Cooperativity between sodium ions and water molecules facilitates lipid mobility in model cell membranes

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Abstract: Cellular membranes are surrounded by an aqueous buffer solution containing various ions, which influence the hydration layer of the lipid head groups. At the same time, water molecules hydrating the lipids play a major role in facilitating the organisation and dynamics of membrane lipids. Employing fluorescence microscopy imaging and fluorescence recovery after photobleaching measurements, we demonstrate that the cooperativity between water and sodium (Na^+) ions is crucial to maintain lipid mobility upon the removal of the outer hydration layer of the lipid membrane. At similar hydration conditions, lipid diffusion ceases in absence of Na^+ ions. We unravel that Na^+ ions strengthen the water clathrate cage around the lipid phosphocholine head group and thus prevent its breaking upon removal of bulk water. Intriguingly, divalent cation Ca^{2+} does not show this effect. In this article we provide a detailed molecular-level picture of ion specific dependence of lipid mobility and membrane hydration properties.

Introduction

Biological membranes are self-assembled structures composed of various lipids embedded with proteins. They act as a dynamic barrier separating intra- and extra-cellular matrices and encapsulate various subcellular organelles. A large variety of lipids in terms of chain length, chain saturation, headgroup structure, and charge is present in biomembranes. The structural lipid heterogeneity, in particular the length mismatch between the hydrophobic tails of the lipids, promotes the formation of lipid domains in response to their unfavourable interactions with the membrane aqueous hydration layer - the so-called “hydrophobic mismatch”. Saturated lipids, such as sphingomyelin (SM) along with cholesterol form more compact liquid ordered (L_o) phase domains in the sea of more fluid liquid disordered phase (L_d) composed predominantly of unsaturated lipids[1]. The Lo domains are believed to be platforms for various important biological processes like the attachment of proteins, cell signalling, ion channel regulation, pathogen entry and many more[2]. The extent of phase separation in membranes is modulated not only by lipid composition and structure, but also by various other physicochemical factors, such as temperature, pH, and the ionic strength of the environment[3]. The latter in particular is an important factor known to alter various structural as well as functional properties of membranes. The interactions between lipids and ions modulate the local and global properties of the lipid bilayer such as thickness, packing, phase transition temperature, acyl chain ordering, headgroup tilt or swelling[4–7], but also take part in the regulation of ion channels, and signal transduction[4,8,9]. Several MD simulations have claimed that various ions have also a profound effect on the dynamics of the lipids in the membrane[10,11]. A number of ions, predominantly Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-, are found at the membrane interface with different intracellular and extracellular concentrations. The asymmetric distribution of lipids with different charge characters, as well as the different concentrations of various ions across the membrane, generate a suitable membrane potential for biochemical reactions[12,13].

Ions are known to affect the structure and dynamics of the hydration layer of lipid headgroups. The presence of Na^+ and Ca^{2+} ions significantly influences the hydration and orientation of the phosphate group of DPPC lipids[14]. Song et al. showed that slowing down of water molecules present within 10 Å

of the hydrophilic surface of lipid vesicles was modulated by the presence of different ions following the order of the well-known Hofmeister series[15]. At the same time, water molecules hydrating the lipids play a major role in determining membrane structure, organization, and lipid dynamics16. Intriguingly, recent MD simulations showed that hydrogen-bonded water network directly hydrating the membrane exhibits both structural and dynamical heterogeneity[17,18]. Clearly, both in native as well as in biomimetic membrane systems water-lipids-ions interactions are strongly interdependent. Hence numerous endeavors were made to elucidate the exact nature of the effect of a particular ion on lipid-water interactions and the resulting alterations of the lipid bilayer properties. Yet, the existing studies are mostly limited to molecular dynamics simulations, except few experimental works[7,14], which mainly addressed the effect of salts on the lipid-water interplay in the excess of water. While most common biological conditions indeed involve full hydration, nature exhibits several phenomena of "anhydrobiosis", where living organisms, such as tardigrades, nematodes, yeasts, bdelloid rotifer, seeds or pollens survive complete dehydration[19–21]. In addition, many biochemical processes, such as cell fusion or adsorption of macromolecules, involve both local variation of ion concentration as well as local and transient membrane dehydration[22–24].

Clearly, it is very important to obtain an explicit picture of how ions affect lipid-water interactions at the molecular level under different membrane hydration conditions. To date, however, the understanding of lipid-ion interplay in the presence and absence of water has remained rather poor due to the unavailability of suitable membrane hydration modulation technique. Exploiting the recently developed protocol of preparation of desiccation-tolerant membranes16, the present work pioneers the experimental study of ion-water-lipid interactions under low hydration conditions.

Herein, using fluorescence imaging and fluorescence recovery after photobleaching (FRAP) experiments we showed how Na^+ and Ca^{2+} ions affect the structure and lipid dynamics of phase-separated solid supported lipid bilayers (SLBs) at fully hydrated and dehydrated conditions. We addressed not only how a specific ion influences the lipid-water interactions, but also focused on how the entire process of lipid dehydration is modulated diffusion of phospholipids (14:1 PC) in membranes at various hydration conditions as well as with varying concentrations of Na^+ ions. We discovered that Na^+ ions play a crucial role in retaining the water hydration layer around the phosphocholine moiety in membranes subjected to dehydration. Surprisingly, Ca^{2+} cation, although comparable in size with Na^+, does not exhibit hydration structure-promoting capabilities. As such our findings highlight the unique characteristic of Na^+ ion-lipid interactions based on its specific charge density, binding affinity and hydration energy. Our study provides molecular level insights into a scarcely studied but important topic of the specificity of the ionic composition of membrane local environment modulating the hydration properties and lipid diffusion within the membrane.

Results and Discussion

Impact of Na^+ ions on the structure of SLBs

To understand how the ion-lipid-water interactions relate to the structure and lipid dynamics in biomimetic cell membranes, SLBs were reconstructed from 14:1 PC, egg SM and cholesterol and characterized with fluorescence imaging and FRAP experiments. At room temperature, the prepared lipid membranes undergo a prominent phase separation due to the considerable difference in 14:1 PC and SM hydrophobic chain lengths and packing. Unsaturated 14:1 PC forms L_d phase, saturated SM forms Lo domains, while cholesterol partitions in both phases, with a strong preference for the L_o phase. The membranes were prepared either in Milli Q water or in a buffer with the addition of 5 mM up to 1.5 M of NaCl. As the pH of the buffer has a prominent effect on the phase separation in lipid membranes[25], 10 mM HEPES buffer was used to keep the pH of the medium constant at pH=5.2, equal

to that of Milli Q water (see experimental section). Representative confocal images of the phase-separated SLBs prepared in Milli Q water as well as in buffers of different composition are shown in Figure 1A. Qualitatively, the size of L_o domains (black patches) for SLB prepared in Milli Q water (sample #1) is the same as for the SLB prepared in 10 mM HEPES buffer (sample #2). Similarly, the domain size of SLB prepared in 150 mM NaCl solution (sample #5) is very similar to that prepared in 10 mM HEPES-150 mM NaCl buffer (sample #6). These observations indicate that HEPES salt itself does not have a noticeable effect on the phase separation of lipids in the reconstructed SLBs. At the same time, the average domain size in SLBs hydrated with buffer containing NaCl (sample #6) is significantly higher than for the SLBs hydrated without the addition of NaCl (sample #2). Quantitative analysis confirms the strong dependence of the phase separation architecture on the NaCl content - the domain size and domain area% (percentage of area covered by the L^o domains relative to the total area of an image) increase significantly with an increase in NaCl concentration (Figure 1B-C). The average size of domains for SLBs prepared in Milli Q and in 10 mM HEPES – 1.5 M NaCl buffer are 0.45 ± 0.15 μm^2 and 3.79 ± 0.36 μm^2 respectively, showing over an eightfold increase. Similarly, the percentage of area occupied by the L_o phase domains increases with an increase in NaCl concentration (Figure 1C). Between SLBs prepared in Milli Q and in 10 mM HEPES – 1.5 M NaCl buffer the area occupied by the L_o phase increases over 3 times.

To further corroborate that the increase in domain size is caused solely by the addition of NaCl, we

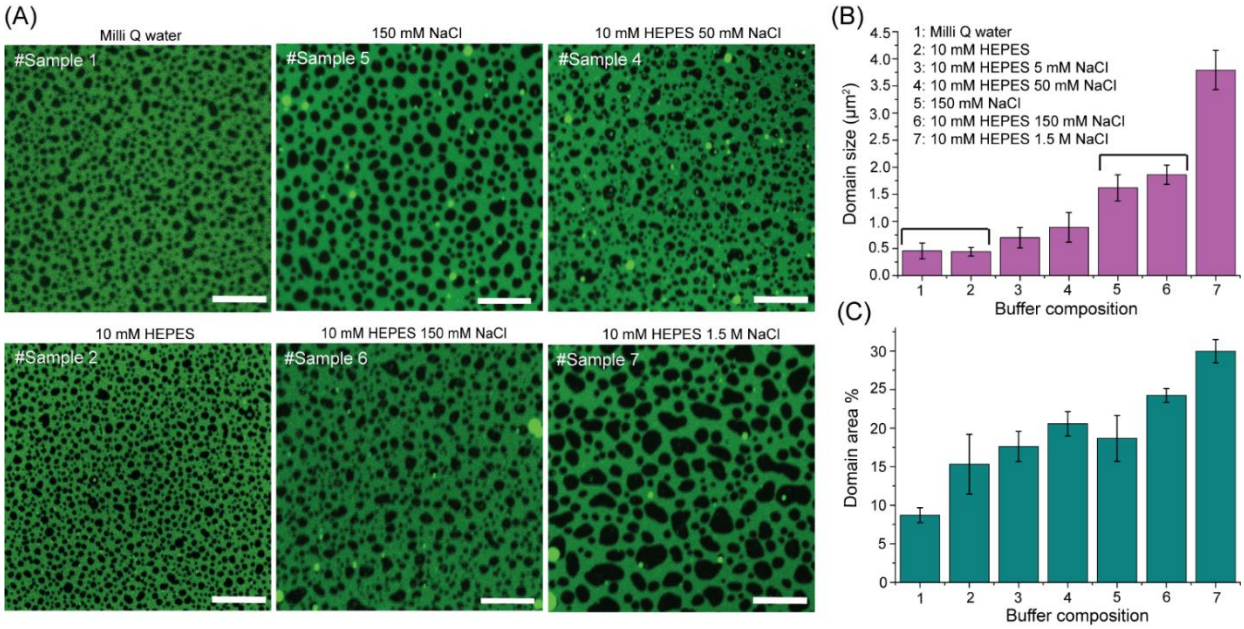

Figure 1. (A) Fluorescence images of phase-separated SLBs (L_d phase - green; Lo domains - black) prepared in Milli Q water and HEPES buffer with various NaCl concentrations. Scale bar represents 10 μm. Dependence of the L_o phase domain size (B) and percentage of area occupied by the L_o phase (C) on the buffer composition. The error bars reflect standard deviations calculated from 10 images (50 x 50 micrometers) from each of at least three samples with a specific buffer composition.

prepared SLB in Milli Q water and imaged it before and after replacing the water by 10 mM HEPES – 150 mM NaCl buffer (Figure S1A). Directly upon buffer replacement, the domain size did not increase considerably, but when imaging after ~20 hours, significantly (nearly 4 times) bigger domains were present. The difference in domain size (~4x) is fully consistent with domain size variation observed in membranes prepared directly in Milli Q water and in HEPES/NaCl buffer (sample #1 vs sample #6, Figure 1B). For the reference sample, which was kept in Milli Q water, the domains grew merely 1.5 times over the same time span. Likewise, the area occupied by domains increased by ~68% upon buffer change implying that more lipids become phase separated after introducing NaCl. The area% of

domains for the reference SLB remained unchanged (Figure S1B) – the existing domains simply merged with no appearance of new domains.

Increase in the L_o domain size and area occupied by L_o phase shows that at higher NaCl salt concentrations lipids exhibit stronger phase separation. On the contrary, at lower salt concentration, lipids have the tendency to be mixed. At the experimental pH (~5.2), HEPES acts as a monoanionic species. Hence the solution around SLBs contains Na^+, Cl^- and $HEPES^-$ ions. MD simulations showed that Cl^- hardly penetrates into the bilayer due to its larger size compared to Na^+. Instead, Cl^- ions remain mostly in the water phase and weakly interact with the choline group of PC lipids[26]. Analogously, $HEPES^-$ is also expected to be prevalent in the aqueous phase without much interaction with the membrane. This is consistent with our observation of no significant difference in domain size for SLBs prepared in the presence and absence of HEPES for a constant NaCl concentration (Figure 1, samples #1 vs #2 and #5 vs #6). Evidently, Na^+ ions are the key players that influence the degree of phase separation in SLBs. It has been suggested that Na+ ions bind to the lipid head groups exposed to water and reduce the electrostatic repulsion and enhance the van der Waals interactions between hydrophobic tails[27,28]. As Na^+ concentration increases, electrostatic repulsion between similar lipids decreases making same type lipid (PC-PC and SM-SM) interactions more favourable, thereby enhancing phase separation at higher Na^+ concentrations (Figure S2 and Supplementary Note 1).

It is clear that in water-rich conditions Na^+ ions modulate phase separation in lipid bilayers. The question arises whether Na^+ ions have the same ability when hydration conditions are altered. To this end, SLBs with varying NaCl concentration were imaged after removing bulk buffer and equilibrating them to relative humidity (RH) of 85% (see Experimental section in SI). In such conditions only a single hydration layer around lipid head groups is present, comprising of about 12 water molecules coordinated by a single PC lipid[16,29]. No structural changes were observed for the SLBs prepared in buffer solutions of different salt concentrations apart from domain size variation similar to that observed for fully hydrated membranes (Figure S3). We note, however, that mechanical stability was lower for dehydrated membranes prepared in buffer with low Na^+ content (see Supplementary Note 2).

Impact of Na^+ ions on the dynamics of lipids in SLBs

Given that structure of membranes and affinity for phase separation is strongly altered by the presence of Na^+ it is expected that it may also have a prominent effect on the lateral dynamics of lipids within the membrane. In earlier FCS studies on fully hydrated, single phase POPC bilayers slowing down of lipid mobility was observed with increasing NaCl concentration[10]. On the other hand, MD simulations of lipid-ion interactions in POPE bilayers suggested that presence of cations leads to decrease in membrane fluidity, likely due to ion-induced lipid dehydration[11]. Despite a few studies investigating the role of ions affecting lipid mobility at fully hydrated conditions, the influence of ions on lipid mobility at lower hydration conditions has so far remained unexplored. We thus examined the dynamics of the L_d phase lipids both in fully hydrated and in dehydrated phase-separated membranes. Exemplary FRAP traces are shown in Figure 2A. For fully hydrated membrane, the diffusion coefficient (D) remained similar within the error bars with increase in NaCl concentration (Figure 2B, magenta squares). The diffusion coefficient varied in the range of 2.3 – 2.8 $\mu m^2/s$ for SLBs prepared in Milli Q water to 10 mM HEPES – 150 mM NaCl buffer. Only for 1.5 M NaCl D was reduced by about 30% of the average value for other buffer compositions. The mobile fraction was high (>90%) and also did not change with NaCl concentration (Figure 2C, magenta squares).

While for fully hydrated membranes presence of Na^+ ions has little to no effect on lipid mobility, for dehydrated membranes the picture is drastically different. The membranes prepared in different

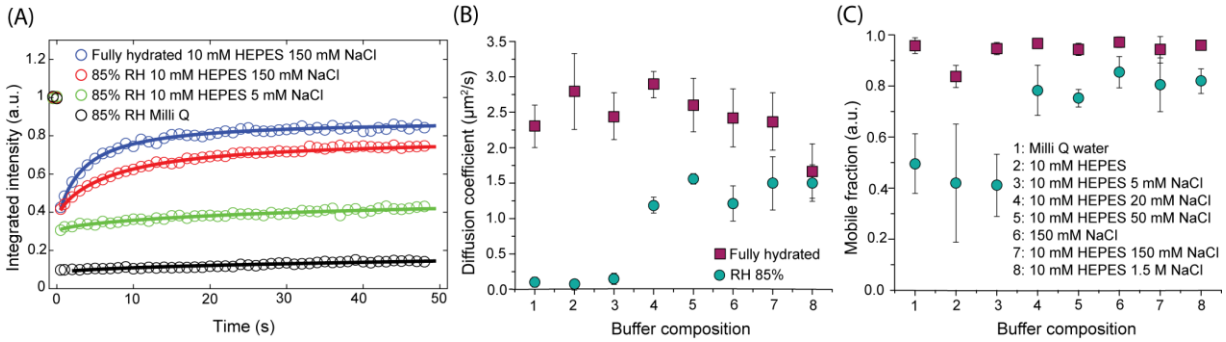

Figure 2. (A) FRAP traces for membrane in full hydration state and for membranes equilibrated to 85% RH. Diffusion coefficient (B) and mobile fraction (C) of L_d phase lipids at fully hydrated condition and at 85% RH condition for SLBs prepared in HEPES buffers of different NaCl concentrations. Each data point is an average of at least 10 values obtained from each of minimum two samples. The error bars denote the standard deviation of an average value.

buffers were exposed to and carefully equilibrated with the environment of high relative humidity (~85%). Remarkably, in the absence of Na^+ ions lipids become nearly immobile, D reaches very low value of <0.2 $\mu m^2/s$ (Figure 2B, green circles). Similarly, L_d lipids exhibit very little mobility in the membrane exposed to buffer containing low (5 mM) concentration of NaCl. However, for the membranes exposed to higher NaCl concentrations (≥20 mM), lipid mobility is significantly higher reaching value of about 1.5 $\mu m^2/s$ (vs ~2.5 $\mu m^2/s$ observed in fully hydrated membranes). A similar trend is observed for the mobile fractions (Figure 2C, green circles). For the membranes containing little or no NaCl, mobile fractions (MF) are considerably lower (MF~45%) than for the membranes containing more NaCl (MF>80%). Here we recall that PC lipids exposed to high RH, close to 100%, are hydrated with a single hydration shell containing about 12 water molecules[29–31]. For the membranes equilibrated with the RH of 85%, this hydration shell starts to be affected and becomes unstable[16]. Based on the changes of D, it is evident that as soon as bulk hydration is removed and the first hydration shell starts to disintegrate, the presence of Na^+ ions is crucial for the lipids to maintain their mobility.

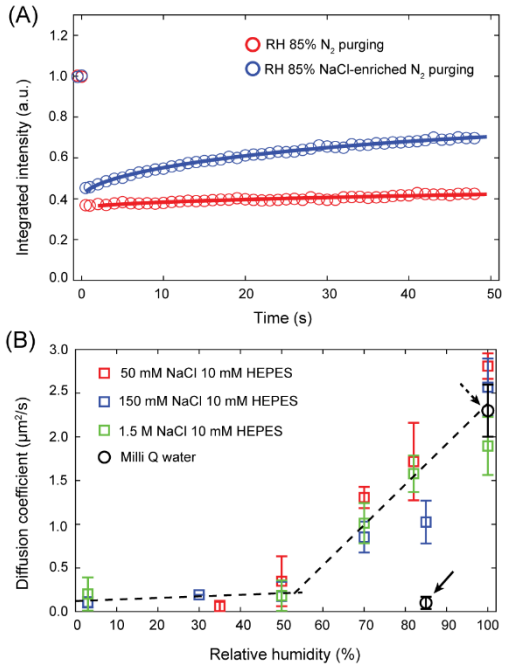

Figure 3. (A) FRAP traces for partially dehydrated SLB prepared in HEPES buffer and subsequently purged with wet N_2 gas enriched with NaCl. (B) Diffusion coefficients of L_d phase lipids at different hydration conditions for SLBs prepared in HEPES buffer with the addition of 50 mM (red), 150 mM (blue) and 1.5 M of NaCl (green). The D values for SLB prepared in Milli Q water at fully hydrated condition and at 85% RH (highlighted by black arrows) are shown by black circles for comparison.

To underpin the key role of Na^+ in promoting the lipid mobility after bulk dehydration, an SLB prepared in 10 mM HEPES buffer was dehydrated to 85% RH followed by 6 h of purging of wet N_2 gas through a buffer solution containing 150 mM NaCl. The wet N_2 gas contains tiny droplets of the buffer, which over time blend with the hydration layer of the membrane. While initially lipids in the SLB exhibited no mobility, after 6 h of purging with the N_2 rich in buffer aerosol, a clear difference in the fluorescence recovery (Figure 3A) was observed with an increase of D from 0.03 ± 0.03 $\mu m^2/s$ to 0.24 ± 0.07 $\mu m^2/s$.

It may seem that the sole presence of Na^+ ions is sufficient for the lipids to maintain their lateral mobility in water scarcity conditions. To verify such a possibility we determined the diffusion coefficients at varying membrane hydration levels for SLBs prepared in HEPES buffer with an addition of 50 mM, 150 mM and 1.5 M of NaCl. Figure 3B shows the extracted diffusion coefficients as a function of membrane hydration level. Interestingly, the change of D follows the same trend, irrespective of the salt concentration. D drops abruptly at the initial stages of dehydration and then below approximately 50% RH it remains largely unchanged. The gradual disintegration of the lipid hydration shell leads to a sharp decline in the lateral diffusion coefficient of PC lipids, underlining that the presence of the hydration shell is absolutely necessary for the lipids to maintain their mobility at mild dehydration conditions, in agreement with our previous work[16]. However, this experiment unambiguously shows that Na^+ ions alone cannot facilitate lipid mobility once dehydration progresses. Instead, Na^+ ions and water molecules need to work in tandem to support lipid mobility when the membrane is subjected to mild dehydration.

To clear up the molecular picture and to find an explanation for the extraordinary ability of Na^+ ions to shape lipid mobility after dehydration, it is important to understand how various ions bind to PC lipids. Based on the different MD simulation studies, it is generally accepted that Na^+ ions can penetrate bilayer interfacial region and localize in the vicinity of the phosphate and carbonyl oxygens of the PC head group[31,32]. Consequently, Na^+ as well as phosphate and/or carbonyl oxygens become partially dehydrated. According to the free energy of ion binding calculations, the most stable state for Na^+ in the vicinity of a fully hydrated lipid bilayer, is to be fully hydrated in bulk water with a hydration coordination number of 5[33]. However, Na^+ can also attach to 4 water molecules and one lipid oxygen with energy higher by only 1-2 kcal mol-1 (Figure 4B). Thus, there is little energetic penalty for the Na+ ions to be (at least partially) dehydrated. Considering other local minima of ion binding free energy, Na^+ ions bound to lipid bilayer can coordinate up to 5-6 oxygen atoms, 1-3 from phosphate/carbonyl oxygen atoms from same or neighbouring lipid molecules and the rest (2-4) of the coordination is filled by water molecules. In fully hydrated conditions, the binding of the Na^+ ions with PC is in a dynamic equilibrium with a maximum residence time of 10-4 s at the polar group[34]. This could explain why at fully hydrated condition, D does not change significantly with a moderate increase in Na^+ concentration. Upon removal of the bulk water due to the unavailability of excess water the binding probability of Na^+ to the lipids and the residence time of Na^+ at the bilayer interface will increase. In order to keep the free energy of binding lowest, Na^+ ion bound to phosphate oxygen (OP) will preferably coordinate 2-5 water molecules to fulfill its coordination instead of binding with other lipid oxygens. Further membrane dehydration leads to increased coordination of Na^+ by lipids' oxygens.

It is widely accepted that for zwitterionic PC lipids, the first hydration shell around the phosphocholine moiety, contains ~6-7 water molecules. This hydration shell is often referred to as water clathrate cage/structure, however given its highly dynamic nature it should be viewed rather as a fluid hydration shell. This hydration layer is held in place through OP-H_2O H-bonds and van der Walls interactions[28]. The water molecules attached to OP-bound Na^+ ion, being polarized and slower[35], also bind to other water molecules through H-bonds. Thus, with the introduction of Coulombic interactions between OP-Na^+-H_2O, together with the OP-H_2O and H_2O-H_2O hydrogen bonds, the PC-Na^+-water clathrate

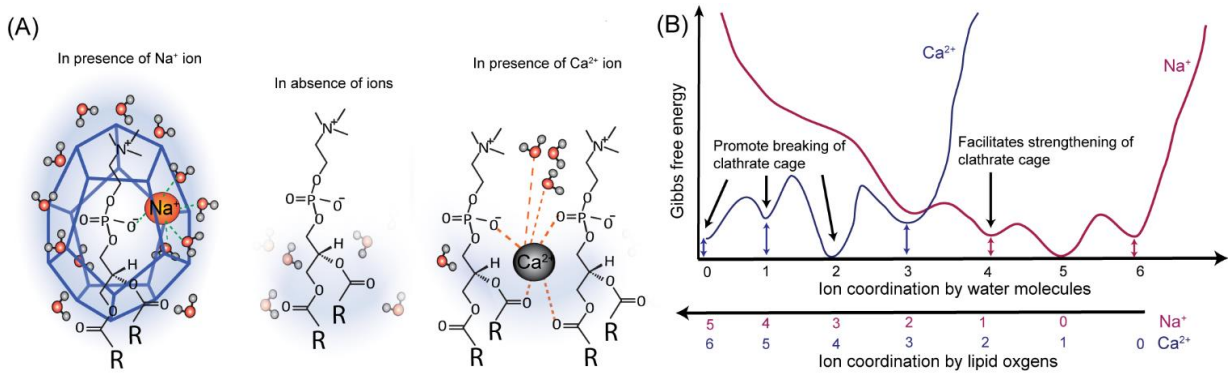

Figure 4. (A) Illustrative pictures of the hydration structures of PC headgroups equilibrated to 85% RH after removal of bulk water in absence of ions as well as in presence of Na^+ and Ca^{2+} ions, (B) Comparative, simplified schematic diagram of free energies of ion hydration for different coordination scenarios (by water molecules and lipid oxygens) for Na^+ and Ca^{2+} ion. The scheme is based on the MD simulations data of free energies for Na^+ and Ca^{2+} ions at various lipid and water coordination by Yang et al.[33].

complex becomes stronger than simple PC-water clathrate complex. In other words, sodium ions strengthen and stabilise the hydration structure around the PC moiety, preventing immediate disintegration of this hydration shell upon bulk dehydration (Figure 4A). As the membrane becomes dehydrated further, Coulombic interactions between lipids, Na^+ ions and water molecules fail in stabilising the hydration layer leading to a sharp decline in lipid mobility. The same trajectory of changes in D, regardless of Na^+ concentration, highlights that the role of Na^+ ions is only related to holding the adequate number of water molecules around the phosphocholine group, but not controlling the lipid mobility directly. Consequently, Na^+ ions themselves do not promote lipid mobility at very low hydration condition (≤50% RH). Hence, both sodium and water molecules complement each other to promote lipid mobility after the removal of the outer hydration layer of the lipids. On the other hand, in absence of Na^+ ions, the hydration structure, bound by only weak van der Waals interactions and H-bonds, falls apart already after the removal of bulk water, resulting in very low lipid mobility at 85% RH (Figure 4A).

Impact of Ca^{2+} ions on the dynamics of lipids in SLBs

An additional reason for high mobility at ~85% RH could be that the presence of ions polarizes the water around the lipid head groups, which in turn shields the electrostatic repulsion between adjacent lipids better. Naturally, in that case, divalent ions, due to higher charge density, should be more efficient in supporting lipid mobility after dehydration. To verify this possibility, we measured the lateral diffusion of lipids at fully hydrated and dehydrated conditions for SLBs prepared in 10 mM HEPES buffer containing 150 mM of $CaCl_2$. Ca^{2+} is a divalent ion but has a very similar ionic radius to Na^+. The average domain size in the SLB prepared in buffer containing 150 mM of CaCl2 was 0.82 ± 0.13 μm^2, which is smaller (approximately by a factor of 2) than the average domain size in SLB prepared with 150 mM of NaCl (Figure 5A).

The D value at fully hydrated condition was found to be 1.18 ± 0.22 $\mu m^2/s$, which is around half of the D observed for the same concentration of Na^+ ions. Consistently, the mobile fraction was also slightly lower (~85%) than that in the presence of NaCl. Strikingly, after the removal of bulk water, lipid mobility was almost ceased already at 85% RH (D = 0.11 ± 0.08 $\mu m^2/s$), similar to the SLB hydrated with Milli Q water (Figure 5B). This indicates that Ca^{2+} ions are unable to contribute to the stabilisation of the hydration layer around lipid head groups. Previous studies showed that the binding constant of Ca^{2+} to the membrane is much higher than for Na^+ and that Ca^{2+} preferentially binds to the lipid oxygens rather than to water molecules, leading to dehydration of the phosphate region[14]. Similar conclusions

have been reached in infrared studies on bulk lipid paste in different hydration conditions and containing various ions[36].

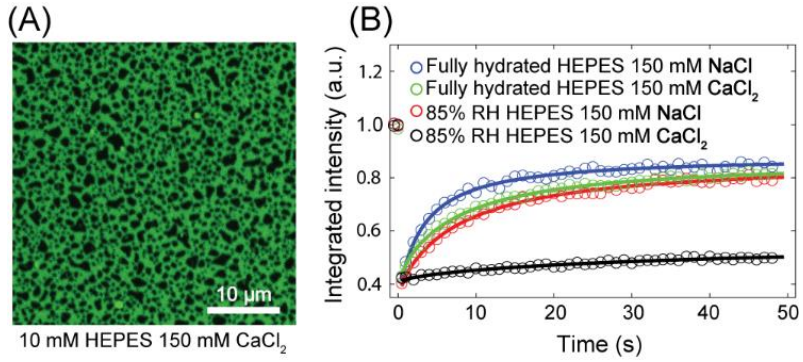

Figure 5. Figure 6. (A) Fluorescence image of the phase-separated SLB prepared with addition of $CaCl_2$. (B) Comparison of FRAP traces for fully hydrated and equilibrated to 85% RH SLBs prepared with NaCl and $CaCl_2$.

Recent MD simulations showed that the lowest free energy state for Ca^{2+} is when it binds to 4 lipid oxygen and 2 water molecules to have its coordination number of 6 filled (see Figure 4B)[33]. The other energy minima, 2-4 kcal mol-1 higher in energy than the global minimum, correspond to binding with 3-5 lipid oxygens with total coordination number 4-6, leaving the number of water molecules attached to Ca^{2+} close to 0. This in return leads to destabilisation of the complex hydration structure, which can no longer anchor to the phosphate oxygens. Hence, it is of no surprise that in the presence of Ca2+ ions the hydration shell disintegrates immediately after bulk water removal causing very low lipid mobility already at 85% RH. Moreover, preferential binding of Ca^{2+} to phosphate and carbonyl oxygens of lipids promotes the formation of Ca^{2+} complex with more than one lipid[7,37,38], which also explains the lower (approximately 2-fold) diffusion coefficient in the presence of 150 mM of Ca^{2+}, already in full hydration conditions.

Evidently, strengthening of lipid hydration structure in the presence of Na^+ is not related to the size or charge of the cation, but it is the hydration energy and the membrane-binding energy of the ion that play the key role here. Last but not least, the divergent action of $CaCl_2$ with respect to NaCl also confirms that Cl^- has no noticeable effect on the dynamics of the membrane constituents, in agreement with the MD simulation, which showed that Cl^- being larger in size mostly resides in the bulk water[31].

Conclusions

In summary, we demonstrated that a cooperativity between water and sodium ions is an essential factor that controls lipid mobility in membranes under water depletion conditions. Na^+ ions reveal their importance already in fully hydrated membranes, in which the extent of phase separation increases significantly with an increase of Na^+ concentration. At the same time, in these conditions, Na^+ ions have no significant effect on the mobility of lipids. In stark contrast, the true capabilities of Na^+ ions are revealed upon membrane dehydration, when they actively penetrate the inter-lipid head group region. There Na^+ ions stabilise the hydration shell structure around the lipid head groups, thereby facilitating lipid diffusion. However, we emphasize that the ability of Na^+ to promote lipid dynamics after membrane dehydration does not nullify the principal role of water in supporting lipid mobility. At very low hydration conditions, where not enough water molecules are present to form the hydration layer, Na+ ions alone fail to retain lipid mobility even at high ion concentration. Clearly, it is a cooperative effect, in which down to a certain dehydration level water and Na^+ ions work in a concerted manner in promoting lipid diffusion. The uniqueness of Na^+ ions is evident when compared to the activity of divalent Ca^{2+} cation, which despite having similar ionic radius, has the tendency to destabilise the hydration structure around lipid head groups due to its greater binding affinity to lipid oxygens than

to remnant water molecules. Clearly, it is not the charge and the ability to polarise the environment but purely the competition between ion hydration and ion binding to lipid oxygens that cause such a divergent activity of Na^+ and Ca^{2+} ions. This work unveils the important and unique role Na^+ ions in modulating the membrane structure as well as lipid dynamics and provides knowledge, which is crucial for understanding the mechanisms of biological processes involving temporary membrane dehydration, such as cell fusion, adsorption of macromolecules, viral entry or fertilisation.

Author Contributions

MC: Conceptualization, methodology, investigation, formal analysis, validation, visualization, writing – original draft; EK: methodology, writing – review and editing; HOR: methodology, writing – review and editing; LP: Supervision, validation, funding acquisition, writing – review and editing.

Conflicts of interest

There are no conflicts of interest to declare.

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