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Adsorption of Ca^{2+} and La^{3+} to bilayer membranes: measurement of the adsorption enthalpy and binding constant with titration calorimetry

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The adsorption of Ca^{2+} and La^{3+} ions to the surface of lipid bilayer membranes was studied with high sensitivity titration calorimetry. Ca^{2+} adsorbs to mixed phosphatidylcholine/phosphatidylglycerol membranes with a reaction enthalpy of $\Delta H \approx 0.1$–0.2 kcal/mol. La^{3+} binds to sonified phosphatidylcholine vesicles with a reaction enthalpy of $\Delta H \approx +1.8$ kcal/mol. Adsorption of La^{3+} to phosphatidylcholine bilayers imparts a net positive charge to the membrane surface which makes the binding of further La^{3+} increasingly more difficult. From the decreasing amplitudes in the calorimetric titration experiment a La^{3+} adorption constant of $K \approx (4.1 \pm 1.1) \cdot 10^3$ M^{-1} was evaluated. Electrostatic effects were corrected for by means of the Gouy-Chapman theory. The adsorption constant of Ca^{2+} was determined previously as $K \approx 10$–20 M^{-1} using the same binding model. Since the reaction enthalpies of Ca^{2+} and La^{3+} adsorption are endothermic, the adsorption of both metal ions to the membrane surface is driven by a distinct change in entropy.

Introduction

Biological membranes contain zwitterionic and negatively charged lipids. Metal ions can be adsorbed to the membrane surface, and the adsorption mechanism can be divided formally into two steps. The first is an electrostatic attraction of metal ions to the negatively charged membrane surface, increasing the interfacial cation concentration compared to that in bulk solution. Next, the metal ion enters the plane of binding forming coordination complexes with one or more of the lipid phosphate groups. In the case of phosphatidylserine, the carboxylate group offers a second site of binding **.

Metal ion-phospholipid equilibria have been investigated extensively by measuring the electrophoretic mobility of lipid vesicles [1–4] and by combining NMR with atomic absorption spectroscopy and ultracentrifugation experiments [5–9]. The electrostatic attraction was analyzed using the Gouy-Chapman theory [10–12], while the 'chemical' binding step was described in terms of a Langmuir adsorption isotherm. Under physiological conditions, i.e., using membranes of moderate surface charge density and composed of lipids with *cis*-unsaturated hydrocarbon chains, metal ion binding was found to be rather weak. After correcting for electrostatic effects, intrinsic adsorption constants of $K \sim 1$ M^{-1} for monovalent metal ions and $K \sim 10$–30 M^{-1} for divalent metal ions were determined.

Different results have been observed, however, for highly charged membranes, e.g., membranes composed of pure phosphatidylglycerol, phosphatidylserine, or cardiolipin with fully saturated hydrocarbon chains. While monovalent ions had rather small effects on these membranes, addition of Ca^{2+} induced an extremely complex phase behavior. Mainly four classes of structural changes were triggered by Ca^{2+}: (i) a liquid crystal-gel transition in which the hydrocarbon chains crystallize into the all-*trans* conformation [13–16], (ii) a phase separation of chemically different lipids into microdomains [16–18], (iii) a reorganisation of the lipid

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Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; SUV, small unilamellar vesicles (sonified); LUV, large unilamellar vesicles (extruded).

** The binding of metal ions to individual lipid sites is short-lived with a residence time of τ 10^{-5} s (as measured with deuterium nmr) and it is difficult to distinguish between physical adsorption and chemical binding. The term 'binding' as used in the present context implies both electrostatic attraction to the membrane surface and short-lived chemical binding to individual sites at the membrane surface.

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packing via a bilayer-hexagonal (H_{II}) phase transition [19–20], and (iv) the fusion of juxtaposed lipid bilayers [21,22]. Calorimetric investigations have revealed relatively large exothermic heats of reaction for processes (i) and (iii). However, the reaction enthalpies must be assigned to structural rearrangments of the lipid molecules and are not due to direct metal ion–phospholipid interactions.

In the present study we were interested in the thermodynamic properties of Ca^{2+} and La^{3+} adsorption to membranes in the absence of phase changes. Calorimetric experiments were performed under conditions in which the membranes remained in the liquid crystalline bilayer phase. Using high sensitivity titration calorimetry, $CaCl_2$ and $LaCl_3$ solutions were titrated into suspensions of phospholipid vesicles and the enthalpy of adsorption was measured. Both the lipid composition and the size of the lipid vesicles were varied. The adsorption enthalpies of the metal ions will be compared with the strong exothermic adsorption/binding enthalpies observed for amphiphilic molecules [23–27] and the near zero adsorption enthalpy of highly charged basic peptides [28].

Materials and Methods

Titration calorimetry

Isothermal titration calorimetry was performed using a Microcal OMEGA titration calorimeter [29]. The calorimeter cell (1.2778 ml cell volume) was filled with lipid vesicle suspension in buffer, the reference cell with buffer only. The lipid concentration was of the order of 10–20 mM. The vesicle suspension was titrated with $CaCl_2$ or $LaCl_3$ solutions (5–20 mM, in the same buffer) by stepwise addition of 10 μl. Typically 10 injections were made. The heat of dilution was determined in separate experiments by injecting the $CaCl_2$ or $LaCl_3$ solutions into buffer. All measurements were made at 27°C.

Vesicle preparations

Unilamellar vesicles were prepared by two different methods. In both preparations 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) (Avanti Polar Lipids, Birmingham, AL), were dissolved in chloroform/methanol and were mixed in the desired ratio (total amount of lipid ~30 mg). The solvent was evaporated under a stream of nitrogen and the lipid dried over night in vacuo. 3 ml buffer ($CaCl_2$ was measured in 10 mM Tris and 100 mM NaCl (pH 7.4); $LaCl_3$ was measured in 10 mM Mes and 100 mM NaCl (pH 5.6)) was added to the dry lipid film, and the suspension was vortexed extensively.

To obtain small unilamellar vesicles (SUV) of diameter $d \sim 30$ nm, the lipid dispersion was sonified under a nitrogen atmosphere for 20–50 min (at 10°C) until an almost clear solution was obtained. The solution was centrifuged in an Eppendorf centrifuge to remove metal debris.

Large unilamellar vesicles (LUV) of diameter $d \sim 50$ nm or 100 nm were prepared by extrusion of the multilamellar lipid suspension through a polycarbonate filter [30]. An extruder with a 10-ml barrel from Lipix Biomembranes (Vancouver, BC) was used. The lipid suspension was extruded under nitrogen pressure through two stacked polycarbonate filters (Nucleopore) by stepwise decreasing the pore size from $d = 400$ over $d = 100$ to $d = 50$ nm, extruding three times per filter.

Analysis of titration isotherms

The basic features of the binding model are the same as described for Ca^{2+} binding to mixed POPC/POPG and POPC/cardiolipin membranes [8,9]. The model takes into account both Ca^{2+} (or La^{3+}) and Na^+ binding/adsorption. The multivalent metal ions are assumed to bind to zwitterionic as well as to negatively charged lipids, Na^+ binding is limited to POPG only [25] (in Refs. 8 and 9, *all* lipids were involved in Na^+ binding). Since the metal ions cannot cross the lipid bilayer, ion adsorption occurs only at the outer half-layer. The electric surface charge density, σ, at the outer leaflet of a POPC/POPG vesicle is then given by:

$$\sigma = (e_o / A_L)(- X_{PG}(1 - X_{Na}) + z_i X_i) \quad (1)$$

where e_o is the electronic charge, A_L is the area per lipid molecule ($A_L = 68$ Å^2 for POPC and POPG), and X_{PG} is the mole fraction of negatively charged lipid. X_{Na} denotes the mole fraction of bound Na^+ referred to the total POPG content (X_{Na} = mole Na^+ bound per mole POPG). X_i is the mole fraction of bound metal ion i (Ca^{2+}, La^{3+}) of valence z_i (X_i = mole bound metal ion per mole *total* lipid).

The surface charge density, σ, creates a surface potential, ψ_o, which can be calculated with the Gouy-Chapman theory [9–12]:

$$\sigma^2 = 2000\epsilon_o\epsilon_r RT\sum_i c_{i,eq}[\exp(- z_i F_o \Psi_o / RT) - 1] \quad (2)$$

where ϵ_o is the permittivity of free space, ϵ_r the dielectric constant of water, $C_{i,eq}$ the equilibrium concentration of ion i in the bulk aqueous phase, F_o the Faraday constant, and RT the thermal energy. The summation is over all ions i in solution, including anions.

The membrane surface charge modifies the ion concentrations at the lipid/water interface: ions of opposite charge are accumulated at the membrane surface, ions of like charge are repelled. Knowledge of the surface potential, ψ_o, allows the calculation of the ion

concentration, $C_{i,I}$, of a particular ion i at the lipid/water interface (i.e., immediately above the plane of binding) according to the Boltzmann relation:

$$C_{i,I} = C_{i,eq} \cdot \exp(-z_i F_o \psi_o / RT) \quad (3)$$

The surface concentration, $C_{i,I}$, determines the adsorption/binding to the lipid surface. Na^+ binding to the negatively charged POPG lipid headgroups was described by a Langmuir adsorption isotherm with:

$$X_{Na}/(1 - X_{Na}) = K_{Na} \cdot C_{Na,I} \quad (4)$$

using a Na^+ binding constant of $K_{Na} = 0.6\ M^{-1}$ [1,2,8,9]. Ca^{2+} and La^{3+} bind to neutral as well as charged lipids. Since the degree of binding, X_i, in the present experiments was small ($X_i < 0.1$) the Langmuir adsorption isotherm degenerates into a simple partition equilibrium:

$$X_i = K_i \cdot C_{i,I} \quad (5)$$

Finally, the measured heat of reaction, Δh, is related to the amount of bound metal ion according to:

$$\Delta h = \Delta H \cdot X_i \cdot C_L^o \cdot V_{cell} \quad (6)$$

ΔH is the *molar* heat of binding of the multivalent metal ion, C_L^o is the lipid concentration in the cell (only the outer layer of the lipid vesicles is considered, corresponding to 60% of the total lipid concentration) and V_{cell} is the calorimetric measuring volume.

Results

La^{3+} and Ca^{2+} binding to non-charged lipid vesicles

Fig. 1A shows the titration of sonified lipid vesicles composed of POPC with a 10 mM $LaCl_3$ solution in buffer (10 mM Mes, 0.1 M NaCl, pH 5.6, 27°C, injection volume 10 μl). The adsorption reaction is *endothermic* and decreases gradually with consecutive injections. The narrow calorimetric traces in Fig. 1 provide evidence that the La^{3+} adsorption equilibrium is readily established and that secondary reactions such as a fusion of juxtaposed vesicles do not occur. The experimentally observed heat of reaction is assigned exclusively to the adsorption of La^{3+}. The cumulative heat of reaction, as evaluated from the areas underneath the titration peaks, is displayed in Fig. 1B. The lipid concentration in the calorimeter cell was 18.3 mM, but only the outer surface of the SUVs ($\sim$ 60% of total lipid) was available for metal ion binding. The total La^{3+} concentration (free plus bound La^{3+}) increased from $\sim$ 80 μM after the first injection to $\sim$ 800 μM at the end of the titration, i.e., the lipid remained always much in excess of La^{3+}. The enthalpy decrease with consecutive injections can be explained

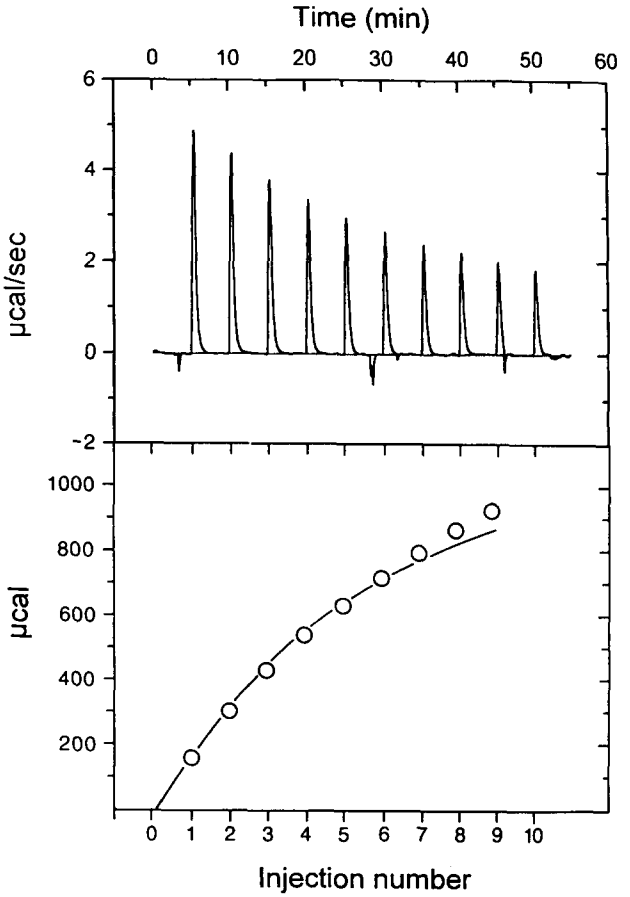

Fig. 1. Titration calorimetry of sonified unilamellar POPC vesicles (18.3 mM) in buffer (10 mM Mes, 100 mM NaCl, pH 5.6, T = 27°C) with a 10 mM $LaCl_3$ solution. 10 μl injection of $LaCl_3$ (in buffer) per injection step. The upper part of the figure shows the calorimeter tracings, the bottom part yields the cumulative reaction enthalpy as a function of the number of injections. The solid line corresponds to the best theoretical fit to the data calculated with $\Delta H = +1.83$ kcal/mol, a surface partition equilibrium constant $K = 4500\ M^{-1}$, and an ion charge $z = 3$. The La^{3+} surface concentration, C_I, was calculated with the Gouy-Chapman theory. The binding/adsorption equilibrium was decribed with a surface partition equilibrium according to $X_b = K \cdot C_I$.

by the gradual accumulation of positive charge on the membrane surface which makes the binding of further La^{3+} ions more and more difficult. A quantitative treatment of this problem is possible via the Gouy-Chapman theory as discussed above. Assuming complete binding of La^{3+} for at least the first injection step, a minimum adsorption enthalpy of $\Delta H = 1.7$ kcal/mol can be evaluated form Fig. 1A. The whole titration experiment can then be described by combining the Gouy-Chapman theory with a surface partition equilibrium characterized by $\Delta H = +1.8$ kcal/mol and a surface partition constant $K = (4.5 \pm 1.1) \cdot 10^3\ M^{-1}$. The solid line in Fig. 1B corresponds to this theoretical prediction.

In a control experiment, the same $LaCl_3$ solution was injected into pure buffer without lipid and a small

exothermic heat of dilution of -5 μcal per injection was noted. The data in Fig. 1B were corrected for this dilution effect.

The analogous titration of POPC vesicles with 5 mM $LaCl_3$ reduced the height of the individual titration peaks by about a factor of 2, but led to a qualitatively similar titration curve as obtained for 10 mM $LaCl_3$ (data not shown). The use of extruded POPC vesicles with $d \sim 100$ nm had a small influence on the adsorption enthalpy. The quantitative analysis of the data is summarized in Table I.

Ca^{2+} titrations of sonified or extruded POPC vesicles with 10 mM or 20 mM $CaCl_2$ (in buffer + 0.1 M NaCl) yielded near zero reaction enthalpies, i.e., the measured heats of reaction were identical to those of the controls. This negative result can be explained by a combination of two effects. First, Ca^{2+} binding/adsorption to neutral POPC vesicles is weak with a binding constant of 10–20 M^{-1} [1–3,6–9]. Secondly, the Ca^{2+} binding/adsorption enthalpy is also small. The latter aspect can be confirmed by measuring Ca^{2+} binding under conditions of increased electrostatic attraction as discussed below.

La^{3+} and Ca^{2+} adsorption to negatively charged lipid vesicles

The binding of metal ions to negatively charged membranes is enhanced by electrostatic attractions. The concentration of metal ions near the plane of binding is larger than that in bulk solution which, in turn, leads to a larger *overall* binding constant. Fig. 2 shows a titration of sonified POPC/POPG vesicles (75:25, mol/mol) with a 10 mM $CaCl_2$ solution. The titration peaks are small with *endothermic* heats of reaction of ~ 9.5 cal per injection. The reaction enthalpies are furthermore rather constant during the whole experiment. The control experiments yield small exothermic heats of dilution of -0.5 cal per injection. From ζ-potential and ultracentrifugation measurements the intrinsic Ca^{2+} binding constant for the system under investigation was found to be 10–20 M^{-1} [1–3,6–9]. Taking into account electrostatic attractions by means of the Gouy-Chapman theory it can be calculated that in the experiment of Fig. 2 about 85%

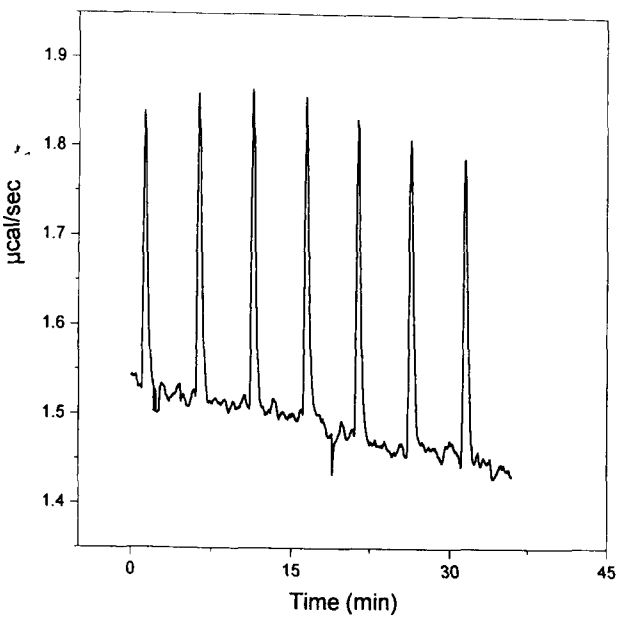

Fig. 2. Titration calorimetry of sonified unilamellar POPC/POPG (75:25, mol/mol) vesicles in buffer (10 mM Mes, 100 mM NaCl, pH 5.6, $T = 27$°C) with a 10 mM $CaCl_2$ solution in the same buffer. 10 μl injection of $CaCl_2$ per injection step. The binding reaction is endothermic and approximately constant per injection step. The surface partition constant of Ca^{2+} binding was taken from the literature as 13 M^{-1} [8,9]. Ca^{2+} binding is dominated by electrostatic attraction and about 80% of the injected Ca^{2+} bind to the lipid membrane (cf. text for calculation).

(1st injection) → 65% (last injection) of the added Ca^{2+} is bound to the membrane surface. In spite of this large extent of Ca^{2+} binding, the heat of reaction is small and the molar adsorption binding enthalpy can be calculated as $\Delta H \approx 0.1$–0.2 kcal/mol.

A different behavior was observed when the same vesicles (POPC/POPG, 75:25) were titrated with a 5 mM $LaCl_3$ solution (Fig. 3). The reaction enthalpy was again *endothermic* but almost 30-fold larger than that observed for 5 mM $CaCl_2$. The molar adsorption enthalpy as calculated from the first injection step (assuming complete La^{3+} binding) was $\Delta H = +2.3$ kcal/mol which is close to the result obtained for pure POPC vesicles ($\Delta H = +1.7$ kcal/mol). Unexpectedly, the reaction enthalpy *increased* during the first five

TABLE I

Thermodynamic parameters for La^{3+} adsorption to POPC vesicles

Concn. $LaCl_3$	Total lipid in calorimeter cell	pH	Vesicle size (nm)	ΔH (kcal/mol)	Partition constant K (M^{-1})
10 mM	16.7 mM POPC	5.6	35	1.9	4800 ± 780
10 mM	18.3 mM POPC	5.6	35	1.7	4490 ± 660
10 mM	14.3 mM POPC	5.6	100	0.8	4400 ± 700
5 mM	15.9 mM POPC	5.6	35	1.65	4570 ± 700
5.2 mM	12.2 mM POPC	5.6	35	1.85	2300 ± 300
5 mM	11.5 mM POPC	5.6	35	1.68	3900 ± 920

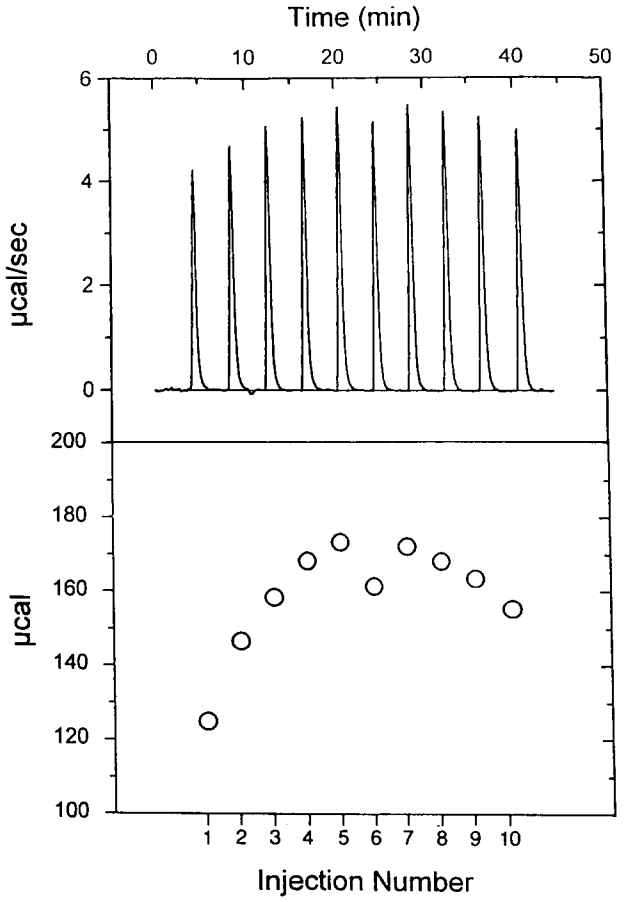

Fig. 3. Titration calorimetry of sonified unilamellar POPC/POPG (75:25, mol/mol) vesicles in buffer (10 mM Mes, 0.1 M NaCl, pH 5.6, $T = 27°C$) with a 5.2 mM $LaCl_3$ solution in the same buffer. 10 μl injection of $LaCl_3$ per injection step. The reaction is endothermic. Assuming complete $LaCl_3$ binding the reaction enthalpy increases from $\Delta H = +2.3$ kcal/mol to 3.3 kcal/mol during the first five injections after which it decreases again.

injections and then started to decrease again (Fig. 3). This unusual behavior suggests the existence of at least two thermodynamically distinct processes. One is the adsorption/binding of the La^{3+} ions which is expected to decrease with increasing loading of the lipid vesicles, the other could be a La^{3+} induced demixing of neutral and negatively charged lipids which would require an additional heat consumption. This result requires further investigation.

We have also investigated the binding of Ca^{2+} and La^{3+} to POPC/POPG vesicles with POPG contents larger than 25 mol%. For all systems studied the heat of reactions were small. Different results were obtained depending not only on the lipid composition and the metal ion employed but also on the concentration of the metal ion and the size of the lipids. No consistent thermodynamic picture emerged at these rather unphysiological concentrations of negatively charged lipid. The results were therefore not included in the present study.

The heat of dilution of lipid vesicles alone is negligible as has been demonstrated experimentally by titrating liposomes into a pure buffer solution [25,26].

Discussion

The binding of multivalent metal ions to membranes containing negatively charged lipids gives rise to a complex phase behavior. Previous studies have been concerned with different aspects of lipid polymorphism and vesicle fusion [13–22]. These multi-step processes are thermodynamically difficult to describe and only a semiquantitative understanding appears to be possible at best. The present analysis was therefore limited to neutral phospholipids (La^{3+} binding to POPC) or mixed membranes with only a small percentage of negatively charged lipid (Ca^{2+} binding to POPC/POPG; 75:25, mol/mol). The phase behavior of the latter system has been investigated extensively with deuterium NMR and differential scanning calorimetry and no unusual phase phenomena was noted for Ca^{2+} binding to membranes of this particular lipid composition [7].

The quantitative analysis was based on the Gouy Chapman theory taking into account the variation of electric charge at the membrane surface. In the La^{3+}/POPC titration experiment the initially neutral membrane becomes positively charged upon La^{3+} binding, making the approach of further La^{3+} ions more and more difficult. The driving force for La^{3+} binding is the large La^{3+} binding constant. In contrast, the main component in Ca^{2+} binding is the electrostatic attraction between the divalent cation and the negatively charged membrane whereas the intrinsic Ca^{2+} binding constant is small.

La^{3+} binding to POPC vesicles

The La^{3+} binding constant to POPC vesicles, after correcting for electrostatic effects, was $K = (4.1 \pm 1.1) \cdot 10^3$ M^{-1} as determined from six different titration experiments. Comparing sonified vesicles ($d \sim 30$ nm) with extruded vesicles ($d \sim 100$ nm) showed only small difference. The La^{3+} adsorption to POPC vesicles could be described by a simple surface partition equilibrium according to $X_b = K \cdot C_I$, with X_b being the molar amount of La^{3+} bound per mole POPC and C_I the interfacial concentration of free La^{3+} immediately above the plane of binding. The *total* La^{3+} concentration in the lipid suspension ranged between 80 μM and 0.8 mM (for a 10 mM $LaCl_3$ stock solution), and the highest X_b value was $X_b \approx 42$ mmol/mol. Under these conditions the La^{3+} ions are sufficiently spaced apart on the membrane surface and direct La^{3+}–La^{3+} interactions can be excluded. The Langmuir isotherm hence degenerates into a surface partition equilibrium.

The equilibrium binding of lanthanide ions to phospholipid membranes has been extensively investigated

with NMR [31–35]. Lanthanide ions such as Eu^{3+} or Pr^{3+} are paramagnetic and shift the position of the phospholipid resonances upon binding. La^{3+} is diamagnetic and exhibits no such effect. However, its binding behavior should be comparable to that of the other members of the lanthanide series because of similar size and charge. Springer and co-workers investigated the binding of Pr^{3+} to 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) vesicles at 52°C where the DPPC membrane is in the liquid crystalline phase. The bulk of the binding data was obtained in the concentration range of 1–10 mM, about 10-fold higher than the equilibrium concentrations employed in the present experiments. It was concluded that the Gouy-Chapman theory could not explain the binding data over the whole concentration range and a cooperative model was proposed instead [35]. However, if the analysis of the Pr^{3+} binding is limited to concentrations of $c < 1$ mM, the binding constant estimated via the Gouy-Chapman theory is $K \sim 10^3$–10^4 M^{-1} (cf. Ref. 35; Fig. 8), in agreement with the present analysis.

Binding of La^{3+} to POPC vesicles was also measured by incubating POPC coarse liposomes with defined amounts of a $LaCl_3$ stock solution. After equilibration the lipid suspension was separated from the bulk solution by ultracentrifugation, and the La^{3+} concentration was measured by a dye assay. The binding equilibrium was analysed in terms of the partition equilibrium/Gouy-Chapman approach and the intrinsic binding constant was determined as $K \approx 4000$ M^{-1}, again in accordance with titration calorimetry [36].

Adsorption enthalpy

Titration calorimetry allows the direct determination of the adsorption/binding enthalpy, ΔH, provided the measuring conditions guarantee a complete binding of the injected metal ions. This holds true for La^{3+} injections into pure POPC or mixed POPC/POPG vesicles due to the large La^{3+} adsorption/binding constant. For Ca^{2+} ions the binding constant is smaller and binding to POPC vesicles is weak. However, for mixed POPC/POPG vesicles (75:25, mol/mol) at least 80% of the added Ca^{2+} ions bind in the initial phase of the titration experiment and the measured ΔH is close to its maximum values. For both metal ions the observed adsorption/binding enthalpies are small and *endothermic* with $\Delta H \approx +0.1$–0.2 kcal/mol for Ca^{2+} and $\Delta H \approx +1.8$ kcal/mol for La^{3+}.

Ca^{2+} binding to cardiolipin vesicles has been studied previously with batch calorimetry [20]. At low Ca^{2+} concentrations (with cardiolipin in excess over Ca^{2+}) an endothermic heat of reaction with $\Delta H \approx +0.2$ kcal/mol was observed. However, in parallel to Ca^{2+} binding a bilayer → isotropic transition of 30% of the lipids and a bilayer → hexagonal H_{II} transition of 10% of the lipids was induced. In an analogous study, the binding of Ca^{2+} and other alkaline earth cations to small and large phosphatidyl*serine* vesicles was monitored with continuous titration calorimetry [37]. For all ions investigated the heat effects were small and endothermic ($\Delta H \leq +1.5$ kcal/mol) as long as the mole ratio of total metal ion to PS was less than 0.35 (i.e., lipid remained in excess over Me^{2+}). Likewise the complexation of Ca^{2+} with inorganic phosphate ligands gives rise to *endothermic* heats of reactions of $\Delta H \approx 3.0$ kcal/mol [38]. Taken together the data suggest that the Ca^{2+} binding/adsorption enthalpy to lipid membranes is zero or endothermic, independent of the actual membrane composition. For La^{3+} the binding enhalpy is also endothermic and slightly larger than that of Ca^{2+}. No comparable La^{3+} binding enthalpies could be found in the literature.

Using titration calorimetry we have previously measured the binding enthalpy of a small basic peptide, pentalysine (Lys_5), to mixed POPC/POPG membranes and obtained a binding enthalpy of about + 1 kcal/mol. The binding was mainly electrostatic with an overall partition constant of 10^4–10^5 [39,40]. In contrast, the binding of small *hydrophobic* drug molecules to sonicated and nonsonicated membranes gives rise to *exothermic* reaction enthalpies of up to $\Delta H \approx -10$ kcal/mol [23–27]. While the latter process is complety enthalpy-driven, the binding of Ca^{2+}, La^{3+}, and Lys_5 to the lipid membrane must be *entropy*-driven.

The free energy of binding/adsorption can be calculated according to $\Delta G = -RT$ ln(55.5 K) where the factor 55.5 accounts for the fact that the ion concentration in the bulk phase is measured in mol/l whereas its concentration at the membrane surface is given as mole fraction (cf. 'cratic contribution' in Ref. 41). For the binding of Ca^{2+} and La^{3+}, free energy values of $\Delta G = -3.9$ kcal/mol and $\Delta G = -7.1$ kcal/mol are evaluated. The entropy term $T\Delta S$ is hence positive and of similar magnitude. The molecular origin of this entropy gain could be a rearrangement of the surface hydration layer of the lipid membrane or a release of water molecules from this layer but the molecular details are unknown.

In conclusion, high sensitivity titration calorimetry is a simple method to determine the binding/adsorption enthalpy of multivalent metal ions to lipid membranes even under conditions of weak binding.

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