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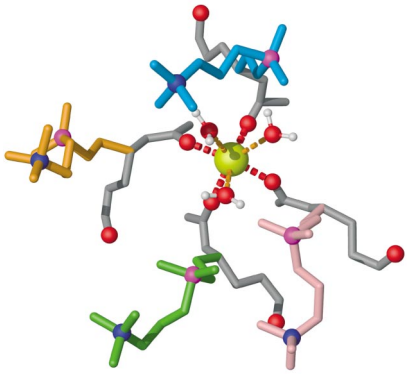

Step by step: The sequential coordination of calcium ions (yellow) to four lipid carbonyl oxygen atoms in neutral zwitterionic phospholipid bilayers has been studied by molecular dynamics simulations.

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Ion–Membrane Interactions

Multistep Binding of Divalent Cations to Phospholipid Bilayers: A Molecular Dynamics Study**

*Rainer A. Böckmann and Helmut Grubmüller**

The specific interaction of cations—particularly divalent cations—with biological membranes is essential not only for the structure, dynamics, and stability of membranes, but also for the binding or insertion of proteins to or into membranes, for membrane fusion, and for the transport of small molecules across membranes. Membrane fusion, for example, has been shown to be triggered by calcium ions.[1,2] Moreover, calcium ions are an integral part of neural signal transduction, and so is their interaction with the neural membrane.[3]

Various experimental methods have been applied in the past to shed light on the interaction of cations with membranes. NMR experiments[4,5] suggested a conformational change in the polar region of dipalmitoylphosphatidylcholine (DPPC) bilayers[4] and a calcium ion/phospholipid stoichiometry of 1:2 at 5 M $CaCl_2$.[5] Additional rearrangements or conformational changes in the carbonyl region of palmitoyloleoylphosphatidylcholine (POPC) lipids were revealed by IR spectroscopy.[6] From neutron diffraction experiments, the distribution of calcium in the lipid headgroup of DPPC bilayers in the liquid-crystalline state could be determined.[7]

Taken together, these experiments suggest a picture in which the calcium ion is predominantly bound to the phosphate moieties of two lipids. However, up to now, there has been no direct evidence for this model and, accordingly, there is no proposal for the structure of the ion–lipid complex. The main experimental obstacle here is the low signal-to-noise ratio, typically resulting from the fact that the two-dimensional lipid–solvent interface comprises only a small fraction of the sample volume. Therefore, the current consensus leaves considerable room for diverging interpretations as, for example, evidenced by a fluorescence study on the influence of anions and cations on the dipole potential of phosphatidylcholine vesicles.[8] In this study—in accord with the available experimental data, but quite in contrast to the above picture—a measured reduction of the overall dipole potential was interpreted in terms of anion binding to the headgroup. More detailed structural information can be expected from recent X-ray diffraction studies on oligolamellar bilayers,[9] which do not suffer from this drawback.

These difficulties on the experimental side give considerable weight to theoretical studies. Indeed, molecular dynamics (MD) simulations of solvated lipid bilayers have yielded information on the conformation and arrangement of the lipids in membranes. In many cases the results were in quantitative agreement with experimental data.[10–13,15]

However, up to now, MD studies of ion binding to lipid bilayers have not been possible because of the apparently slow kinetics of ion binding to and dissociation from lipid membranes. Typically the simulation systems do not reach sufficient equilibration within the nanosecond simulation time spans.[14] Only very recently, we were able to carry out a simulation sufficiently long to observe the binding of sodium ions to the carbonyl oxygens of phospholipids.[15] This study confirmed that even the binding of monovalent ions is unexpectedly slow, and it predicted a reduced self-diffusion coefficient for the lipids under the influence of sodium chloride, which was subsequently confirmed by fluorescence correlation spectroscopy.[15]

The above-mentioned simulation, however, would have been too short to yield sufficiently converged results for calcium binding, which called for a further increase of simulation lengths. Here we present 200-ns MD simulations of calcium binding, which are sufficiently converged for the analysis of calcium binding. These simulations were also suitable for elucidating the differences between the binding of monovalent and divalent cations to phospholipid bilayers. Finally, our simulations enabled us to characterize the different binding kinetics.

Two simulation systems (A and B) were studied, each comprising ca. 20000 atoms (Figure 1). Both systems contained 128 POPC lipids arranged in a bilayer and approximately 5000 water molecules. To system A we added 10 sodium and 10 chloride ions, and to system B, 8 calcium and 16 chloride ions. All ions were randomly placed within the water phase. The sodium chloride simulation system has been described.[15] In the present study the simulation was extended to 200 ns. The simulation time for system B was also 200 ns.

We note that the system had to be relatively small in order to attain sufficiently long simulation times. However, for the chosen concentration of calcium ions (0.089 M), the Debye–Hückel length of $\lambda_D = 5.9$ Å is much smaller than the size of the periodic box ($62 \times 62 \times 80$ Å^3). Therefore, the interactions

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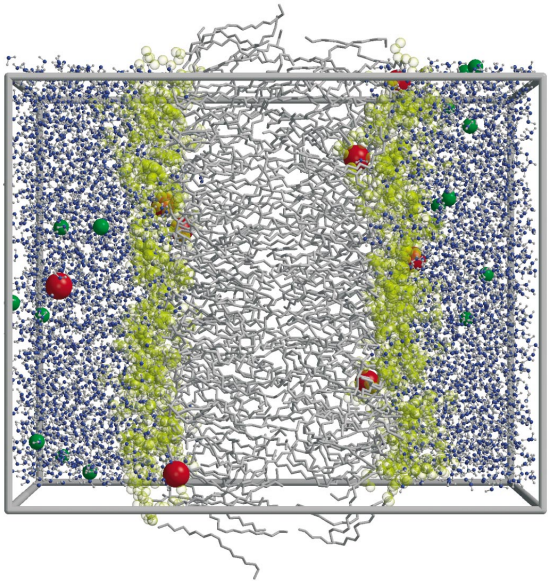

Figure 1. Snapshot of simulation system B after 100 ns (128 POPC lipids; carbon chains = gray, water = light blue, Ca^{2+} = red, Cl^- = green, hydrophilic headgroups = yellow).

between ions in adjacent periodic units should be small, even at the very end of the simulation when all the calcium ions are bound to the membrane ($\lambda_D = 10.2$ Å). This nearly equilibrated end state thus corresponds to a state with a rather low concentration of calcium ions in solution, close to physiological conditions.

Figure 2 shows snapshots from our simulations of the binding of monovalent (top row) and divalent cations (bottom row) to lipid carbonyl oxygens. The progression of the equilibration of the cations is monitored by the averaged number of coordinating water oxygens (black lines) and lipid carbonyl oxygens (red) as a function of simulation time (Figure 3). Monoexponential fits (blue lines) to these curves yield binding times of 23 ns and 86 ns for sodium and calcium

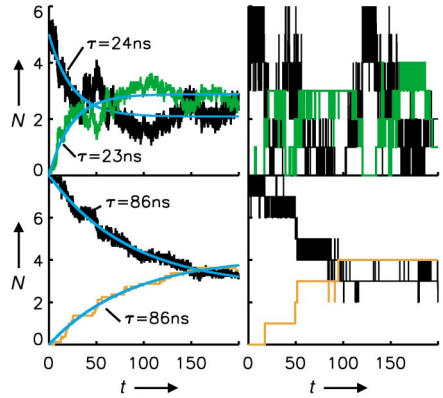

Figure 3. Total number N of water oxygens (black) and lipid carbonyl oxygen atoms (green, orange) within 2.8 Å of the sodium ions (upper panel) and within 3 Å of the calcium ions (lower panel) as a function of simulation time t. The relaxation times τ from the exponential fits (blue) to the data are also given. The right row shows the quantities for representative single cations.

ions, respectively. The fits yield also equilibrium coordination numbers for the binding of lipid carbonyl oxygens of 2.9 for Na^+ and 4.2 for Ca^{2+}.

In the simulations a sequential binding of the lipid carbonyl oxygens to the calcium ions is observed (see Figure 4). Binding of the first carbonyl oxygen requires penetration of the Ca^{2+} ions through the hydrophilic headgroups of the lipids and occurs, for the concentration chosen, within 30–40 ns (red line). After 200 ns of simulation time,

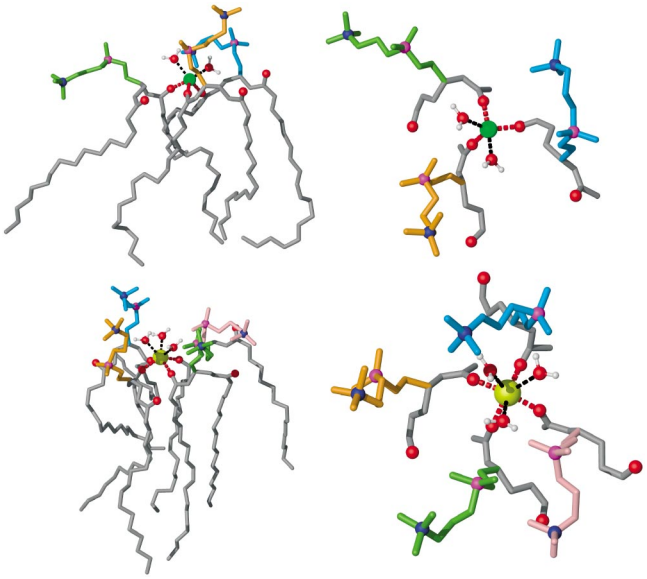

Figure 2. Typical snapshots of the coordination of Na^+ (top row) and Ca^{2+} ions (bottom row) by lipid carbonyl oxygens (dotted lines in red) and by water oxygens (dotted lines in black). Left: side view; right: top view.

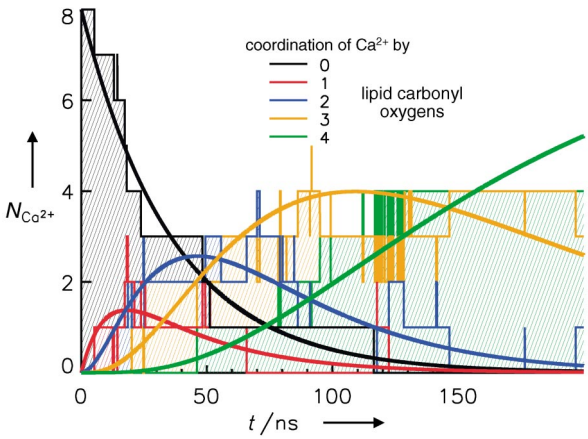

Figure 4. Coordination of Ca^{2+} by lipid carbonyl oxygens as a function of simulation time t ($N_{Ca^{2+}}$ is the number of Ca^{2+} ions with a given coordination number). The colors differentiate between coordination by only water oxygens (black) and by one (red), two (blue), three (orange), and four lipid carbonyl oxygens (green). Also shown is a fit of the simulation data (thick lines) assuming a multilevel process for the sequential binding of the calcium ions to finally four lipid carbonyl oxygens (see text).

about half of the ions reached the preferred coordination by at least four phosphatidylcholines (green line). The red, blue, and orange traces show the population of intermediates of one-, two-, and threefold lipid coordination, respectively.

The observed sequence of intermediates suggests that the binding of the lipid carbonyl oxygens to the Ca^{2+} ions can be described by a sequential rate equation [Eq. (1)]. Since in our

$$Ca^{2+} \xrightarrow{k_0} Ca^{2+}\cdot L \xrightarrow{k_1} Ca^{2+}\cdot 2L \xrightarrow{k_2} Ca^{2+}\cdot 3L \xrightarrow{k_3} Ca^{2+}\cdot 4L \qquad (1)$$

simulations the flux of binding ions is significantly larger than the flux of dissociating ions, we simplify the treatment by neglecting the back reactions. The bold lines in Figure 4 show fits of the solution of these rate equations to the coordination numbers obtained from the simulations, which yield the corresponding rate coefficients k_i and binding times $\tau_i = 1/k_i$. The agreement is well within the statistical error of the finite number of observed events.

After the calcium ion binds to the first lipid carbonyl oxygen ($\tau_0 = 36^{+16}_{-11}$ ns[*]), the second coordination is faster ($\tau_1 = 10^{+5}_{-4}$ ns). In contrast, coordination by the third and fourth lipid carbonyl oxygen is much slower again, as can be seen from the significantly increased decay times ($\tau_2 = 31^{+14}_{-10}$ ns, $\tau_3 = 107^{+90}_{-50}$ ns). Closer inspection of the structure of the complexes (cf. Figure 2) shows that binding of the third and fourth lipid requires major reorientation and fine-tuning of the lipid–ion packing. Accordingly, the slow kinetics can be explained in terms of entropic barriers, which is also the main determinant of the slow kinetics of the whole binding process. Due to saturation effects we expect a deviation from the above binding times for higher salt concentration.

Like our previous findings for sodium ions,[15] complex formation for calcium ions here is also found to reduce lipid self-diffusion. In addition we predict a decreased rotational diffusion coefficient for the lipid headgroups, as evidenced by the increase of the half-life $t_{1/2} \propto 1/D_R$ of the rotational correlation function [Eq. (2)]. For the vector $\boldsymbol{p}$ connecting

$$C_2(t) = \frac{1}{2}\langle 3[\boldsymbol{p}(t)\,\boldsymbol{p}(0)]^2 - 1\rangle \qquad (2)$$

the phosphate and the nitrogen atom in the headgroup, the half-life increases from 0.96 ns for the case without ions to approximately 1.83 ns in the presence of 330 mM $CaCl_2$ (1.11 ns at 110 mM NaCl, 1.58 ns at 330 mM NaCl).

The different binding of monovalent and divalent cations to the lipid bilayer entails differences in the lipid headgroup conformation (see Figure 2). In particular, the tight packing of lipids around the Ca^{2+} ions leads to a smaller angle ($\approx 60°$) between the dipole moment of the lipid headgroup and the membrane normal (63.5° on average vs. 69.8° for the case without ions[15]). Additionally, a significant increase of the order of the lipid acyl chain is observed.

In conclusion, our simulations provide an atomistic model for the binding of potassium and calcium ions to neutral, zwitterionic POPC lipid bilayers. Key features of these models are a sequential multistep binding and coordination of the cations by three and four lipid carbonyl oxygens, respectively. These predictions will likely be tested in the near future by X-ray diffraction studies on oligolamellar bilayers and by solid-state NMR experiments. Our results should enable construction and/or refinement of quantitative models for biological processes involving calcium binding such as neural signal transduction and membrane fusion.[16]

[*] The exponents and indices on these numbers correspond to the values of the error bars.

Experimental Section

Both simulations were carried out with the GROMACS suite[17] using the force field devised by Berger et al.;[18] parameters for the unsaturated carbons were taken from the GROMOS87 force field. Application of the Lincs[19] and Settle[20] methods allowed for an integration step size of 2 fs. We note that the force field used is not a polarizable one and may provide an inaccurate description of the ion–carbonyl interactions. Therefore, the actual binding rates may differ from those reported here, most likely roughly by a common scaling factor. The Particle-Mesh Ewald (PME) method[21] was used, and an NPT ensemble was applied in the simulations, with separate coupling of the membrane and the solvent to a 300 K heat bath, and a semiisotropic pressure coupling in the lateral direction and perpendicular to the membrane surface, as described.[15] Coordination numbers for the ions were calculated from the cumulative radial distribution function at distances of 2.8 Å (Na^+) and 3.0 Å (Ca^{2+}) as a function of simulation time.

Error bars (1σ) for the rate coefficients k_i were determined from multiple Monte Carlo simulations of Equation (1) using the values for k_i given above and eight calcium ions, as in the MD simulation. Many, statistically independent replica of the data shown in Figure 4 were obtained. From each of these replica, four rate coefficients k_i were computed using the same fitting procedure as was used for the MD data. The statistical scatter of these sets of k_i provided the statistical error expected in the rate coefficients derived from the MD simulation. Note that the scatter was computed on a logarithmic scale, which is more appropriate for rates; for this reason the error bars are asymmetric.

The simulations were performed on a dual-processor PC with two Athlon MP 2200+ processors; each ns of simulation time required about 26 h of computation time.

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