Investigation of Phospholipid Area Compression Induced by Calcium-Mediated Dextran Sulfate Interaction

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ABSTRACT The association of anionic polyelectrolytes such as dextran sulfate (DS) to zwitterionic phospholipid surfaces via Ca^{2+} bridges results in a perturbation of lipid packing at physiologically relevant Ca^{2+} concentrations. Lipid area compression was investigated in 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) multilamellar bilayer dispersions by ^{2}H-NMR and in monolayer studies. Binding of DS to DMPC surfaces via Ca^{2+} results in denser lipid packing, as indicated by higher lipid chain order. DMPC order parameters are homogeneously increased throughout the lipid bilayer. Higher order translates into more extended hydrocarbon chains and decreased average lipid area per molecule. Area compression is reported as a function of DS concentration and molecular weight. Altering the NaCl and Ca^{2+} concentrations modified electrostatic interactions between DS and phospholipid. A maximal area reduction of $\Delta A = 2.7$ Å^2 per DMPC molecule is observed. The lipid main-phase transition temperature increases upon formation of DMPC/Ca^{2+}/DS-complexes. Lipid area compression after addition of DS and Ca^{2+} to the subphase was also observed in monolayer experiments. A decrease in surface tension of up to 3.5 mN/m at constant molecular area was observed. DS binds to the lipid headgroups by formation of Ca^{2+} bridges without penetrating the hydrophobic region. We suggest that area compression is the result of an attractive electrostatic interaction between neighboring lipid molecules induced by high local Ca^{2+} concentration due to the presence of DS. X-ray diffraction experiments demonstrate that DS binding to apposing bilayers reduces bilayer separation. We speculate that DS binding alters the phase state of low-density lipoproteins that associate with polyelectrolytes of the arterial connective tissue in the early stages of arteriosclerosis.

INTRODUCTION

A key molecular process in the pathogenesis of arteriosclerosis is the association of low-density lipoproteins (LDL) with glycosaminoglycans (GAG) of the arterial connective tissue. The GAG molecules can be exposed to the bloodstream at defects of the endothelium of the arterial walls (Rudel et al., 1986; Camejo et al., 1985; Camejo, 1982). Attractive forces arise from electrostatic interactions between clusters of positively charged amino acids of the protein component of LDL and negatively charged sulfate groups of the GAG molecules (Arnold et al., 1989; Cardin and Weintraub, 1989; Weisgraber and Rall, Jr. 1987; Iverius, 1972). It has been shown that the zwitterionic phospholipids of LDL also contribute to the association by formation of Ca^{2+} bridges to the GAGs (Kim and Nishida, 1979; Srinivasan et al., 1975; Nishida and Cogan, 1970). The Ca^{2+}-mediated interaction between zwitterionic phospholipids and sulfated polyelectrolytes must be a rather strong contribution since pure phosphatidylcholine (PC) liposomes and micelles form large aggregates with GAG at physiological Ca^{2+} concentrations, as found in the extracellular space (2–3 mM) (Steffan et al., 1994; Krumbiegel and Arnold, 1990; Arnold et al., 1990; Kim and Nishida, 1977).

Although the biophysical properties of LDL/Ca^{2+}/GAG and phospholipid/Ca^{2+}/GAG complexes are studied rather well (Arnold, 1995; Krumbiegel and Arnold, 1990; Arnold et al., 1990; Kim and Nishida, 1977, 1979; Gigli et al., 1992; Cardin et al., 1989), little is known about consequences of this association on the lipid packing. Fluorescence measurements revealed lipid surface dehydration upon Ca^{2+}-mediated GAG association (Steffan et al., 1994). Lateral diffusion of phospholipid molecules in the LDL monolayer is restricted by GAG binding (Fenske and Cushley, 1990). In a recent study we described the response of the PC headgroup to Ca^{2+}-mediated adsorption of dextran sulfate (DS) (Huster and Arnold, 1998). ^{2}H-NMR detected a small reorientation of the phospholipid headgroup toward the membrane surface as a consequence of DS binding. Ca^{2+}-mediated DS adsorption to PC surfaces was described as a complex equilibrium between attractive forces, caused by Ca^{2+} bridge formation between lipid headgroups and DS molecules, and repulsive forces between adsorbed DS strands due to electrostatic interactions. These electrostatic forces are screened by higher NaCl concentrations, which results in weaker binding of the polyelectrolyte through fewer Ca^{2+} bridges. However, because the lateral repulsive forces between adsorbed DS strands are also screened, the amount of adsorbed DS to the PC surface is larger at higher NaCl concentration.

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Although there is agreement that GAG binding involves the phospholipid component of LDL, it is unknown how this interaction influences the lipid packing in the phospholipid monolayer as well as in the core of LDL particles. In a DSC study it was established that the core lipids of LDL (cholesterylesters, triglycerides) form a fluid isotropic phase at body temperature. However, after binding to GAG via Ca^{2+} these lipids form a liquid ordered state (Bihari-Varga et al., 1981). The mechanism that leads to this phase transition is not clear at the moment.

In this paper we investigated the influence of Ca^{2+}-mediated GAG binding on phospholipid packing in a model system. We studied the influence of Ca^{2+}-mediated DS adsorption on PC chain packing in both monolayers and bilayers. We suggest that changes in lipid packing could be responsible for the phase transition in the LDL core lipids. Indeed, it was demonstrated that lipid-lipid interaction is sensitive to binding of peptides and proteins in both monolayers and bilayers (Diederich et al., 1996; Maksymiw et al., 1987; Gawrisch et al., 1995; Roux et al., 1989). ^{2}H-NMR on deuterated lipids has been shown to be a very sensitive method for detection of changes in lipid chain packing (Davis, 1983; Seelig, 1977). Chain order parameters can be reproduced with a precision of 0.2% (Holte et al., 1996). From order parameter changes, differences in average area per lipid molecule in the bilayer are estimated with similar precision (Koenig et al., 1997).

Lipid monolayers represent a well-suited system to study the interaction of polyelectrolytes with phospholipid molecules at the lipid/water-interface, because lipid density as well as subphase properties can be easily modified. Penetration of polyelectrolyte into the monolayer is indicated by an increase of the surface tension, while interaction with the surface often reduces the surface tension. Interface and lattice properties, as well as lateral packing of anionic phospholipid monolayers, are strongly influenced by interaction with cationic polyelectrolytes (de Meijere et al., 1997) or model peptides (Johnson et al., 1991).

Here, we report quantitative investigations of area compression in DMPC monolayers and bilayers induced by Ca^{2+}-mediated polyelectrolyte binding. DS was used as a model compound for GAG that is available in a variety of rather well-defined chain lengths. Area condensation was investigated as a function of mono- and divalent cation concentration that modify electrostatic interactions at the membrane surface.

MATERIALS AND METHODS

Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-d_{54}-*sn*-glycero-3-phosphocholine (DMPC-d_{54}) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and used without further purification. Dextran sulfate 500 kDa (DS 500) was purchased from Serva, Feinbiochemica, Heidelberg, Germany; dextran sulfate 40 kDa (DS 40) from ICN Biochemicals (Cleveland, OH), dextran sulfate 8 kDa (DS 8) from Sigma Chemical Co. (St. Louis, MO), and dextran sulfate 1 kDa (DS 1) from Pfeifer and Langen, Dormagen, Germany.

NMR and x-ray sample preparation

For each lipid sample, 10 mg lipid powder was weighed into plastic containers and hydrated in 1 ml buffer (10 or 100 mM NaCl, 10 mM Hepes, pH 7.4, buffer prepared in deuterium-depleted water) containing aliquots of Ca^{2+} added from a 0.1 M $CaCl_2$ stock solution resulting in multilamellar lipid vesicles. Preparations were vortexed, followed by five freeze-thaw cycles to ensure homogeneous ion distribution between the bilayers. DS was added to the suspensions from 100 mg/ml stock solutions. Mixtures were subjected to intense vortexing and 10 additional freeze-thaw cycles to equilibrate phospholipid/DS preparations. The suspensions were centrifuged at 20,000 $\times$ *g*, and the pellet was transferred into 5-mm glass tubes and sealed for NMR measurement or into glass capillaries (1 mm diameter) and sealed for x-ray diffraction.

NMR measurement

^{2}H-NMR spectra were recorded on a Bruker DMX300 NMR spectrometer (Billerica, MA) operating at 46.1 MHz using a high-power probe equipped with a 5-mm solenoid sample coil. Spectra were accumulated by applying a phase-cycled quadrupolar echo pulse sequence (Davis et al., 1976) using 2.1-μs 90° pulses separated by a 50-μs delay, a spectral width of 200 kHz, and a recycle delay of 500 ms. First spectral moments were calculated from the lineshape of the ^{2}H-NMR powder pattern according to

$$M_1 = \frac{\int_0^\infty f(\omega)\omega\, d\omega}{\int_0^\infty f(\omega)\, d\omega} \tag{1}$$

^{31}P-NMR spectra were accumulated at a resonance frequency of 121.5 MHz using a Hahn-echo pulse sequence with a 90° pulse length of 1.6 μs, a 39 μs delay between pulses, a spectral width of 125 kHz, and a relaxation delay of 1 s. Broadband proton decoupling was used during the pulse and acquisition periods. Unless stated otherwise, all NMR measurements were carried out at 37°C.

Order parameter profiles and area per lipid molecule

^{2}H-NMR powder spectra were dePaked (Sternin et al., 1983) using the algorithm of McCabe and Wassall (1995). Smoothed order parameter profiles (Lafleur et al., 1989) were determined from the relation

$$\Delta\nu_Q = \frac{3}{4}\frac{e^2qQ}{h}S(n) \tag{2}$$

with e^2qQ/h being the quadrupolar coupling constant (167 kHz for deuterons in the C—^{2}H bond) and $S(n)$ the chain order parameter for carbon number n. Average order parameters $\langle S\rangle$ are the mean average of all chain methylene group order parameters. The order parameter of the terminal methyl group was replaced by the order parameter of a hypothetical methylene group by extrapolating from the order parameter of nearest neighbor methylenes.

It has been shown that the effective hydrocarbon chain length of a saturated lipid chain is proportional to the average order parameter (Nagle, 1993; Bloom et al., 1991; Seelig and Seelig, 1974). The average projection of the chain length on the bilayer normal $\langle L\rangle$ is calculated from the average order parameter according to

$$\langle L\rangle = l(0.5 + \langle S\rangle). \tag{3}$$

In Eq. 3, l is the length of an all-*trans* chain calculated from $l = m \cdot 1.27$ Å, where m is the number of C—C bonds and 1.27 Å the distance between neighboring carbons in an all-*trans* chain (Bunn, 1939).

Lipid chain volumes were calculated according to

$$V_{\text{Chains}} = 2(V_{CH_3} + xV_{CH_2}) \tag{4}$$

where x is the number of methylene groups in the chain and $V_{CH3} = 54$ Å^3 and $V_{CH2} = 27$ Å^3 (Marsh, 1992). Changes in area per molecule, ΔA, were calculated from differences in average chain lengths ($\langle L_1 \rangle$, $\langle L_2 \rangle$) by

$$\Delta A = \frac{V_{\text{Chains}}}{\langle L_1 \rangle \langle L_2 \rangle} (\langle L_1 \rangle - \langle L_2 \rangle). \quad (5)$$

X-ray diffraction

X-ray diffraction patterns were obtained using a pinhole camera with nickel-filtered CuK(α) radiation (30 mA/40 kV). The intensity was detected with a linear position sensitive detector system (MBraun GmbH, München, Germany). Samples were placed within a gas-tight, home-built diffraction cell, which was thermostated by a Julabo F10 water bath thermostat (Julabo Labortechnik GmbH, Seelbach, Germany). X-ray measurements were carried out at 37°C. The 2θ angle was calibrated in the range of small-angle x-ray scattering (SAXS) using the diffraction pattern of Ag-behenate, which exhibits a repeat distance of $d = 58.376$ Å.

Monolayer studies

Measurements of monolayer surface tension were carried out on a Krüss K12D process tensiometer (KRÜSS GmbH Hamburg, Germany) equipped with a Wilhelmy balance for surface pressure measurements. Aliquots of DMPC in chloroform/hexane were spread on buffer to achieve a surface pressure of 32 mN/m. After the monolayer had equilibrated, indicated by constant surface pressure, DS and Ca^{2+} solutions were added to the subphase using a Hamilton syringe, and the change in film pressure was recorded. Monolayer experiments were carried out at a temperature of 30°C.

RESULTS

Characterization of DMPC/Ca^{2+}/DS 500 complex structure

According to the anisotropy of chemical shift of the ^{31}P-NMR powder spectra, all preparations investigated at 37°C were in a lamellar liquid-crystalline phase (data not shown).

Fig. 1 shows x-ray repeat spacings for DMPC in the presence of 1 mg/ml DS 500 as a function of the Ca^{2+} concentration. Due to the Ca^{2+}-mediated DS binding to DMPC, a drastic decrease in the x-ray repeat spacing is observed yielding similar repeat spacings for all investigated Ca^{2+} concentrations.

Ca^{2+} concentration dependence

In Fig. 2, typical ^{2}H-NMR powder spectra of DMPC-d_{54}/DS 500 mixtures are shown. In the absence of Ca^{2+}, no influence of DS 500 on lipid chain order is detected, as indicated by identical average order parameters of $\langle S \rangle = 0.167$. With increasing Ca^{2+} concentration an increase in the lipid chain quadrupolar splittings is observed, indicating higher lipid chain order as a result of Ca^{2+}-mediated DS binding to DMPC. No changes in the average order parameters (within experimental error (± 0.002)) were observed in the absence of DS 500 at Ca^{2+} concentrations up to 15 mM. Smoothed chain order parameter profiles of the spectra in Fig. 2 are shown in Fig. 3 *A*. To evaluate the order changes along the chain, difference order parameter profiles were plotted (Nezil and Bloom, 1992). Fig. 3 *B* shows the difference between the chain order parameters of the DMPC/DS 500 mixtures in the presence and absence of different Ca^{2+} concentrations. As seen from the plot, there is a rather uniform order increase over the entire chain due to Ca^{2+}-mediated binding of DS to DMPC.

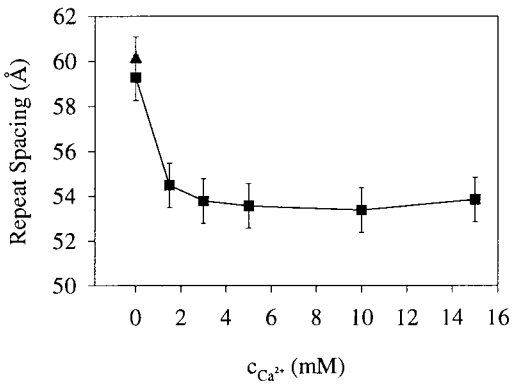

FIGURE 1 X-ray repeat spacings for DMPC multilamellar vesicles in the absence (▲) and presence (■) of 1 mg/ml DS 500 as a function of the Ca^{2+} concentration at 37°C. The buffer concentration was 10 mM NaCl, 10 mM Hepes, pH 7.4.

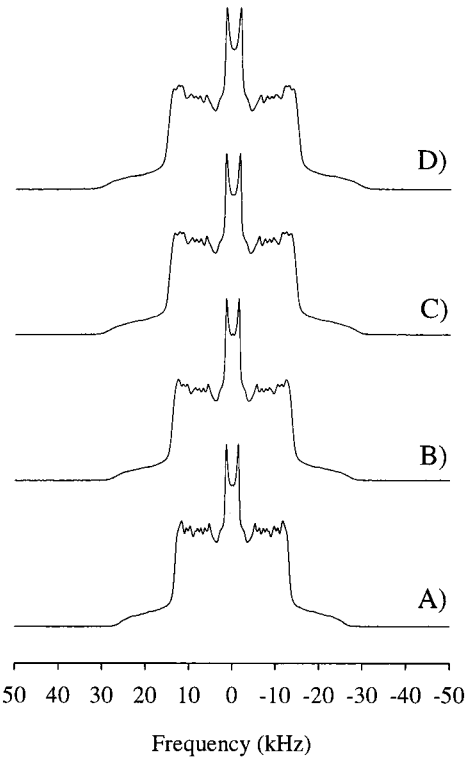

FIGURE 2 Typical series of ^{2}H-NMR spectra of DMPC-d_{54}/DS 500 dispersion at varying Ca^{2+} concentrations recorded at 37°C. (*A*) No Ca^{2+}, (*B*) 1.5 mM Ca^{2+}, (*C*) 3 mM Ca^{2+}, and (*D*) 15 mM Ca^{2+}. The DS concentration was 1 mg/ml, the buffer contained 10 mM NaCl, 10 mM Hepes, pH 7.4. A DS concentration of 1 mg/ml translates into a molar ratio of roughly one sulfate group per two DMPC molecules.

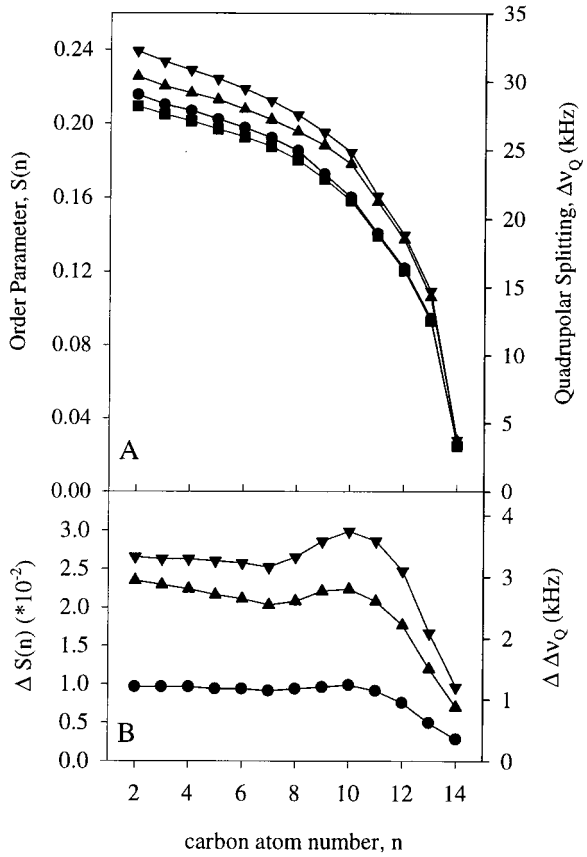

FIGURE 3 (*A*) ^{2}H-NMR order parameter profiles of the spectra of DMPC-d_{54} shown in Fig. 2. The Ca^{2+} concentrations in these samples were 0 mM (■), 1.5 mM (●), 3 mM (▲), and 15 mM (▼) and the DS 500 concentration 1 mg/ml. (*B*) Absolute difference order parameter profiles obtained by subtracting the order parameter profile of DMPC/DS 500 in the absence of Ca^{2+} from those in the presence of various Ca^{2+} concentrations. Difference plots are shown for the spectra of Fig. 2 at 1.5 mM Ca^{2+} (●), 3 mM Ca^{2+} (▲), and 15 mM Ca^{2+} (▼).

In Fig. 4, lipid area reduction in DMPC/DS 500 mixtures and average order parameters are recorded as a function of Ca^{2+} concentration at 10 and 100 mM NaCl. Ca^{2+}-mediated binding of DS 500 to DMPC surfaces increases lipid chain order, which is analogous to a reduction in the average lipid area per molecule (see Eq. 3). Lipid area reduction upon Ca^{2+}-induced DS 500 binding is of larger magnitude at 10 mM NaCl compared to 100 mM NaCl. A rather steep decrease in area per molecule is observed at Ca^{2+} concentrations from 0 to 5 mM, while only small changes are detected at higher Ca^{2+} concentrations.

DS molecular weight dependence

The interaction of DMPC with DS of varying molecular weight was investigated at 3 mM Ca^{2+}, the approximate extracellular concentration of Ca^{2+}. Again, experiments were carried out at two different electrostatic screening lengths that were adjusted by 10 or 100 mM NaCl. Average order parameters and reduction in lipid area per molecule

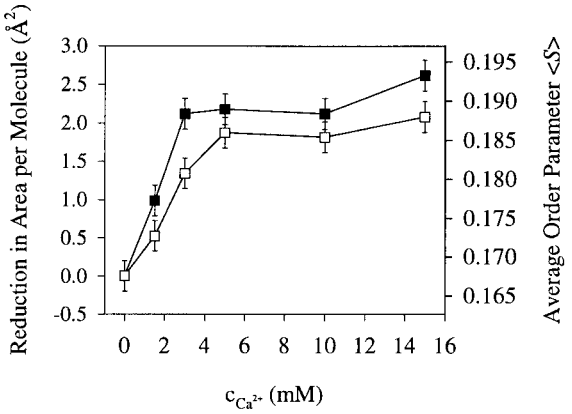

FIGURE 4 Average lipid chain order parameter and reduction of average area per lipid molecule upon Ca^{2+}-induced interaction of DMPC with DS 500 at 10 mM (■) and 100 mM (□) NaCl buffer concentrations as a function of Ca^{2+} concentration calculated from ^{2}H-NMR data.

are shown in Fig. 5. Within experimental error, the short chain DS 1, consisting of only four sulfated glucose subunits, does not induce an area change, suggesting that no interaction with the DMPC surface occurs. DS of longer chain lengths, however, induce a significant reduction in lipid area per molecule starting at molecular weights ≥8000. Almost identical area reduction is observed at 10 mM NaCl for all DS with longer chains. At 100 mM NaCl a moderate area decrease due to DS 8 and approximately uniform area compression of DMPC due to DS 40 and DS 500 is detected.

Surface pressure in DMPC monolayers

Within experimental error, no changes of the surface pressure of pure buffer solution due to DS in the presence or absence of Ca^{2+} were detected suggesting that DS alone is not surface-active at the concentration used in this study. However, substantial changes of DMPC monolayer surface

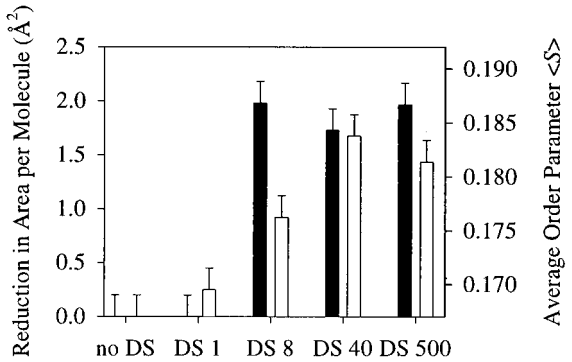

FIGURE 5 Average lipid chain order parameter and reduction of average area per lipid molecule upon Ca^{2+}-induced interaction of DMPC with DS of varying molecular weights. The Ca^{2+} concentration was 3 mM and the DS concentration 1 mg/ml. Experiments were conducted at NaCl buffer concentrations of 10 mM (*filled bars*) and 100 mM (*open bars*).

pressure as a result of Ca^{2+}-mediated interaction with DS 500 were investigated. The initial surface pressure was adjusted to 32 mN/m in all monolayer experiments. In the presence of Ca^{2+}, addition of DS to the subphase leads to a decrease of monolayer surface pressure of several mN/m within a few minutes. Fig. 6 shows the reduction of DMPC surface pressure upon Ca^{2+}-induced DS 500 association as a function of Ca^{2+} concentration. At 10 mM NaCl, a steep decrease of surface pressure is observed, reaching a plateau value at Ca^{2+} concentrations of ~4 mM. A smaller surface pressure decrease is observed at 100 mM NaCl.

Finally, surface pressure changes were recorded for the four DS chain lengths investigated in this study at 3 mM Ca^{2+} and salt concentrations of 10 and 100 mM NaCl. Results are shown in Fig. 7. Within experimental error, no surface pressure reduction is observed for the short DS 1. At 10 mM NaCl, the monolayer is clearly compressed by higher molecular weight DS with increasing magnitude from DS 8 to DS 500. At 100 mM NaCl no change in surface tension is recorded for DS 1 and DS 8, while at higher molecular weight a small reduction in surface pressure is found.

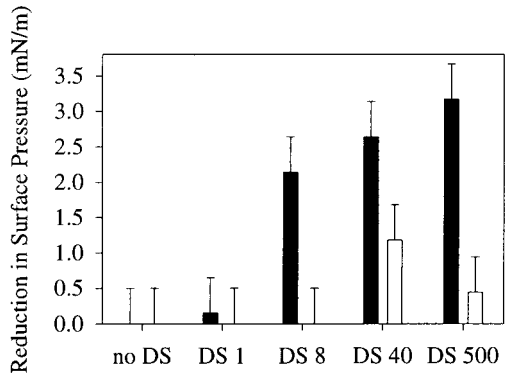

FIGURE 7 Reduction of monolayer surface pressure due to Ca^{2+}-mediated association of DS of various chain lengths to the DMPC monolayer. The subphase concentrations of DS and Ca^{2+} were 1 mg/ml and 3 mM, respectively. Buffer salt concentrations were 10 mM NaCl (*filled bars*) and 100 mM NaCl (*open bars*).

DMPC phase transition

The influence of Ca^{2+}-mediated DS 500 association on the DMPC phase transition was investigated by ^{2}H-NMR. Spectral first moments of ^{2}H-NMR powder spectra of DMPC-d_{54} liposomes in the presence of 15 mM Ca^{2+} (10 mM NaCl) are plotted in Fig. 8. In the absence of DS 500, a rather sharp phase transition of DMPC-d_{54} at ~20.5°C is indicated by a sudden decrease of the first spectral moment. In the presence of 1 mg/ml DS 500, the phase transition is much broader, with a midpoint of 26.7°C. At 5 mg/ml DS 500 the phase transition is narrow again and occurs at ~28.6°C. At these ion and DS 500 concentrations, a large increase of the chain order parameters in the fluid phase is observed ($\langle S \rangle = 0.197$) that corresponds to an area compression of $\Delta A = 2.74$ Å^2 per lipid molecule.

DISCUSSION

Lipid chain order

The interaction of anionic GAGs with zwitterionic phospholipids is the result of formation of Ca^{2+} bridges between sulfate groups of DS and phosphate groups of the zwitterionic phospholipids that only involves the phospholipid headgroup (Fenske and Cushley, 1990; Steffan et al., 1994; Iverius, 1972; Kim and Nishida, 1977). In a previous paper we investigated DS binding to DMPC in the presence of Ca^{2+} (Huster and Arnold, 1998). At 1 mg/ml DS, as in the bilayer experiments of this study, the molar ratio of anionic charge to DMPC is ~1 to 2.3, assuming two sulfate groups per DS monomer. At 10 mM NaCl and 3 mM Ca^{2+}, the molar DMPC/Ca^{2+}/DS monomer ratio is 10:1.7:2.2, and at 15 mM 10:3.3:2.2. Such molar ratios translate into local

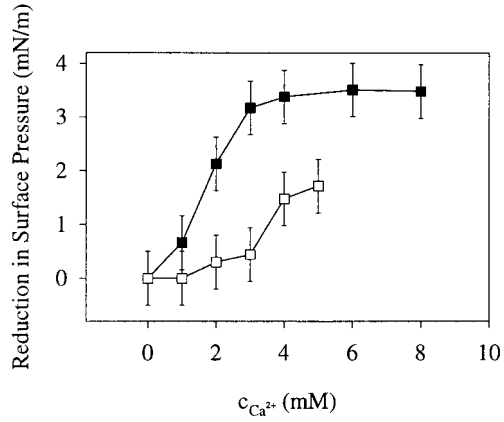

FIGURE 6 Relative decrease in monolayer surface pressure as a function of the Ca^{2+} concentration in the subphase at 30°C. The subphase DS 500 concentration was 1 mg/ml and the salt concentration was 10 mM (■) and 100 mM (□) NaCl.

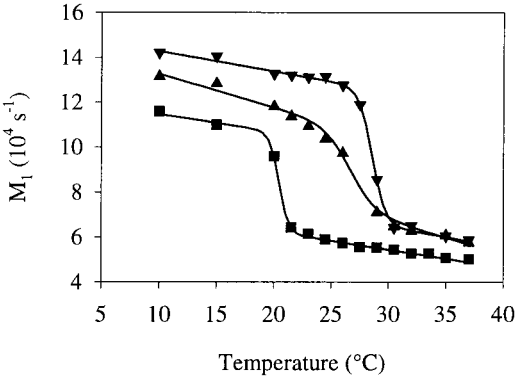

FIGURE 8 Temperature dependence of the ^{2}H-NMR first spectral moments for DMPC/Ca^{2+}/DS 500 complexes for preparations containing no DS 500 (■), 1 mg/ml DS 500 (▲), and 5 mg/ml DS 500 (▼). The Ca^{2+} concentration was 15 mM and buffer salt concentrations 10 mM NaCl.

Ca^{2+} concentrations in the lipid headgroup region of the order of 1 M.

The binding of DS to zwitterionic phospholipid surfaces via Ca^{2+} bridges increases phospholipid chain order (Fig. 3). The magnitude of the ordering increases with Ca^{2+} concentrations (Fig. 4). Clearly, membrane perturbation is a function of the number of Ca^{2+} bridges formed. At 100 mM NaCl, compared to 10 mM NaCl, electrostatic screening of the charges and binding competition between Na^{+} and Ca^{2+} ions result in formation of fewer Ca^{2+} bridges. The increase in chain order is the result of the presence of Ca^{2+} ions and DS. Ca^{2+} ions alone have no measurable effect on lipid chain order up to concentrations of 15 mM. Measurable membrane packing perturbations require Ca^{2+} concentrations higher than 25 mM (Zidovetzki et al., 1989).

DS induced lipid ordering occurs over the entire length of the lipid chains (Fig. 3 *B*). In contrast, a partial penetration of the bilayer by DS would have disordered mostly chain segments in the center of the hydrophobic core. Therefore, a penetration of DS into the upper hydrophobic region near the lipid/water interface is unlikely (Barry and Gawrisch, 1995). The influence of DS binding on lipid packing is similar to previously published data on the influence of binding of cationic polypeptide chains to acidic bilayers (Laroche et al., 1990). For example, binding of polylysine to phosphatidic acid bilayers increased lipid chain order and raised the phospholipid phase transition temperature. From that perspective, the more complicated mechanism of binding the polyelectrolyte via Ca^{2+} bridges is comparable to the purely electrostatic attraction between oppositely charged membrane and polyelectrolyte.

DS of lower molecular weight interact only weakly with PC membranes. This is attributed to binding energy provided by the relatively few Ca^{2+} bridges, which is insufficient to counterbalance repulsion by thermal fluctuation and configurational entropy losses of an aligned polyelectrolyte chain. Therefore, no effect of DS 1 on the order parameters is seen (Fig. 5). Sufficient adsorption energies accumulate, however, for longer chain DS, resulting in strong binding as reflected by increased lipid chain order parameters.

Reduction of lipid area per molecule

Ca^{2+}-mediated binding of DS to DMPC membranes resulted in a decrease of average area per lipid molecule in the bilayer (Figs. 4 and 5). The lipid area is determined by an equilibrium of attractive and repulsive forces between lipid molecules (Marsh, 1996; Evans and Skalak, 1980; Israelachvili et al., 1980). Attractive forces between lipids arise from the hydrophobic effect and the van der Waals interactions between the atoms. Repulsive forces are due to the electric charges of the lipid headgroups, headgroup hydration, and steric repulsion between atoms. Furthermore, at close approach the hydrocarbon chain motional degrees of freedom are severely restricted, which is entropically unfavorable. In equilibrium, the repulsive forces are counterbalanced by attractive interactions and the hydrophobic effect resulting in a tension-free state of the lipid bilayer. The decrease in average area per lipid molecule in response to DS binding via Ca^{2+} could be the result of a reduction in magnitude of the repulsive forces, an increase of the attractive forces, or, most likely, a combination of both.

We have evidence that the presence of DS raises the Ca^{2+} concentration between the lipid bilayers by orders of magnitude (Huster and Arnold, 1998). The increase of Ca^{2+} concentration in the lipid headgroup region due to the presence of the polyelectrolyte can be explained by the Manning condensation theory (Manning, 1978). Binding of Ca^{2+} ions to the lipid phosphate groups occurs, and Ca^{2+} ions may also form bridges between two neighboring phospholipid molecules, which establishes an attractive electrostatic interaction. This attractive force may reduce the area per lipid molecule. A similar effect on the packing of PC bilayers has been observed at high Ca^{2+} concentrations without the presence of DS (Huster et al., 1997; Zidovetzki et al., 1989). Furthermore, the binding of DS to the lipid via Ca^{2+}-induced electrostatic interactions may result in an energetically favorable structural arrangement of phospholipids, calcium, and polyelectrolyte with fast ion exchange between multiple binding sites and formation of Ca^{2+} bridges. Because DS itself has no surface activity and does not penetrate into the hydrophobic region of the bilayer, an influence of DS on the hydrophobic effect is unlikely but cannot entirely be ruled out.

The attractive forces between the lipid molecules, induced by Ca^{2+}-mediated DS binding, stabilize the more tightly packed lipid gel phase, as seen by an increase of the lipid phase transition temperature. As the lateral tension increases, the chain melting is shifted toward higher temperatures. Similar shifts in the phase transition in PC membranes at high Ca^{2+} concentration have been reported (Blatt and Vaz, 1986). Increased lipid phase transition temperatures, due to divalent cation mediated GAG binding, have also been measured by DSC (Voszka et al., 1989; Steffan et al., 1994).

DS bridging between lipid surfaces

In the presence of DS and Ca^{2+}, the x-ray repeat spacing of DMPC bilayers is reduced (Fig. 1). In contrast, the presence of Ca^{2+} alone results in electrostatic repulsion between lipid bilayers that increases the repeat spacing (Lis et al., 1981). The reduction in spacing can be explained by the binding of DS strands to both bilayer surfaces via Coulomb interactions, a process that has been called "bridging of the polyelectrolyte" (Akesson et al., 1989; Podgornik, 1991, 1992) (see Fig. 9). The bridging mechanism provides an attractive force that causes the lipid surfaces to approach each other. The attractive bridging forces counterbalance repulsive hydration, electrostatic, and steric forces.

The x-ray repeat spacing is the sum of bilayer and water layer thickness. The determination of the bilayer thickness

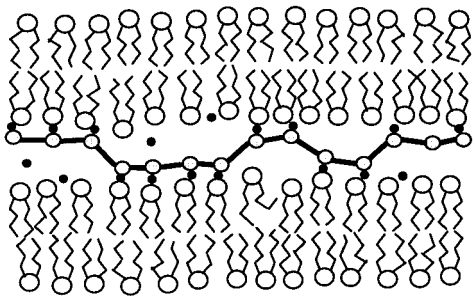

FIGURE 9 DS binds to apposing bilayer surfaces by Coulomb interactions that are mediated by Ca^{2+} ions (●). Due to the attractive bridging pressure of the polyelectrolyte the surfaces approach to close proximity, squeezing out the order of 30% of the water from the space between bilayers. Further approach of the lipid surfaces is prevented by repulsive hydration, electrostatic and steric forces, and the reduction of polyelectrolyte chain configurational entropy.

from the repeat spacings was shown to be model-dependent (Petrache et al., 1998; Lemmich et al., 1999; Janiak et al., 1976). Because lipid chains are extended as a result of DS binding (as revealed by an increase of the lipid chain order parameter, see Eq. 3) the observed reduction in the x-ray repeat spacing must be the result of a changed thickness of the water/DS layer between the lipid bilayers. For example, at 5 mM Ca^{2+} and 1 mg/ml DS 500, the thickness of the water layer is reduced by 6.8 Å. Assuming an average area per DMPC molecule of 59.5 $Å^2$ (Koenig et al., 1997), the reduction in x-ray repeat spacing translates into a loss of ~7 water molecules per lipid.

According to this rough estimate, DS bridging results in a close approach between lipid surfaces squeezing out ~30% of the water molecules from the space between bilayers (assuming that the number of waters per lipid molecule at full hydration is 23 (Arnold and Gawrisch, 1993)). Fluorescence measurements by Steffan et al. (1994) on GAG binding to bilayer surfaces via Ca^{2+} also suggested bilayer dehydration. This raises the question whether lipid dehydration is a primary effect of DS interaction or a secondary result of the presence of strong DS-mediated attractive forces between bilayers. Membrane dehydration also results in increased lipid chain order and decreased area per lipid molecule (Gawrisch and Holte, 1996; Holte and Gawrisch, 1996). We consider the observed dehydration a secondary effect of Ca^{2+}/DS binding to the lipid. Because both DS and Ca^{2+} alone are likely to attract water to the membrane interface because of their high charge density, the gain in free energy from bridging must be large enough to overcompensate the repulsive forces. An even closer approach is prevented by a steep increase in hydration repulsion, but also by a loss in configurational entropy of the polyelectrolyte. This model is supported by preliminary magic angle spinning NMR results that suggest a strong reduction in mobility of lipid-bound DS (unpublished results).

Monolayer surface pressure reduction

The equilibrium of attractive and repulsive forces in a lipid bilayer results in a tension-free state. Lipid monolayers require application of an outside surface pressure for stability. The pressure equivalent to a bilayer was reported to be ~30 to 35 mN/m (Marsh, 1996). Therefore, the monolayer experiments in this study were carried out at a surface pressure of 32 mN/m.

Ca^{2+}-mediated binding of DS to DMPC monolayers reduced the surface tension at constant area (Figs. 6 and 7). This confirms that DS molecules do not penetrate deep into the lipid monolayer; otherwise, an increased monolayer surface pressure would have been observed (de Meijere et al., 1997; Demel et al., 1989). The decrease of surface tension is consistent with the reduction in lipid area per molecule that was calculated from the lipid chain order parameters. Very recent experiments on DS interactions with DPPE monolayers via Ca^{2+} also observed denser lipid packing (de Meijere et al., 1998).

Biological implication

Our findings may be related to modifications of the thermotropic properties of LDL core lipids in the pathogenesis of arteriosclerosis. The denser lipid packing induced by GAG binding to the lipid headgroup increases the pressure inside the lipoprotein particle. Surface tension (τ) and internal isotropic pressure (p) of a colloidal particle are related by the Laplace equation

$$\Delta\tau = \frac{\Delta p r}{2}. \tag{6}$$

An increase of the surface tension by 3.5 mN/m as observed in the monolayer experiments translates into an internal pressure increase of 700 kPa for an LDL particle with a typical radius (r) of 10 nm. This increased internal pressure may be sufficient to shift the phase transition of the core lipids to higher temperature, which may result in crystallization. We speculate that the change in thermotropic properties of the LDL core lipids in the pathogenesis of the arteriosclerosis may be mediated by surface interactions between LDL phospholipids and GAG.

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