Journal of Colloid and Interface Science 358 (2011) 635–638

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Short Communication

Substrate effects on interactions of lipid bilayer assemblies with bound nanoparticles

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ARTICLE INFO

Article history:
Received 7 January 2011
Accepted 25 February 2011
Available online 12 March 2011

Keywords:
Nanoparticles
Self-assembly
Lipid bilayer
Biosensors

ABSTRACT

Understanding the interactions of nanoparticles with lipid membranes is crucial in establishing the mechanisms that govern assembly of membrane-based nanocomposites, nanotoxicology, and biomimetic inspired self-assembly. In this study, we explore binding of charged nanoparticles to lipid bilayers, both as liposomes and substrate supported assemblies. We find that the presence of a solid-support, regardless of curvature, eliminates the ability of zwitterionic fluid phase lipids to bind charged nanoparticles.

The manipulation of protein assemblies and ordered domains within lipid bilayer hosts accounts for much of the exquisite functionality observed in cellular membranes. Researchers are now exploring the extent to which this functionality can be exploited in supported lipid bilayers for applications in sensors, energy storage, biomaterials, and separations media. In artificial systems the desire is to replace fragile proteins with more robust species, such as functional nanoparticles, to create programmable or reconfigurable nanocomposites. Critical issues that impact the development of such nanocomposites include understanding: (1) the conditions required to promote nanoparticle adsorption and/or insertion into lipid bilayer hosts, and (2) the impact that nanoparticle interactions have on key host properties such as head group packing, lipid mobility, domain formation, phase separation, and component segregation. For example, previous work on phosphatidylcholine (PC) bearing zwitterionic liposome/nanoparticle mixtures indicates that adsorption of anionic nanoparticles can induce the formation of gel phase domains into fluid lipid phases, while adsorption of cationic particles can create fluid domains within gel phase systems [1–3]. For supported lipid bilayers, a third parameter must also be considered, which is how lipid–support interactions mediate lipid behavior. Here, we demonstrate that interactions between the lipids and the substrate can have a dramatic influence on both particle adsorption and the phase behavior of the resulting nanoparticle–lipid composite.

Substrate-supported lipid bilayer assemblies (LBAs) were prepared on atomically flat mica substrates by standard vesicle fusion techniques. Lipid bilayers were prepared using both the zwitterionic lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1,2-dioleoyl-3-trimethyammonium-propane (DOTAP), which has a cationic head group. These supported LBAs were then exposed to solutions containing polystyrene nanoparticles (24 nm in diameter) that were either cationic (amine terminated surfaces) or anionic (carboxyl terminated). The extent and nature of nanoparticle adsorption was monitored using atomic force microscopy (AFM). On supported LBAs of the cationic DOTAP, no cationic nanoparticles were adsorbed, while adsorption of the anionic nanoparticles was extensive as expected based on simple electrostatic interactions (Fig. 1c and d). However, no adsorption was observed on LBAs consisting of zwitterionic POPC for either cationic or anionic particles (Fig. 1a and b). The total absence of particle adsorption on zwitterionic POPC LBAs is in sharp contrast to what is reported for POPC liposomes, where extensive adsorption of the exact same cationic and anionic particles has been observed [1–3].

Are the dramatic differences in nanoparticle adsorption on supported and unsupported membranes real, and if so, how can these differences be rationalized? A good starting point for understanding the role of the substrate in mediating particle adsorption involves examining the mechanisms that have been proposed to explain nanoparticle adsorption onto zwitterionic liposomes [3]. Although the net charge on the POPC head group is zero, the polar head group contains both positive and negative sites that can potentially interact with charged nanoparticles. It has been postulated that when the zwitterionic head group is approached by charged nanoparticles, the head group can reorient itself such that either the positively charged choline group or the negatively charged phosphatidic acid group terminates the surface, depending on whether the approaching particle is negatively or positively

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doi:10.1016/j.jcis.2011.02.063

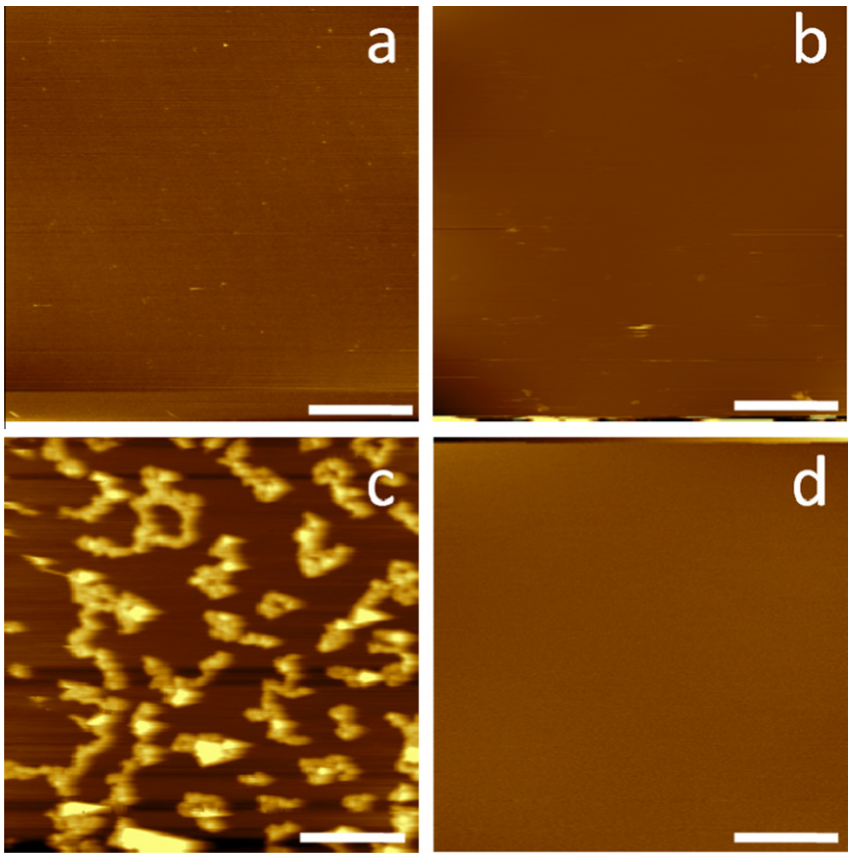

Fig. 1. AFM topography images of POPC (a and b) and DOTAP (c and d) LBAs on mica. LBAs were exposed to anionic (a and c) and cationic (b and d) polysterene nanoparticles. Scale bars represent 5 μm for all images.

charged, respectively [3]. This reorientation is accompanied by a change in the effective "size" of the head group and thus produces an overall change in lipid packing. With a negative nanoparticle, electrostatic interactions should straighten out the head group, allowing the lipids to pack more efficiently and occupy less surface area per molecule. In liposomes, this efficient packing results in a ~20% shrinkage in the liposome diameter and has been shown to stabilize gel phases relative to fluid phases [3]. Conversely, positive nanoparticles should bend the head group over, increase the area occupied per head group, expand the lipid bilayer, and stabilize fluid phases relative to gel phases. Key questions involve understanding how the presence of a substrate could influence any of these lipid rearrangement processes.

Substrate effects that could influence lipid reconfigurations include: (1) steric crowding of the head groups associated with having the lipids deployed on a flat substrate vs. the curved surfaces present in spherical liposomes, and (2) specific lipid–substrate interactions that suppress the volume changes required to allow for head group reorientations. To identify the dominant factor, we have directly compared the extent of nanoparticle adsorption on lipid bilayers deployed in three distinct geometries: (1) LBAs adsorbed on flat mica substrates (as described above), (2) lipid bilayers deployed in liposomes (with a diameter of 450 nm), and (3) LBAs adsorbed on nanoparticles having the same effective diameter as our liposomes (500 nm diameter silica beads). As direct imaging of nanoparticle adsorption is difficult for the latter two geometries, we have utilized the optical technique of fluorescence resonance energy transfer (FRET) to obtain an indirect measure of the extent of particle adsorption and the influence of such adsorption on lipid phase behavior [4].

For our FRET measurements, we have used fluorescence lifetimes to monitor the interactions between the same two lipid bearing dye molecules that were used in previous liposome–nanoparticle studies [3]: (1) the optical donor dye NBD, which partitions into gel-like lipid phases, and (2) the optical acceptor Rhodamine B, which is confined within fluid phases [5]. In the absence of particle adsorption, both donor and acceptor dyes are expected to coexist within a single fluid POPC phase, resulting in extensive donor–acceptor interactions, efficient FRET from donor to acceptor, and a decrease in donor fluorescence lifetime. Conversely, the adsorption of anionic particles is expected to induce formation of a gel phase (see above). Since only the donor partitions into the gel phase, the donor and acceptor are separated from each other resulting in an increased fluorescence lifetime for the donor. Thus, the fluorescence lifetime of the donor can be used as an indicator of nanoparticle binding and induced gel–fluid lipid phase formation and separation.

Fig. 2 compares the fluorescence lifetimes observed for the NBD donor dye in both liposomes and LBAs supported on silica beads. In the "native" materials, donor lifetimes are essentially identical for both liposomes and LBAs (around 4.7 ns), indicating that the lipid environments are quite similar for both the supported and unsupported membranes. As expected, addition of the acceptor Rhodamine B dye decreases the fluorescence lifetimes in both materials. As the lifetimes observed in the presence of donor–acceptor interactions are similar for both supported at unsupported membranes (1.4 ns and 1.8 ns, respectively), the concentrations and mobilities of the dyes appear to be comparable. However, when membranes containing both donor and acceptor dyes are exposed to anionic nanoparticles, dramatic differences are observed

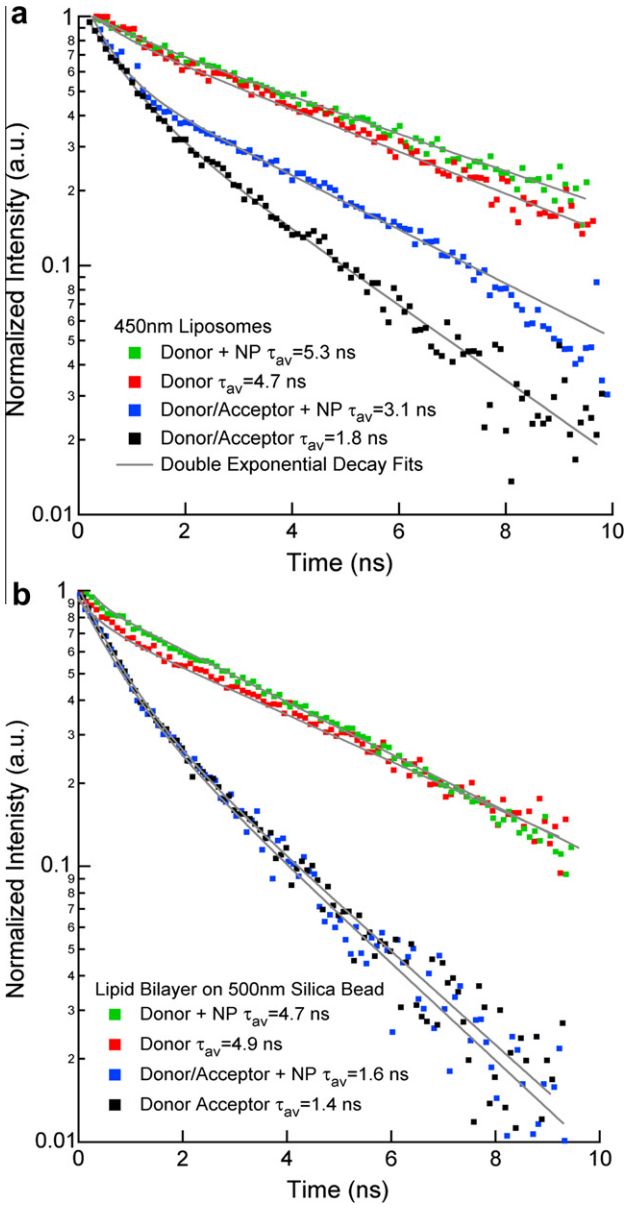

Fig. 2. Fluorescence lifetime data for lipid bilayers as liposomes (a) and supported on silica beads (b). Fluorescence intensity is shown vs. time for the donor and donor/acceptor systems, both with and without exposure to anionic nanoparticles.

between the supported and unsupported membranes. For liposomes, the donor lifetime increases from 1.8 ns to 3.1 ns, consistent with the creation of a gel phase as induced by nanoparticle adsorption, while for the supported LBA, no significant increase in lifetime is observed. These results indicate that solid supports prevent the rearrangements of lipid head groups required to promote particle adsorption regardless of the radius of curvature of the substrate. For zwitterionic lipids such as POPC, it appears that particle adsorption only occurs on unsupported membranes such as liposomes.

Our results show that the presence of a substrate on one side of a lipid bilayer can have a profound influence on how materials such as nanoparticles interact with the other side. We identify two primary factors that limit charged nanoparticle adsorption on solid-supported LBAs are as follows:

As stated above, the reorientation of the phosphatidylcholine headgroup induces localized gel-like behavior and densification of the lipids in close proximity to an anionic nanoparticle. This change in molecular packing effectively shrinks the surface area of lipid molecules in the LBA. A 20% contraction of the supported lipid bilayer due to adsorption of anionic particles would require the disruption of the layer and the creation of bare patches, both of which are energetically unfavorable. From a simple thermodynamics perspective, this is unfavorable for high surface energy substrates such as glass or mica, which are commonly used for LBA deposition [6]. The energy needed into increase the area of the substrate/solution interface must be directly offset by energy gained by nanoparticle binding. However, the binding energy of anionic nanoparticles to a phosphatidylcholine LBA is only measured at ~20 k_BT [3], which does not seem to be high enough to overcome the increase in interfacial energy of the system. Conversely, head group "expansion" would require a compression of the layer, which Langmuir trough experiments show could require working against surface pressures as high as 20 mN/m [7].

In addition to reorientation of the lipid headgroups directly in contact with a nanoparticle, lipids in the distal leaflet that directly oppose the nanoparticles may also reorganize. The idea of phase change in one leaflet of a LBA being mirrored in the opposite leaflet is known as domain registration and has been explored both experimentally [8,9] and theoretically [10,11]. It was recently found that registry between gel phase domains is hindered in substrate-supported LBAs and depended heavily on the spacing between the substrate and LBA [9,12]. Registry only occurred when the LBA was separated by ~6 nm using a hydrated polymer cushion. In contrast, LBAs in direct contact with a substrate were concluded to have significant coupling between the substrate and its proximal leaflet. Assuming the same phenomenon is present in our system, reorientation of lipid headgroups in direct contact with the substrate may be reduced due to substrate coupling and/or possible steric constraints. Therefore, if nanoparticle binding requires reorientation of lipids in both leaflets, binding will not occur for substrate-supported LBAs.

In conclusion, the work presented indicates that the nanoparticle-induced LBA rearrangements previously reported [1–3] are limited to bubble-like liposomes. The solid supports, regardless of the curvature of the membrane or fluidity of the system resulted in lack of nanoparticle induced lipid rearrangements. Such surface support effects must be considered when moving from liposome to solid support systems and thus in many biomimetic platforms.

Acknowledgments

This work was performed, in part, at the Center for Integrated Nanotechnologies, a U.S. Department of Energy, Office of Basic Energy Sciences user facility. Sandia National Laboratories is a multi-program laboratory operated by Sandia Corporation, a Lockheed-Martin Company, for the U S Department of Energy under Contract No. DE-AC04-94AL85000. Los Alamos National Laboratory, an affirmative action equal opportunity employer, is operated by Los Alamos National Security, LLC, for the National Nuclear Security Administration of the U.S. Department of Energy under contract DE-AC52-06NA25396.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcis.2011.02.063.

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