Interaction of Chitosan with Cell Membrane Models at the Air−Water Interface

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In this paper we employed phospholipid Langmuir monolayers as membrane models to probe interactions with chitosan. Using a combination of surface pressure−area and surface potential−area isotherms and rheological measurements with the pendent drop technique, we observed that chitosan interacts with phospholipid molecules at the air−water interface. We propose a model in which chitosan interacts with the phospholipids mainly through electrostatic interactions, but also including H-bonding and hydrophobic forces, depending on the phospholipid packing density. At large areas per molecule, chitosan in the subphase adsorbs onto the monolayer, expanding it. At small areas per molecule, chitosan is located in the subsurface. Indeed, a mixed chitosan−phospholipid monolayer can be transferred onto solid supports, even at high surface pressures. The effects of chitosan on the viscoelastic properties of phospholipid monolayers may be taken as evidence for the ability of chitosan to disrupt cell membranes.

Introduction

Chitosan has been widely used in cosmetics,[1,2] biotechnology,[3] and medicine[4,5] mainly because of its physicochemical properties such as biodegradability and polycationic nature in acidic solutions.[6,7] However, the mechanisms responsible for chitosan activity, including interactions with membrane phospholipids, are not known in detail. This interaction is by no means simple, for its charges cause chitosan to interact strongly with oppositely charged[8] and neutral[9−15] phospholipids in biomembranes. One possible mechanism for the action of chitosan in human bodies is a combination of electrostatic and hydrophobic interactions between chitosan and phospholipid droplets in the intestine, which is supported by reports describing chitosan effects on phospholipids in micelles[16] and liposomes.[15] Evidence for hydrophobic interactions comes from the ability of chitosan to form gels above a given concentration.[17] The properties of dilute chitosan solutions are well-known,[18−21] but relatively few studies have been made on its surface properties at the air−water interface.[9,11,16,22−26] Chitosan has very little (if any) surface activity. Li et al.[26] reported a small decrease of surface tension for a high chitosan concentration (5 g L^{-1}), probably related to viscous effects. Qun and Ajun showed that chitosan caused only slight changes in surface tension with concentration, ionic strength, and degree of acetylation.[27] Surface activity is normally increased with modified derivatives such as (carboxymethyl)chitosan,[28−32] alkylated chitosan,[33] or surfactant−chitosan complexes.[8,16,31,32,34,35] Interestingly, surface activity is enhanced when an oppositely charged surfactant is used in surfactant−polymer complexes,[34] forming micellelike aggregates.

The investigation of molecular-level interactions with the whole cell membrane is not realistic with the present technology, but one can learn a great deal using simplified membrane models, such as Langmuir monolayers of phospholipids.[36] The advantages of using Langmuir monolayers are primarily related to the control of phospholipid packing, which can mimic various stages of the cell membrane structuring. Though chitosan is not surface active, it does interact with phospholipid monolayers, as already shown in other works.[11,24,37] Still lacking is a correlation between the physicochemical properties of chitosan and the model membrane stability. In this paper, we investigate the interaction between chitosan and monolayers made with the negatively charged dipalmitoylphosphatidylglycerol (DPPG) and the zwitterionic dipalmitoylphosphatidylcholine (DPPC), using surface pressure and surface potential isotherms. We also used the axi-symmetric drop shape analysis for measuring the dynamic surface tension and rheological properties to learn how chitosan affects the mechanical properties of the phospholipid layers. To ensure that chitosan is bound to the phospholipid molecules, even in closely packed monolayers, we transferred Langmuir−Blodgett (LB) films from chitosan mixed with DPPC and DPPG.

Experimental Details

DPPC and DPPG were purchased from Sigma Chemical Co. and used as received. Chitosan was obtained from deacetylation of chitin extracted from shrimp shells,[38] with a degree of acetylation of 15%, as determined using H-RMN, according to the method of Signini and Campana.[39] The molecular weight, M_n (108 700), and polydispersity index (6.2) were determined by size exclusion chromatography (SEC). Langmuir and LB films were fabricated with a KSV 5000 Langmuir trough housed in a class 10 000 clean room. The trough is equipped with a surface pressure sensor (Wilhelmy method) and a Kelvin probe to measure the surface potential. Aliquots of a chloroform (Mallinckrodt) phospholipid solution, 0.5−1.0 mg mL^{-1}, were spread on an

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aqueous subphase containing Theorell−Stenhagen buffer (NaOH, citric acid, boric acid, phosphoric acid, whose pH was adjusted to 3.0 with addition of HCl, 2 mol L^{-1}). Water for preparing the buffer solution was supplied by a Milli-RO coupled to a Milli-Q purification system from Millipore, resistivity 18.2 MΩ cm, pH ≈ 6. The chitosan samples were dissolved in the buffer mentioned, using concentrations varying between 0.050 and 0.300 mg mL^{-1}, and employed as the subphase for phospholipid monolayers. The ionic strength was kept at 0.03 mol L^{-1}, at which chitosan is believed to adopt a random coil conformation.[40] Compression was performed with a speed of 0.04 $(\Delta A_T/A_T)$/min (where A_T is the total area of the trough) using movable barriers. Surface pressure−area ($\pi-A$) and surface potential−area ($\Delta V-A$) isotherms were measured simultaneously at 23 ± 0.1 °C. From the surface pressure isotherms, we obtained the surface compressional modulus, C_s^{-1}, defined as $-A(\partial\pi/\partial A)$ and also referred to as the equilibrium in-plane elasticity.[41]

The kinetics of chitosan adsorption at various phospholipid packing densities (corresponding to different initial surface pressures) was studied with the axisymmetric drop analysis method (OCA-20 from Dataphysics Instruments GmvH, Germany), with the oscillating drop accessory ODG-20 as described in other works.[42,43] In this measurement, a solution of ca. 10^{-4} mol L^{-1} DPPG or DPPC was gently touched on the surface of the drop, which was formed with the buffer solution, with or without chitosan. The drop was expanded up to a desired surface tension (or surface pressure). The subsequent changes in surface pressures due to chitosan adsorption were plotted against time. The dynamic surface elasticity data were obtained after the surface tension reached a constant value, by using a periodic drop oscillation of amplitude 0.1 mm (relative area variation $\Delta A/A$ of 5.5%) and frequency 1.0 Hz. The viscous effect (imaginary elasticity) for the surface elasticity was estimated from the phase angle.

The transfer of phospholipid or phospholipid−chitosan monolayers onto solid supports (AT-cut quartz crystal coated with Au, Stanford Research Systems Inc., fundamental frequency of ca. 5 MHz, cleaned with hydrogen peroxide and hydrochloride acid) was performed at a constant surface pressure of 40 mN m^{-1}, a temperature of 23 ± 0.1 °C, and a deposition rate of 5.0 mm min^{-1}. This pressure was chosen because if deposition were successful, one would ensure that chitosan was not completely expelled from the interface even at surface pressures above 30 mN m^{-1}. The presence of both phospholipid and chitosan in the LB films was inferred by nanogravimetry analysis with a quartz crystal microbalance (QCM; Stanford Research Systems Inc.).

Results and Discussion

Surface Pressure−Area and Surface Potential−Area Isotherms. Figure 1 shows the influence of chitosan in the subphase on DPPC and DPPG monolayers at the air−water interface. For the pure DPPC monolayer (Figure 1A), the pH of the buffer was 3.0, which is sufficiently low to dissolve chitosan and causes insignificant modification in the isotherm in comparison to that for DPPC on pure water (pH ≈ 6).[44−46] Typically, a plateau is observed around 5.0 mN m^{-1} in the range between 65 and 85 $Å^2$, corresponding to the liquid-expanded-to-liquid-condensed transition. Collapse occurs at 44 $Å^2$ and 62 mN m^{-1}, with the minimum area, taken as the extrapolation of the more condensed region of the curve to a zero surface pressure, being 52 $Å^2$. For pure DPPG (Figure 1B), the isotherm is relatively condensed, consistent with those reported in the literature.[47,48] Also observed is a phase transition at ca. 4 mN m^{-1} due to the buffer (in pure water the transition is close to zero). The minimum area was calculated as 46 $Å^2$, and collapse occurs at 41 $Å^2$ and 63 mN m^{-1}.

In the polysaccharide concentration range used in this work, i.e., between 0.05 and 0.3 mg mL^{-1}, chitosan is considered diluted with no steep increase in bulk viscosity. No surface activity was detected, as the surface tension was not altered when the barriers (without a phospholipid film) were compressed with chitosan in the subphase, which may be explained by the high solubility of chitosan at low pH values. With chitosan in the subphase, the isotherms for both DPPC and DPPG are shifted to larger areas per molecule, and the liquid-expanded-to-liquid-condensed plateau is less defined when the chitosan concentration in the subphase is increased. In the most compressed state, the areas per molecule for mixed chitosan−DPPC and pure DPPC Langmuir films practically coincided. For mixed chitosan−DPPG monolayers, expansion occurs at higher molecular areas. Higher concentrations (up to 0.5 mg mL^{-1}) did not change the isotherms significantly (results not shown) for both DPPG and DPPC.

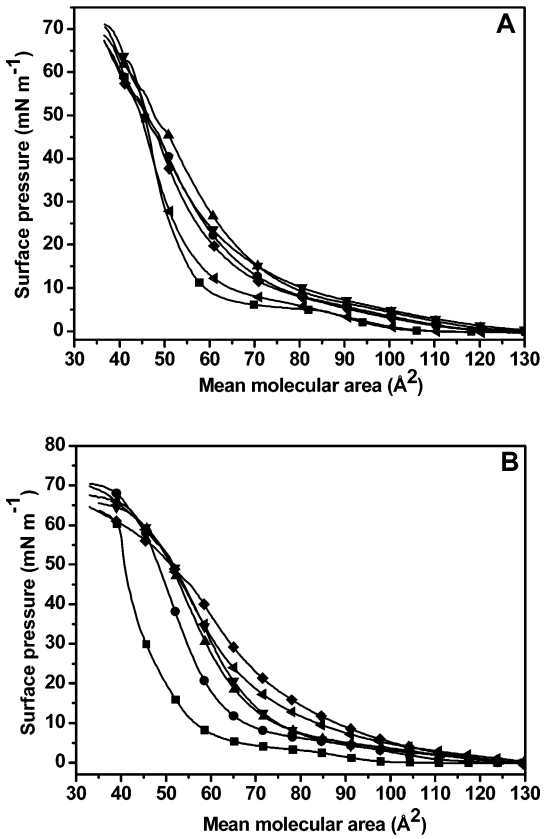

Figure 1. Surface pressure isotherms for DPPC (A) and DPPG (B) on buffer (pH 3.0) solution and chitosan: ■, 0 mg mL^{-1}; ●, 0.05 mg mL^{-1}; ▲, 0.075 mg mL^{-1}; ▼, 0.1 mg mL^{-1}; ◆, 0.2 mg mL^{-1}; left-pointing sideways triangle, 0.3 mg mL^{-1}.

The presence of chitosan also affects the in-plane elasticity of DPPC and DPPG monolayers, as indicated in Figure 2. Two features are highlighted: (i) the pure phospholipid monolayer reaches higher values of C_s^{-1} with compression (low molecular areas), and (ii) mixed chitosan−phospholipid monolayers do not exhibit a relevant reduction of elasticity in the phase transition region, unlike the pure phospholipid. The insets in Figure 2 show that the elasticity increases monotonically with surface pressure for the pure phospholipid, but displays a maximum at ca. 25−30 mN m^{-1} (for DPPC) and at ca. 35−45 mN m^{-1} (for DPPG) for the mixed chitosan−phospholipid monolayer. At low surface pressures (i.e., large areas per molecule) elasticity is higher for the mixed DPPC−chitosan monolayer. For DPPG, C_s^{-1} tends to be lower for the mixed monolayers after the phase transition (i.e., above 4 mN/m), probably because chitosan hinders the close packing of DPPG molecules. Overall, the effects on C_s^{-1} and on the overall

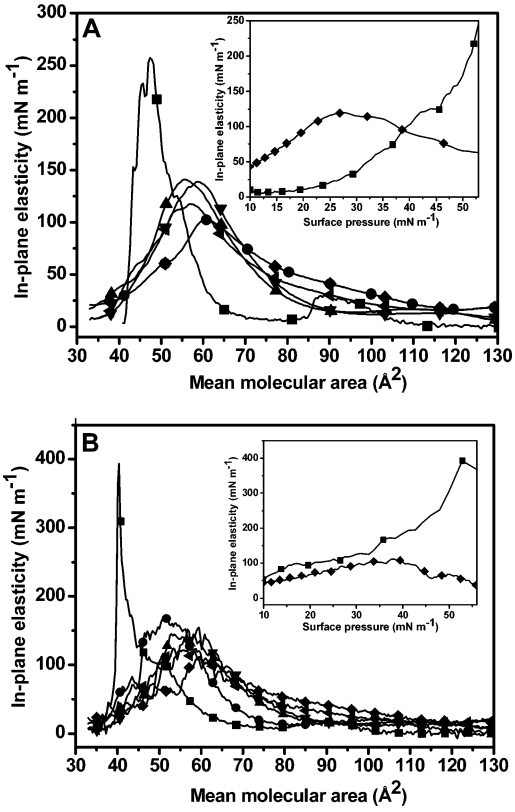

Figure 2. In-plane elasticity for DPPC (A) and DPPG (B) on buffer (pH 3.0) solution and chitosan: ■, 0 mg mL^{-1}; ●, 0.05 mg mL^{-1}; ▲, 0.075 mg mL^{-1}; ▼, 0.1 mg mL^{-1}; ◆, 0.2 mg mL^{-1}; left-pointing sideways triangle, 0.3 mg mL^{-1}. The insets show in-plane elasticity vs surface pressure for two chitosan concentrations: ■, 0 mg mL^{-1}; ◆, 0.2 mg mL^{-1}.

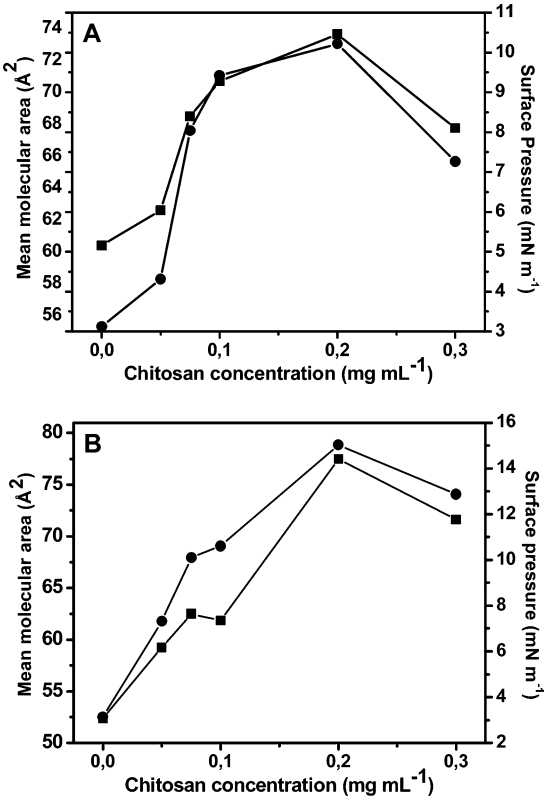

Figure 3. Area per phospholipid molecule at 17 mN m^{-1} (●) and surface pressure at 80 Å^2 (■) vs chitosan concentration in the subphase for DPPC (A) and DPPG (B) monolayers.

behavior of the mixed monolayers vary little with the chitosan concentration.

These results point to an effective interaction of chitosan with the phospholipids at the air−water interface. Though chitosan has no surface activity at a bare interface, it can interact with other surfactants,[8,31,32,34,35] including phospholipids.[10−14] The effects are caused mainly by the change in the activity coefficient of chitosan, acting as a counterion due to electrostatic interactions with the phospholipids. Another possibility is a preferential orientation of the less hydrophilic acetylated groups, causing an additional decrease in surface tension. Chitosan expands the phospholipid monolayers. However, at higher surface pressures there is progressively less expansion, and close to the collapse, the areas per molecule are practically the same as those for pure DPPC. For DPPG, even close to collapse, there is an expansion in terms of molecular areas. The smaller elasticity of the mixed monolayers at a high surface pressure may be attributed to the chitosan−phospholipid polar head contacts that make the film more flexible, which is typical of phospholipids interacting with macromolecules at the air−water interface.[49]

Figure 3 shows the effect of the concentration of chitosan in the subphase, taking either a fixed area of 80 Å^2 or a fixed pressure of 17 mN m^{-1}. Because the isotherms are expanded with chitosan at low concentrations in the subphase, both the area per molecule and surface pressure increase with the chitosan concentration up to 0.2 mg mL^{-1}, above which the area and the pressure decrease. An excess of chitosan therefore affects the monolayer structure, causing it to be more condensed due to chain coiling for chitosan at the interface. Overall, chitosan causes the area per molecule to increase because of its adsorption on the phospholipid monolayer and interaction with the polar headgroups of the phospholipid.

Analogously to the surface pressure isotherms, the introduction of chitosan caused the surface potential isotherms to become more expanded, but there was practically no difference in surface potential for the condensed monolayers (maximum value for the surface potential). Figure 4 shows surface potential−area isotherms for pure DPPC and DPPG and for mixed chitosan−phospholipid monolayers.

Such expansion confirms that chitosan is able to adsorb onto a phospholipid monolayer at large molecular areas, and at low molecular areas, little effect of chitosan is noted. The increase in surface potential may arise from the chitosan dipole moment or the formation of an electric double layer owing to the positive charge of chitosan. Significantly, we observed in subsidiary experiments that chitosan on its own does not lead to a measurable surface potential. The increase in surface potential at 130 Å^2 varies with the chitosan concentration as indicated in the inset of Figure 4, again pointing to a maximum interaction between chitosan and DPPC and DPPG for a concentration of 0.2 mg mL^{-1}. In this area, no changes can be seen in the surface pressure isotherms, which are not sensitive to such small surface densities. One should note that the data were collected at pH 3. For a charged DPPG monolayer, the surface potential differs from that of a pure water surface because the negative contribution from the double layer is smaller due to the lower dissociation of the headgroups. Indeed, the maximum surface potential increased from 190 mV (for pure water, pH 5.6[50]) to 510 mV in the presence of the buffer (pH 3, in this work).

Compression−decompression curves were performed for pure DPPC and DPPG and also for mixed chitosan−phospholipid monolayers. For pure DPPC and DPPG monolayers little hysteresis is observed in the surface pressure−area isotherms (not shown), whereas for mixed chitosan−phospholipid mono-

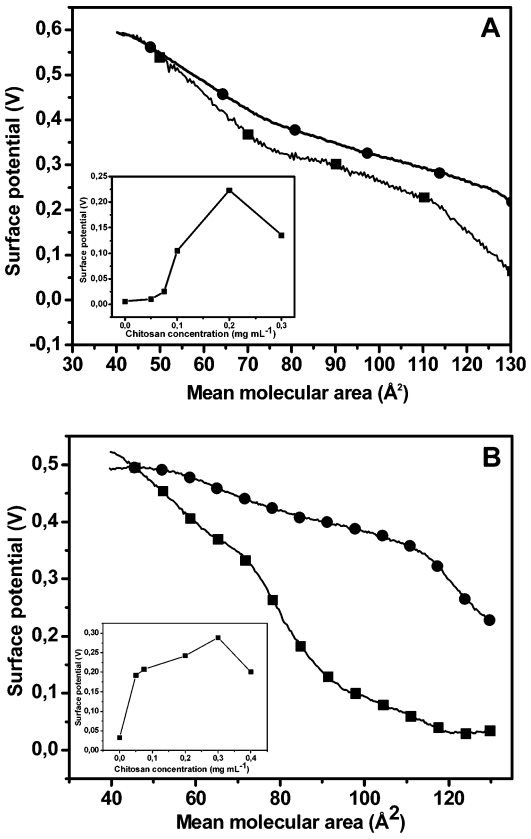

Figure 4. Surface potential−area isotherms for DPPC (A) and DPPG (B) on a pH buffer in the absence (■) and with (●) 0.2 mg mL^{-1} chitosan in the subphase. The inset shows the surface potential at 130 Å^2 vs chitosan concentration.

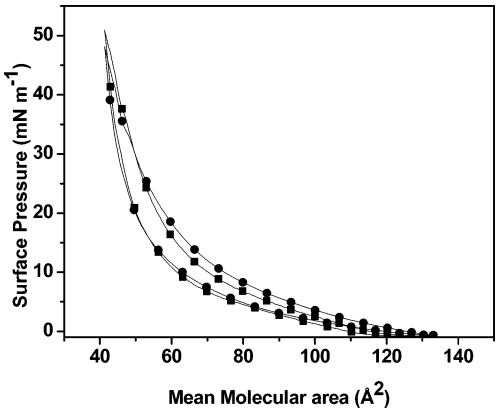

Figure 5. Cycle of compression−decompression for mixed chitosan (0.2 mg mL^{-1}) phospholipid monolayers: (■) DPPC and (●) DPPG (target pressure 50 mN m^{-1}).

layers, a significant hysteresis is observed in Figure 5, probably owing to irreversible formation of chitosan−phospholipid aggregates at the air−water interface. The similar effects observed for chitosan on DPPC and on DPPG monolayers indicate analogous interactions of the polymer with the phospholipid, regardless of the polar head charge.

Dynamic Measurements. To evaluate the kinetics of adsorption for chitosan onto phospholipid monolayers, the pendent drop technique[42,43] was used. A drop of chitosan solution was formed, and a phospholipid solution was touched on the surface of the drop, according to the schematic diagram of Figure 6. The drop area was increased until the desired initial surface pressure, with any change in surface pressure being ascribed to chitosan adsorption. Without chitosan in the drop, after expansion of the phospholipid-containing drop, there was no significant change in the surface pressure with time.

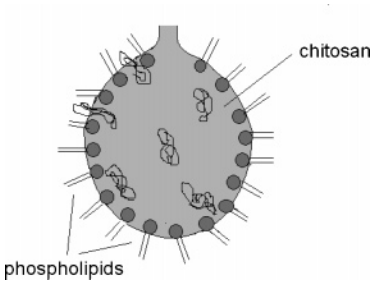

Figure 6. Schematic diagram for the measurements of dynamic surface tension and dynamic dilatational elasticity for phospholipid−chitosan monolayers.

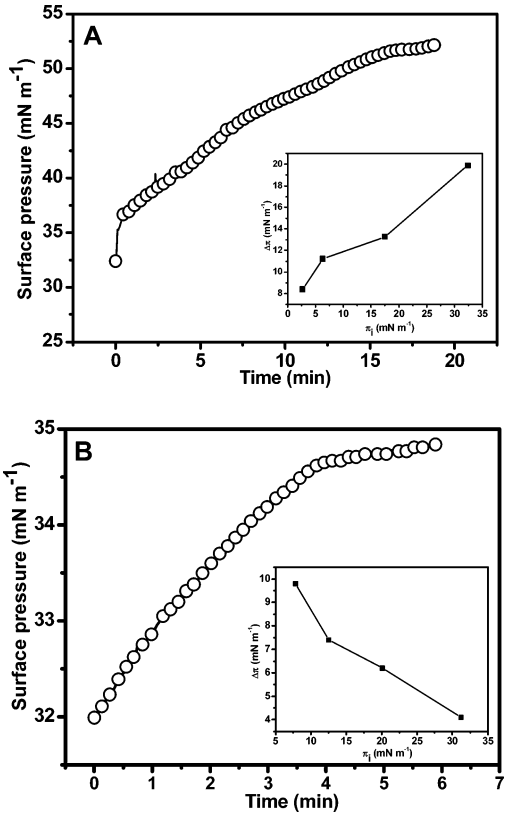

Figure 7. Adsorption kinetics for chitosan on DPPC (A) and DPPG (B) monolayers at an initial surface pressure of 32 mN m^{-1}. The inset shows the increase in surface pressure ($\Delta\pi$) caused by chitosan adsorption (0.3 mg mL^{-1}) as a function of the initial surface pressure (π_i).

Several initial surface pressures were tried, and surface pressure−time curves were obtained for DPPC and DPPG. For all initial pressures, π_i, at which only the phospholipid is present at the interface, there is a quick increase in surface pressure, reaching equilibrium in a few minutes (less than 20 min). There is no induction time, which means that chitosan adsorption is not diffusion-dependent. Since chitosan is not surface active on its own, its practically instantaneous adsorption confirms the high affinity with the phospholipids. Figure 7A shows the adsorption kinetics for an initial surface pressure of 32 mN m^{-1} as an example. The change in surface pressure due to chitosan adsorption ($\Delta\pi$) increases for DPPC with the initial pressure (π_i), as indicated in the inset of Figure 7A. Therefore, a close packing of DPPC does not prevent chitosan from interacting with the phospholipid molecules, in contrast to other cases in the literature where adsorption of macromolecules does not

Table 1. Dynamic Elasticity for DPPC and Mixed Chitosan−DPPC Monolayers Chitosan Concentration 0.075 mg mL^{-1})

DPPC		DPPC + chitosan		
π_i (mN/m)	ϵ (mN/m)	$\Delta\pi$ (mN/m)	ϵ (mN/m)	ϵ_i (mN/m)
8 ± 1	28.4	7.8	42.9	0.6
17 ± 2	46.7	10.0	45.0	6.6

Table 2. Dynamic Elasticity for DPPG and Mixed Chitosan−DPPG Monolayers (Chitosan Concentration 0.075 mg mL^{-1})

DPPG		DPPG + chitosan		
π_i (mN/m)	ϵ (mN/m)	$\Delta\pi$ (mN/m)	ϵ (mN/m)	ϵ_i (mN/m)
8 ± 1	51.3	15.0	140	2.7
17 ± 2	67.8	11.2	261.8	33.9

occur at high surface packing.[51−54] Indeed, one usually defines an "exclusion surface pressure",[36,51,55] at which $\Delta\pi$ becomes zero and the guest molecule can no longer interact with the phospholipids at the interface.

The kinetics of adsorption for chitosan on a DPPG monolayer (Figure 7B) indicates no induction time, with equilibrium being reached in less than 5 min. Now, $\Delta\pi$ decreases with the initial surface pressure, with chitosan adsorption leading to smaller pressure changes than for DPPC. These smaller changes are probably due to strong interactions between chitosan and the charged DPPG heads, which hinder chitosan penetration in the film and cause the equilibrium pressure to be reached faster.

Tables 1 and 2 show the data for dynamic dilatational elasticity, ϵ, for pure DPPC and DPPG monolayers and DPPC and DPPG monolayers mixed with chitosan. The values depicted in these tables are the complex elasticity modulus as defined by Lucassen and van den Tempel.[56]

Chitosan solutions exhibited negligible elasticity (less than 0.5 mN m^{-1}) in the concentration range used (0.05−0.3 mg mL^{-1}). However, when introduced in the subphase on which DPPC or DPPG monolayers were spread, chitosan caused significant changes, as shown in the tables for a chitosan concentration of 0.075 mg mL^{-1}. For 0.2 and 0.3 mg mL^{-1}, the same trend was observed (results not shown). The presence of chitosan in the subphase causes ϵ to increase at an initial surface pressure (π_i) of 8 mN m^{-1}. This imparts a higher ability to restore a surface pressure condition when submitted to a deformation, which may be explained by an increase of DPPC molecular packing in the presence of chitosan. The process of chitosan adsorption is faster than the frequency imposed to the oscillating drop. Hence, it is likely that the relaxation process due to the area change should be related to chitosan molecular rearrangements at the interface rather than diffusion or adsorption.

In the equilibrium measurements (Figure 2A), except for the plateau and next-to-collapse regions, the in-plane elasticity, C_s^{-1}, for pure DPPC monolayers progressively increased with surface pressure. In the presence of chitosan, C_s^{-1} noticeably increased at surface pressures lower than 37 mN m^{-1}, which indicates that the film becomes more elastic upon chitosan adsorption on the DPPC monolayer until a limit is reached.

Although in-plane elasticity, C_s^{-1}, and dynamic dilatational elasticity (in the drop), ϵ, can be related to each other,[42] the phenomena prevailing in the two measurements are different. For C_s^{-1} the measurement is performed with an equilibrium phenomenon, while the surface elasticity in the drop, ϵ, is a nonequilibrium property in which dynamic processes are involved. Chitosan adsorption in the first steps of adsorption may be undetected in surface pressure−area curves. Moreover, for a monolayer in a Langmuir trough, chitosan adsorption occurs at a low state of packing (0 mN m^{-1}), and higher surface pressures are attained by compression. On the other hand, in the oscillating drop, DPPC is spread at an aqueous interface, after formation of a chitosan solution drop. Expanding and compressing the drop will form dynamically new interfaces, and processes of adsorption/desorption of chitosan may occur. One should be aware that surface rheology parameters measured in the presence of soluble and/or associating components may depend on the geometry of the system.[57] The high dynamic surface elasticity for mixed chitosan−DPPC monolayers may be explained by the rapid adsorption of chitosan to the interface, at a higher state of packing (7−17 mN/m) and in a system with a reduced area/volume ratio provided by the new monolayer composition (chitosan−phospholipid) formed. During the expansion/compression process of the drop with DPPC monolayers at the air−water interface, chitosan adsorbs rapidly, leading to larger changes in surface pressure. As elasticity is defined as $d\gamma/d(\ln A)$, a sudden compression of the DPPC monolayer, due to fast penetration of chitosan, yields a higher surface elasticity. Since the compressional modulus is smaller for equilibrium measurements (surface pressure−area isotherms), it is possible that after adsorption chitosan is rearranged due to monolayer relaxation. Moreover, the imaginary part (ϵ_i), in which the viscous effect can take part, increased at a high π_i (see Table 1), thus confirming the high affinity of chitosan for DPPC interfaces, even at high surface pressures and a short time of formation of new interfaces.

Table 3. Nanogravimetry Data for Phospholipids Transferred from the Air−Water Interface to Quartz Crystals (Surface Pressure of Deposition 30 mN m^{-1})

	frequency variation (−Hz)	total mass transferred (ng)
DPPC	45	303
DPPC + chitosan, 0.2 mg mL^{-1}	116	781
DPPG	44	297
DPPG + chitosan, 0.2 mg mL^{-1}	120	809

From Table 2, the dynamic elasticity markedly increased for DPPG with chitosan in the subphase. The imaginary contribution (ϵ_i) has a higher effect when compared to that of chitosan−DPPC, as analyzed from the corresponding component of the elastic modulus. Since this contribution is associated with loss, one could attribute it to the stronger electrostatic interactions between DPPG and chitosan, which would inhibit the regeneration of the drop shape during excitation. This is clear at high surface pressures, in which ϵ_v reaches 33.9 mN m^{-1} at an initial surface pressure of 17 mN m^{-1}. Although also present for DPPC monolayers, this effect is smaller owing to its zwitterionic nature. Note that, for an initial pressure equal to or above 30 mN m^{-1}, the mixed chitosan−phospholipid systems cannot withstand gravitational forces and the drop fell down during the oscillation experiment for both DPPC and DPPG mixed monolayers. This does not occur for the pure phospholipid monolayer at the same surface pressure, which is further evidence for the presence of chitosan even at high surface pressures.

In summary, from the surface pressure−area and surface potential−area isotherms, chitosan was inferred to be expelled from the interface when the monolayer was compressed to high surface pressures. However, with the results from the dynamics

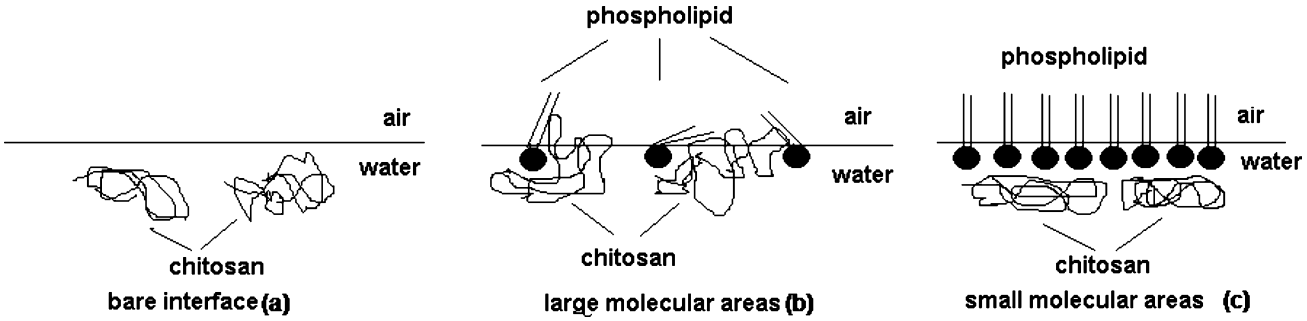

Figure 8. Model for chitosan and phospholipid interaction.

experiments presented here, it is reasonable to assume that chitosan molecules are kept at the interface even at a high surface pressure due to their high affinity for DPPC and DPPG, probably forming a subsurface.

LB Films. To demonstrate that chitosan is not expelled from the interface at high surface pressures, but lies in a subsurface interacting mainly with phospholipid polar heads, we performed sythesized one-layer LB films of a phospholipid or mixed phospholipid−chitosan monolayer. The solid support used was a hydrophilic quartz crystal covered with gold as described in the Experimental Details. The transfer ratios were 0.89 for pure DPPG, 0.91 for pure DPPC, 0.86 for mixed DPPG−chitosan, and 0.92 for mixed DPPC−chitosan, thus confirming an effective deposition. Table 3 shows the nanogravimetry results, indicating the amount of phospholipid or chitosan transferred from the air−water interface to the solid support. This mass was calculated with the Sauerbrey equation,[58] which relates the frequency decrease to the deposited mass. For the mixed chitosan−phospholipid monolayers, a mass increase of approximately 400 ng was measured for the mixed chitosan−DPPG and chitosan−DPPC LB films. This indicates the transfer not only of the phospholipid, but also of chitosan. As the transfers were performed at 40 mN m^{-1}, these results unequivocally prove that chitosan is present at the air−water interface even at high surface pressures, and therefore, it was not expelled during compression.

Model for Chitosan−Phospholipid Interaction at the Air−Water Interface. On the basis of the results presented in this paper, we propose a model illustrated in Figure 8, which is described as follows.

(1) Chitosan has no surface activity at a bare interface and therefore does not affect the surface pressure and surface potential in isotherms obtained with the subphase and no phospholipid at the air−water interface. The surface of a chitosan solution displays negligible surface dilatational elasticity. Therefore, in Figure 8a there is no surface excess for a chitosan solution.

(2) With introduction of phospholipids at the air−water interface, chitosan displays significant surface activity, as indicated by changes in surface pressure−area and surface potential−area isotherms and by an increase in surface pressure with introduction of chitosan (see the adsorption kinetics, Figure 7). Moreover, chitosan disturbs the packing of the phospholipid monolayer structure (changes in C_s^{-1} and ϵ), revealing a disrupting effect on the phospholipid membranes. The effects from chitosan depend on the phospholipid packing as follows.

(2.1) At large areas per molecule (or low surface pressures), chitosan moieties are believed to penetrate among phospholipid chains, thus resulting in hydrophobic interactions, as depicted in Figure 8b, but with most molecules still interacting with the phospholipid polar heads. The combination of hydrophobic and electrostatic interactions appears at small surface densities of the phospholipid. This rationale is based on the shifts of surface pressure−area isotherms for large molecule areas, the higher surface potential for mixed phospholipid monolayers with chitosan in the subphase, and also the increase in dynamic and equilibrium elasticity. Expansion would be not only due to adsorption of chitosan at the interface, but mainly due to interaction between the ammonium salt of chitosan under acidic conditions and the choline moieties on the DPPC headgroups, which cause the acetylated groups of chitosan to orientate themselves toward the interface.

(2.2) At small areas per molecule in the DPPC or DPPG monolayers, the high surface density may favor complete expelling of chitosan moieties that would otherwise interact with the hydrophobic groups at the interface. This conclusion is supported by the lack of an effect from chitosan for the closely packed monolayers. However, chitosan is not completely expelled to the bulk solution: it forms a subsurface interacting with phospholipid polar heads. That a subsurface as illustrated in Figure 8c is formed was inferred from the following results: (a) chitosan in the subphase caused an increase in surface pressure for DPPC and DPPC monolayers at a high π_i (see Figure 7); (b) it caused the drop to fall down during oscillation experiments (too elastic); (c) mixed films of chitosan and phospholipids can be transferred onto solid supports even at 40 mN m^{-1}. At high surface pressures, chitosan should interact with phospholipid polar heads through hydrogen bonding in addition to electrostatic interactions. Hydrophobic interactions should be discarded at high phospholipid surface densities, because monolayer compression causes chitosan to be located at the subsurface.

Finally, the effect from chitosan on phospholipid monolayers at distinct surface pressures can be related to that in cell membranes. According to Marsh,[49] for partitioning of molecules into the membrane or for conformational changes of proteins and polysaccharides at the membrane interface, the relevant quantity is the compressional modulus (i.e., in-plane elasticity and dynamic elasticity) rather than the lateral pressure itself. The effect of membrane fluctuations is to reduce the compressional modulus for the change in membrane area, because these changes are absorbed by alterations in thermal fluctuations. This favors interaction of certain macromolecules.[49,59] Many proteins cause reduction of elasticity during insertion, though maintaining the membrane structure. Under the conditions used in this work, chitosan increased the dynamic elasticity, which is believed to disrupt the membrane organization. Finally, this work demonstrates that chitosan can "stick" on lipid monolayers mainly by Coulombic interactions, similarly to what occurs in layer-by-layer films.[60]

Conclusions

We have demonstrated that chitosan has a strong affinity for phospholipids at the air−water interface, despite its lack of surface activity. Chitosan promotes a local distortion of the phospholipid tails, in a process governed by a combination of electrostatic, dipole, and hydrophobic interactions. As a consequence, chitosan causes disruption of the phospholipid layer, from which a possible implication is that chitosan may affect the stability of cell membranes. Taking together data from various techniques employed with Langmuir monolayers and LB films and from the pendent drop method, we proposed a model in which the surface activity of chitosan was promoted by the presence of a phospholipid at the air−water interface. At large phospholipid areas per molecule, chitosan is located at the interface, interacting with the phospholipid molecules via electrostatic and hydrophobic interactions. At small areas per phospholipid molecule, on the other hand, interaction is predominantly electrostatic, with chitosan forming a subsurface, with negligible contribution to the surface pressure or surface potential.

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