Article

# Specific Ion Binding at Phospholipid Membrane Surfaces

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Ⓢ *Supporting Information*

ABSTRACT: Metal cations are ubiquitous components in biological environments and play an important role in regulating cellular functions and membrane properties. By applying metadynamics simulations, we have performed systematic free energy calculations of Na^+, K^+, Ca^{2+}, and Mg^{2+} bound to phospholipid membrane surfaces for the first time. The free energy landscapes unveil specific binding behaviors of metal cations on phospholipid membranes. Na^+ and K^+ are more likely to stay in the aqueous solution and can bind easily to a few lipid oxygens by overcoming low free energy barriers. Ca^{2+} is most stable when it is bound to four lipid oxygens of the membrane rather than being hydrated in the aqueous solution. Mg^{2+} is tightly hydrated, and it shows hardly any loss of a hydration water or binding directly to the membrane. When bound to the membrane, the cations' most favorable total coordination numbers with water and lipid oxygens are the same as their corresponding hydration numbers in aqueous solution, indicating a competition between ion binding to water and lipids. The binding specificity of metal cations on membranes is highly correlated with the hydration free energy and the size of the hydration shell.

Na$^+$ coordinated to water oxygens and lipid oxygens
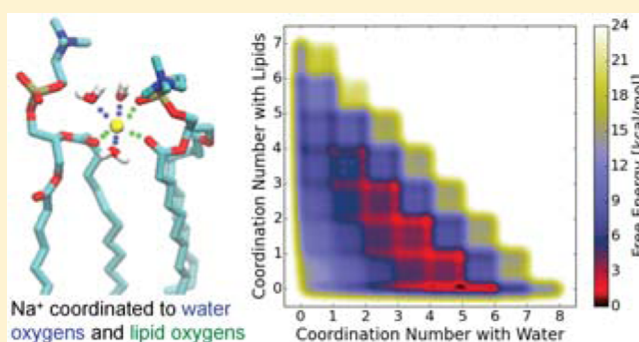
1. INTRODUCTION

Specific ion effects on biological systems have attracted great attention in recent decades.[1] In general, specific effects for cations are less pronounced than those for anions when ion–water interactions are dominant.[2] However, for biological membranes in physiological environments, the interactions between metal cations and charged lipid headgroups are also essential and significant. Metal cations bound to membranes have been found to regulate membrane properties and membrane functioning, and such regulations deeply depend on ion specificity.[3,4] Therefore, understanding the specific interactions of metal cations with membranes is of great fundamental importance.

Several experiments suggest that metal cations are bound to the phosphate and carbonyl regions of phospholipid membranes.[5−8] While bound to membranes in aqueous solution, a hydrated metal cation will lose one or more water molecules from its first hydration shell, resulting in several possible bound configurations.[9,10] Although the binding constant and Gibbs free energy of metal cations can be estimated from experiments,[9] it is difficult to study in detail the relative stabilities of different bound states. Molecular dynamics (MD) simulations have been widely employed to investigate the role of metal cations in solvated phospholipid membranes from an atomic point of view.[8,10−16] Nevertheless, the theoretical study of ionic

binding to membranes is a computationally demanding task due to the long simulation times required to probe ion–membrane association and dissociation events. In addition, various bound configurations are separated by high free energy barriers, making it difficult for MD simulations to sample them adequately.[17] Free energy calculations using enhanced sampling techniques provide a method to address this problem. However, despite the significant progress in free energy calculations achieved in recent years,[18] to the best of our knowledge, the binding free energy landscape of metal cations on membrane surfaces is still lacking. This is partially due to the difficulty in applying appropriate sampling techniques to address this problem and is also because of the complexity of membrane environments, making the determination of the proper collective variables a difficult challenge.[19]

In this work, we have performed a systematic free energy calculation of the binding states of metal cations at phospholipid membrane surfaces. The specific interactions of biologically relevant cations Na^+, K^+, Ca^{2+}, and Mg^{2+} with membranes are revealed and interpreted from a free energy perspective. The competition between ion–water and ion–membrane binding is also studied. Although such competition
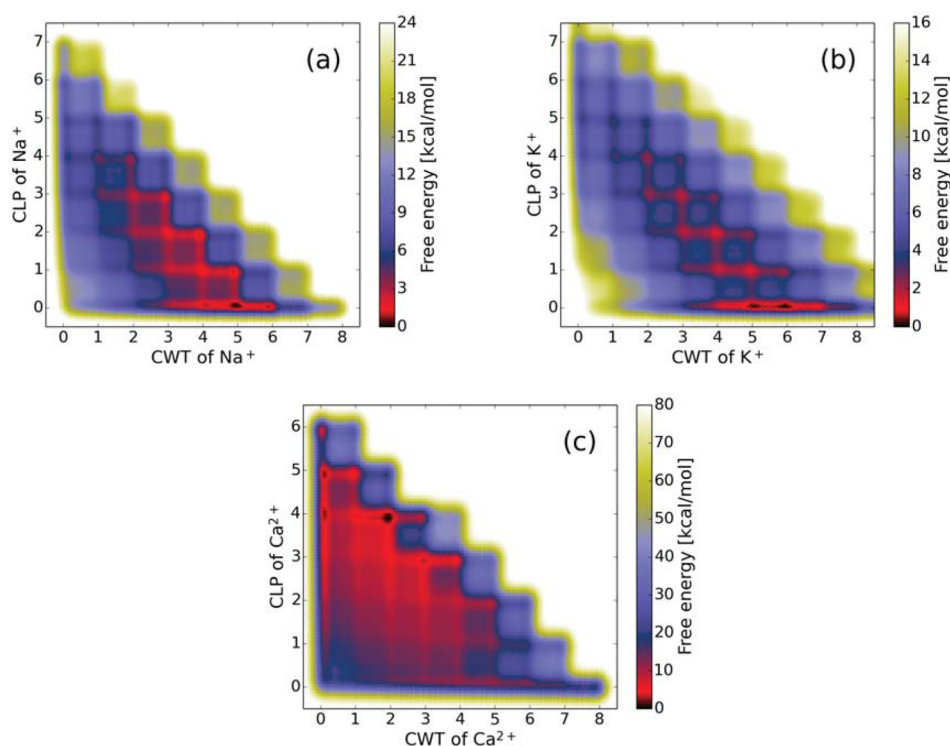
Figure 1. Free energy surface as a function of the coordination number of water oxygens (CWT) and the coordination number of lipid oxygens (CLP) for Na^+ (a), K^+ (b), and Ca^{2+} (c) at DMPC membrane surfaces.

has been discussed before,[12,14] here we provide, for the first time, a quantitative free energy characterization of the relevant ion binding states to water and lipids. Our work provides a general methodology to explore the free energy landscapes for ions at complex biological interfaces that can be extended to study other interactions of interest between ions and charged headgroups in colloidal chemistry and biology.[20]

2. METHODS

Applying proper enhanced sampling methods and defining proper collective variables (CVs) are essential for the success of free energy calculations. Recently, Jämbeck et al. showed that missing an essential CV in the construction of free energy profiles for membrane partitioning of a solute can lead to divergences in the permeability of the solute.[21] While the normal direction to the plane of the membrane, Z, has been typically adopted as the reaction coordinate for the calculation of solute partitioning[21,22] and ionic permeation,[23,24] the problem of ion binding to membranes is more complex. Membranes are composed of a large number of lipid molecules with numerous conformations, and each lipid molecule has several binding sites at its headgroup, making it a challenge to define proper CVs to describe the interaction between ions and membranes. Moreover, ions are hydrated by water molecules, and the hydration level depends on the location of ions at the membranes' surface. Therefore, taking the interactions between ions and both lipid and water molecules into consideration could be a rational way to define CVs. A number of experiments[5−8] and simulations[8,10−16] have shown that metal cations directly coordinate with the oxygen atoms of phosphate (PO_4^-) and carbonyl ($C=O$) groups of lipid molecules, indicating that the oxygens in the lipid headgroup are the binding sites for metal cations. Accordingly, in this work, we defined two CVs to describe ion−membrane binding: the

coordination number between a metal ion and lipid oxygens (CLP) and the coordination number between a metal ion and water oxygens (CWT). Details of the definition of the coordination numbers are given in the Supporting Information.

Well-tempered metadynamics,[25] a variant of metadynamics[17,26] capable of enhancing the sampling of coordination numbers in multiple CV dimensions, was employed to calculate the free energy landscape of ion binding to the membrane. Four sets of two-dimensional (2D) well-tempered metadynamics simulations based on the above CVs were performed to calculate the free energy surfaces of Na^+, K^+, Ca^{2+}, and Mg^{2+} ions at neutral zwitterionic phospholipid membranes. CHARMM-GUI[27,28] was employed to generate four sets of lipid bilayer systems. Each system consisted of 50 dimyristoylphosphatidylcholine (DMPC) lipid molecules,[29,30] 2500 TIP3P water molecules,[31] a metal cation, and one or two Cl^- anions to neutralize the system. All simulations were performed using NAMD 2.9[32] together with PLUMED2 plugin[33] and the CHARMM36 force field.[34] The simulation parameters are described in the Supporting Information.

3. RESULTS

The resulting 2D free energy surfaces (FES) of metal cations bound to DMPC membranes are shown in Figure 1. A staircase pattern is present in the FES of Na^+ in Figure 1a. The Na^+ ion is considered to be in the aqueous solution at CLP = 0, where it can be hydrated by at most eight water molecules. When it is fully dehydrated (CWT = 0), Na^+ can be bound to at most seven lipid oxygens. Between these two extreme cases, Na^+ is bound to oxygens of both water and lipids. The fact that the states at local free energy minima are those with integer numbers for CLP and CWT validates our choice of CVs to explore the binding processes. The pattern shown in Figure 1a is a consequence of the competition between the binding of the

ion to lipids and to water. Indeed, paths of approximately the same binding free energy are found for integer values of CLP and CWT with the same total coordination number (CLP + CWT). The global minimum of the FES is at the (CWT = 5, CLP = 0) state, revealing that being hydrated by five water molecules in the aqueous solution is the most favorable state for Na$^+$. When Na$^+$ is bound to the membrane, the stable bound states are located in the region with CLP $\in$ [1,4] and CWT $\in$ [1,5], with the total coordination number (CLP + CWT) $\in$ [4,6]. A representative snapshot of a bound state of Na$^+$ coordinated with six oxygens is shown in Figure 2a.
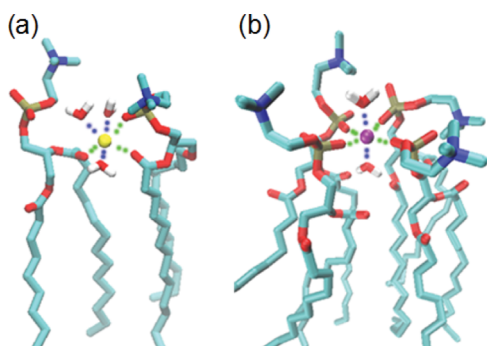

Figure 2. (a) A representative bound state for Na$^+$ coordinated to three lipid oxygens and three water molecules. (b) The most stable bound state for Ca^{2+} coordinated to four lipid oxygens and two water molecules. Na$^+$ is in yellow, and Ca^{2+} is in purple. The binding to lipid oxygens and to water oxygens is shown in green and blue, respectively.

Similar patterns are also present in the 2D FES of K$^+$ in Figure 1b, revealing similar binding behaviors of K$^+$ and Na$^+$ at aqueous membrane interfaces. Because of its larger atomic size, K$^+$ has a larger first coordination shell but a relatively lower surface charge density. The global minima states are at (5,0) and (6,0). Therefore, being hydrated by five or six water

molecules in the aqueous solution is most favorable for K$^+$. When bound to membrane surfaces, K$^+$ can be coordinated to more water oxygens and lipid oxygens than Na$^+$. The stable bound states for K$^+$ are in the region with CLP $\in$ [1,4] and CWT $\in$ [2,6], with the total coordination number (CLP + CWT) $\in$ [5,7].

While staying in the aqueous solution is most favorable for the monovalent metal cations, i.e., Na$^+$ and K$^+$, being bound to the membrane surface is the most stable state for Ca^{2+}. The global free energy minimum at (2,4) in Figure 1c indicates that Ca^{2+} is preferably bound to four lipid oxygens (Figure 2b) rather than being hydrated in the aqueous solution. In contrast to the stable bound states of Na$^+$ and K$^+$ with CLP $\in$ [1,4], those for Ca^{2+} are located in the region with CLP $\in$ [2,6], which suggests a significantly greater affinity of Ca^{2+} to lipid oxygens than that of monovalent metal cations.

The case of Mg^{2+} is different from the other ions considered. The bound states of Mg^{2+} to lipid oxygens (CLP $\geq$ 1) are separated from the unbound states in the aqueous solution (CLP = 0) by high free energy barriers and could not be appropriately sampled by our calculations. This is attributed to the high energy required for the partial dehydration of the first hydration shell of Mg^{2+}, which is shown in Figure 3d, where only the case with CLP = 0 is considered. The strong binding of Mg^{2+} to its hydration layer has been also reported recently by Allnér et al. They calculated the potential of mean force between Mg^{2+} and water oxygens and that between Mg^{2+} and phosphate oxygens and showed extremely high free energy barriers for water dehydration and direct phosphate binding.[35]

A quantitative representation of the above FES is given in Figure 3, where the free energies are plotted as a function of CWT at various CLP, and in Table 1, where the most relevant binding states are selected. As shown in Figure 3a, several hydration free energy basins have been found for CLP = 0, where Na$^+$ is unbound to membranes. The most stable hydration state for Na$^+$ is hydration with five water molecules,
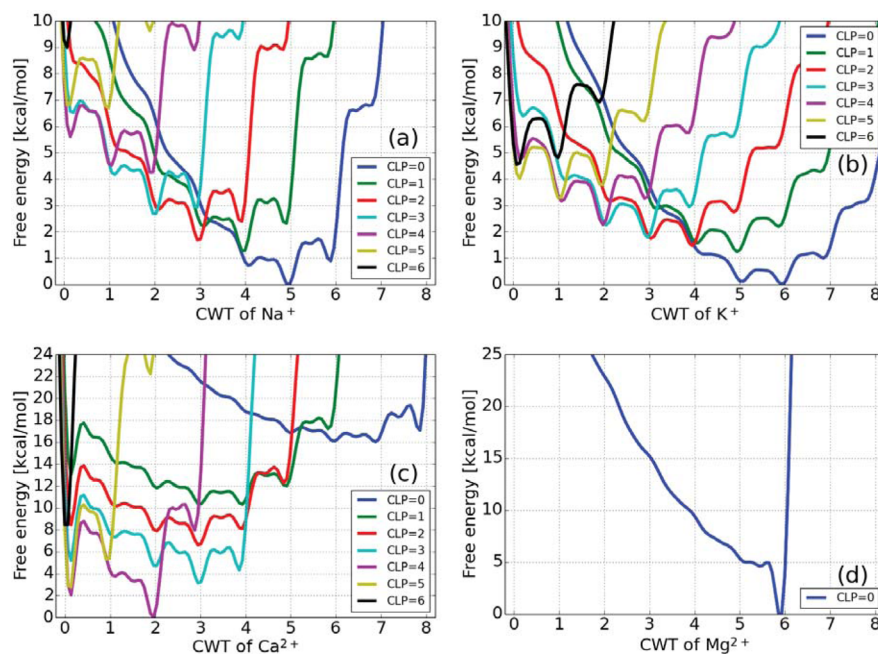

Figure 3. Free energy as a function of CWT at various CLP for Na$^+$ (a), K$^+$ (b), Ca^{2+} (c), and Mg^{2+} (d). The data for Na$^+$, K$^+$, and Ca^{2+} are extracted from Figure 1. For Mg^{2+}, only the case with CLP = 0 is considered.

Table 1. Free Energies for the Lowest States of Na$^+$, K$^+$, and Ca^{2+} with Respect to the Global Minimum

ion	binding state			F [kcal/mol]
	CLP	CWT	CLP + CWT	
Na$^+$	0	5	5	0.0
	1	4	5	1.28
	2	3	5	1.69
	3	2	5	2.69
K$^+$	0	6	6	0.0
	0	5	5	0.11
	1	5	6	1.23
	2	4	6	1.46
	3	3	6	1.78
	4	2	6	2.26
Ca^{2+}	4	2	6	0.0
	4	0	4	2.07
	5	0	5	2.74
	3	3	6	3.13

in agreement with the hydration number measured by experiments.[36,37] The ion is considered to be bound to the membrane when CLP > 0. For Na$^+$ at low binding, i.e., for CLP = 1, 2, 3, the corresponding minimum free energies are located at hydration levels CWT = 4, 3, 2, respectively, with 5 being the total coordination number, which is the same as the experimental hydration number.[36,37] At high binding, i.e., for CLP = 4, 5, 6, the most stable hydration levels are CWT = 2, 1, 0, respectively, with 6 being the total coordination number. The states with CLP = 1, 2, 3 and a total coordination number of 5 or 6 are the lowest free energy bound states, which are 1 to 2 kcal/mol higher than the global minimum at CLP = 0. A quantitative characterization of the free energies of those states is given in Table 1. Consequently, these are the most likely bound states of Na$^+$ that one can expect to find in a DMPC membrane immersed in a NaCl aqueous solution.

As shown in Figure 3b, the profiles for K$^+$ are similar to those for Na$^+$. Hydration with 5 or 6 water molecules is the most stable state for K$^+$ at CLP = 0, in agreement with the experimental hydration number.[36,37] Free energy barriers constraining K$^+$ at the stable hydration states (CWT = 5, 6) are smaller than those for Na$^+$ (CWT = 5), indicating that K$^+$ has a weaker affinity for water molecules. At low binding, i.e., for CLP = 1, 2, 3, the corresponding minimum free energies are located at hydration levels CWT = 5, 4, 3, respectively, resulting in a total coordination number of 6, which is the same as the experimental hydration number for K$^+$.[36,37] Note that the free energy barriers for the lowest energy bound states of K$^+$ are also smaller than those for Na$^+$, indicating that it releases more easily from lipid binding sites.

Although being hydrated in aqueous solution (CLP = 0) is the most favorable state for Na$^+$ and K$^+$, for Ca^{2+}, that state is ~16 kcal/mol higher than the global minimum, as shown in Figure 3c. This shows that staying in the aqueous solution is greatly unfavorable for Ca^{2+} compared to being bound to the membrane. In aqueous solution, the most stable hydration states for Ca^{2+} are CWT = 6, 7, consistent with the experimental hydration number.[38,39] The most stable bound state for Ca^{2+} is at (CWT = 2, CLP = 4), where the total coordination number is 6. Other stable bound states are at CLP = 3, 4, 5, with a total coordination number of 4−6, which are 2−4 kcal/mol higher than the global minimum (Table 1). These bound states are also the global minima of the FES,

which are 12−16 kcal/mol lower than the lowest energy unbound states at CLP = 0. For Ca^{2+}, we obtained a significantly stronger binding to phospholipid membranes over aqueous solution, in contrast to that for Na$^+$ and K$^+$, where the binding is weak, in agreement with experiment.[7] For the ions considered, the ion binding free energy has its minimum at the same total coordination number (CLP + CWT), irrespective of CLP or CWT, as shown in Table 1 and Figure S1 of the Supporting Information, and coincides with the hydration number in solution. This is a clear indication of the competition between ion binding to water and lipids and is responsible for the correlation between ion hydration and ion binding specificity at the membranes.

Figure 3d shows that in aqueous solution the hydration with six water molecules is the most stable state for Mg^{2+}, in accordance with experiments.[38,39] However, in contrast to the other cations that have several hydration free energy basins in solution, Mg^{2+} has only one basin at CWT = 6, and the corresponding hydration free energy barrier is very high (~5 kcal/mol) compared with that for Na$^+$, K$^+$, and Ca^{2+}. This is due to the strong binding of water molecules to Mg^{2+}, which makes the change of its hydration state to other hydration states (CWT < 6) and bound states (CLP > 0) a rare phenomenon.

4. CONCLUSIONS

In summary, we have provided a quantitative characterization of the binding states of biologically relevant metal cations (Na$^+$, K$^+$, Ca^{2+}, and Mg^{2+}) at DMPC phospholipid membranes. With the help of well-tempered metadynamics simulations, we have calculated the free energies of the binding states, defined by the ion coordination to lipid (CLP) and water (CWT) oxygen atoms. Our results indicate that Na$^+$ and K$^+$ are more likely to stay in the aqueous solution and can easily bind to 1−3 lipid oxygens by overcoming free energy barriers of 1 to 2 kcal/mol. Ca^{2+} is most stable when it is bound to four lipid oxygens of the membrane, and the corresponding bound basin is ~16 kcal/mol lower than the unbound states in the aqueous solution. Mg^{2+} has a strong affinity to hydration water, which makes direct binding to the membrane difficult. When bound to the membrane, the cations' most favorable total coordination numbers with water and lipid oxygens are 5, 6, and 6 for Na$^+$, K$^+$, and Ca^{2+}, respectively. Such coordination numbers coincide with their corresponding hydration numbers in bulk, suggesting a competition between ion binding to water and lipids.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jctc.5b00540.

Detailed simulation parameters, free energy as a function of total coordination number (CLP + CWT), and free energy convergence (PDF).

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