Contribution of the Hydration Force to Vesicle Adhesion on Titanium Oxide

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S *Supporting Information*

ABSTRACT: Titanium oxide is a biocompatible material that supports vesicle adhesion. Depending on experimental parameters, adsorbed vesicles remain intact or rupture spontaneously. Vesicle rupture has been attributed to electrostatic attraction between vesicles and titanium oxide, although the relative contribution of various interfacial forces remains to be clarified. Herein, we investigated the influence of vesicle surface charge on vesicle adsorption onto titanium oxide and observed that electrostatic attraction is insufficient for vesicle rupture. Following this line of evidence, a continuum model based on the DLVO forces and a non-DLVO hydration force was applied to investigate the role of different interfacial forces in modulating the lipid−substrate interaction. Within an experimentally significant range of conditions, the model shows that the magnitude of the repulsive hydration force strongly influences the behavior of adsorbed vesicles, thereby supporting that the hydration force makes a strong contribution to the fate of adsorbed vesicles on titanium oxide. The findings are consistent with literature reports concerning phospholipid assemblies on solid supports and nanoparticles and underscore the importance of the hydration force in influencing the behavior of phospholipid films on hydrophilic surfaces.
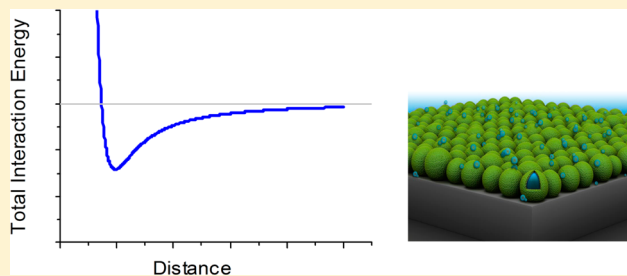
INTRODUCTION

Molecular self-assembly of phospholipids on solid supports can guide the organization of macromolecular assemblies that mimic biological membranes.[1,2] Typically, the self-assembly process involves the adhesion of lipid vesicles on hydrophilic solid supports.[3] Depending on material substrate properties along with vesicle characteristics and experimental conditions, adsorbed vesicles may either remain intact (e.g., titanium oxide) or rupture to form a planar lipid bilayer (e.g., silicon oxide) (see refs 4 and 5 and references therein). The deformation of adsorbed vesicles strongly influences the rate of rupture,[6] and the extent of vesicle deformation is commonly studied by using adsorbed vesicles on titanium oxide as a model system.[7,8] In order to form a planar bilayer on titanium oxide, experimental methods have also been devised, including peptide-induced vesicle rupture[9] and enhancement of the vesicle−substrate interaction.[10−14]

The incorporation of negatively charged lipids promotes vesicle rupture on titanium oxide in the presence of divalent cations.[12] In addition to divalent cations acting as bridging ligands and also influencing vesicle−vesicle interactions, Zhu et al.[14] demonstrated that zwitterionic lipid vesicles can rupture on titanium oxide surfaces in the presence of calcium ions. Hence, it appears that divalent cations may generally modulate the lipid−substrate interaction. The rupture of zwitterionic lipid vesicles has also been achieved by changing the solution pH in order to promote electrostatic attraction.[12,13] Besides changing

the solution pH, vesicles containing a high fraction of positively charged lipids (50 mol % or greater) may rupture on titanium oxide due to electrostatic attraction as well.[11,13] Additional interfacial forces have also been identified to promote bilayer formation, including the van der Waals force on rutile titanium oxide surfaces.[15,16]

Within the milieu of empirical findings and theoretical approaches (e.g., continuum models based on DLVO and non-DLVO forces[16−18] and phenomenological models[19−21]), the relative contribution of different interfacial forces (e.g., van der Waals and double-layer electrostatic forces) to the lipid−substrate interaction remains to be clarified. The problem is interesting because lipid−substrate interactions are reported to be appreciably stronger on titanium oxide versus silicon oxide.[16] Considering that lipid−substrate interactions directly influence vesicle deformation, it is therefore difficult to comprehend why vesicles typically rupture on silicon oxide but not on titanium oxide. Certainly, additional factors must be important, although there has been limited theoretical examination of experimental results[16,18] in this context. Herein, we investigated the adsorption kinetics of charged lipid vesicles onto titanium oxide under monovalent salt conditions and performed corresponding theoretical calculations based on a continuum
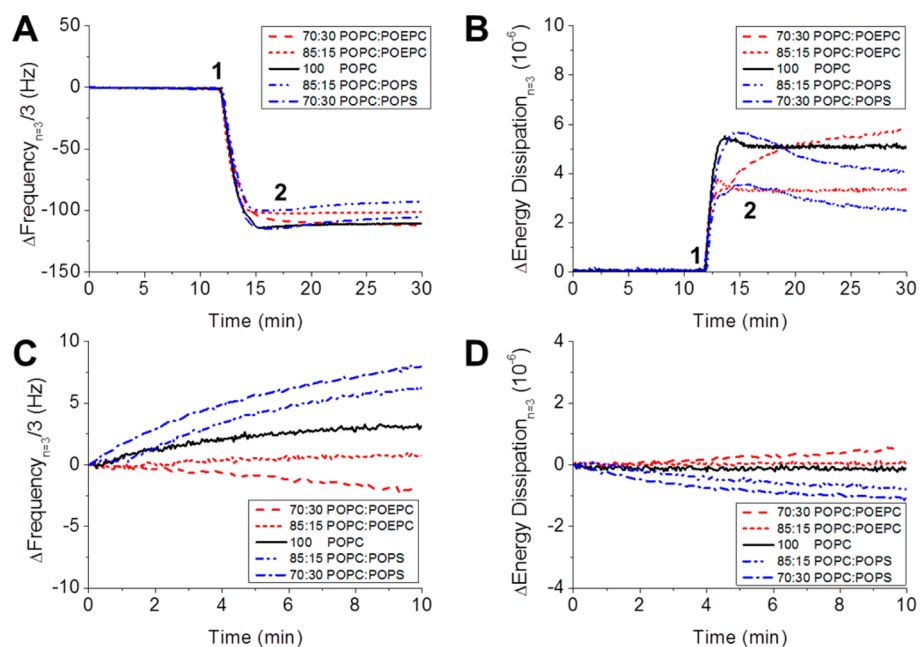
Figure 1. Influence of vesicle surface charge on vesicle adsorption onto titanium oxide. QCM-D monitoring was employed to track the adsorption kinetics of vesicles on titanium oxide by measuring changes in (A) frequency and (B) energy dissipation as functions of time. Markers 1 and 2 denote vesicle addition and the onset of the buffer wash, respectively. Panels (C) and (D) show magnified results from panels (A) and (B), respectively, and correspond to buffer washing. For comparative analysis of the different lipid compositions, the measurement values were normalized based on the start of the buffer wash. The initial time corresponds to the onset of the buffer wash (from marker 2 onward).
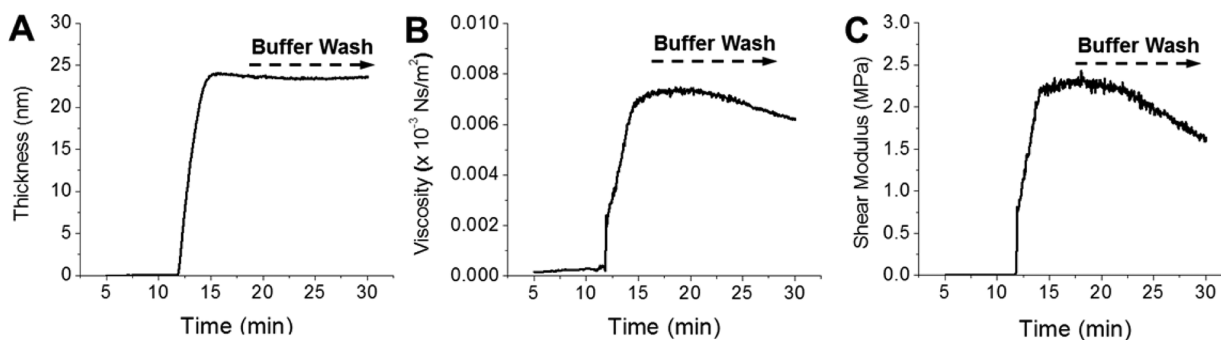
Figure 2. Structural transformation of the adsorbed vesicle layer. Viscoelastic properties of the 70/30 POPC/POPS vesicle adlayer were characterized in response to buffer washing. Specifically, effective film properties as a function of time were calculated by using the Voigt−Voinova model. Changes in the(A) effective film thickness, (B) viscosity, and (C) shear modulus as functions of time were determined. The black dotted line corresponds to the period of buffer washing.

model that included the DLVO forces and a non-DLVO hydration force. The aim of this work was to identify how the interplay of different interfacial forces may influence vesicle adhesion on titanium oxide, with particular focus on the lipid−substrate interaction.

■ RESULTS AND DISCUSSION

Small unilamellar vesicles (ca. 55 nm average diameter) containing zwitterionic 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) lipid along with up to 30 mol % negatively charged 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) or positively charged 1-palmitoyl-2-oleoyl-*sn*-glycero-3-ethylphosphocholine (POEPC) lipid, respectively, were prepared by the extrusion method.[22] Using the quartz crystal microbalance with dissipation (QCM-D) monitoring technique,[23] the adsorption kinetics of vesicles onto a titanium oxide-coated sensor chip were characterized in the neutral pH regime (10 mM Tris [pH 7] with 200 mM NaCl). In all cases, vesicles

adsorbed until reaching a saturation coverage that corresponded to changes in frequency and energy dissipation of approximately -110 ± 10 Hz and $4 \pm 1 \times 10^{-6}$, respectively (Figure 1A,B). Adsorbed vesicles did not rupture even when there was electrostatic attraction. By contrast, in the acidic pH regime, similarly sized POPC lipid vesicles have been reported to rupture in the presence of an even weaker attractive electrostatic interaction[10] (Figure S1). Hence, we conclude that there must be additional factors which mediate vesicle rupture. Nevertheless, vesicle surface charge did influence vesicle adhesion, as determined by a buffer wash.

Depending on the lipid composition, the structural arrangement of adsorbed vesicles could be influenced by a buffer wash (Figure 1C,D). While POPC and POPC/POEPC vesicle adlayers were largely unaffected, buffer washing caused a more appreciable change in the physical properties of POPC/POPS vesicle adlayers, as indicated by an increase in frequency and a corresponding decrease in the energy dissipation. Indeed,
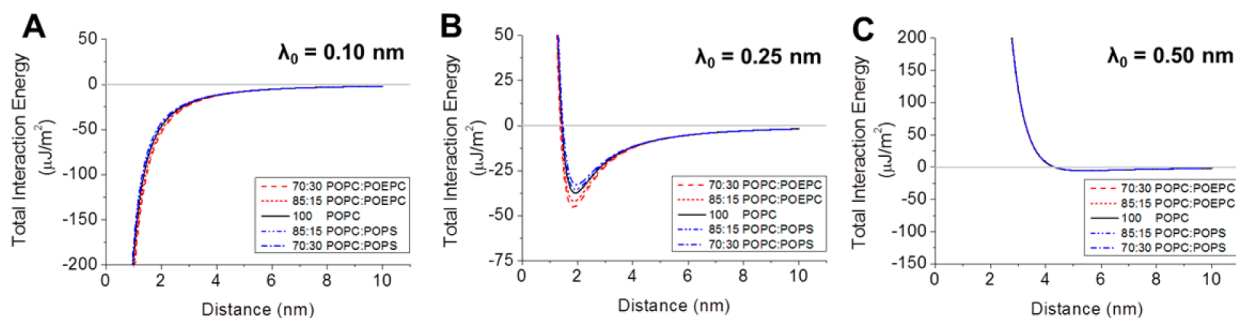
Figure 3. Total interaction energy of vesicle−substrate interactions on titanium oxide. The total interaction energy as a function of separation distance between the adsorbed vesicle layer and the substrate was estimated based on the continuum model (DLVO and non-DLVO hydration forces). In the model calculations, different values corresponding to the decay length of the hydration force were tested, including (A) 0.10, (B) 0.25, and (C) 0.50 nm. The graphs presented do not depict the short-range, attractive interaction which typically occurs at separation distances below 0.3 nm.

based on an increase in the slope of the relative changes in energy dissipation versus frequency,[24] there is likely a structural rearrangement of negatively charged, adsorbed vesicles on the substrate.

Voigt−Voinova model analysis corresponding to the 70/30 POPC/POPS vesicle adlayer provided insight into the viscoelastic effects of the structural rearrangement (Figure 2). Upon buffer wash, there was no change in the adlayer's thickness (Figure 2A), but there were changes in the viscoelastic properties of the adlayer, including decreases in the viscosity and shear modulus (Figure 2B,C). Reviakine et al.[7] previously identified via QCM-D monitoring that the addition of a small number of vesicles to an adsorbed vesicle layer influences the amount of trapped solvent, and can cause significant changes in the adlayer's viscoelastic properties without appreciable changes in vesicle surface coverage detected by atomic force microscopy. In the present system under consideration, the observed trends are consistent with the desorption of some vesicles, which leads to a lower vesicle surface coverage. Additional trapped solvent would likely replace the desorbed vesicles. As a result, there would be no change in the effective film thickness although the viscoelastic properties of the adlayer are affected. Implicit in this analysis is the assumption that adsorbed POPC/POPS vesicles are mobile. While Monte Carlo simulations indicate that zwitterionic vesicles are immobile due to surface pinning points,[25] the observed behavior and repulsive electrostatic interactions support that negatively charged POPC/POPS vesicles adhere more weakly to the substrate. Nevertheless, arguments based on the double-layer electrostatic force alone are insufficient to explain why vesicles do not rupture for all tested lipid compositions.

Considering the aforementioned points, we next investigated the relative strength of lipid−substrate interactions by using a continuum model that includes the DLVO forces and a non-DLVO hydration force, as described in previous works.[18,26] In this model, the total interaction energy is governed by the balance of three interfacial forces, namely, the van der Waals, double-layer electrostatic, and hydration forces. By taking into account the experimental conditions and material properties, the model is useful for estimating the total interaction energy between vesicles and the substrate as a function of separation distance. In our calculations, we assumed that the contact area between vesicles and the substrate can be represented as two planar surfaces, and the details of the calculations are presented in the Supporting Information. Herein, we discuss general

trends related to the model calculations while focusing on effects of the hydration force.

The hydration force accounts for the short-range, repulsive interaction between two hydrophilic surfaces.[18] In general, it occurs appreciably only at small separation distances below 2 nm and is typically represented as an exponential decay function with a characteristic decay length.[18] The decay length corresponding to our system is not known explicitly, so we estimated three values: 0.10, 0.25, and 0.50 nm. The values were selected on the condition that the range of appreciable hydration interaction energy does not exceed a separation distance of 2 nm and is in the range of experimentally obtained values.[27] Depending on the decay length, variations in the lipid−substrate total interaction energy as a function of separation distance were significant and, strikingly, followed the same general trends largely independently of lipid composition and accordingly vesicle surface charge (Figure 3).

For a 0.10 nm decay length, it was predicted there would always be an attractive lipid−substrate interaction (Figure 3A). At separation distances below 3 nm, the interaction was exponentially attractive due to the strong van der Waals force and weak hydration force. By contrast, for a 0.25 nm decay length, the interaction was attractive only at separation distances beyond 1.5 nm; the hydration force was dominant at shorter distances (Figure 3B). For all lipid compositions, there was an equilibrium separation distance of approximately 1.8 nm. However, the total interaction energy corresponding to the equilibrium separation distance was appreciably greater for positively charged POPC/POEPC vesicles (i.e., −46 μJ/m^2), as compared to negatively charged POPC and POPC/POPS vesicles (i.e., −33 μJ/m^2). At this decay length, the electrostatic force is clearly an important factor, as compared to other tested decay length values where the electrostatic force has a marginal effect. For a 0.50 nm decay length, the interaction was always repulsive at separation distances below 4.5 nm (Figure 3C). Under such a condition, vesicle adsorption is predicted to be energetically unfavorable, and consistent findings have been observed on silica when there is an icelike hydration layer on the substrate at high pH.[17] Collectively, the continuum model analysis suggests that the hydration force plays a governing role in modulating vesicle adhesion on titanium oxide. Additionally, within a certain range of decay length values (0.17 to 0.28 nm), the electrostatic force has an appreciable effect on the magnitude of the total interaction energy that is associated with the equilibrium separation distance (Figure S5). Therefore, the full interplay of interfacial forces is important to

consider when estimating how adsorbed vesicles will behave on solid supports, including titanium oxide.

The importance of the hydration force is further substantiated by previous works related to planar bilayer formation on silicon oxide, including on planar substrates[28] and nanoparticles.[29] There is also anecdotal empirical evidence that supports the contribution of the hydration force to vesicle adhesion on titanium oxide. Tero et al.[16] have previously reported vesicle rupture on atomically flat rutile titanium oxide surfaces in the absence of divalent cations (as also reported earlier in ref 15). While most vesicle adsorption studies on titanium oxide have used oxygen-plasma-treated, sputter-deposited surfaces,[7–12,14,30–33] their study used a single-crystal, step-and-terrace rutile $TiO_2(100)$ surface that was prepared by wet treatment and thermal annealing. The processing techniques cause an appreciable disassociation of surface hydroxyls (40% or greater loss of hydroxyl groups),[34] which may attenuate the hydration force because a lower density of surface hydroxyls would be less capable of forming a hydrogen-bonded water layer. In turn, vesicle rupture on thermally annealed titanium oxide surfaces could be possible depending on the other interfacial forces (e.g., strong van der Waals attraction; see Figure S6). Overall, the findings indicate that the hydration force strongly contributes to vesicle adhesion on titanium oxide. In order to optimize model membrane fabrication strategies, due consideration of the full spectrum of interfacial forces in a particular set of environmental conditions (e.g., ionic strength, solution pH) is warranted for the experimental system under consideration.

CONCLUSIONS

Herein, we have investigated the influence of vesicle surface charge on vesicle adsorption onto titanium oxide. Compared to other experimental approaches used to tune the magnitude of the double-layer electrostatic force between vesicles and the substrate (e.g., changing solution pH), our approach varied the electrostatic force across a wide range while additional interfacial forces and corresponding material substrate properties remained constant. A key advantage of this approach is that a theoretical analysis of the corresponding lipid–substrate interaction can be performed within one set of fixed experimental conditions. Following the experimental observation that electrostatic attraction is insufficient to promote vesicle rupture, continuum model analysis of the vesicle–substrate interaction was performed by accounting for the DLVO forces and non-DLVO hydration force, and it showed that the total interaction energy was particularly sensitive to the hydration force. Retrospectively, empirical evidence from the literature is consistent with the model calculations. Taken together, the findings support that the hydration force plays an important, and potentially governing, role in controlling vesicle adhesion on titanium oxide, including promoting bilayer formation under appropriate conditions. The development of an experimental system to test our prediction via control of the magnitude of the hydration force and its corresponding effect on the lipid–substrate interaction would be advantageous. Such knowledge may have practical utility in developing methods for controlling the interaction between natural or synthetic phospholipid assemblies and hydrophilic surfaces, including biomaterials such as nanoparticle therapeutics and medical implants.

ASSOCIATED CONTENT

Ⓢ Supporting Information

More detailed information about experimental methods, continuum model calculations (Table S1 and Figures S1–S6), and substrate characterization by atomic force microscopy (Figure S7). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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