Ca^{2+}-Mediated Interaction between Dextran Sulfate and Dimyristoyl-*sn*-Glycero-3-Phosphocholine Surfaces Studied by ^2H Nuclear Magnetic Resonance

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ABSTRACT The binding of dextran sulfates (DSs) with varying chain lengths to phosphatidylcholine multilamellar vesicles was investigated as a function of polyelectrolyte, NaCl, and Ca^{2+} concentration. Attractive forces between negatively charged polyelectrolytes and zwitterionic phospholipids arise from the assembly of calcium bridges. The formation of calcium bridges between the sulfate groups on the dextran sulfate and the phosphate group of the lipid results in increased calcium binding in mixtures of DS and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC). At high NaCl concentration, the plateau adsorption of DS 500 is increased. The strength of dextran sulfate binding to DMPC is reflected in the changes of the ^2H NMR quadrupolar splittings of the headgroup methylenes. Association forces increase with the number of calcium bridges formed. Low-molecular-weight DS does not bind to DMPC surfaces whereas longer-chain DSs strongly influence headgroup structure as a result of strong association. DS binding increases with increasing concentration; however, further association of the polyelectrolyte can be promoted only if negative charges are sufficiently screened. DS binding to lipid bilayers is a complicated balance of calcium bridging and charge screening. From our data we postulate that the structure of the adsorbed layer resembles a lattice of DS strands sandwiched between the bilayer lamellae.

INTRODUCTION

Surfaces of cells, viruses, and lipoproteins are exposed to a large variety of contacts with ions, proteins, and other molecules in the aqueous medium. Such contacts are of both structural and functional importance but may also be related to pathological processes. One example for the latter is the adhesion of plasma low-density lipoproteins (LDLs) to components of the extracellular matrix of the connective tissue of the arterial walls. Contacts between LDLs and connective tissue are possible when the innermost cell layer of the arterial wall is damaged and the glycosaminoglycans (GAGs) of the extracellular matrix are exposed to the bloodstream. The association of LDLs with anionic GAGs leads to the formation of insoluble complexes, which is thought to be the key molecular process in atherogenesis (Camejo, 1982; Camejo et al., 1985; Rudel et al., 1986).

The interaction of LDLs with GAGs has been suggested to result from an electrostatic attraction between negatively charged sulfate groups of the GAGs and clusters of positively charged amino acids on the protein component of the LDLs (Arnold et al., 1989; Cardin and Weintraub, 1989; Iverius, 1972; Weisgraber and Rall, 1987). It was also shown that additional adhesion forces involve the phospholipid component of the LDLs (Kim and Nishida, 1979; Krumbiegel et al., 1990; Nishida and Cogan, 1970; Srinivasan et al., 1975). At millimolar Ca^{2+} concentrations, as found in the extracellular space of tissue, a precipitation of pure phosphatidylcholine (PC) vesicles with GAGs can be achieved resulting in insoluble complexes (Kim and Nishida, 1977). Attractive forces are generated by Ca^{2+} ions that are capable of forming bridges between negatively charged phosphate groups of the phospholipids and the sulfate groups on the GAG molecules (Kim and Nishida, 1977; Srinivasan et al., 1970). In precipitated lysolecithin micelles, a lipid/calcium/dextran sulfate ratio of 2/1/3 was found.

Most of the previous studies on PC/cation/GAG complexes focused on the global biophysical properties of the complexes as a whole (Arnold, 1995; Arnold et al., 1990; Kim and Nishida, 1977). Binding of GAG to PC mediated by calcium ions results in negative zeta potential of increasing magnitude (Arnold et al., 1990; Krumbiegel and Arnold, 1990). The size of the liposome/Ca^{2+}/GAG complexes increases with increasing GAG concentration until a turning point is reached and complexes tend to deaggregate again (Arnold et al., 1990; Kim and Nishida, 1977). Apparently, adhesion forces are not strong enough to counterbalance electrostatic repulsion of negatively charged vesicle surfaces (Arnold et al., 1990). At very high GAG concentrations, vesicles are completely deaggregated again and covered by strands of GAG, which is apparent by the negative zeta potential of the vesicles. It was shown by fluorescence measurements that the cation-induced GAG binding causes a dehydration of the phospholipid surfaces, which might be responsible for the increase of the lipid chain phase transition due to GAG binding (Steffan et al., 1994; Zschörnig et al., submitted for publication).

Only little is known about structural changes of the lipid component of LDLs in response to GAG association and the structure of the adsorbed layer itself. Two NMR studies investigated lipid/calcium/GAG complexes. In a ^{31}P NMR

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study, the authors have detected a reduction of the lateral diffusion rate of phospholipids in the monolayer of the LDLs as a result of GAG binding (Fenske and Cushley, 1990). Line-width analysis of deuterated GAGs in LDL/calcium/GAG complexes revealed restricted motions of associated GAG molecules (Fenske and Cushley, 1988).

In our study, we continue investigations of molecular details of the PC/Ca^{2+}/GAG interaction in multilamellar vesicles (MLVs). The role of the phospholipid headgroup as carrier of electric charge in the adsorption of GAG to lipid surfaces is particularly interesting as this interaction is described to be purely electrostatic. Solid-state ^2H NMR of headgroup deuterated phospholipids is known to be a sensitive method for monitoring surface electrostatics (Scherer and Seelig, 1989; Seelig et al., 1987). Binding of ions and charged molecules to the vesicle surface causes a structural modification of the headgroup dipole (molecular voltmeter) that can be monitored by contradirectional changes in the residual quadrupolar splittings of the two choline headgroup methylenes.

In this paper, the response of the PC headgroup to Ca^{2+}-mediated adsorption of DS is described. Electrostatics were modified by altering the NaCl and Ca^{2+} concentrations. We used DS as a model substance for GAG because of its similar structure, high charge density, and the availability of different chain lengths. Combined with measurements of Ca^{2+} and DS association constants, analysis of the quadrupolar splittings of the lipid headgroup provide information about structural details of the Ca^{2+}-mediated DS association.

MATERIALS AND METHODS

Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine-1,1,2,2-d4 (DMPC-d4) was purchased from Avanti Polar Lipids (Alabaster, AL). The deuterated methylene groups are denoted as α for the P-O-CD_2 group and β for the CD_2-N$(CH_3)_3$ group of the PC headgroup. Lipids were delivered as fluffy powders and used without further purification. Deuterium-depleted water was purchased from Aldrich Chemical Co. (Milwaukee, WI); DS 500 kDa (DS 500) from Serva, Feinbiochemica, Heidelberg, Germany; DS 40 from ICN Biochemicals, Cleveland, OH; DS 8 from Sigma Chemical Co., St. Louis, MO; and DS 1 from Pfeifer and Langen, Dormhagen, Germany.

Sample preparation

For each lipid sample, 10 mg of lipid powder was weighed into plastic containers and hydrated in 1 ml of 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, frozen in liquid nitrogen, and thawed in a water bath at 45°C five times to ensure equal ion distribution between the bilayers. DS was added to the suspensions from 100 mg/ml stock solutions. Mixtures were subjected to intense vortexing and an additional 10 freeze-thaw cycles to equilibrate lipid DS mixtures. The suspensions were centrifuged at 20,000 × *g*, and the pellet was transferred into 7-mm glass tubes and sealed for NMR measurement. The aqueous supernatant was used for chemical analysis as described below.

NMR spectroscopy

^2H NMR spectra were recorded on a Bruker (Rheinstetten, Germany) MSL300 NMR spectrometer operating at 46.1 MHz using a high-power probe equipped with a 7.5-mm solenoid sample coil. Spectra were accumulated using the quadrupole echo pulse sequence (Davis et al., 1976) at a spectrum width of 125 kHz. The 2.8-μs 90° pulses were separated by a 50-μs delay; recycle delay was 150–200 ms. Typically, 20,000–40,000 scans were accumulated for a decent signal-to-noise ratio. A line broadening of 10 Hz was applied before Fourier transformation.

Proton-decoupled ^{31}P NMR spectra were accumulated at a resonance frequency of 121.5 MHz using a single 3.4-μs 90° pulse, a relaxation delay of 1 s, and a sweep width of 50 kHz. According to the anisotropy of chemical shift of the ^{31}P NMR powder spectra, all preparations investigated in this study were in a lamellar liquid-crystalline phase state (data not shown). All NMR measurements were carried out at a temperature of 37°C.

Ca^{2+} and DS binding studies

Both Ca^{2+} and DS binding studies were carried out by measuring the concentration of nonadsorbed calcium and DS in the supernatant of centrifuged lipid suspensions prepared for NMR measurements. Free calcium was measured using a Ca^{2+} electrode, model 97-20 (Orion Research, Beverly, MA). Calcium binding was analyzed empirically by means of a Langmuir adsorption isotherm of the form

$$\frac{X_b}{1 - nX_b} = K_{app} c_{Ca^{2+}}, \quad (1)$$

where X_b is the number of associated calcium ions per lipid or DS monomer (mol/mol) and n the number of lipids or DS monomers bound by one calcium ion (Seelig and Macdonald, 1989). K_{app} denotes the apparent binding constant and $C_{Ca^{2+}}$ the total Ca^{2+} concentration. Electrostatic interactions were not taken into account more specifically.

The concentration of free DS was measured using an assay probing the binding of Alcian Blue to the acidic sulfate group (Whiteman, 1973). The DS-binding isotherm was modeled by

$$\frac{\Gamma}{1 - n\Gamma} = K_{app} c_{DS\,500}, \quad (2)$$

where Γ denotes the number of adsorbed DS molecules per lipid (mol/mol) and n the number of lipids interacting with one DS molecule. Again, no further electrostatics was taken into account, and K_{app} is the apparent binding constant and c_{DS500} the total DS 500 concentration.

RESULTS

Binding studies

We started our investigation studying the Ca^{2+} binding to DMPC, DS 500, and the mixture of DMPC and DS 500. Calcium-binding isotherms are plotted in Fig. 1 (10 mM NaCl in the medium). Experimental data were fitted using Eq. 1, and fit parameters are given in Table 1. Pure DMPC MLVs bind Ca^{2+} rather weakly with an apparent binding constant of $K_{app} = 37.7\ M^{-1}$. More than three lipid molecules are associated with one calcium ion ($n = 3.4$). DS also binds calcium with a small binding constant ($K_{app} = 24.6\ M^{-1}$), but the binding plateau is not reached within the concentration investigated in our study. Approximately three calcium ions are associated with one sulfate group on DS ($n = 0.3$). In the mixture of DMPC and DS 500, the association constant of calcium is 2.4-fold increased

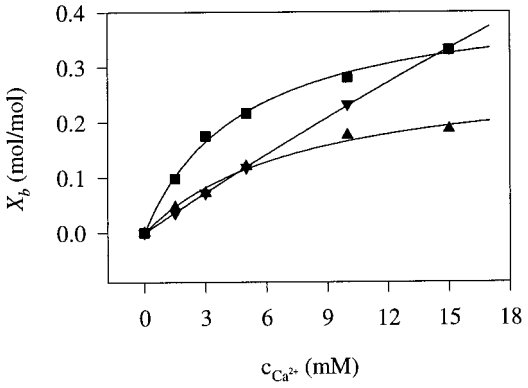

FIGURE 1 Calcium-binding isotherms of pure DMPC bilayers (▲), pure DS 500 molecules (▼), and the mixture of DMPC and DS 500 (■) at 10 mM NaCl. The lines represent fits of Langmuir-type adsorption isotherms according to Eq. 1.

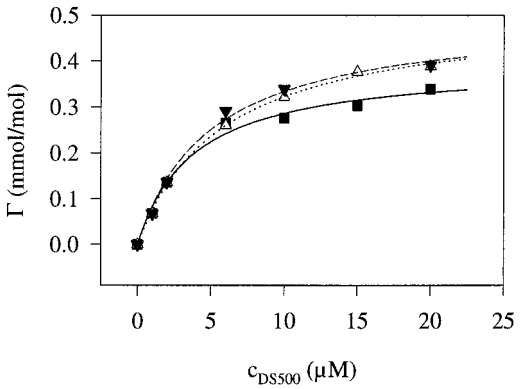

FIGURE 2 Langmuir-type binding isotherms of DS 500 to DMPC surfaces in the presence of 3 mM Ca^{2+}, 10 mM NaCl (■, ——), 3 mM Ca^{2+}, 100 mM NaCl (△, · · ·), and 15 mM Ca^{2+}, 10 mM NaCl (▼, - - -).

(K_{app} = 89.1 M^{-1}). The ratio of charged groups and calcium ions is 2.3.

The association of DS 500 to DMPC bilayers in the presence of Ca^{2+} could be described according to Eq. 2. Fig. 2 shows plots of the experimental binding curves at various salt concentrations. Binding constants of DS 500 are approximately similar for all Ca^{2+} and NaCl concentrations ($K_{app} \approx 90$–$100\ M^{-1}$). However, the binding plateau values are rather different. At 3 mM Ca^{2+}, the amount of DS 500 bound to the DMPC bilayers is much smaller at 10 mM NaCl than at the much higher salt concentration of 100 mM NaCl (solid versus dotted line in Fig. 2). DS 500 binding at low salt concentration can be enhanced by higher Ca^{2+} concentrations resulting in the largest plateau values in Fig. 2 (dashed line). The numbers of lipids associated with one DS 500 molecule varies between 2000 and 2500 (Table 2).

Calcium concentration dependence

We studied the influence of the Ca^{2+} concentration on the lipid-DS association using ^2H solid-state NMR. Fig. 3 shows a series of ^2H powder spectra of DMPC-d4 MLVs in the presence of 1 mg/ml DS 500. The calcium concentration increases from bottom to top in Fig. 3. In the absence of Ca^{2+}, quadrupolar splittings of the α- and β-methylenes are identical, and only one splitting is resolved (Fig. 3 *a*). Additionally, no differences in the quadrupolar splittings of pure DMPC and DMPC in the presence of DS 500 is observed. With increasing concentration of calcium in the preparation, resolved quadrupolar splittings can be detected for the two methylene groups. We assign the larger splitting to the α- and the smaller splitting to the β-methylene group (Roux and Bloom, 1990; Roux et al., 1988, 1989; Ulrich and Watts, 1994). The changes in the size of the quadrupolar splittings are plotted in Fig. 4. Up to a Ca^{2+} concentration of 3 mM, the α splitting increases and eventually reaches a plateau value of approximately 7.5 kHz. The β splitting decreases very steeply also up to 3 mM Ca^{2+} and continues to decrease at higher calcium concentrations at a more shallow slope. These experiments were carried out at a salt concentration of 10 mM NaCl.

DS chain length dependence

The physiological extracellular calcium concentration of 3 mM was chosen to investigate the influence of the dextran sulfate on the lipid headgroup's quadrupolar splittings. In Fig. 5, typical series of ^2H NMR powder spectra of DMPC-d4 are shown for preparations with DSs with varying molecular weights. Spectra were recorded at electrolyte concentrations of 10 mM NaCl (left column) and 100 mM NaCl (right column). In the absence of DS, only one quadrupolar splitting is resolved for DMPC-d4 (Fig. 5 *a*). The addition of the low-molecular-weight DS 1 does not result in significant changes of the powder pattern (Fig. 5 *b*). However, DSs of larger molecular weight strongly interact with lipid surfaces, which is apparent by a drastic change of the spectra and the appearance of two distinct quadrupolar splittings, characteristic for the α- and β-methylenes in the

TABLE 1 Apparent calcium binding to DMPC, DS 500, and DMPC/Ca^{2+}/DS 500 complex

System	K_{app} (M^{-1})	*n**
DMPC	37.7	3.4
DS 500	24.6	0.3
DMPC/calcium/DS 500 complex	89.1	2.3

* *n* denotes the number of the lipid's phosphate and/or DS sulfate groups associated with one Ca^{2+} ion.

TABLE 2 Apparent calcium-mediated DS 500 binding to DMPC liposomes

System	K_{app} (M^{-1})	*n**
3 mM Ca^{2+}, 10 mM NaCl	103.7	2526
3 mM Ca^{2+}, 100 mM NaCl	88.6	1964
15 mM Ca^{2+}, 10 mM NaCl	101.1	1999

* *n* denotes the number of lipids interacting with one DS 500 molecule.

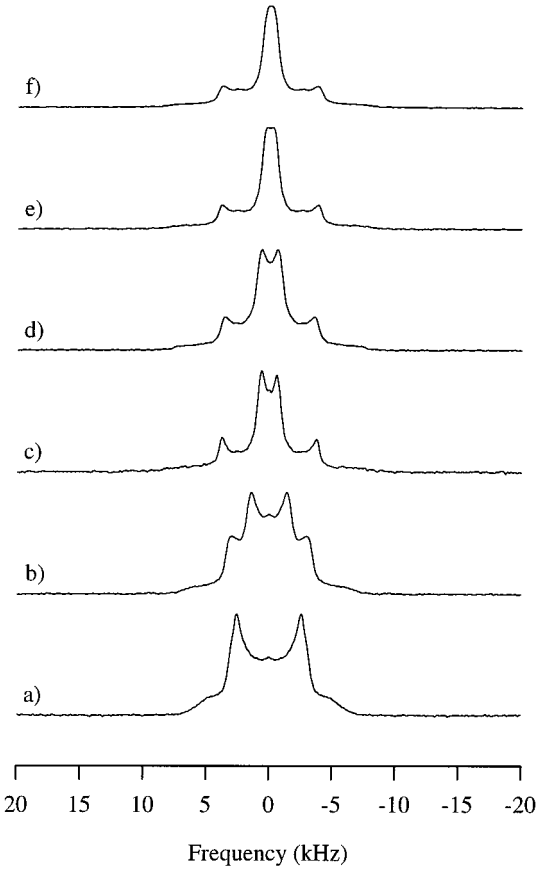

FIGURE 3 ^{2}H NMR spectra of DMPC-d4/Ca^{2+}/DS 500 complexes at 1 mg/ml DS 500 in buffer (10 mM NaCl, 10 mM HEPES, pH 7.4). The Ca^{2+} concentrations of the samples were 0 mM (*a*), 1.5 mM (*b*), 3 mM (*c*), 5 mM (*d*), 10 mM (*e*), and 15 mM (*f*).

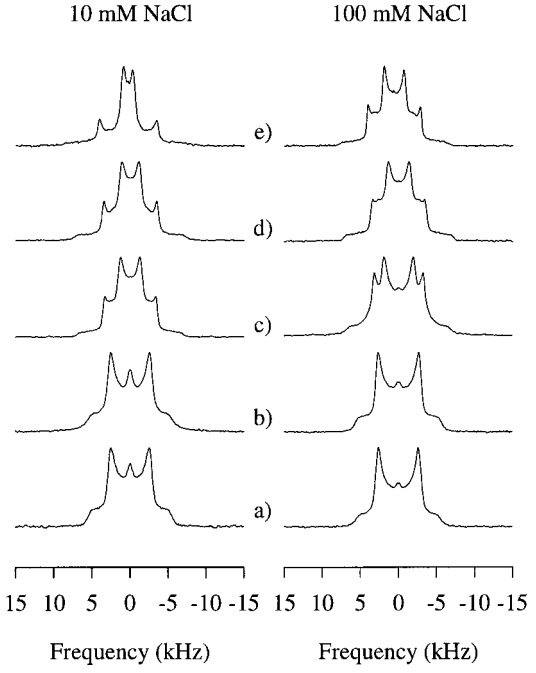

FIGURE 5 ^{2}H NMR powder spectra of DMPC-d4 MLV in the presence of 3 mM Ca^{2+} and dextran sulfates of varying chain lengths at 10 mM (*left column*) and 100 mM (*right column*) NaCl concentration in the buffer at 37°C. Preparations contained no dextran sulfate (*a*), 1 mg/ml DS 1 (*b*), 1 mg/ml DS 8 (*c*), 1 mg/ml DS 40 (*d*), and 1 mg/ml DS 500 (*e*).

PC headgroup (Fig. 5, *c–e*). These effects are qualitatively similar at high and low ionic strength.

The quantitative behavior of the quadrupolar splittings as a function of DS chain length is shown in Fig. 6. No change of the quadrupolar splittings occurs when the short-chain DS 1 is added to the preparation. Quadrupolar splittings increase, however, when longer-chain DSs interact with the lipid surfaces. Due to the interaction of DS 8, DS 40, and DS 500, the α splittings increase by 30, 36, and 50% at low and by 21, 30, and 31% at high ionic strength, respectively. A decrease of the β splittings by 50, 57, and 74% at low and by 28, 48, and 50% at high ionic strength is caused by the interaction of DS 8, DS 40, and DS 500, respectively. It is interesting to note that at high salt concentration both α and β quadrupolar splittings remain constant for DSs of 40 kDa and 500 kDa whereas a further increase and decrease for DS 500 is observed at 10 mM NaCl.

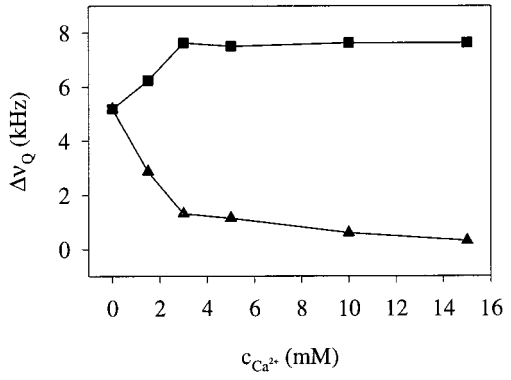

FIGURE 4 Variation of the α (■) and β (▲) quadrupolar splittings of DMPC-d4 as function of Ca^{2+} concentration in 10 mM NaCl buffer. DS 500 concentration was 1 mg/ml.

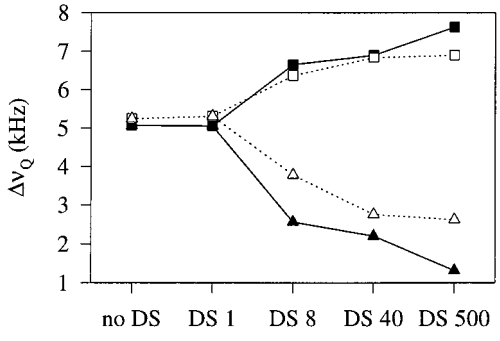

FIGURE 6 Development of α (□ and ■) and β (△ and ▲) quadrupolar splittings at 100 mM (□ and △) and 10 mM NaCl (■ and ▲) in the presence of 3 mM Ca^{2+} as a function of chain length of dextran sulfates present in the preparations.

Calcium-mediated DS 500 binding to DMPC

Fig. 7 displays the variation of the quadrupolar splittings as a function of DS 500 concentration. As clearly seen, the quadrupolar splittings are not directly proportional to the amount of DS added to the suspensions. Higher DS 500 concentrations appear to decrease the influence on the PC headgroups. At 3 mM Ca^{2+} and 10 mM NaCl, addition of DS 500 leads to a very steep increase (decrease) of the α (β) quadrupolar splitting that reaches a maximum at 1 mg/ml DS 500. Additional increases of DS 500 gradually decreases (increases) the α (β) quadrupolar splittings. A similar behavior is observed at 100 mM NaCl and 3 mM Ca^{2+}; however, maximal (minimal) values of the α (β) splittings of DMPC-d4 at 1 mg/ml are smaller (larger) compared with low NaCl concentration. At high concentrations of DS 500, the α quadrupolar splitting is approximately identical at low and high NaCl concentration whereas the β quadrupolar splittings are slightly smaller at high ionic strength. The α-β plot (Seelig et al., 1988) of the DS 500 concentration dependence on the quadrupolar splittings according to

$$\Delta\nu_\beta = -m\Delta\nu_\alpha + n \qquad (3)$$

shows a linear correlation between α and β splittings with a slope of $m = -1.5$ (plot not shown).

A third set of quadrupolar splittings as a function of DS 500 concentration is also shown in Fig. 7. These data points represent DMPC/Ca^{2+}/DS 500 complexes prepared at a Ca^{2+} concentration of 15 mM and a salt concentration of 10 mM NaCl. Both α and β quadrupolar splittings reach more extreme values under these circumstances and the β-methylenes even show a negative quadrupolar splitting at 5 mg/ml DS 500. The revision of the sign of this quadrupolar splitting is a consequence of the counterdirectional changes of the headgroup quadrupolar splittings as a response to changes of its average orientation. If the α splitting is increasing, the β splitting consequently must decrease and can even reverse its sign. As experimentally only absolute values are measured, the assignment of positive or negative signs is arbitrary, although the revision of the sign of $\Delta\nu_\beta$ is an experimental fact (Altenbach and Seelig, 1984). In contrast to the data at 3 mM Ca^{2+}, the α (β) quadrupolar splittings increase (decrease) up to a DS 500 concentration of 5 mg/ml and decrease (increase) beyond this concentration.

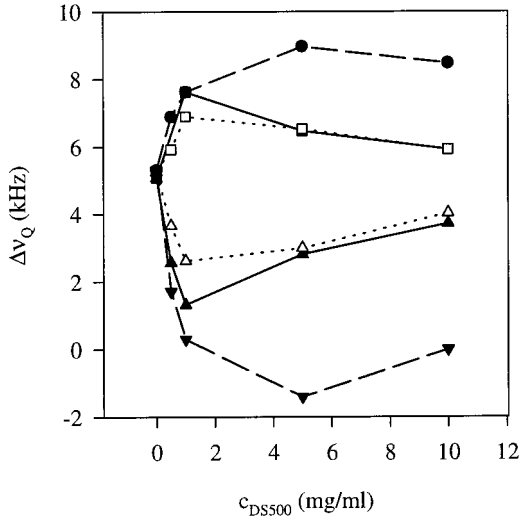

FIGURE 7 Dependence of the quadrupolar splittings on the DS 500 concentration. Data points represent quadrupolar splittings of α (□ and ■) and β (△ and ▲) segments of DMPC-d4 as a function of the DS 500 concentration in the presence of 3 mM Ca^{2+} at NaCl concentrations of 10 mM (■ and ▲) and 100 mM (□ and △). The other data set shows α (●) and β (▼) quadrupolar splittings of DMPC/Ca^{2+}/DS 500 complexes prepared at 15 mM Ca^{2+} and 10 mM NaCl concentrations.

DISCUSSION

Calcium-mediated DS-phospholipid interaction

Calcium is known to bind only weakly to PC membranes (Altenbach and Seelig, 1984; Ohki et al., 1982). We could verify that, in the presence of DS 500, calcium binding of the lipid-DS complex is drastically enhanced as seen by the apparent binding constants as well as in the plateau values of calcium association curves (Fig. 1; Table 1).

As known from microelectrophoresis, the zeta potential of zwitterionic phospholipid vesicles and LDLs turns to strong negative values upon calcium-mediated DS and GAG association (Arnold et al., 1990; Krumbiegel and Arnold, 1990; Krumbiegel et al., 1990). Therefore, we conclude that increased Ca^{2+} binding of DMPC-DS 500 complexes is due to the formation of calcium bridges between the lipid's phosphate groups and the sulfate groups of DS as well as the increased accumulation of calcium in the diffuse double layer at the vesicle-DS plane according to the Gouy-Chapman theory.

Pure DS 500 molecules in dilute aqueous solutions bind calcium with a low apparent binding constant, but within the investigated calcium concentration, no saturation of binding was observed (Fig. 1). The condensation theory developed by Manning (1978) predicts that counterions of the charge Z will condense on the polyelectrolyte as long as the charge density parameter ξ is larger than Z^{-1}. For DS 500 ξ was calculated to be 2.8 (Hlady, 1984), which explains the affinity of calcium to DS by counterion condensation. Condensed counterions compensate a part of the negative charge of the DS molecules (Lerner and Torchia, 1986; Manning, 1978).

According to Seelig and colleagues (Scherer and Seelig, 1989; Seelig et al., 1987), the lipid headgroups are sensitive probes of membrane surface electrostatics. A counterdirectional change of the α and β quadrupolar splittings is characteristic of the response of the PC headgroup to surface charge. In our experiments, we observed an increase of the α and a decrease of the β quadrupolar splittings upon binding of DS (Figs. 4, 6, and 7). According to the molecular voltmeter model, such changes are interpreted as an orientation of the PC dipole toward the membrane surface in response to calcium-mediated association of the anionic DS.

It is conceivable that such a conformational change of the PC headgroup facilitates the assembly of the calcium bridges.

It must be added that the assignment of the quadrupolar splittings of DMPC-d4 is not unambiguous as spectra consist of the superposition of α- and β-methylenes. Our assignment is based on the counterdirectional changes of the quadrupolar splittings induced by negative charge (Marassi and Macdonald, 1992; Roux et al., 1988, 1989; Seelig et al., 1987). The α-β plot of the DS concentration dependence gave slopes of $m = -1.5$, which is typical for negative charge, confirming our assignment (Scherer and Seelig, 1989). In the following we will use the magnitude of changes in the quadrupolar splittings as a measure for the strength of DS binding to PC via calcium bridges.

DS chain length

DSs of low molecular weight (1000) do not or only weakly bind to DMPC surfaces in the presence of calcium concentrations used in our study (Fig. 6). However, DSs of higher molecular weights ($\geq$8000) adsorb to phospholipid membranes and modify their headgroup structure. Obviously, calcium-mediated DS binding to phospholipid surfaces is a highly cooperative effect. The adsorption energy of one binding site (i.e., one calcium bridge) may be small (of the order of kT), but cumulatively for n segments of DS, substantial adsorption energies $n \times kT$ will occur for larger polyelectrolytes (Goddard, 1990). Compared with DS 1, the number of possible DS binding sites increases by a factor of 8, 40, and 500 for DS 8, DS 40, and DS 500, respectively, assuming the same degree of sulfation for all DS species. As seen from the quadrupolar splittings, the longer the DS chain the stronger is the influence on the phospholipid headgroup structure (Fig. 6). In accordance with theoretical considerations, the molecular weight dependence is more pronounced at low ionic strength (Roe, 1973).

Our results are in agreement with biological studies that also indicate that chain length of the polyelectrolyte as well as the degree of sulfation is an important determinant of the extent of GAG binding to LDLs in the presence of divalent cations (Cardin et al., 1989; Gigli et al., 1992). These studies, however, explain GAG binding to LDLs exclusively by GAG-LDL-protein interactions and neglect the contribution of the phospholipid phase in the presence of calcium.

Effect of NaCl on the DS 500 adsorption

As the largest effects were observed for DS 500, it was investigated in more detail. We could show that the influence of DS 500 binding to DMPC surfaces is modified by the presence of both mono- and divalent metal ions. At 3 mM Ca^{2+} and 10 mM NaCl, a much stronger influence on the PC headgroups is revealed by the quadrupolar splittings especially for DS 500 concentrations $\leq$1 mg/ml (Fig. 7). Screening of negative charges by higher Na^+ concentration results in weaker binding of DS as seen by smaller changes of the quadrupolar splittings. Beyond the concentration of 1 mg/ml, a reduction of the salt effect is observed at 3 mM Ca^{2+} and differences in headgroup structures between high and low NaCl are negligible. At 15 mM Ca^{2+}, however, this turning back of the quadrupolar splittings is observed at higher DS 500 concentrations (Fig. 7). The magnitude of the DS association upon the different ion concentrations was explained by different binding affinities of Na^+ and Ca^{2+} (Ohki et al., 1982; Steffan et al., 1994; Zschörnig et al., submitted for publication). As Na^+ is not capable of establishing ion bridges between GAG/DS and PC (Kim and Nishida, 1977), the binding competition of cations to the PC surfaces modifies the strength of DS 500 adsorption. At the largest Ca^{2+} concentration, the most calcium bridges are formed, which results in very strongly influenced PC headgroup conformations as seen by maximal changes of the quadrupolar splittings (Figs. 3 and 4).

The adsorption isotherms of DS 500 to DMPC in the presence of Ca^{2+} are well rounded and not of the high-affinity type (Fig. 2). The apparent binding constants are approximately similar for all Ca^{2+} and salt concentrations used ($K_{app} \approx 90$–$100\ M^{-1}$). Plateau values of DS 500 adsorption, however, are very different. At 3 mM Ca^{2+}, the least DS 500 adsorption is observed at 10 mM NaCl. As higher NaCl concentration screens the repulsive forces between the like charges on the DS molecules and the negatively charged DS/liposome surface as well as the repulsive lateral forces between adsorbed DS molecules, higher plateau adsorption is consequently promoted at 100 mM NaCl. Similarly, high plateau values of adsorbed DS 500 are reached at 15 mM Ca^{2+} (10 mM NaCl). The divalent calcium is much more efficient to screen the negative charges of adsorbed DS than the monovalent sodium.

This consideration may explain the turning back of the quadrupolar splittings upon adsorption of DS 500 in Fig. 7. The binding of DS 500 can be increased only if the negative charges at the surface and between DS segments are screened by positive counterions. If little DS 500 is bound, all available Ca^{2+} ions are used to build calcium bridges. At 3 mM Ca^{2+}, this point is reached at approximately 1 mg/ml DS 500; at 15 mM Ca^{2+}, it is reached at approximately 5 mg/ml. Additional DS can be bound only if electrostatic repulsion between like charges of the DS and between adsorbed strands of the polyelectrolyte are screened. For that reason, we postulate that some calcium bridges are opened again and the released Ca^{2+} may screen the negative charges as well as bind additional DS. Fewer calcium bridges will still provide sufficient adhesion forces to bind the DS to the vesicle surface. However, the influence of fewer calcium bridges on the lipid headgroups is attenuated as seen in the turning back of the quadrupolar splittings in Fig. 7.

Adsorption of the polyelectrolyte on the lipid surfaces

Finally, the question arises how the DS molecules are associated with the lipid surface. In the terminology of polymer adsorption to surfaces adsorption in loops, trains and tails are differentiated. Chemical analysis reveals the stoichiometry of the complex using the apparent binding of both Ca^{2+} and DS 500. Using these data, we find that in the saturated complex 10 phospholipid molecules are associated with approximately 9–10 calcium ions and 7–8 DS monomers in the presence of 15 mM Ca^{2+}. These numbers correlate with a rather high coverage of DS on the vesicle surface. Between 2000 and 2500 phospholipid molecules are involved in the binding of one DS 500 molecule, which roughly represents the number of monomers in a DS 500 molecule (Table 2).

Judging from the influence of polyelectrolyte association on the phospholipid headgroups, it can be assumed that the DS is rather closely attached to the surfaces at low DS concentrations. This would be consistent with an adsorption of the polyelectrolyte in trains. Bridging of the polyelectrolyte between membrane stacks is also likely to occur as the DS coverage is not too high. As binding of DS increases, the influence on the lipid headgroups is decreased, which we explained by breaking of calcium bridges between lipids and DS. This would be more consistent with the formation of some more or less distinct loop and tail structures of DS 500 at the surface. However, as there is only limited space between the lamellae, we assume a rather close attachment of DS to the vesicle surface.

We compare the structure of the DMPC/calcium/DS 500 complexes with aggregates of cationic liposomes with DNA that recently have been characterized (Lasic et al., 1997; Mitrakos and Macdonald, 1996; Rädler et al., 1997). In these complexes, the polyelectrolyte (DNA) is ordered in smectic layers sandwiched between the bilayer lamellae. This structural assembly appears to be applicable for the cation-mediated DS-DMPC interaction. As DS molecules are more flexible than DNA, a somewhat more disordered structure of the polyelectrolyte lattice is expected.

Our model of the calcium-mediated DS adsorption to phospholipid membranes is shown in Fig. 8 in a very simplified cartoon. Many calcium bridges are formed between lipids and sulfate groups of the polyelectrolyte, which align the DS in a train-like structure on the lipid surface. Other parts show loop and tail structures where no calcium bridges are formed between lipid and DS. Free calcium is attracted by the negative surface charge of the lipid/DS surface and thus screens the surface charges.

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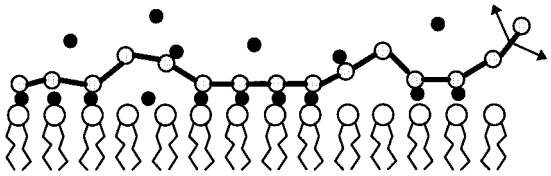

FIGURE 8 Long-chain dextran sulfate binds to zwitterionic lipid surfaces via calcium. Calcium bridges are formed between the anionic phosphate groups of the lipid and the anionic sulfate group of the DS molecules. Depending on the amount of adsorbed DS, the association may occur in train-like, loop-like, and tail-like structures. Free calcium is accumulated near the surface in a diffuse double layer and thus screens the negative charges of the dextran sulfate. Some Ca^{2+} ions are bound to the sulfate group of DS as well as to the phospholipid phosphate group. If one end of the polyelectrolyte desorbs from the lipid surface, it is held back by the other segments that are still associated to lipids via calcium bridges.

REFERENCES

Altenbach, C., and J. Seelig. 1984. Ca^{2+} binding to phosphatidylcholine bilayers as studied by deuterium magnetic resonance: evidence for the formation of a Ca^{2+} complex with two phospholipid molecules. *Biochemistry.* 23:3913–3920.

Arnold, K. 1995. Cation-induced vesicle fusion modulated by polymers and proteins. *In* Structure and Dynamics of Membranes. R. Lipowsky and E. Sackmann, editors. Elsvier Science, Amsterdam. 903–957.

Arnold, K., J. Arnhold, O. Zschœrnig, D. Wiegel, and M. Krumbiegel. 1989. Characterization of chemical modifications of surface properties of low density lipoproteins. *Biomed. Biochim. Acta.* 48:735–742.

Arnold, K., S. Ohki, and M. Krumbiegel. 1990. Interaction of dextran sulfate with phospholipid surfaces and liposome aggregation and fusion. *Chem. Phys. Lipids.* 55:301–307.

Camejo, G. 1982. The interaction of lipids and lipoproteins with the intercellular matrix of arterial tissue: its possible role in atherogenesis. *Adv. Lipid. Res.* 19:1–53.

Camejo, G., A. Lopez, F. Lopez, and J. Quinones. 1985. Interaction of low density lipoproteins with arterial proteoglycans: the role of charge and sialic acid content. *Atherosclerosis.* 55:93–105.

Cardin, A. D., R. L. Jackson, B. Elledge, and D. Feldhake. 1989. Dependence on heparin chain-length of the interaction of heparin with human plasma low density lipoproteins. *Int. J. Biol. Macromol.* 11:59–62.

Cardin, A. D., and H. J. R. Weintraub. 1989. Molecular modeling of protein-glycosaminoglycan interactions. *Arteriosclerosis.* 9:21–32.

Davis, J. H., K. R. Jeffrey, M. Bloom, M. I. Valic, and T. P. Higgs. 1976. Quadrupolar echo deuteron magnetic resonance spectroscopy in ordered hydrocarbon chains. *Chem. Phys. Lett.* 42:390–394.

Fenske, D. B., and R. J. Cushley. 1988. Soluble complex formation between low-density lipoprotein and glycosaminoglycans: a ^{2}H- and ^{31}P-NMR and quasi-elastic light scattering study. *Chem. Phys. Lipids.* 49:15–29.

Fenske, D. B., and R. J. Cushley. 1990. Insoluble complex formation between low density lipoprotein and heparin: a ^{31}P-NMR study. *Chem. Phys. Lipids.* 54:9–16.

Gigli, M., A. Consonni, G. Ghiselli, V. Rizzo, A. Naggi, and G. Torri. 1992. Heparin binding to human plasma low-density lipoproteins: dependence on heparin sulfation degree and chain length. *Biochemistry.* 31:5996–6003.

Goddard, E. D. 1990. Polymer/surfactant interaction. *J. Soc. Cosmet. Chem.* 41:23–49.

Hlady, V. 1984. Adsorption of dextran and dextran sulfate on precipitated calcium oxalate monohydrate. *J. Colloid Interface Sci.* 98:373–384.

Iverius, P. H. 1972. The interaction between human plasma lipoproteins and connective tissue glycosaminoglycans. *J. Biol. Chem.* 247: 2607–2613.

Kim, Y. C., and T. Nishida. 1977. Nature of interaction of dextran sulfate with lecithin dispersions and lysolecithin micelles. *J. Biol. Chem.* 252: 1243–1249.

Kim, Y. C., and T. Nishida. 1979. Nature of the interaction of dextran sulfate with high and low density lipoproteins in the presence of Ca^{2+}. *J. Biol. Chem.* 254:9621–9626.

Krumbiegel, M., and K. Arnold. 1990. Microelectrophoresis studies of the binding of glycosaminoglycans to phosphatidylcholine liposomes. *Chem. Phys. Lipids.* 54:1–7.

Krumbiegel, M., H. Machill, O. Zschornig, D. Wiegel, and K. Arnold. 1990. Microelectric measurements of LDL complexed by PEG and dextran sulfate. *Stud. Biophys.* 136:71–80.

Lasic, D. D., H. H. Strey, M. C. A. Stuart, R. Podgornik, and P. M. Frederik. 1997. The structure of DNA-liposome complexes. *J. Am. Chem. Soc.* 119:832–833.

Lerner, L., and D. A. Torchia. 1986. A multinuclear NMR study of the interactions of cations with proteoglycans, heparin, and Ficoll. *J. Biol. Chem.* 261:12706–12714.

Manning, G. S. 1978. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q. Rev. Biophys.* 11:179–246.

Marassi, F. M., and P. M. Macdonald. 1992. Response of the phosphatidylcholine headgroup to membrane surface charge in ternary mixtures of neutral, cationic, and anionic lipids: a deuterium NMR study. *Biochemistry.* 31:10031–10036.

Mitrakos, P., and P. M. Macdonald. 1996. DNA-induced lateral segregation of cationic amphiphiles in lipid bilayer membranes as detected via ^{2}H NMR. *Biochemistry.* 35:16714–16722.

Nishida, T., and U. Cogan. 1970. Nature of the interaction of dextran sulfate with low density lipoproteins of plasma. *J. Biol. Chem.* 245: 4689–4697.

Ohki, S., N. Düzgünes, and K. Leonards. 1982. Phospholipid vesicle aggregation: effect of monovalent and divalent ions. *Biochemistry.* 21: 2127–2133.

Rädler, J. O., I. Koltover, T. Salditt, and C. R. Safinya. 1997. Structure of DNA-cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes. *Science.* 275: 810–814.

Roe, R.-J. 1973. Multilayer theory of adsorption from a polymer solution. *J. Chem. Phys.* 60:4192–4207.

Roux, M., and M. Bloom. 1990. Ca^{2+}, Mg^{2+}, Li^{2+}, Na^{+}, K^{+} distribution in the headgroup region of binary membranes of phosphatidylcholine and phosphatidylserine as seen by deuterium NMR. *Biochemistry.* 30: 7089.

Roux, M., J.-M. Neumann, M. Bloom, and P. F. Devaux. 1988. ^{2}H and ^{31}P NMR study of pentalysine interaction with headgroup deuterated phosphatidylcholine and phosphatidylserine. *Eur. Biophys. J.* 16:267–273.

Roux, M., J.-M. Neumann, R. S. Hodges, P. F. Devaux, and M. Bloom. 1989. Conformational changes of phospholipid headgroups induced by a cationic integral membrane peptide as seen by deuterium magnetic resonance. *Biochemistry.* 28:2313–2321.

Rudel, L. L., J. S. Parks, F. L. Johnson, and J. Babiak. 1986. Low density lipoproteins in atherosclerosis. *J. Lipid Res.* 27:465–474.

Scherer, P. G., and J. Seelig. 1989. Electric charge effects on phospholipid headgroups: phosphatidylcholine in mixtures with cationic and anionic amphiphiles. *Biochemistry.* 28:7720–7728.

Seelig, A., P. R. Allegrini, and J. Seelig. 1988. Partitioning of local anesthetics into membranes: surface charge effects monitored by the phospholipid head-group. *Biochim. Biophys. Acta.* 939:267–276.

Seelig, A., and P. M. Macdonald. 1989. Binding of a neuropeptide, substance P, to neutral and negatively charged lipids. *Biochemistry.* 28: 2490–2496.

Seelig, J., P. M. Macdonald, and P. G. Scherer. 1987. Phospholipid head groups as sensors of electric charge in membranes. *Biochemistry.* 24: 7535–7541.

Srinivasan, S. R., A. Lopez, B. Radhakrishnamurthy, and G. S. Berenson. 1970. Complexing of serum pre-beta and beta-lipoproteins and acid mucopolysaccharides. *Atherosclerosis.* 12:321–334.

Srinivasan, S. R., B. Radhakrishnamurthy, and G. S. Berenson. 1975. Studies on the interaction of heparin with serum lipoproteins in the presence of Ca^{2+}, Mg^{2+}, and Mn^{2+}. *Arch. Biochem. Biophys.* 170: 334–340.

Steffan, G., S. Wulff, and H. J. Galla. 1994. Divalent cation-dependent interaction of sulfated polysaccharides with phosphatidylcholine and mixed phosphatidylcholine/phosphatidylglycerol liposomes. *Chem. Phys. Lipids.* 74:141–150.

Ulrich, A. S., and A. Watts. 1994. Molecular response of the lipid headgroup to bilayer hydration monitored by ^{2}H-NMR. *Biophys. J.* 66: 1441–1449.

Weisgraber, K. H., and S. C. Rall, Jr. 1987. Human apolipoprotein B-100 heparin-binding sites. *J. Biol. Chem.* 262:11097–11103.

Whiteman, P. 1973. The quantitative measurement of Alcian Blue-glycosaminoglycan complexes. *Biochem. J.* 131:343–350.