Cationic Bilayers on Polymeric Particles: Effect of Low NaCl Concentration on Surface Coverage

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The interaction between dioctadecyldimethylammonium bromide (DODAB), chloride (DODAC), or acetate (DODAAc) cationic bilayer fragments (BF) and polystyrene sulfate (PSS) particles (2×10^{11} particles/mL) was evaluated at and above equivalence of total surface areas for bilayers and particles (A_b/A_p) over a range of low NaCl and lipid concentrations, by means of zeta-potential, ζ, and particle diameter, D_z, analysis. In pure water, lipid addition at and above $A_b/A_p = 1$ rapidly increased D_z and ζ, which reached stable plateau values above those expected at bilayer coverage. By addition of small NaCl concentrations (0.05−5.00 mM), D_z decreased with ζ still positive. Therefore, the effect of low salt concentration on the particle coverage with lipid bilayer fragments was surface rearrangement of bilayer fragments on the surface from uneven coverage with some bilayer fragments adsorbed vertically, to perfect bilayer coverage upon addition of small amounts of NaCl. This occurred over a broad range of lipid concentration (0.04−1.0 mM DODA), possibly due to NaCl-induced sealing of adjacent bilayer patches adsorbed on particles. At 0.2 mM lipid, the effect of salt, counterion, and bilayer type (BF or large vesicles, LV) on surface tension at the air−water interface (γ) complemented the evaluation of bilayer behavior at a hydrophobic interface. The γ decay rate and total extent increased with counterion size, NaCl concentration, and frequency of hydrophobic defects in the lipid bilayer. Upon addition of 2 mM NaCl to 0.2 mM DODAB BF or LV, γ decay was much faster for BF, showing the importance of hydrophobic defects in the bilayer to induce its fusion to the air−water interface.

Introduction

Substrate-supported lipid arrays[1−4] are finding many interesting uses in basic and applied research areas such as biosensors development;[5,6] simulation of biological substrates for cellular receptor engagement; response and immune cell adhesion;[7−9] targeted drug delivery and drug nanoencapsulation;[10,11] reconstitution and study of protein structure and function;[12−14] production of G protein-coupled receptors microarrays where binding affinity, relative potency, and selectivity of compounds with potential as drugs could be screened;[15] protein adsorption studies;[16,17] or bilayer-protein interactions.[18,19] A variety of flat or spherical, hydrophilic or hydrophobic substrates have been used as supports, though major focus has been placed on flat hydrophilic surfaces.[20] Among the bilayers, cationic bilayers have found several uses in biology and medicine for drug, gene, and vaccine delivery or as antimicrobial agents,[20] but their interaction with hydrophobic surfaces remained poorly understood.[11]

Recently, interactions between hydrophobic polymeric films (spin-coated polystyrene sulfate (PSS)) and dioctadecyldimethylammonium (DODAB) vesicles were characterized by means of in situ ellipsometry.[21] In water, in situ DODAB adsorption monotonically increased yielding a final, 1.6−1.8 nm thick, deposited lipidic layer. This thickness is in disagreement with the 5 nm expected for bilayer deposition.[21] Thus, at early stages in pure water, DODAB adsorption linearly increased with the square root of time, indicating a vesicle diffusion controlled process with ca. 1.0×10^{-11} m^2 s^{-1} as the vesicle diffusion coefficient (D) in nice agreement with reported D for similar vesicles.[21] However, the dynamics for the lipid/surface interaction completely changed by adding small amounts of NaCl to the system. Upon addition of NaCl (50 mM final concentration) to a DODAB lipid dispersion (0.2 mM DODAB), adsorption from vesicles on PSS films immediately yielded a 6.0 nm thick DODAB layer that remained stable as a function of time. The hydrophobic attraction between salt-induced defects on the bilayer and the film surface determined bilayer deposition with very fast adsorption kinetics.[21]

The production of bilayer covered-polystyrene microspheres was previously described.[22,23] In this work, the effect of low NaCl concentration on surface coverage of PSS particles by cationic bilayers was evaluated by means of particle sizing and zeta-potential determinations over a range of lipid (0.04−1.0 mM) and NaCl concentration (0.05−5.0 mM). Our strategy involved bilayer deposition from cationic bilayer fragments (BF).[24,25] Over a range of DODA lipid concentrations, BF addition to the oppositely charged particles increased mean particle size and changed the zeta-potential sign from negative to positive. The increase in particle size was not large enough to indicate particle clustering, flocculation, or bridging by the bilayer fragments so a model including both flat and vertical BF adsorption on particles was proposed. Upon addition of NaCl (0.05−5.00 mM final concentrations) to the BF/PSS mixture, these unevenly adsorbed bilayer fragments rearranged (with possibly some desorption), which resulted in perfect surface coverage as a bilayer. Final sizes and zeta-potentials indicated coverage of particles with one bilayer over a range of lipid concentrations.

The importance of the hydrophobic interaction between hydrophobic defects on the bilayer and hydrophobic interface was demonstrated from NaCl and DODA lipid concentration

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TABLE 1: Mean Zeta-average Diameters and Zeta-potentials for Mixtures of Bilayer Fragments of DODA Lipids with X as Counterion (DODAX) and 2×10^{11} PSS Particles/mL over a Range of DODAX Concentrations[a]

X^-	[DODAX]/mM	D_z/nm (0.0)	ζ/mV (0.0)	D_z/nm (0.05)	ζ/mV (0.05)	D_z/nm (0.5)	ζ/mV (0.5)	D_z/nm (5.0)
	0.0	115 ± 1**	-31 ± 4	115 ± 1	-31 ± 4	112 ± 1	-31 ± 4	111 ± 1
		100 ± 5*						
Br^-	0.05	170 ± 1	41 ± 2					
	0.10	171 ± 1	31 ± 1					
	0.20	167 ± 1	39 ± 1	118 ± 1	38 ± 5	109 ± 1	30 ± 3	112 ± 1
	0.40	165 ± 1	43 ± 2					
	0.60	164 ± 1	36 ± 1					
	0.80	163 ± 1	34 ± 1	121 ± 1	21 ± 4	108 ± 1	26 ± 5	108 ± 1
	1.00	157 ± 1	30 ± 1					
Cl^-	0.05	131 ± 1	58 ± 1					
	0.10	131 ± 1	60 ± 1					
	0.20	132 ± 1		118 ± 1	31 ± 6	109 ± 1	28 ± 3	112 ± 1
	0.40	128 ± 1	47 ± 2					
	0.60	144 ± 1	57 ± 3					
	0.80	147 ± 1	47 ± 1	113 ± 1	38 ± 8	103 ± 1	32 ± 2	109 ± 1
	1.00	134 ± 1	45 ± 6					
Ac^-	0.05	148 ± 1	54 ± 2			114 ± 1	34 ± 2	117 ± 1
	0.10	155 ± 1	58 ± 3					
	0.20	145 ± 1	63 ± 2					
	0.40	135 ± 1	60 ± 1					
	0.60	135 ± 1	58 ± 2					
	0.80	128 ± 1	58 ± 1	122 ± 1		116 ± 1		115 ± 1
	1.00	129 ± 1	53 ± 2					

[a] DODAX concentrations at 0.0, 0.05, 0.5, and 5.0 mM NaCl (given between parentheses). Before adding NaCl, mixtures interacted for 24 h and thereafter. Bare particle size was determined by transmission electron microscopy (*) or by dynamic light scattering (**).

effects on γ decay at the air−water interface over a range of low salt and lipid concentrations. Upon addition of 0.5 mM NaCl, γ decay for DODAB BF was much faster than the kinetics for DODAB LV, emphasizing the role of hydrophobic defects in determining dynamics of the bilayer particle coverage.

Experimental Section

Materials. Dioctadecyldimethylammonium bromide (DODAB), 99.9% pure, was obtained from Sigma and used as such without further purification. Dioctadecyldimethylammonium chloride (DODAC) or acetate (DODAAc) was obtained by ion exchange from DODAB using AMBERLYSTA-26 from E. Merck (Darmstatd, Germany) in the chloride or acetate form, respectively, as previously described.[26] Small DODA lipid bilayer fragments (BF), (Vieira and Carmona-Ribeiro,[24] Moura and Carmona-Ribeiro[25] and references therein) were prepared by sonication with tip in MilliQ water at 1.0 mM DODAB as previously described.[27,28] Mean BF sizes and zeta-potentials were determined at 4.0 mM lipid yielding ca. 73, 64, and 35 nm mean diameter for DODAB, DODAC, and DODAAc, respectively, with 42, 49, and 50 mV zeta-potential, respectively. Large DODAB vesicles (LV), ca. 500 nm mean diameter and 55 ±1 mV of mean zeta-potential were obtained by vortexing the DODAB powder in pure water at 60 °C.[29] DODAB concentrations were analytically determined by microtitration.[30] Anionic sulfate polystyrene (PSS) particles, nominal mean diameter of 100 nm, 5.7×10^5 cm^2g^{-1} specific surface area, area per charge group of 17.6 nm^2, 0.9 $\mu C\ cm^{-2}$, and -31 ± 4 mV mean zeta-potential (Table 1) were purchased from Interfacial Dynamics Corporation (Portland, OR, United States). NaCl and all other reagents were analytical grade. Water was MilliQ quality.

Determination of Mean Diameters, Size Distributions, and Zeta-potentials for the Dispersions. Particle size (mean diameter D_z), size distribution, and zeta-potential (ζ) for dispersions were determined using the ZetaPlus- ZetaPotential Analyzer (Brookhaven Instruments Corporation, Holtsville, NY), which was equipped with a 677 nm laser and dynamic light-scattering (PCS) at 90° for particle sizing. The value of ζ was determined from electrophoretic mobility μ either in pure water and from the Smoluchowski's equation: $\zeta = \mu\eta/\epsilon$, where η is the medium viscosity and ϵ the medium dielectric constant.

Determination of Surface Tension Kinetics. Surface tension at the air−water interface was measured at 25 °C as a function of time after adding NaCl to a 0.2 mM DODA dispersion prepared in pure water were determined with a Du Nouy ring as previously described.[31]

Results

NaCl -induced Rearrangements of Bilayer Fragments on Polymeric Particles. The mean particle diameter for PSS particles in pure water was ca. 105−115 nm (Table 1). Deposition of one single DODA bilayer on each polymeric particle should yield ca. 115−125 nm for the mean diameter. The increase in particle size for PSS particles upon addition of DODA BF was shown from D_z kinetics over a range of DODAB, DODAC and DODAAc concentrations (Figure 1). This increase in particle size was well above figures expected for even bilayer deposition on each particle. In general, sizes stood above 125 nm. Aging had no effect in improving the situation; mean diameters did not decrease over longer periods of time. At 24 h of interaction time, sizes remained above those expected for bilayer deposition inside the 130−170 nm range for the three DODA lipids tested (Figure 1). When mixed with PSS particles in pure water, the largest bilayer fragments (73 nm mean diameter), which were those composed of DODAB, gave the largest mean diameters (155−170 nm), whereas the smallest DODAAc fragments (35 nm) yielded the smallest sizes, 130−155 nm mean diameter (Figure 1, Table 1).

NaCl addition to each BF/PSS mixture in Figure 1 almost immediately reduced mean sizes to values in the 108−122 nm range in nice agreement with the expected theoretical increase in diameters due to bilayer deposition, namely, 115−125 nm (Figure 2, Table 1). Determination of mean zeta-potentials for

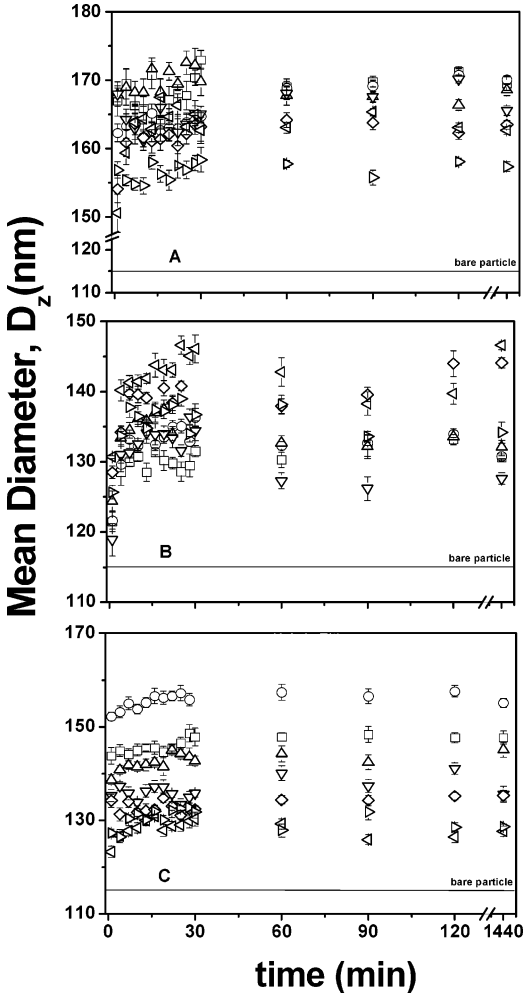


Figure 1. Effect of DODAB (A), DODAC (B), or DODAAc BF concentration (C) on kinetics of mean D_z at 2×10^{11} PSS particles/mL. DODA salt final concentration was 0.05 (□), 0.1 (○), 0.2 (△), 0.4 (▽), 0.6 (◇), 0.8 (triangle pointing left), and 1.0 mM (triangle pointing right).

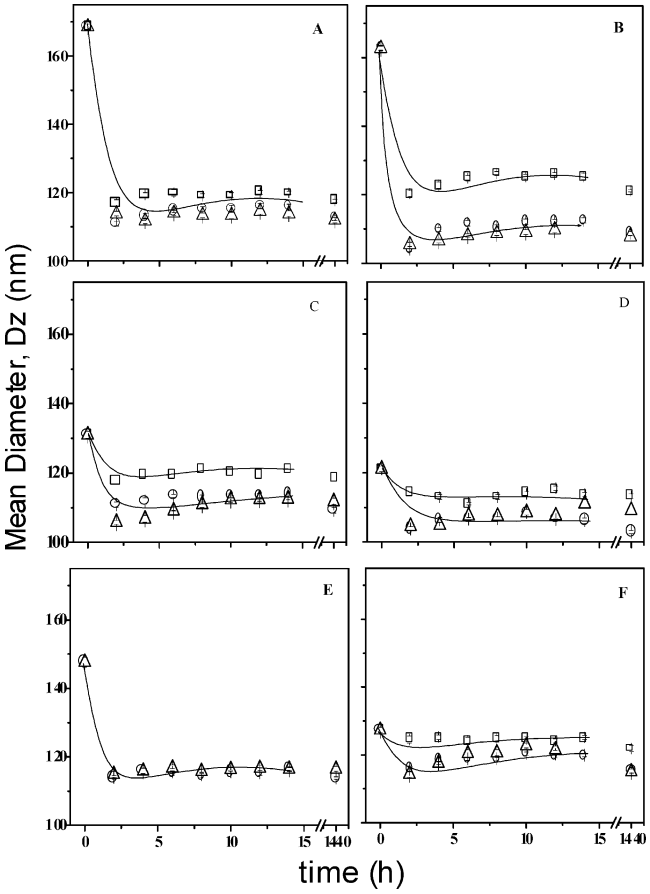


Figure 2. Effect of NaCl concentration on kinetics of mean D_z for mixtures of DODA BF/PSS particles. DODA BF and particles (2×10^{11} particles/mL) interacted for 24 h before adding 0.05 (□) or 0.5-(○) e 5.0 mM final concentrations of NaCl (△). Two different DODA concentrations were used in each subfigure: 0.2 (A) and 0.8mM DODAB (B); 0.2 (C) and 0.8 mM DODAC (D); 0.05 (E) and 0.8 mM DODAAc (F).

these samples gave positive values that were inside the range expected for bilayer-covered particles (Table 1). NaCl in the dispersions did not cause DODA desorption from particles. Instead, over a range of low concentrations, it caused improved rearrangement of bilayer fragments and bilayer coverage on particles. Most importantly, this occurred over a broad range of lipid concentrations (0.04−1.0 mM DODA) and for the three DODA lipids tested, independently of the ratio between total surface areas for bilayers (A_b) and particles (A_p). One should notice that the results were obtained at $A_b/A_p \geq 1$, an experimental condition that allowed sufficient bilayer surface area to cover all particles present in the dispersions.

In pure water, zeta-potentials for DODAB, DODAC and DODAAc bilayer fragments were 42, 49, and 50 mV, respectively (see Materials and Methods section) whereas DODA/PSS mixtures yielded 30−43 (DODAB), 45−60 (DODAC), and 53−63 mV (DODAAc) as zeta-potential ranges depending on counterion type and DODA lipid concentration (Table 1). For bilayer fragments alone in dispersion or bilayer fragments/PSS mixtures, going from bromide to chloride to acetate as counterions, zeta-potentials increased.

Figure 3 showed size distributions at 0.04 mM DODA lipid and 2×10^{11} part./mL ($A_b/A_p = 1$), before and after adding NaCl to a final concentration of 0.5 mM. Similar to results shown in Figure 2, sizes decreased upon NaCl addition (Figure 3). In Table 2, the positive zeta-potential measurements at 0.5 mM NaCl suggested that this small amount of salt did not cause bilayer desorption from the microspheres. The zeta-potential values were smaller in the presence of 0.5 mM salt than those in water (Table 2), possibly due to rearrangement of adsorbed BF at the particle surface and/or salt screening effect on the electric double layer of the charged supported bilayers.

TABLE 2: Mean Zeta-average Diameters (D_z) and Zeta-potentials (ζ) for DODAX BF/PSS Mixtures at 0.04 mM DODAX and 2×10^{11} PSS Particles/mL after 24 h Interaction before and after Adding 0.5 mM NaCl[a]

DODAX	D_z/nm PSS + DODAX	ζ/mV	D_z/nm PSS + DODAX + salt	ζ/mV
DODAB	125 ± 1	41 ± 2	113 ± 1	+29 ± 1
DODAC	117 ± 1	47 ± 2	115 ± 1	+35 ± 1
DODAAc	126 ± 1	58 ± 3	110 ± 1	+34 ± 1

[a] X represents the counterion employed, which was bromide (DODAB), chloride (DODAC), or acetate (DODAAc). DODAX concentration was slightly above the one required to cover surface area on PSS particles with one bilayer.

Fusion of Cationic Bilayer Fragments with the Air−water Interface and their Sealing on Particles at Low NaCl Concentrations. At 0.2 mM DODA lipid in the form of BF, over a range of NaCl concentrations (1−50 mM), there was a decrease in surface tension at the air−water interface as a function of time (Figure 4). Possibly at the air−water interface,

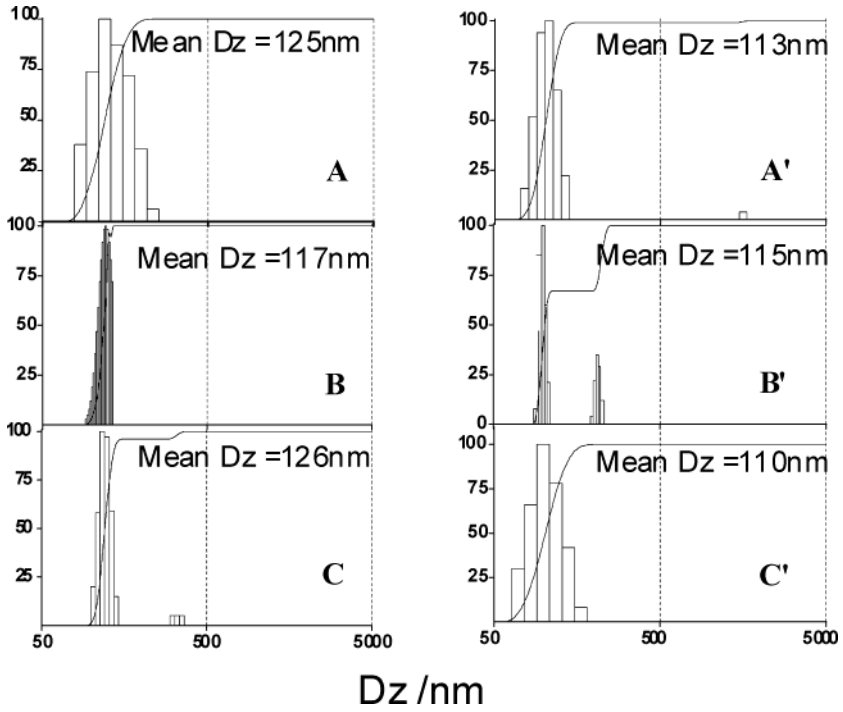


Figure 3. Size distribution for DODA BF/PSS mixtures at 0.04 mM DODA BF and 2×10^{11} PSS particles/mL after 24 h of interaction before (A, B, C) and after adding 0.5 mM NaCl (A′, B′,C′). DODA concentration was slightly above the one required to cover the surface area on PSS particles with one bilayer ($A_b/A_p = 1.5$). DODA counterion was bromide (A), chloride (B), or acetate (C).

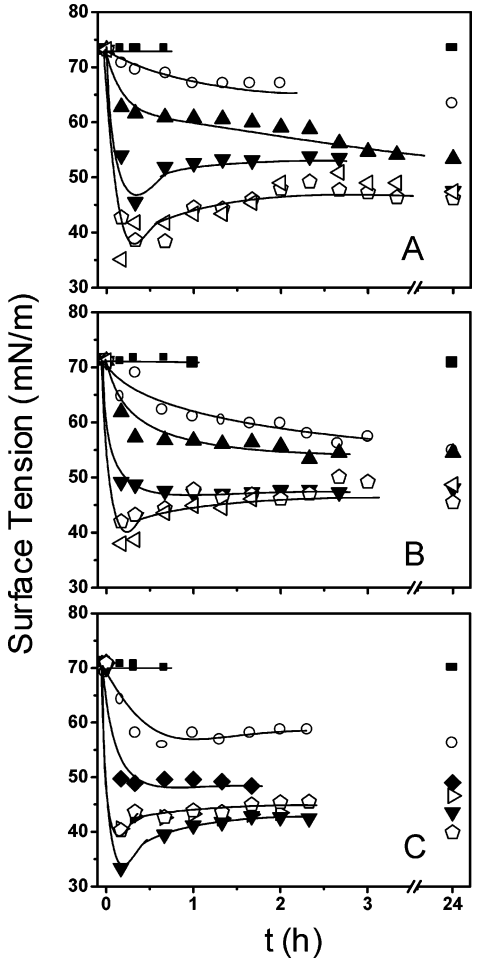


Figure 4. Effect of NaCl concentration and DODA counterion on kinetics of surface tension at the air−water interface at 0.2 mM DODAB (A), DODAC (B), and DODAAc BF (C). NaCl concentration was 0 (■), 1 (○), 2 (▲), 10 (▼), 20 (pentagon), and 50 mM (triangle pointing left).

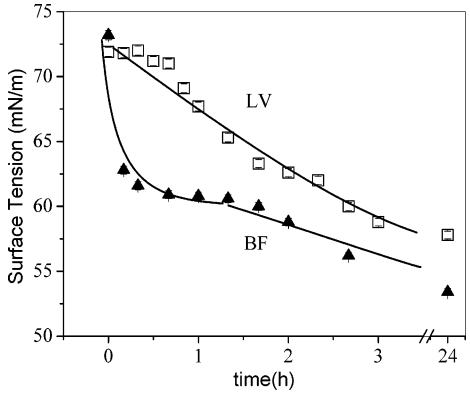


Figure 5. Effect of DODAB dispersion method on surface tension kinetics at the air−water interface. Measurements started upon addition of 2 mM NaCl to 0.2 mM DODAB BF (▲) or LV (□).

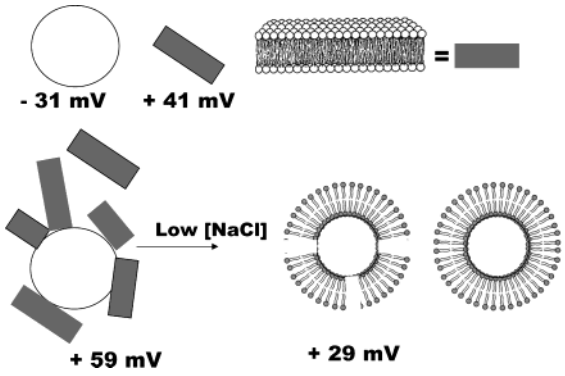
there was fusion of bilayer fragments with formation of a DODA salt monolayer.[21] Another example of the NaCl effect on DODA lipid dispersions was vesicle-vesicle fusion.[32] Eventually, adding NaCl to supported bilayer fragments on particles would also promote fusion between adsorbed and adjacent BF forming a continuous supported bilayer.

At 2 mM NaCl, DODAB BF fusion to the air−water interface was more rapid than that obtained for DODAB LV (Figure 5). Destabilization of extensive hydrophobic regions at BF edges upon salt addition might have been responsible for this result.

Discussion

The large mean sizes for adsorbed bilayer fragments on polymeric particles (Figure 1) could be understood assuming two modes for adsorption of bilayer fragments: (1) upright positioned, hydrophobically driven adsorption; (2) flatly positioned, electrostatically driven adsorption (Scheme 1). Another possibility would be a certain extent of interparticle aggregation where bilayer fragments could behave as bridges between

SCHEME 1: Model for the Interaction between DODA BF and Sulfate Polystyrene Particles in Pure Water and at Low NaCl Concentration[a]



[a] Low [NaCl] would destabilize upright BF adsorption on particles from BF hydrophobic edge, thereby improving flat bilayer coverage and fusion between adjacent bilayer fragments.

particles. However, from the mean diameters for the BF/PSS mixtures the later possibility could be eliminated since sizes were not large enough to indicate colloidal instability such as particle clustering, flocculation, or bridging of particles. There was a clear NaCl effect at small concentrations enhancing surface coverage with flatly adsorbed BF as depicted from the immediate mean size decrease upon NaCl addition (Figure 2). The explanation for this BF rearrangement at the particle surface could be related to the immediate fusion of bilayer fragments with the air−water interface upon addition of 2 mM NaCl, seen as rapid decrease of surface tension with time (Figure 5). In fact, destabilization of hydrophobic edges of adsorbed BF on PSS particles could have promoted removal of upright BF by free BF in the dispersion and sealing of adjacent, flatly adsorbed BF with perfect bilayer coverage upon addition of small amounts of NaCl. Both phenomena would be responsible for lowering mean size and zeta-potential of the DODA-covered particles (Figures 2 and 3, Table 1). The positive zeta-potentials measured for the system after NaCl addition suggested that total BF desorption was not a possibility.

Regarding zeta-potentials, Table 1 showed that values for DODA lipid/PSS samples followed the expected trend for the three counterions, namely, lowest binding at the adsorbed bilayer surface for acetate as counterion yielding the largest zeta-potentials; highest binding for bromide yielding the lowest zeta-potentials.[26] Zeta-potentials were dominated by bilayer properties at the level of different counterion binding (Table 1). Addition of 0.5 mM NaCl to the three different systems apparently normalized zeta-potentials that became the same for the three DODA lipids, ca. +31 mV on particles with mean size at a minimum (ca. 108−122 nm mean diameter). If one considers the bare particle size as 100−115 nm, which are sizes measured from TEM and light-scattering, respectively, in all cases one would have coverage of particles with a 4-nm DODAX bilayer in the presence of 0.5 mM NaCl over the all range of DODA salt concentration added to produce the bilayer covered particles (0.04−1.0 mM DODA).

It is interesting to compare BF adsorption on flat[21] and curved PSS substrates as are the PSS particles employed in this work. A much lower range of NaCl concentration (0.5−5.0 mM NaCl) was required for bilayer coverage on PSS particles (Figure 2; Table 1) than the one needed for bilayer adsorption on flat PSS films, which was around 50 mM NaCl.[21] Possibly, this reflected the preference of the DODA lipid bilayer to deposit on curved surfaces. Due to the DODAX molecular shape of truncated cone, DODAX bilayers would prefer to cover curved surfaces whereas other lipid bilayers formed by cylinder shaped-lipids would prefer to cover flat substrates.[28]

This work provided a recipe for improving bilayer coverage on particles: (1) produce DODAX bilayer fragments in the gel state (BF) by ultrasonically dispersing the lipid(s) with a macrotip; (2) at a lipid concentration equal to or higher than the one required for covering all particles, mix BF with oppositely charged, hydrophobic polymeric particles; (3) allow interaction for 10 min; (4) add a minute amount of NaCl (0.05−5.0 mM) to produce fusion between adjacent, flatly adsorbed BF on particles.

Conclusions

The effect of low NaCl concentration on particle coverage with lipid bilayers from bilayer fragments was surface rearrangement of bilayer fragments, from uneven coverage with some bilayer fragments adsorbed vertically to perfect bilayer coverage upon addition of small amounts of NaCl. This occurred over a broad range of lipid concentration (0.04−1.0 mM DODA), possibly due to NaCl-induced sealing of adjacent bilayer patches adsorbed on particles. At 0.2 mM of lipid, the effect of salt, counterion, and bilayer type (BF or LV) on surface tension at the air−water interface (γ) complemented the evaluation of bilayer behavior at an hydrophobic interface. The γ decay rate and total extent increased with counterion size and hydration, NaCl concentration, and frequency of hydrophobic defects in the lipid bilayer. Upon addition of 2 mM NaCl to 0.2 mM DODAB bilayer fragments or large vesicles, γ decay was much faster for bilayer fragments, showing the importance of hydrophobic defects in the bilayer to induce its fusion to the air−water interface.

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