Competing for the same space – protons and alkali ions at the interface of phospholipid bilayers

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Abstract (150 to 250 words)

Maintaining gradients of solvated protons and alkali metal ions such as Na^+ and K^+ across membranes is critical for cellular function. Over the last few decades both the interactions of protons and alkali metal ions with phospholipid membranes has been studied extensively and the reported interactions of these ions with phospholipid headgroups are very similar, yet few studies have investigated the potential interdependence between proton and alkali metal ion binding at the water-lipid interface. In this short review, we discuss the similarities between the proton–membrane and alkali ion–membrane interactions. Such interactions include cation attraction to the phosphate and carbonyl oxygens of the phospholipid headgroups that form strong lipid-ion and lipid-ion-water complexes. We also propose potential mechanisms that may modulate the affinities of these cationic species to the water-phospholipid interfacial oxygen moieties. This review aims to highlight the potential interdependence between protons and alkali metal ions at the membrane surface and encourage a more nuanced understanding of the complex nature of these biologically relevant processes.

Keywords: protons, hydronium ions, alkali ions, ion lipid interactions, membranes, lipid bilayers

1. Introduction

Both protons and alkali metal ions are critical in a wide range of cellular processes such as energy production and metabolism, the import of nutrients, osmotic regulation, nerve conduction and cell signalling. Maintaining a proton gradient across membranes in an energy efficient manner requires the protons to be confined to a restricted space close to the bilayer preventing them from diffusing to extra- or intracellular space. The molecular mechanism of (solvated) protons moving along membrane surfaces has been investigated for many decades (Agmon et al., 2016; Gutman & Nachliel, 1990; Heberle, 2000; Mulkidjanian & Cherepanov, 2006; Mulkidjanian et al., 2006). Similarly, the interactions of alkali ions with membranes has been studied extensively (Leontidis, 2017). While there is still an ongoing controversy whether alkali ions interact with membranes at physiological concentrations (Catte et al., 2016), there is growing evidence that they can alter the morphology and fluidity of membranes (Binder & Zschornig, 2002; Böckmann et al., 2003; Garcia-Manyes et al., 2005; Vorobyov et al., 2014) and thus indirectly affect cellular processes.

Both protons and alkali ions are small cations that have been shown to bind to the water-lipid interface of phospholipid membranes and, through these water-ion-lipid interactions, have the capacity to change the structure and physico-chemical properties of membranes (Böckmann et al., 2003; Cordomí et al., 2009; Cranfield et al., 2016; Deplazes et al., 2017; Garcia et al., 2019; Garcia-Manyes et al., 2005, 2006; Koynova & Caffrey, 1998; Petelska & Figaszewski, 2002; Piantanida et al., 2017; Reif et al., 2017; Vácha et al., 2009) . As we will outline in this review, in many cases the reported findings on how protons and alkali ions interact with phospholipid membranes demonstrate remarkable similarities. Yet, to the best of our knowledge, there is very little research connecting these findings and even less on understanding the potential interdependence between the binding of protons and alkali ions to membranes. Note that the focus of this review is to highlight the potential overlap in the site at which protons and alkali ions bind to the water-lipid interface. A detailed discussion of the many, and often conflicting studies investigating the mechanism of proton diffusion along membranes, as well as the challenges in measuring this process is beyond the scope of this short review. For this, the reader is referred to reviews dedicated to these topics (Agmon et al., 2016; Leontidis, 2017; Medvedev & Stuchebrukhov, 2011; Mulkidjanian & Cherepanov, 2006; Mulkidjanian et al., 2006).

The remainder of this review is structured as follows. In sections 2 and 3, we separately discuss the interaction of protons and alkali ions to phospholipid membranes with a particular focus on the molecular mechanism and the 'site' of interaction. In section 4, we propose a competitive relationship between protons and alkali ions at the water-lipid interface and describe several potential mechanisms for how this competition may manifest itself. In addition, we discuss the implications of this competitive binding for experiments aimed at understanding proton migration or alkali ion–membrane interactions.

2. Interactions of (solvated) protons with membranes

Migration of protons along membranes has been observed in purple membranes and membranes reconstituted with bacteriorhodopsin (Alexiev et al., 1995; Brändén et al., 2006; J. Heberle & Dencher, 1992; Joachim Heberle et al., 1994; Nachliel et al., 1996; Scherrer et al., 1994) as well as phospholipid monolayers, bilayers and phsopholipid vesicles (Amdursky et al., 2019; Antonenko & Pohl, 2008; Gabriel et al., 1994; Gabriel & Teissie, 1996; Ojemyr et al., 2010; Sandén et al., 2010; Serowy et al., 2003). It has also been suggested that compared to the movement along the surface, the transfer of protons into the bulk is delayed (Alexiev et al., 1995; Cherepanov et al., 2004; Nachliel & Gutman, 1996; Scherrer et al., 1994; Zhang et al., 2012) . However, what constitutes the main energy barrier for surface-to-bulk release of protons remains unclear (Agmon et al., 2016) . While the detailed mechanism of this proton migration is yet to be determined, studies have suggested that the process is facilitated by one or more of the following: i) ionisable groups on the membrane surface, which can either be phospholipid head groups or residues on proteins, collectively referred to as immobile buffers (Ädelroth & Brzezinski, 2004; Alexiev et al., 1995; Brändén et al., 2006; J. Heberle & Dencher, 1992; Joachim Heberle et al., 1994; Jones & Jackson, 1989; Junge & McLaughlin, 1987; Nachliel & Gutman, 1996; Scherrer et al., 1994; Tocanne & Teissié, 1990); ii) interfacial water and other molecules acting as a mobile buffers (Cherepanov et al., 2004; Springer et al., 2011; Tocanne & Teissié, 1990). As discussed in Agmon et al (Agmon et al., 2016) and demonstrated by Springer et al (Springer et al., 2011) proton transfer does not necessarily require the presence of immobile buffers yet proton diffusion is affected by the phospholipid composition of the membrane (Amdursky et al., 2019).

Independent of the detailed mechanisms, proton migration involves the spatial restriction of the proton to the membrane surface. The solvated proton can exist in a number of forms

including the hydronium cation (H_3O^+) where the proton is located on single water molecule, the Zundel cation ($H_5O_2^+$) where the excess proton is shared by two water molecules and the Eigen cation ($H_9O_4^+$) where the H_3O^+ forms hydrogen bonds with three surrounding water molecules. The affinity of (solvated) protons for the surface of phospholipid membranes has been demonstrated both with 'wet-lab' experiments (Brändén et al., 2006; Cranfield et al., 2016; Sandén et al., 2010; Springer et al., 2011; Weichselbaum et al., 2017) and simulations (Smondyrev & Voth, 2002; Wolf et al., 2014; Yamashita & Voth, 2010). It is also worth noting that simulations of hydrated protons at the water-lipid interface are one of the most complex and challenging tasks in computational biophysics and the predictions of proton diffusivity often differ by orders of magnitudes from experimental values. Nevertheless, data from simulations have provided molecular level insight into structure of lipid-ion complexes formed. Combined results from these simulations studies suggest that the hydronium and Zundel cation can interact with the headgroups of phospholipids and sit in the cavities formed by neighbouring phospholipid molecules (Deplazes et al., 2017; Smondyrev & Voth, 2002; Wolf et al., 2014; Yamashita & Voth, 2010). For example, Yamashita et al (Yamashita & Voth, 2010) investigated the configurations of solvated protons at the water-phospholipid interface of both zwitterionic and anionic phospholipid bilayers. In both types of membranes, hydronium ions are coordinated by phosphate oxygens as well as water molecules sitting in the lipid headgroups (Fig 1A). In comparison, distorted Zundel-like cations are located deeper in the interfacial region (i.e. closer to the hydrophobic core of the membrane) where they bind to phosphate and carbonyl oxygens in the phospholipid headgroup (Fig 1B). Lipid-ion complexes of similar configurations were demonstrated by other simulations of solvated protons with zwitterionic phospholipid bilayers (Deplazes et al., 2017; Smondyrev & Voth, 2002; Wolf et al., 2014). In addition Wolf and coworkers (Wolf et al., 2014) showed that the hydronium ion can also reside in '*lipid binding cavities*' in a less tightly bound configuration where the cation is surrounded by clusters of water molecules that are bridging the cation to the phosphate and carbonyl oxygens. The authors also used free energy calculations to demonstrate that the hydronium ion is attracted to the phospholipid headgroup with an energy minima closer to the hydrophobic core compared to the energy minima of water molecules (Fig 1C). The affinity of hydronium ions has also been shown in other simulations where hydronium ions that were randomly placed in the bulk solution accumulated at the water-lipid interface where they formed strong interactions with phospholipid headgroups (Deplazes et al., 2017). Interestingly, these simulations also showed that the interactions of hydronium ions with the phospholipid headgroups resulted in a reduced area per phospholipid and increased membrane thickness,

consistent with experimental data (Cranfield et al., 2016). In addition, a number of studies have demonstrated that low pH alters the physico-chemical properties of membranes including phase transition temperature (Koynova & Caffrey, 1998), mechanical and electrical properties (Zhou & Raphael, 2007), interfacial tension (Petelska & Figaszewski, 2002) and morphology (Cranfield et al., 2016).

In summary, both experimental measurements and simulations show that solvated protons show strong affinity for water-lipid interfaces where they interact with the phosphate and carbonyl oxygen in the phospholipid headgroups and form stable lipid-ion and lipid-ion water complexes.

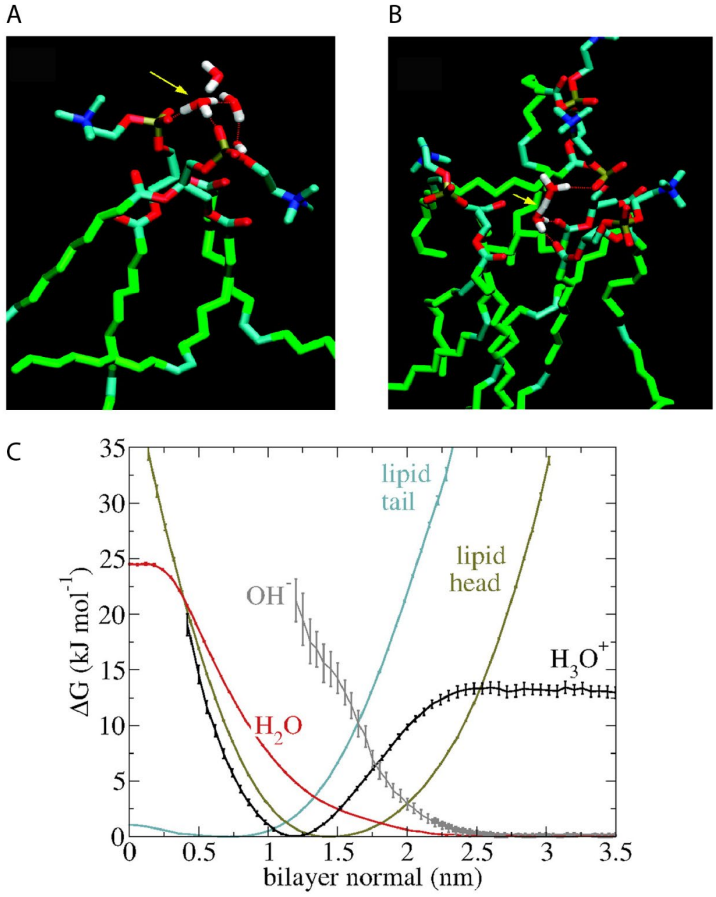

Figure 1. Interactions of protons with phospholipid membranes from simulations studies. (A) Structure of a hydronium ion coordinated by phosphate oxygen and water. (B) Structure of a Zundel cation coordinated by phosphate and carbonyl oxygen. (A,B) adapted with permission from (Yamashita & Voth, 2010). Copyright 2010 American Chemical Society. (C) Free energy profiles of the hydronium ion (H_3O^+), hydroxyl ion (OH^-), water and the phospholipid head

and tail along the bilayer normal of the upper leaflet of the membrane. 0 nm is the centre of the membrane (i.e. the centre of the hydrophobic core) and 3.5 nm is the water layer above the lipid head groups. The free energy, ΔG, was obtained from $\Delta G(z) = -RT \ln p(z)$ where $p(z)$ is the normalised number density of a given system component at position z along the bilayer normal, calculated from MD simulations of a proton near the surface of a hydrated DMPC lipid bilayer. Reprinted from (Wolf et al., 2014) with permission from Elsevier.

3. Interactions of alkali ions with membranes

The extent of the interaction between zwitterionic phospholipid membranes and monovalent ions is still disputed. Generally, it is considered that interactions between monovalent cations and zwitterionic phospholipid bilayers are relatively weak. This was supported by several experimental studies reporting little effect on physico-chemical properties of phospholipid membranes at sub-molar ion (Akutsu & Seelig, 1981; Binder & Zschornig, 2002; Brown & Seelig, 1977; Clarke & Lupfert, 1999; Cunningham et al., 1988; Cunningham et al., 1986; Petrache et al., 2006) PMID: 4642226. However, there are divergent findings using different experimental techniques demonstrating significant changes in the properties of the bilayer such as hydration at the interface, changes in bilayer mechanical strength and the phase transition induced by sub-molar concentrations of monovalent ions (Garcia et al., 2019; Garcia-Manyes et al., 2005, 2006; Piantanida et al., 2017). In addition, simulations have demonstrated changes to the area per lipid, order parameters, diffusion coefficients and orientations of the headgroup dipole (Böckmann et al., 2003; Cordomí et al., 2008; Gurtovenko & Vattulainen, 2008; Reif et al., 2017; Vácha et al., 2009).

Similar to the study of proton-membrane interactions, molecular details on where and how alkali ions bind to phospholipids has predominantly been obtained from MD simulations. A large number of studies demonstrated the binding of Na^+ and/or K^+ ions to the water-lipid interface of zwitterionic and anionic phospholipid bilayers at physiologically relevant salt concentrations (Böckmann et al., 2003; Cordomí et al., 2008, 2009; Gambu & Roux, 1997; Gurtovenko & Vattulainen, 2008; Javanainen et al., 2017; Jurkiewicz et al., 2012; Klasczyk & Knecht, 2011; Lee et al., 2008; Mao et al., 2013; Mukhopadhyay et al., 2004; Pabst et al., 2007; Pandit et al., 2003; Reif et al., 2017; Vácha et al., 2010; Vácha et al., 2009; Vorobyov et al., 2014). Combined results from these studies suggest that alkali ions form stable interactions

with the phosphate and carbonyl groups in the phospholipid headgroups (Böckmann et al., 2003; Cordomí et al., 2008, 2009; Gambu & Roux, 1997; Javanainen et al., 2017; Jurkiewicz et al., 2012; Lee et al., 2008; Mukhopadhyay et al., 2004; Pandit et al., 2003; Vácha et al., 2010). In much the same way as protons, alkali ions are found in the *'binding cavity'* formed by neighbouring phospholipids and can interact with the carbonyl or phosphate oxygens either directly or via bridging water molecules. In studying these interactions using MD simulations, the exact binding mode (i.e. the number of phospholipids or water molecules coordinated to the ion) and the preference for the phosphate or carbonyl oxygen depends on the force field and other simulation parameters used. However, there is a consensus that the alkali ions accumulate deep at the interfacial region located close to phosphate and carbonyl groups, while the corresponding negative counter ions (usually Cl^-) reside in the region closer to the bulk water.

Simulations have also been used to predict the relative binding affinities of Na^+ and K^+ for phospholipid bilayers (Gambu & Roux, 1997; Klasczyk & Knecht, 2011; Mao et al., 2013; Melcr et al., 2018; Vácha et al., 2009). The accurate calculation of binding affinities is challenging and in the case of alkali ions the predicted affinities are often overestimated (Catte et al., 2016) This is further complicated by conflicting results from experimental measurements (Melcr et al., 2018). In some studies, Na^+ appears to show stronger binding and forming more stable lipid-ion complexes than K^+ (Cordomí et al., 2008; Gurtovenko & Vattulainen, 2008; Mao et al., 2013; Vácha et al., 2010; Vácha et al., 2009). However, some studies also reported no significant difference in the affinities of the two ions (Vorobyov et al., 2014), while other simulations predicted that water molecules are preferred to Na^+ (and Cl^-) at the water-lipid interface (Melcr et al., 2018). The lower binding affinity of K^+ has been attributed to the larger size, smaller ionic surface charge, and less ordered hydration shell (Gurtovenko & Vattulainen, 2008). Irrespective of its predicted lower affinity, K^+ reportedly associates with the negatively charged carbonyl and phosphate oxygen moieties, much like Na^+ (Gambu & Roux, 1997). It should be noted that an accurate prediction of binding affinities for biomolecular systems is very challenging. The choice of force fields for the phospholipids and the water model used, as well as other simulations parameters, significantly affect the predicted affinities (Cordomí et al., 2009; Gurtovenko & Vattulainen, 2008; Melcr et al., 2018).

Another similarity between the alkali ions and protons is their capacity to alter the properties of the membrane. Studies in the presence of alkali ions at physiologically relevant

concentrations have reported significant changes to the physical properties of membranes (Binder & Zschornig, 2002; Böckmann et al., 2003; Garcia-Manyes et al., 2005; Vorobyov et al., 2014). Generally, ion binding to phospholipid membranes increases lipid ordering (Garcia-Manyes et al., 2005), area per phospholipid (Cornell et al., 1983) and area compressibility, with a concurrent reduction in elasticity (Reif et al., 2017). Much like the reported difference in binding affinities for Na^+ and K^+ to the lipid interface, relative to Na^+, K^+ induces a weaker effect on membrane structure (Petrache et al., 2006). The authors reported no structural alterations in the phosphatidylcholine bilayer with K^+, which they determined was due to minimal headgroup binding. However, effects of membrane fluidity in the presence of K^+ and Na^+, were reported to be indistinguishable (Kagawa et al., 2013; Zimmermann et al., 2012). As in MD simulations, the alkali ions seem to modulate the physical properties of membranes, but determining precise binding affinities and ion-specific modulation of the physical properties of the membrane remains an experimental challenge.

In summary, even though both experiment and simulations have demonstrated that Na^+ and K^+ interact with phospholipid bilayers at sub-molar concentrations, and induce subsequent alterations to the physico-chemical properties of the bilayer, there remains significant debate regarding these assertions. Nevertheless, across a large number of studies, spanning more than two decades, there appears at least a consensus that Na^+ and K^+ are attracted to the water-lipid interface, where they coordinate with the phosphate and the carbonyl oxygen moieties to form ion-lipid or ion-lipid-water complexes.

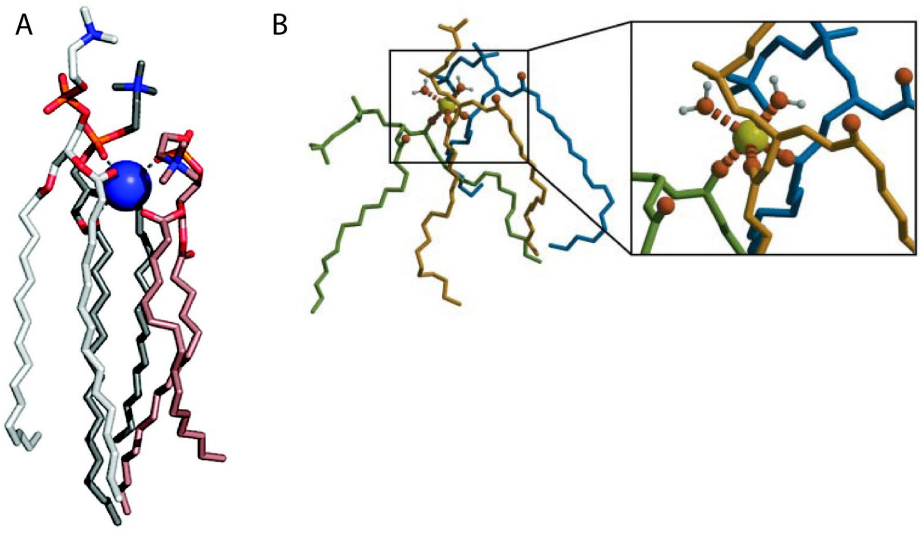

Figure 2. Interactions of Na^+ ions with phospholipid membranes from simulations studies. (A) Structure of a Na^+ - lipid complex where the ion is coordinated by phosphate and carbonyl oxygens from three lipids. Reprinted with permission from (Cordomí et al., 2009). Copyright 2009 American Chemical Society. (B) Typical structure of a Na^+ ion coordinated by the carbonyl oxygen of two lipids and water molecules. Reprinted from (Böckmann et al., 2003) with permission from Elsevier.

4. Competing for the same space? The relationship between proton-membrane and ion-membrane interactions and its implications

The studies outlined above were collected using a wide range of experimental techniques, membrane structures (e.g. multilamellar and unilamellar vesicles, supported bilayers) and simulations. They demonstrate that protons and alkali ions accumulate at the interfacial region of phospholipid membranes. In addition, results from simulations suggest that both protons and alkali ions form ion-lipid or ion-lipid-water complexes where the ion can be coordinated by the phosphate or carbonyl oxygens from neighbouring phospholipid headgroups as well as water molecules. Based on this evidence, it is conceivable that protons and alkali ions would participate in competitive binding.

To the best of our knowledge, the paper by Tocanne and Tessié (Tocanne & Teissié, 1990) published almost 30 years ago, is one of the few papers that provides an in-depth discussion of both proton migration and alkali ion–membrane interactions. The authors also point to the complex interplay between these processes noting that the ionisation state of phospholipids at the water-lipid interface depends, among other parameters, on the alkali-ion lipid binding constants. The paper also reports the pKa values for a range of phospholipids from various studies showing that the pKa depends on the type and concentration of salt used as a buffer.

Interestingly, many studies reporting the affinity or the effect of alkali ions on zwitterionic phospholipid bilayers fail to buffer their solutions to ensure a neutral pH. Similarly, studies investigating proton migration do not consider whether the measurements depend on the type or concentration of the salt buffer. Buffering solutions may be unsuited to the experimental techniques reported in these articles, but one is unable to ignore the fact that these reports have been used as evidence for limited monovalent cation binding to zwitterionic phospholipid bilayers (Catte et al., 2016). Considering the likelihood of proton accumulation at the surface

of the phospholipid bilayer it is impossible not to question the relevance of this experimental confounder, especially when determining alkali ion interactions at physiologically relevant concentrations.

The idea that a simple competitive relationship may exist at the water/lipid interface between monovalent cations and solvated protons may constrain other potential aspects that are associated with proton accumulation at the surface. One of these is the capacity to alter the water structure at the interface (Nguyen et al., 2018; Vácha et al., 2007). The deliberate arrangement of the water structure by specific ions has been previously described in terms of 'structure makers' and 'structure breakers' (Collins, 1995). While it is believed to be a short-range effect, local changes in the structure of interfacial water due to the accumulation of solvated protons at the membrane surface may impose a barrier to free diffusion of ions that do not form complementary water structures. This potential higher energy cost associated with ion movement from bulk water into the lipid binding cavity will exist when the water structure around the ion differs from the interfacial water structure at the membrane surface. In this case, modulation of ion interactions with the phospholipid bilayer surface could also be associated with potential changes in the interfacial water structure (Boström et al., 2005).

Direct competition and localised structural changes in water are unable to exist without concomitant changes in the structural properties of the phospholipid headgroups. The fact that bulk pH has the capacity to modulate the packing density of a phospholipid bilayer (Cranfield et al., 2016; Deplazes et al., 2017, 2018) may introduce a structural component to the affinity of alkali ions to the membrane. The coordination of alkali ions at the lipid/water interface involves the carbonyl and phosphate oxygens of the lipid headgroup indicating that structural changes to this coordination pocket, such as rigidification or changes to the radial size of the *'binding cavity'* itself can occur. It is reasonable to propose that the capacity of the solvated proton to increase phospholipid packing would produce a far more constrained *'binding cavity'*, both in terms of flexibility and size, within the headgroup region. This in itself may alter the comparative affinity of the monovalent ions at the phospholipid surface.

Conclusion

A new perspective has emerged in which understanding of proton and ion interactions at membrane surface are interwoven. The potential interdependence between protons and alkali ions at the membrane surface, which has so far mostly been overlooked, means that studies

disregarding bulk pH may be obscuring the interactions of physiologically relevant ions at the water/phsopholipid interface. This may well lead to a significant underestimation of the affinity of these ions to the membrane interface. In turn, the capacity of protons to migrate along the phospholipid bilayer surface may be modulated by other ionic species at the surface, if they are competing for the same ionisable groups. The interdependence of pH and ion/membrane interactions is an important topic of investigation that may reconcile some of the disparate reported results and provide a significant step forward towards a richer understanding of the complex nature of the water/lipid interface.

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