Characterization of Mixed Monolayers of Phosphatidylcholine and a Dicationic Gemini Surfactant SS-1 with a Langmuir Balance: Effects Of DNA

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ABSTRACT Monolayers of a cationic gemini surfactant, 2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*;*N*-dimethyl-ammonium)butane dibromide (abbreviated as SS-1) and its mixtures with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were studied using a Langmuir balance. More specifically, we measured the force-area (π-*A*) curves and determined the elastic area compressibility modulus (C_s^{-1}) as a function of lateral packing pressure and the mole fraction of the cationic lipid ($X_{SS\text{-}1}$), with and without DNA in the subphase. Both SS-1 and POPC exhibited smooth compression isotherms, indicating their monolayers to be in the liquid expanded state. Even low contents ($X_{SS\text{-}1} < 0.05$) of SS-1 in a POPC monolayer condensed the film dramatically, up to 20% at 30 mN/m. This effect is suggested to reflect reorientation of the P^--N^+ dipole of the POPC headgroup. Accordingly, the magnitude of the condensing effect diminishes with $X_{SS\text{-}1}$ and is not observed for mixed films of dioleoylglycerol and SS-1. Reorientation of the P^--N^+ dipole is further supported by the pronounced increase in monolayer dipole potential ψ due to SS-1. The presence of DNA in the subphase affected the mixed POPC/SS-1 monolayers differently depending on the constituent lipid stoichiometry as well as on the DNA/SS-1 charge ratio. At a DNA concentration of 0.63 μM (in base pairs) condensation of neat POPC monolayers was evident, and this effect remained up to $X_{SS\text{-}1} < 0.5$, corresponding to DNA/SS-1 charge ratio of 1.25. An expansion due to DNA, evident as an increase in ΔA/molecule, was observed at $X_{SS\text{-}1} > 0.5$. At a higher concentration of DNA (1.88 μM base pairs) in the subphase corresponding to DNA/SS-1 charge ratio of 3.75 at $X_{SS\text{-}1} = 0.5$, condensation was observed at all values of $X_{SS\text{-}1}$.

INTRODUCTION

Cationic liposomes represent perhaps the most promising vehicle for use in gene therapy because of the advantages they offer compared with viral vectors. Accordingly, they lack the immunogenicity and biohazards inherent to viruses and allow for introducing larger DNA fragments into target cells (Felgner et al., 1987; Lasic, 1997). Although complexes formed by cationic lipids and DNA have been extensively studied, our understanding of the structure and relevant physicochemical properties yielding maximal transfection efficiencies is, however, still limited (Tang and Szoka, 1998; Zantl et al., 1999; Subramanian et al., 2000). Complex formation by cationic lipids and negatively charged phosphates of DNA is driven by electrostatic attraction (Köiv and Kinnunen, 1994; Kennedy et al., 2000) while also other interactions seem to contribute, as demonstrated using different derivatives of sphingosine, a natural cationic amphiphile (Kõiv et al., 1994). Binding of cationic lipids to DNA causes its condensation and formation of dense structures (Rädler et al., 1997), and several models for the organization of DNA/cationic lipid complexes have been suggested (e.g., Sternberg et al., 1994; Huebner et al., 1999). For transfection, the DNA-cationic lipid complexes must be able to either fuse with the cell membrane or somehow transfer DNA through the membranes into the cell (Felgner and Ringold, 1989; Zabner et al., 1995). Proper lipid phases and packing properties can be anticipated to be important for the above membrane processes (Phillips et al., 1975; Kinnunen and Laggner, 1991).

We have recently initiated a series of experiments on the use of a novel dicationic lipid, 2,3-dimethoxy-1,4-bis(*N*-hexadecyl *N*;*N*-dimethylammonium)butane dibromide, abbreviated as SS-1 (Cerichelli et al., 1996), as a transfection vector (S. J. Ryhänen, V. M. J. Säily, T. Paukku, S. Borocci, G. Mancini, J. M. Holopainen, and P. K. J. Kinnunen, in preparation). In structural studies, the three-dimensional nature of the cationic lipid-DNA aggregates complicates any experimental approach and interpretation of the data. To avoid some of the inherent problems involved in solution studies, we used a Langmuir balance to characterize SS-1 and its binding to DNA. The interactions of interest are thus confined within the two-dimensional monomolecular layer, thus avoiding the mesophasic structural changes that often

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Abbreviations used: *A*, area per molecule; $A_{DNA,}$ area per molecule recorded with DNA in the subphase; C_s^{-1}, elastic modulus of area compressibility; DOG, dioleylglycerol; LE, liquid expanded state; LC, liquid condensed state; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SS-1, 2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*;*N*-dimethyl-ammonium)butane dibromide; X_A, mole fraction of compound A; π, surface pressure; π_{Cs}-1_{max}, surface pressure corresponding to the compressibility modulus maximum; ψ, dipole potential.

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occur in other model biomembrane systems (Brockman, 1999). Moreover, with this method, lipid-lipid interactions in a range of molecular areas known to occur in membrane systems can be investigated in a systemic manner, with force-area (π-A) isotherms and compressibility providing precise indicators of changes in the structure of the film (Brockman, 1999). Examining the interfacial elastic moduli of area compressibility C_s^{-1} as a function of lateral packing pressure (i.e., π vs. C_s^{-1}) further provides a simple approach to determine the onset and completion pressures of possible transitions (Smaby et al., 1996). Further information on the electric properties of the film can be derived from measurement of surface dipole potential ψ (Brockman, 1994).

The in-plane interactions of different SS-1/POPC compositions both with and without DNA in the subphase were compared by measuring π-A and ψ-A curves and calculating π vs. C_s^{-1}. Already low contents of the cationic SS-1 in a POPC monolayer are reflected as dramatic changes in the properties of the film. At $X_{SS\text{-}1} = 0.05$ and 30 mN/m, the POPC film condensed by ~20%, and maximal effect is seen at $X_{SS\text{-}1} = 0.38$. The presence of DNA in the subphase had a condensing effect on POPC monolayers. When SS-1 was contained in the films, the effects of DNA did depend on both $X_{SS\text{-}1}$ as well as on the concentration of the nucleic acid.

MATERIALS AND METHODS

Materials

POPC, DOG, calf thymus DNA, HEPES, and EDTA were from Sigma Chemical Co. (St. Louis, MO). The dicationic gemini surfactant SS-1 (see Fig. 1 for structure) was synthesized as described previously and its purity verified by NMR (Cerichelli et al., 1996). The purity of other lipids was checked by thin-layer chromatography on silicic acid coated plates (Merck, Darmstadt, Germany) using chloroform/methanol/water (65/25/4, v/v) as a solvent system. Examination of the thin-layer chromatography plates after iodine staining revealed no impurities. Lipid concentrations were determined gravimetrically by using a high-precision electrobalance (Cahn, Cerritos, CA). DNA concentrations (in millimolar base pairs) were determined by absorbance at 260 nm (ϵ = 6600 L/mol cm). Freshly deionized filtered water (Milli RO/Milli Q, Millipore, Jaffrey, NH) was used in all experiments.

Monolayer measurements

A computer-controlled Langmuir-type film balance (μThrougS, Kibron, Helsinki, Finland) was used to record compression isotherms (π-A). All glassware used was rinsed thoroughly with ethanol and purified water (Millipore). The indicated lipids were mixed in chloroform and spread in this solvent onto the surface of 14 ml of 5 mM HEPES, 0.1 mM EDTA, pH 7.4 buffer at ~26°C. To ensure complete evaporation of the solvents the films were allowed to settle for 4 min before the recording of π-A isotherms. The monolayers were compressed by two symmetrically approaching barriers at a rate of ~4 Å^2/molecule/min, so as to allow for the reorientation and relaxation of the lipids in the course of the compression. Surface pressure was measured by the Wilhelmy technique with a small-diameter alloy probe in the air/water interface and hanging from a high-sensitivity microbalance (KBN 502, Kibron). Surface pressure π is defined as

$$\pi = \gamma_0 - \gamma,$$

where γ_0 is the surface tension of the air/buffer interface and γ is the value for surface tension in the presence of a lipid monolayer compressed at varying packing densities. When indicated, calf thymus DNA was included in the subphase. The concentration of DNA is given in micromolar base pairs, which allows for a direct comparison of the DNA/SS-1 charge ratios. Monolayer dipole potential ψ (Brockman, 1994) was measured using the vibrating plate method (μSpot, Kibron). All experiments were repeated at least twice.

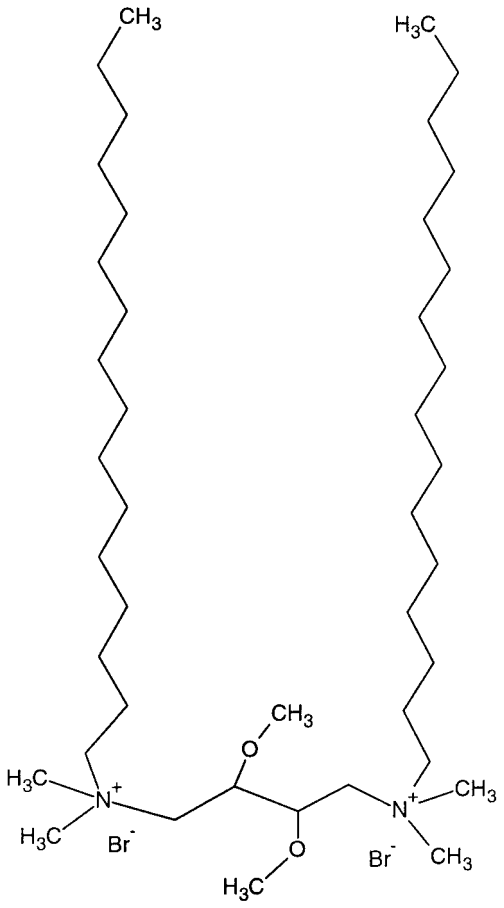

FIGURE 1 Chemical structure of the dicationic lipid SS-1.

Analysis of isotherms

The value for monolayer compressibilities (C_s) for the indicated film compositions at the given surface pressures (π) were obtained from π-A data using

$$C_s = (-1/A_\pi)(dA/d\pi)_\pi,$$

where A_π is the area per molecule at the indicated surface pressure π. To identify the phase transition points we further analyzed our data in terms of the reciprocal isothermal compressibility, i.e., the elastic modulus of area compressibility (C_s^{-1}) as described previously (Smaby et al., 1996). Accordingly, the higher the value for C_s^{-1} the lower was the interfacial elasticity.

RESULTS

Force-area isotherms for SS-1/POPC monolayers

In a parallel series of experiments on the use of SS-1 to deliver plasmid DNA into cultured cells we could show that deviating from most other cationic lipids the efficiency of SS-1 as a transfection vector was enhanced by POPC and did not require the presence of lipids such as diacylglycerol or dioleylphosphoethanolamine, forming inverted phases (S. J. Ryhänen, V. M. J. Säily, T. Paukku, S. Borocci, G. Mancini, J. M. Holopainen, and P. K. J. Kinnunen, in preparation). Accordingly, it was of interest to study the properties of mixed films composed of SS-1 and POPC. The saturated dicationic lipid SS-1 formed stable monolayers at an air/water interface, and its compression isotherms revealed a smooth π-A curve, lacking indications for structural transitions and indicating the film to be in the liquid expanded state, similarly to POPC (Fig. 2). At a limiting mean molecular area of ~52 Å^2 and at a surface pressure of 44 mN/m the SS-1 films collapsed. Subsequently, we measured compression isotherms for the mixed POPC/SS-1 films, varying systematically the monolayer composition. Compression isotherms for POPC/SS-1 monolayers at $X_{SS\text{-}1} < 0.5$ were smooth and revealed no discontinuities indicative of phase transitions. At $X_{SS\text{-}1} = 0.5$, however, a discontinuity in the π-A curve was evident at a surface pressure of 27 mN/m (Fig. 2 *B*). This discontinuity became less prominent at higher contents of SS-1 yet remained at the same lateral pressure. Analysis of the mean molecular areas revealed that already at $X_{SS\text{-}1} = 0.05$ the films were condensed and at 10 mN/m for instance a reduction by ~15% was evident in the mean molecular area, from 92 to 78 Å^2 (Fig. 4 *A*). Maximal condensation was seen at $X_{SS\text{-}1} \approx 0.38$. After this minimum the area/molecule increased with increasing $X_{SS\text{-}1}$ and the monolayers slowly expanded back toward the isotherm of neat SS-1. The condensing effect of SS-1 ($X_{SS\text{-}1} = 0.05$) on POPC monolayers did depend on the phosphocholine headgroup and was absent for the neutral DOG monolayers (Fig. 3). Accordingly, instead of condensation, film expansion was observed, in keeping with additional electrostatic repulsion in the presence of SS-1. Qualitatively similar results were obtained when using dipalmitoylglycerol (data not shown).

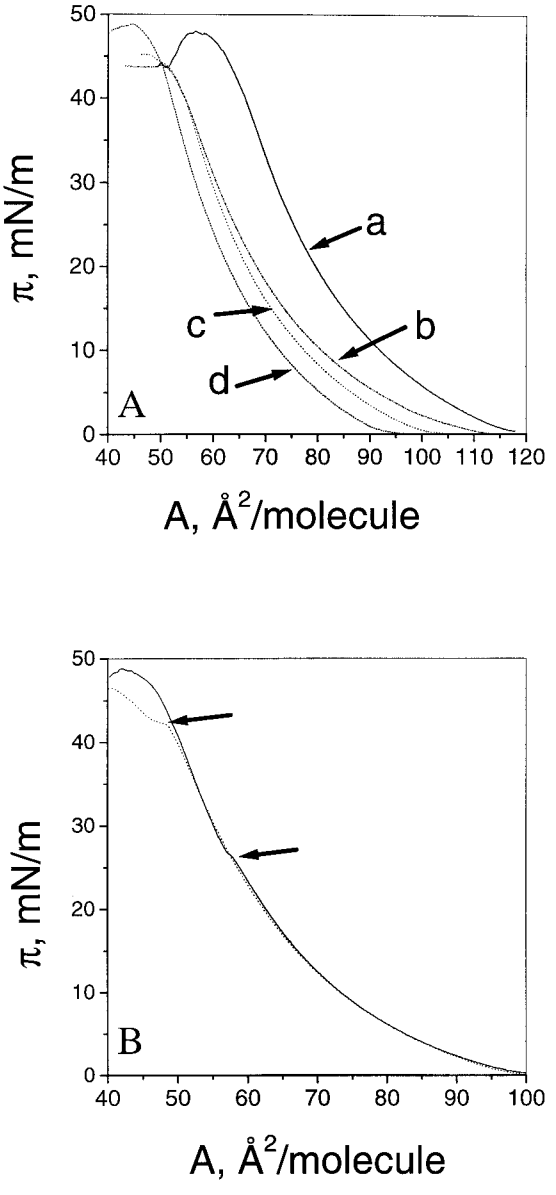

FIGURE 2 (*A*) Representative π-A isotherms for POPC (*a*), SS-1 (*b*), and their mixed monolayers recorded on a subphase of 5 mM HEPES, 0.1 mM EDTA, pH 7.4. The content of SS-1 in the isotherms of the binary films shown was $X_{SS\text{-}1} = 0.05$ (*c*) and $X_{SS\text{-}1} = 0.13$ (*d*). (*B*) Force-area isotherms for binary POPC/SS-1 films at $X_{SS\text{-}1} = 0.5$ (——), revealing a discontinuity (marked by an *arrow*) at ~27 mN/m, and a similar measurement with 0.63 μM DNA (in base pairs), in the subphase, corresponding to DNA/SS-1 charge ratio of 1.25 (·····).

Effects of DNA

As a strongly anionic biopolymer DNA can be readily expected to bind to SS-1-containing cationic films. To observe the effects of DNA on the POPC/SS-1 monolayers we repeated the above compression isotherm measurements but with varying concentrations of DNA included into the subphase. Interestingly, DNA condensed neat POPC monolayers, for example, at 10 mN/m from 92 to 80 Å^2/molecule (Fig. 4). The discontinuity observed in the π-A isotherms at $X_{SS\text{-}1} > 0.5$ became barely distinguishable already in the presence of 0.063 μM DNA (data not shown). A condensing effect due to DNA was evident also in the presence of the gemini surfactant and did depend on the DNA/SS-1 charge ratio. Accordingly, at 0.63 μM DNA, film condensation remained, up to $X_{SS\text{-}1} < 0.5$, corresponding to a DNA/SS-1 charge ratio of ~1.25 (Fig. 4 *B*). At $X_{SS\text{-}1} > 0.5$, expansion was observed. However, when the concentration of DNA was increased to 1.88 μM base pairs, yielding saturation of the charges of SS-1 (DNA/SS-1 charge ratio =

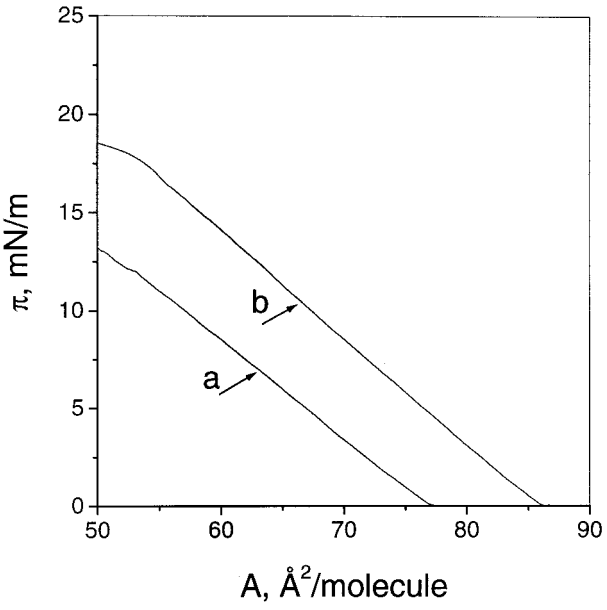

FIGURE 3 Compression isotherms for a DOG monolayer (*curve a*) and a DOG/SS-1 monolayer ($X_{SS\text{-}1} = 0.05$, *curve b*) recorded on a subphase of 5 mM HEPES, 0.1 mM EDTA, pH 7.4. Temperature was ~26°C.

1.88 at $X_{SS\text{-}1} = 1.0$), the films were condensed irrespective of $X_{SS\text{-}1}$ (Fig. 4 *C*). To better illustrate the effects of DNA these data are shown also as the area difference (ΔA/molecule) between isotherms measured in the presence (π vs. A_{DNA}, either 0.63 or 1.88 μM DNA) and absence of DNA in the subphase (π vs. *A*, Fig. 5, *A* and *B*, respectively). At 0.63 μM DNA the relative decrement became more pronounced at higher pressures, being ~14 Å^2/molecule at 40 mN/m. At $X_{SS\text{-}1} > 0.5$, with insufficient DNA to screen the charges of SS-1, the films were expanded, evident as an increase in ΔA/molecule. Although expansion due to DNA was more pronounced at low pressures ($\pi < 10$mN/m) it was measured also at 40 mN/m. Maximum increment was observed between $X_{SS\text{-}1}$ from 0.63 to 0.88. Between these mole fractions of SS-1 the area/molecule increased above the value for a neat POPC film with 0.63 μM DNA in the aqueous phase (Fig. 4 *B*). When $X_{SS\text{-}1}$ was further increased ΔA/molecule continued to increase, reaching its highest value for a neat SS-1 monolayer. A different pattern was evident in the presence of 1.88 μM DNA, yielding saturation of the positive charges of the gemini surfactant also at $X_{SS\text{-}1} = 1.0$. Accordingly, the average values for ($A_{DNA} - A$) remained relatively constant, irrespective of $X_{SS\text{-}1}$.

In the light of the above, it was of interest to express the data also in terms of changes in the area occupied by POPC in the monolayers (Fig. 6). The steep initial decrease observed upon introducing SS-1 to $X_{SS\text{-}1} = 0.05$ was followed by a continuous decrement in ΔA/POPC to $X_{SS\text{-}1} = 0.4$ (Fig. 6 *A*). In the range of $X_{SS\text{-}1}$ from 0.4 to 0.7 the value for ΔA/POPC decreased less, being ~5 Å^2/molecule smaller at 30 mN/m than for a neat POPC monolayer. Upon exceeding $X_{SS\text{-}1} = 0.7$ the value for ΔA/POPC decreased further, reaching its maximum when approaching a neat SS-1 monolayer. In the presence of 0.63 μM DNA in the subphase a decrease of ~2 Å^2 in the ΔA/POPC at 30 mN/m was evident when increasing $X_{SS\text{-}1}$ to 0.1. Thereafter, ΔA/POPC remained nearly constant up to $X_{SS\text{-}1} = 0.3$. In the range of $X_{SS\text{-}1}$ from 0.3 to 0.7 (i.e., charges of SS-1 exceeding those of DNA) a decrease of ~3 Å^2 at 30 mN/m in the area was observed, with a maximum for $X_{SS\text{-}1}$ between 0.6 and 0.7. At $X_{SS\text{-}1} > 0.7$ the reduction in area per POPC decreased almost reciprocally to its increase in the range of $0.3 <$

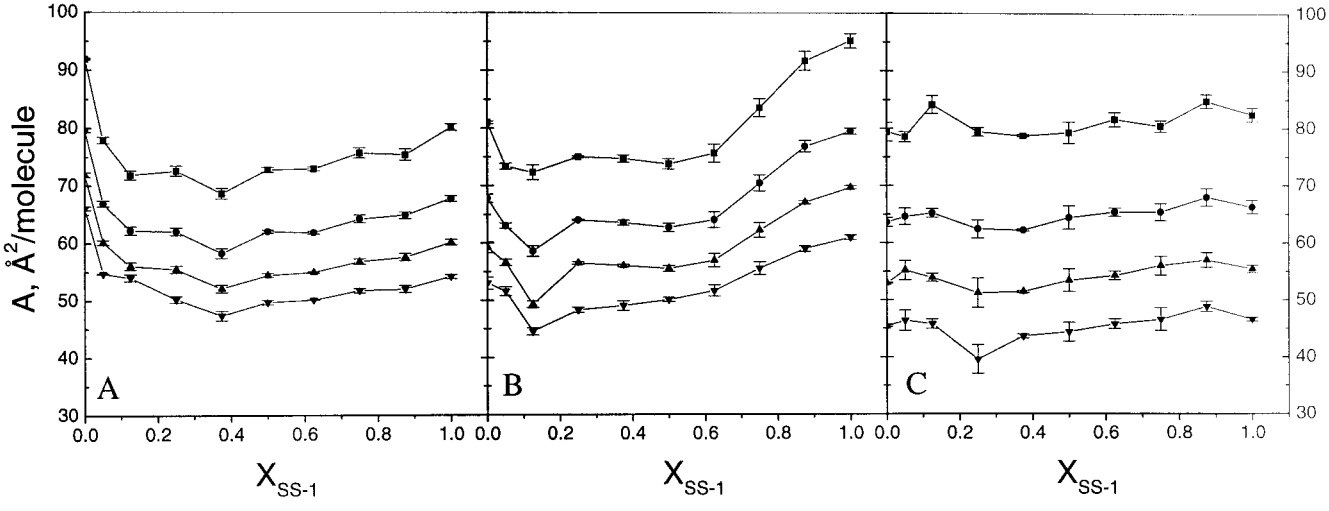

FIGURE 4 (*A*) The effect of increasing $X_{SS\text{-}1}$ on the area/molecule in compression isotherms of mixed POPC/SS-1 films. The values of π were 10 (■), 20 (●), 30 (▲), and 40 (▼) mN/m. The subphase was 5 mM HEPES, 0.1 mM EDTA, pH 7.4. Temperature was ~26°C. (*B* and *C*) Similar data recorded in the presence of 0.63 and 1.88 μM DNA (in base pairs) in the subphase, respectively. In this as well as all subsequent figures the illustrated data points represent the mean from three to five measurements, with the respective error bars.

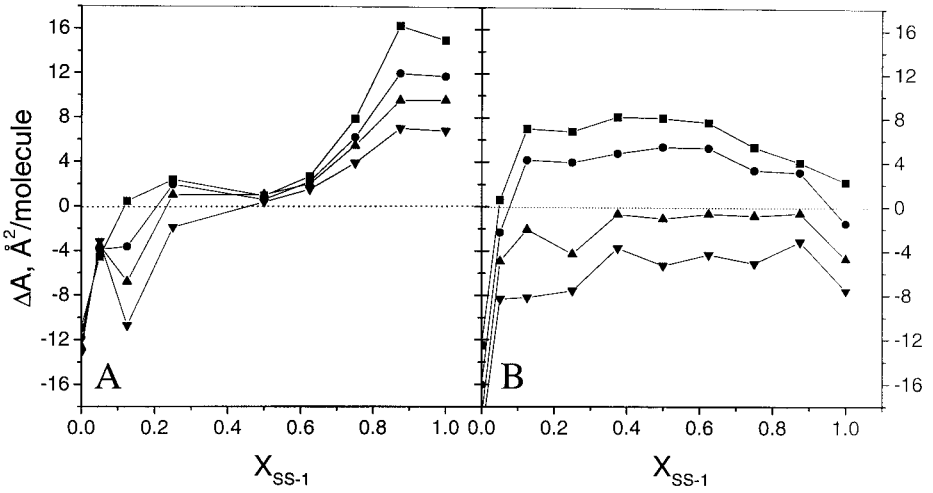

FIGURE 5 The difference in the area $A_{DNA} - A$ (Å^2/molecule) as a function of X_{SS-1}. The values for A_{DNA} were recorded with 0.63 (*A*) or 1.88 μM (*B*) DNA (in base pairs) in the subphase and those for *A* without DNA. The values of π were 10 (■), 20 (●), 30 (▲), and 40 mN/m (▼). The subphase was 5 mM HEPES, 0.1 mM EDTA, pH 7.4. Temperature was ~26°C.

$X_{SS-1} < 0.7$ reaching 2 Å^2 at $X_{SS-1} = 0.9$ and at 30 mN/m. The changes in the ΔA/POPC were more pronounced at low pressures (<10mN/m) but were measured at 40 mN/m as well. With 1.88 μM DNA in the subphase (yielding DNA/SS-1 charge ratio of 1.88 at $X_{SS-1} = 1.0$), only a relatively small decrement at $X_{SS-1} > 0.6$ was observed.

Changes in monolayer dipole potential ψ

Taking into account the highly charged nature of the monolayers containing SS-1 and the binding of the polyanionic DNA to these films, one can readily anticipate alterations in the monolayer dipole potential (Brockman, 1994). These data were recorded as a function of X_{SS-1} and are depicted at varying molecular densities in Fig. 7. The presence of SS-1 had a significant impact on the monolayer dipole potential (Fig. 7 *A*), and already at $X_{SS-1} = 0.05$ a pronounced increase in ψ was evident, at 127 pmol/cm^2 from 240 to 340 mV. This effect of the dicationic gemini surfactant saturated at $X_{SS-1} > 0.5$ with little difference between $X_{SS-1} = 0.5$ and 1.0. In the presence of a charge-saturating concentration of DNA in the subphase (1.88 μM, corresponding to DNA/SS-1 charge ratio of 1.88 at $X_{SS-1} = 1.0$) the cross-increment in ψ upon increasing X_{SS-1} from 0 to 0.5 remained (Fig. 7 *B*). To better illustrate the impact of DNA these data are shown also as a function of X_{SS-1} as the recorded voltage difference $\Delta\Psi$ for the monolayers with

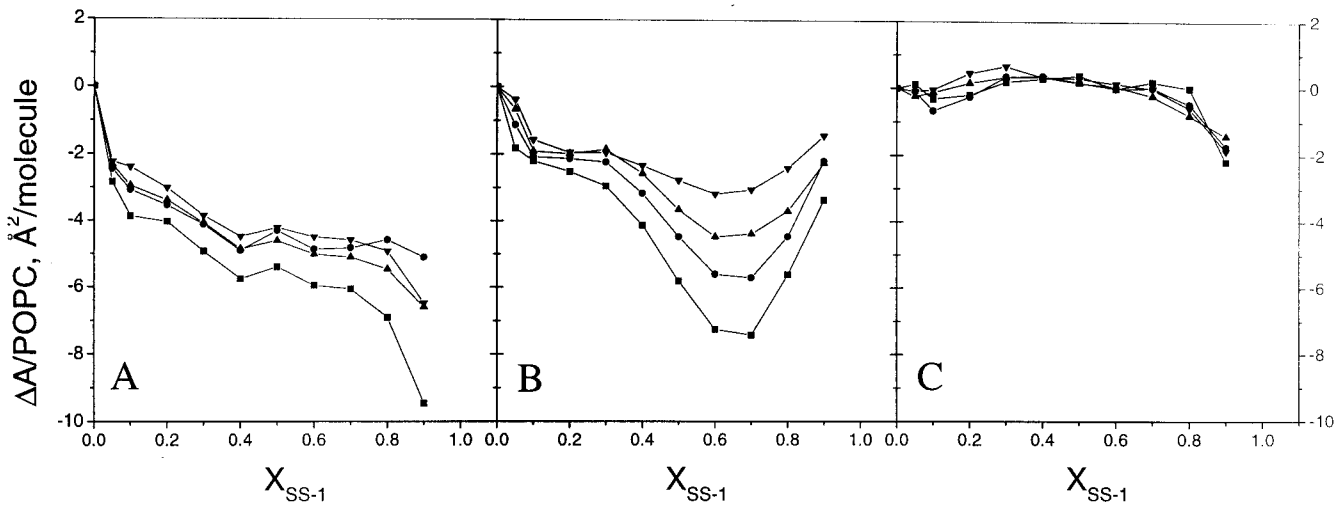

FIGURE 6 (*A*) The condensing effect of SS-1 on POPC monolayers expressed as a reduction in the mean molecular area per POPC as a function of X_{SS-1}. The values of lateral pressure π were 10 (■), 20 (●), 30 (▲), and 40 (▼) mN/m. (*B* and *C*) Similar data recorded in the presence of either 0.63 or 1.88 μM DNA, respectively, in the subphase.

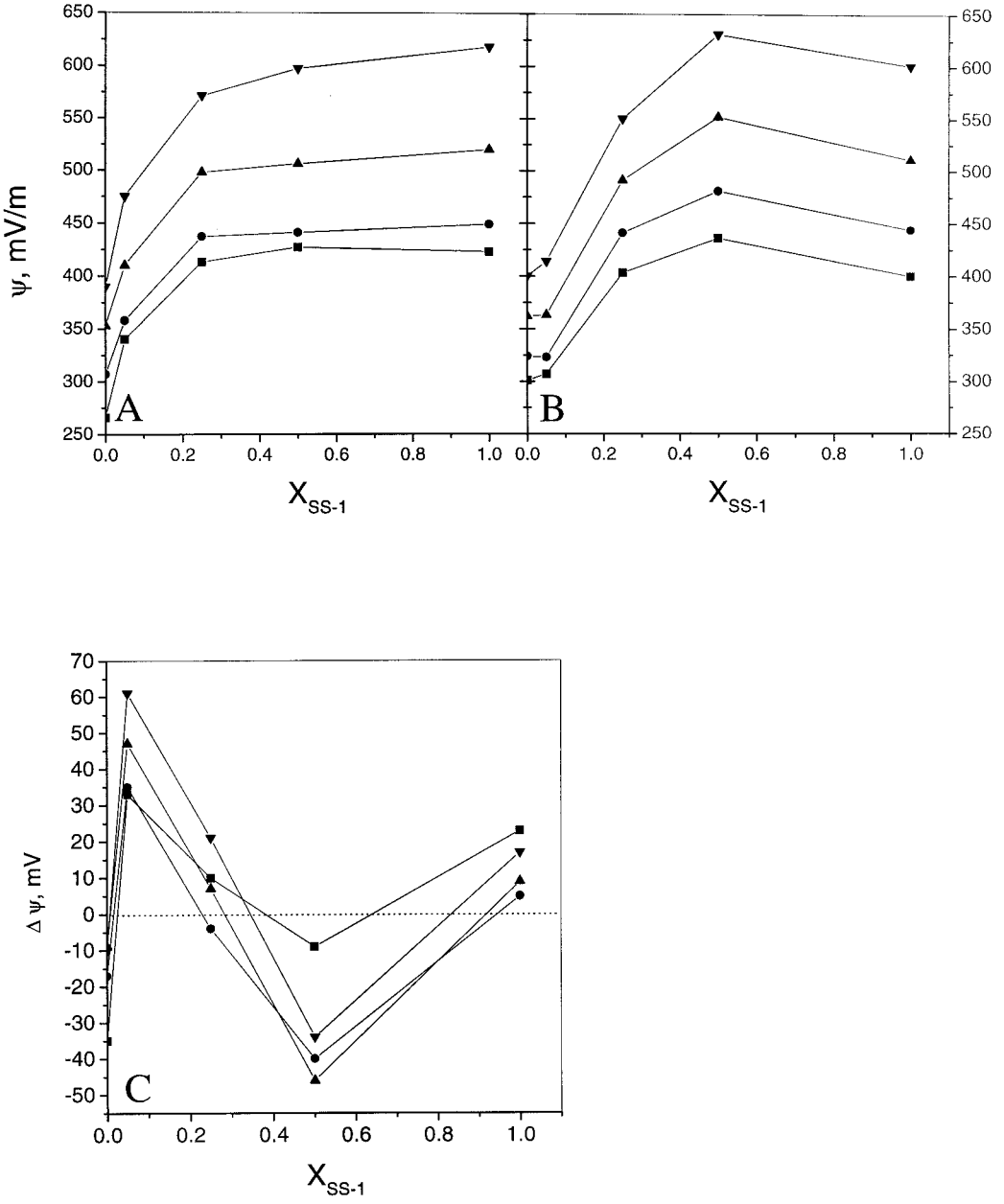

FIGURE 7 Values for monolayer dipole potential derived from compression isotherms for POPC/SS-1 monolayers as a function of X_{SS-1} and recorded both without (*A*) and with (*B*) 1.88 μM DNA in the subphase. The data are shown at varying molecular densities, at 127 (■), 185 (●), 237 (▲), and 332 (▼) pmol/cm^2. Temperature was ~26°C. Also shown is the voltage difference between the above data points, representing the impact of DNA binding to the monolayers (*C*).

and without DNA in the subphase (Fig. 7 *C*). The observed pattern is complex, namely, an initial steep increase until $X_{SS-1} \approx 0.05$ and followed by a decline up to $X_{SS-1} \approx 0.5$. Thereafter, a second increase in $\Delta\Psi$ is evident.

Monolayer compressibility modulus

To gain further insight into the surface properties of the SS-1/POPC monolayers we analyzed the above compression isotherms in terms of the compressibility modulus C_s^{-1} as a function of π and X_{SS-1}. Representative π vs. C_s^{-1} data recorded at $X_{SS-1} = 0.5$ both without and with DNA in the subphase are illustrated in Fig. 8. In the absence of DNA the compressibility modulus C_s^{-1} reveals a decrease by ~30% at 27 mN/m (Fig. 8), corresponding to the discontinuity in the π-*A* isotherm. The film behavior was very different when 0.63 μM DNA was present, and a discontinuity in C_s^{-1} vs. *A* was evident at a pressure slightly above 40

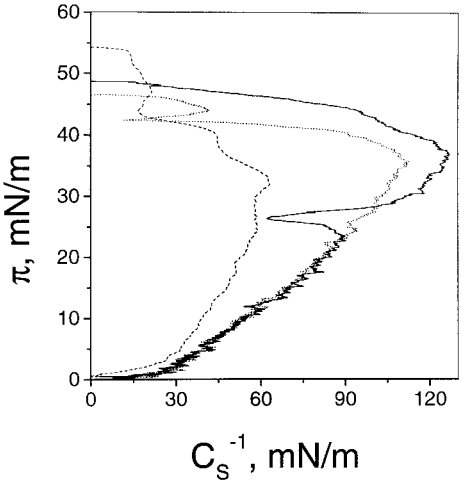

FIGURE 8 π versus elastic moduli C_s^{-1} for monolayers at $X_{SS\text{-}1} = 0.5$ (——) and similar measurements but with either 0.63 (· · ·) or 1.88 μM (– – –) DNA (in base pairs) in the subphase of 5 mM HEPES, 0.1 mM EDTA, pH 7.4.

mN/m, close to the collapse pressure of this monolayer (Fig. 8). At a higher DNA concentration resulting in DNA/SS-1 charge ratio of 1.88 (at $X_{SS\text{-}1} = 1.0$) the values for C_s^{-1} were significantly reduced. The values for the maximum in compressibility modulus ($C_s^{-1}{}_{max}$) as a function of $X_{SS\text{-}1}$ and both with and without DNA in the subphase are depicted in Fig. 9 *A*. Already low contents of SS-1 ($X_{SS\text{-}1} = 0.05$) increased the $C_s^{-1}{}_{max}$ by ~30 and 20 mN/m in the absence and presence of DNA, respectively. Without DNA the highest value for $C_s^{-1}{}_{max} = 130$ mN/m, which was evident at $X_{SS\text{-}1} = 0.63$. Thereafter, with increasing $X_{SS\text{-}1}$ the value of $C_s^{-1}{}_{max}$ diminished (i.e., the elasticity of the film increased) progressively with $X_{SS\text{-}1}$, being ~112 mN/m for the neat cationic lipid. In the presence of 0.63 μM DNA the maximum in C_s^{-1} of ~122 mN/m was seen at $X_{SS\text{-}1} = 0.63$, corresponding to DNA/SS-1 charge ratio of 1.0. At higher values of $X_{SS\text{-}1}$ there was a progressive decrement in $C_s^{-1}{}_{max}$ to ~103 mN/m, reached at $X_{SS\text{-}1} = 1.0$. Under conditions with saturation of the charges of SS-1 by those of DNA (1.88 μM concentration in the subphase) the values for $C_s^{-1}{}_{max}$ were significantly reduced and remained rather constant irrespective of $X_{SS\text{-}1}$.

The values of π corresponding to $C_s^{-1}{}_{max}$ as a function of increasing $X_{SS\text{-}1}$ are illustrated in Fig. 9 *B*. The elasticity minimum of the POPC film shifted to lower pressures when low contents of SS-1 were included in the films, with the lowest value, ~33 mN/m at $X_{SS\text{-}1} = 0.25$. Thereafter, an increment by ~4 mN/m with increasing $X_{SS\text{-}1}$ was observed until after $X_{SS\text{-}1} = 0.75$, when the values for $\pi_{Cs^{-1}max}$ decreased to ~35 mN/m at higher contents of SS-1. When 0.63 μM DNA was included in the subphase the pressure yielding minimum in film elasticity decreased to 34 mN/m with neat POPC and decreased further to ~27 mN/m at $X_{SS\text{-}1} = 0.05$. Thereafter, a progressive increment to 37

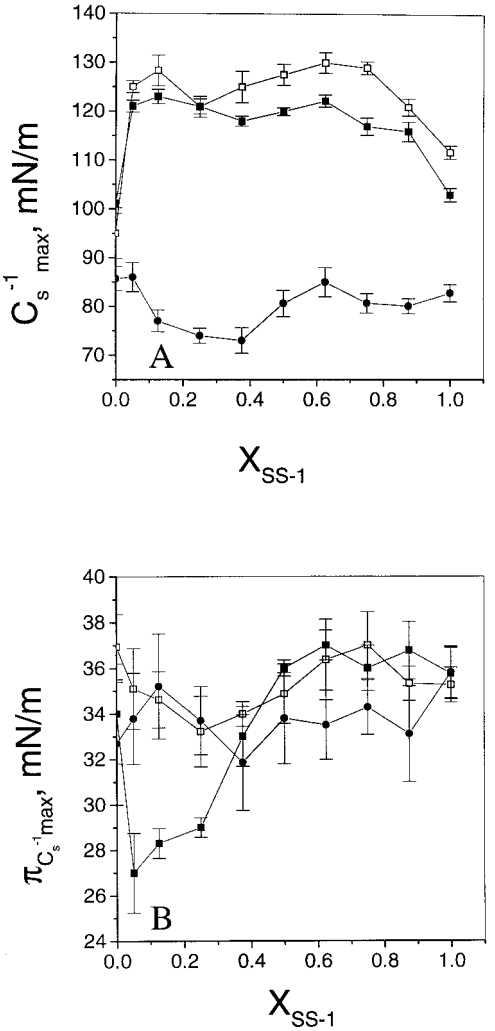

FIGURE 9 (*A*) The dependence of C_s^{-1} on $X_{SS\text{-}1}$ in mixed SS-1/POPC monolayers, recorded in the absence (□) and in the presence of either 0.63 (■) or 1.88 (●) μM DNA (in base pairs) in the subphase (5 mM HEPES, 0.1 mM EDTA, pH 7.4) at ~26°C. (*B*) Surface pressures π corresponding to the compressibility modulus maxima C_s^{-1} measured in the absence (□) and in the presence either 0.63 (●) or 1.88 (●) μM DNA in the subphase.

mN/m at $X_{SS\text{-}1} = 0.63$ was observed. With higher concentration of DNA the values for $\pi_{Cs^{-1}max}$ were closer to those measured for lipids without DNA.

DISCUSSION

Our concomitant studies on SS-1 revealed this cationic lipid to represent a well tolerated and nontoxic transfection vector for the delivery of DNA into cultured cells (S. J. Ryhänen, V. M. J. Säily, T. Paukku, S. Borocci, G. Mancini, J. M. Holopainen, and P. K. J. Kinnunen, in preparation). Moreover, SS-1 exhibited good transfection efficiencies with POPC, not requiring the presence of lipids forming inverted non-lamellar phases. It was therefore of interest to

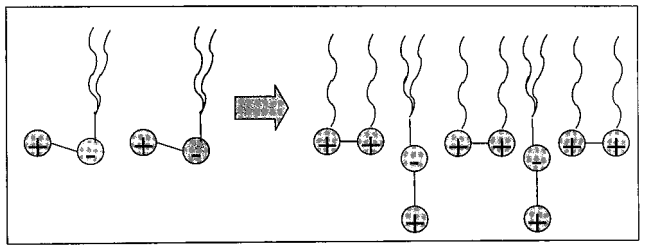

FIGURE 10 A schematic illustration of the postulated change in the orientation of the P^--N^+ headgroup dipole of POPC, induced by SS-1. See Discussion for further details.

characterize the surface properties of SS-1 and its mixed monolayers with POPC.

SS-1 as such formed stable monolayers exhibiting smooth, continuous π-A isotherms, similarly to POPC, indicating the film to be in the in the liquid expanded state. An intriguing finding was that already low contents of SS-1 ($X_{SS\text{-}1} = 0.05$) caused strong condensation of POPC films. This is somewhat unexpected as introduction of the dicationic SS-1 increases the charge density of the monolayers. Condensation was not observed when SS-1 was added into monolayers of either saturated or unsaturated diacylglycerol. Accordingly, this effect is likely to be of electrostatic origin, as further supported by the pronounced effect of SS-1 on dipole potential (Fig. 7). A possible explanation is that SS-1 causes a reorientation of the dipole (P^--N^+) of the phosphocholine moiety. More specifically, upon introduction of the two positive charges with each SS-1 the dipole of the PC headgroup could turn from a parallel to vertical orientation with respect to the plane of the monolayer, so as to maximize the distance between the positive charges of SS-1 and that associated with the choline moiety, as schematically illustrated in Fig. 10. Reorientation of the P^--N^+ dipole of POPC induced by a cationic lipid has been previously observed in NMR studies on liposomes (Scherer and Seelig, 1989). Similar results have been recently obtained by molecular dynamic simulation (Banduopadhyay et al., 1999) and in DSC and Langmuir balance studies (Zantl et al., 1999). Our data further support the notion that the binding of DNA to the monolayer at SS-1/POPC stoichiometries $<$ 1:2 could primarily involve coulombic interaction between the phosphates of DNA and the cationic choline moiety of POPC.

Due to coulombic repulsion the SS-1 molecules in mixed monolayers should adopt a distribution maximizing their distances. Accordingly, we may assume miscibility of POPC and SS-1. The condensing effect of SS-1 first increased up to $X_{SS\text{-}1} = 0.4$ when calculated as reduction in the mean molecular area per POPC (Fig. 6 *A*). This supports the notion that maximal average angle between the P^--N^+ dipole and membrane surface would be achieved at this content of SS-1. Subsequently, in the range of $X_{SS\text{-}1} \sim 0.4-0.7$ the condensing effect remained constant (Fig. 6 *A*), yielding an average of ~5 Å reduction in the surface area of POPC at 30 mN/m, suggesting the reorientation of the dipole to remain unaltered. At $X_{SS\text{-}1} > 0.7$ the behavior of the film should be determined by the charges of the cationic lipid with insufficient screening of the charges of SS-1 by the negative charge of the phosphate moiety of POPC, resulting in film expansion. The above mechanisms underlying the condensing effect of the cationic lipid would also cause augmented chain-chain interactions between the lipids, in keeping with the pronounced reduction in the interfacial elasticity of the film evident already at $X_{SS\text{-}1} = 0.05$ (Fig. 9 *A*). This possibility is further supported by the finding that the elasticity minimum is observed at slightly lower pressures when SS-1 is included in the monolayer (Fig. 9 *B*). The value for C_s^{-1} remains high at increasing $X_{SS\text{-}1}$, until when approaching 1.0, in agreement with enhanced coulombic repulsion between SS-1 molecules. The molecular origin of the discontinuity observed in the π-A curve at $X_{SS\text{-}1} \geq 0.5$ remains uncertain at the moment. One possibility could be that it involves a transition between two lattice structures involving different charge distribution patterns and with minor difference in the interaction potentials involved. A discontinuity is evident also in the π vs. C_s^{-1} isotherms recorded at $X_{SS\text{-}1} \geq 0.5$ (Fig. 8).

Of particular interest are the effects of DNA on the SS-1/POPC films. Intriguingly, the inclusion of DNA into the subphase underneath a POPC monolayer had a significant condensing effect. It is possible that similarly to the condensing effect of SS-1, the strongly anionic polymer associates weakly due to electrostatic interactions to the monolayer, causing the P^--N^+ dipole to reorient with respect to the membrane plane (i.e., from approximately parallel to vertical), decreasing the projected surface area of the lipid in the monolayer. Subsequently, with increasing $X_{SS\text{-}1}$ to 0.5 this condensing effect by 0.63 μM DNA disappears, and upon exceeding $X_{SS\text{-}1} = 0.5$ (exceeding DNA/SS-1 charge ratio of one), an expansion of the film becomes evident. The latter could arise from an efficient electrostatic association of the cationic SS-1 with DNA, which would diminish the average extent of orientation of P^--N^+ dipole of POPC parallel to the plane normal. In addition, because of the persistence length of DNA (reflecting its stiffness), microscopic lateral heterogeneity in the lateral lipid distribution (Kõiv et al., 1994; Subramanian et al., 2000) could arise in the film, evident as film expansion. This mechanism would be compatible with the slight yet consistent decrement in the values of C_s^{-1} when measured with 0.63 μM DNA in the subphase (Fig. 9 *A*). More specifically, in the presence of 0.63 μM DNA and at $X_{SS\text{-}1}$ between 0.05 and $<$0.4 the maximum in C_s^{-1} decreased to lower lateral pressures (Fig. 9 *B*) whereas at $X_{SS\text{-}1} \geq 0.4$ the presence of this concentration of DNA had no effect. The lower values for C_s^{-1} and the condensing effect of SS-1 at $X_{SS\text{-}1} < 0.4$ in the presence of DNA compared with its absence (Fig. 6, *A* and *B*) are in keeping with the assumption that DNA itself can

cause the direction of P^--N^+ dipole to tilt with respect to the membrane plane. Due to the decrement in coulombic repulsion between SS-1 molecules caused by the negative charges of DNA the reduction in the mean molecular area per POPC is enhanced at $X_{SS\text{-}1}$ between 0.4 and 0.7 (Fig. 6 *B*). This mechanism is independent of the angle of the P^--N^+ dipole and so takes place in the range of SS-1 contents where the binding of DNA to the membrane is augmented and the maximal average angle between the P^--N^+ dipole and the membrane is reached. With 0.63 μM DNA in the subphase and at $X_{SS\text{-}1} > 0.7$ the net positive charge of the membrane increases and an expansion of the films is observed. In the presence of a higher DNA concentration (1.88 μM) sufficient to saturate the charges of the gemini surfactant, no expansion is observed.

To conclude, the impact of DNA on the organization and properties of mixed SS-1/POPC monolayers strongly depends on the constituent lipid stoichiometry as well as DNA/SS-1 charge ratio. Both mechanical and electrical properties are influenced by the content of the cationic lipid in the monolayers. Finally, it is interesting to note that the cellular transfection efficiency correlates to the content of SS-1 in POPC liposomes, increasing significantly at $X_{SS\text{-}1} > 0.5$ (S. J. Ryhänen, V. M. J. Säily, T. Paukku, S. Borocci, G. Mancini, J. M. Holopainen, and P. K. J. Kinnunen, in preparation). Although the physical basis underlying the improved transfection at $X_{SS\text{-}1} > 0.5$ remains open at this stage it is intriguing to note that also the behavior of the monolayers below and above this critical value are very different, both with and without DNA. Molecular level understanding of the above processes requires detailed description of electrostatics of the involved highly charged supramolecular systems. As pointed out by Gelbart et al. (2000) these issues are surprisingly complex and still remain elusive, exhibiting somewhat counterintuitive behavior. Finally, it is important to note that calf thymus DNA was used in this study whereas plasmid DNA was utilized in the transfection experiments (S. J. Ryhänen, V. M. J. Säily, T. Paukku, S. Borocci, G. Mancini, J. M. Holopainen, and P. K. J. Kinnunen, in preparation). Although the persistence length of DNA is large, ~500 Å (Bloomfield, 1998), and its interactions with the dicationic lipid reported here are confined to a two-dimensional surface, there may well be differences in the properties of these two types of DNA. Further studies on the characterization of SS-1 are in progress in our laboratory.

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