig. 5).

The silica-supported membranes demonstrated overall good mechanical and chemical stability. No substantial loss of lipid coverage was observed after 1 week storage of the silica suspensions in PBS. Also, the silicas could be removed from the solution (e.g. by centrifugation) and re-suspended back in the solution phase

without any notable loss of lipids and changes in the membrane structure. In the presence of melittin, no desorption of lipid was observed up to melittin–lipid molar ratios ~1:15. At melittin–lipid ratios greater than 1:10, the membranes lost their integrity due to the interactions with melittin and desorbed from silicas.

To conclude, we described the method of preparation of lipid membranes supported on ~20 and 30 nm silicas. The lipid membranes supported on nanosilicas combined the properties of solid-supported membranes (easy handling and improved stability) and those of lipid vesicles (high surface area and optical transparency) providing a convenient model system for characterization of membrane-protein interactions using conventional techniques.

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Appendix A. Supplementary material

The complete experimental details of preparation and characterization of the lipid-coated silicas are given as supplement. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcis.2010.11.064.

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