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Measured Depletion of Ions at the Biomembrane Interface

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From biological to voltaic cells, ions at interfaces pose outstanding theoretical and experimental questions.[1−3] Ion-specific effects in halophilic bacterial growth,[4] water purification,[5] and biomatter production of atmospheric contaminants[6] force us to recognize physical interactions beyond double-layer electrostatics.[3] While halide distributions at the air/water interface can be studied by spectroscopic techniques,[7,8] measurements of salt distributions near fuzzy biomembrane interfaces have long eluded experiments. Here, we demonstrate that an unexpectedly simple yet accurate method reveals the extent of halide ion (Cl^- and Br^-) depletion next to lecithin lipids, the scaffold of biological membranes. We find that multilamellar lipid aggregates sink in low salt, but float in salt solutions that are much less dense than the lipid itself. The density mismatch allows us to evaluate the salt deficit and the spatial extent of exclusion layers. By manipulating bath and membrane composition, using heavy water and varied lipid chain length, we obtain previously inaccessible exclusion curves over a wide range of salt concentrations. Matching the extent of water ordering measured by NMR,[9] the exclusion layer is of constant width at low salt as long as the Debye screening length is large, consistent with the dominance of ion−surface interaction. At high salt concentrations, the exclusion layer essentially follows the screening length, indicating that ion−ion interaction near the membrane surface is a more appropriate description in this regime. Consistent with interfacial binding of polarizable ions,[10−12] we find that Br^- salts are excluded much less than Cl^-. With a calculated binding energy of $\sim 2k_BT$, one Br^- ion per 60 lipid headgroups ($\sim$40 nm^2) is adsorbed in the interfacial region in the presence of 100 mM salt. These measurements of salt partitioning at membrane interfaces over a broad range of concentrations open new ways to examine ion−biomembrane interactions.

Our measurement of salt exclusion is based on a simple, benchtop experimental observation. Mixed with water, common neutral phosphatidylcholine (PC) lipids spontaneously form colloidal, multilamellar vesicles (MLVs) of bilayers, separated by water layers of 20−30 water molecules per lipid.[13] Forming visible white sediments, these denser-than-water MLVs sink.[14] However, when MLVs are formed in sufficiently concentrated salt solutions, the lipid aggregates float (Figure 1). At some particular salt concentration, the bath and MLV densities match and a state of "neutral buoyancy" is achieved (MLVs neither sink nor float). The matching density, ρ^*_{bath} is unique to a given lipid and salt type.

In Figure 1, we plot the specific volume (inverse density) of KCl and KBr salt solutions to compare with specific volumes of common phospholipids. If salt ions were equally distributed inside and outside of MLVs, neutral buoyancy would occur at the intersection between salt and lipid curves. Experimentally, however, we systematically find that MLVs float in salt solutions that are much less dense than the lipids themselves, as indicated by the buoyancy symbols in Figure 1. The MLVs are lighter because there is a salt deficit within the MLVs, or equivalently, there is water excess. Salt is *excluded* from the vicinity of membranes because interfacial solvation of ions differs from bulk.

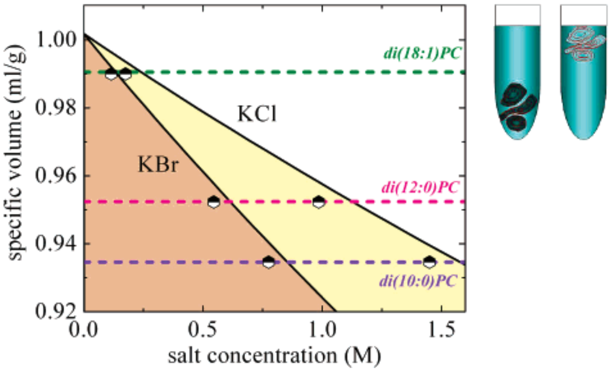

Figure 1. Lipid multilayers sink in low salt and float in high salt. Specific volumes of pure KCl and KBr salt solutions at 20 °C versus concentration are shown with solid lines. Lipid-specific volumes are indicated by dashed lines. For each lipid, neutral buoyancy (symbols) occurs at salt concentrations lower than those matching lipid densities (intersection of solid and dashed lines).

The effective number, $N^{\mathrm{ex}}_{\mathrm{W}}$, per lipid of "excluding water molecules" incapable of solvating ions[15] can be exactly calculated at the neutral buoyancy point. With known lipid density, ρ_{L}, we have

$$N^{\mathrm{ex}}_{\mathrm{W}} = \frac{V_{\mathrm{L}}}{V_{\mathrm{W}}}\frac{\rho_{\mathrm{L}} - \rho^*_{\mathrm{bath}}}{\rho^*_{\mathrm{bath}} - \rho_{\mathrm{W}}} = \frac{M_{\mathrm{L}}}{M_{\mathrm{W}}}\frac{1 - \rho^*_{\mathrm{bath}}/\rho_{\mathrm{L}}}{\rho^*_{\mathrm{bath}}/\rho_{\mathrm{W}} - 1}$$

$$\rho^*_{\mathrm{bath}} = \frac{M_{\mathrm{L}} + N^{\mathrm{ex}}_{\mathrm{w}} M_{\mathrm{W}}}{V_{\mathrm{L}} + N^{\mathrm{ex}}_{\mathrm{W}} V_{\mathrm{W}}} \qquad (1)$$

where V_{L} and V_{W} are molecular volumes,[16] and M_{L} and M_{W} are the molecular weights of lipid and water, respectively. Note that the calculation does not require knowledge of interlamellar water spacings. To determine $N^{\mathrm{ex}}_{\mathrm{W}}$ versus salt concentrations, we manipulate ρ^*_{bath} by adding heavy water (D_2O) and alter lipid density by changing acyl chain length. Combined results for three different phospholipids (with 10, 12, and 18 carbon segments[17]) and two salt solutions (KCl and KBr) at 20 °C are shown in Figure 2.

Our results show that salt exclusion is practically independent of chain length; it is mainly determined by the interactions between PC headgroups, interfacial water, and salt ions. Figure 2 also shows the thickness, $D^{\mathrm{ex}}_{\mathrm{W}}$, of the exclusion layer, calculated by dividing the exclusion layer volume by the known cross-sectional area per lipid.[13] $D^{\mathrm{ex}}_{\mathrm{W}}$ is practically constant at low salt until it becomes comparable with the salt-screening length. At higher salt concentrations, the exclusion layer thickness then approximately follows the change of screening length with salt concentration.

Typical of Hofmeister ranking,[1−3,18−20] there is a clear distinction between Cl^- and Br^-, with Br^- being less excluded than Cl^-. At

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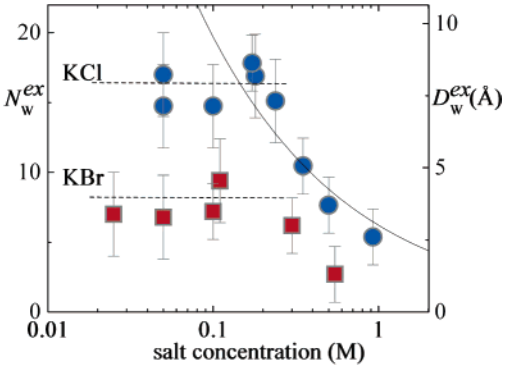

Figure 2. Number of salt-excluding water molecules per lipid (left axis) and corresponding exclusion layer thickness (right axis) versus salt concentration. Solid line shows the Debye screening length to compare with the excluding layer thickness.

low salt, about 15 interfacial water molecules per lipid are unavailable to solvate the ions of KCl, but only 7 water molecules are unavailable to KBr. Reflecting the competition between salt and membrane surface for the available water, N_W^{ex} decreases with increasing salt concentration, for example, $N_W^{ex} = 5$ for 1 M KCl.

Salt exclusion can be thought of as competitive dehydration of headgroups and of salt ions. Measured by X-ray diffraction and by ^{2}H NMR spectroscopy, PC membranes that are dehydrated to less than 15 water molecules per lipid are shown to undergo strong structural modifications.[9] These changes are seen in both deuterated and protonated water with practically no difference in bilayer structure and interactions. (Note, however, that deuteration of lipid acyl chains does modify lipid interactions, as indicated by substantial shifts of phase transition temperatures.)

Neglecting water-deuteration effects, the interfacial ordering of water measured by solid-state ^{2}H NMR can be described as two water shells of 3 Å thickness, where each layer is occupied by about six water molecules.[9] As shown in Figure 2, the inner water shell is readily accessible to KBr but not to KCl. Only at 600 mM can KCl begin to dissolve into Br-accessible water shells.

Calculating the free energy (entropic) cost of keeping salt out of the interfacial region allows us to estimate Br^- binding energy. Consider, for example, 100 mM salt solutions, where N_W^{ex} decreases by $\Delta N_W^{ex} = 9 \pm 1$, from 16 in KCl to 7 in KBr. To change N_W^{ex} by $\Delta N_W^{ex} = 9$ requires 0.03 k_BT per lipid, as calculated from the integral of $d\Delta G = \Delta N_W^{ex} d\mu_W$, where μ_W is the water chemical potential. In 100 mM salt solutions, for each 9 water molecules, there are $9/554 = 0.016$ anions. The number of Br ions corresponding to $\Delta N_W^{ex} = 9$ (per lipid) is then one Br^- ion per ~60 lipid headgroups, giving an apparent Br binding constant of 0.2 ± 0.02 M^{-1} and a binding energy of ~2 k_BT.

A number of experimental issues must be considered. First, the effect of deuteration on interfacial water arrangement is unknown, but is likely of no measurable consequence to the magnitude of salt exclusion. Second, our reported N_W^{ex} values should be corrected for any modification of lipid densities by salt. However, the dehydration effect of salt most likely causes an increase of lipid density in eq 1, implying that our reported N_W^{ex} values are possibly underestimates! Similarly, N_W^{ex} values should be adjusted if interfacial water density is considered to be different from that of bulk. Third, one might worry about equilibration of salt inside MLVs. We have verified equilibration by density and X-ray measurements of samples with various histories: from pre-equilibration in water to formation in salt solutions, addition of ionophores, and temperature cycling. Results were robust and reproducible.

Competing with interfacial water, salt ions interfere with membrane molecular arrangement, possibly affecting cellular communication and biological signaling. By screening charge fluctuations, interlamellar salt weakens the van der Waals attraction between membranes,[21] increasing the energy barrier for membrane fusion. Ion exclusion, however, could act to alleviate this effect. The extent of salt exclusion shown here makes it relevant to evaluation of interactions at the membrane−water interface, an outstanding question in biological systems.

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