Cation effects on phosphatidic acid monolayers at various pH conditions

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Abstract

The impact of pH and cations on phase behavior, stability, and surface morphology for dipalmitoylphosphatidic acid (DPPA) monolayers was investigated. At pH < 10, DPPA monolayers on water are predominantly populated by neutral species and display the highest packing density. Cations are found to expand and stabilize the monolayer in the following order of increasing magnitude at pH 5.6: $\text{Na}^+ > \text{K}^+ \sim \text{Mg}^{2+} > \text{Ca}^{2+}$. Additionally, cation complexation is tied to the pH and protonation state of DPPA, which are the primary factors controlling the monolayer surface behavior. The binding affinity of cations to the headgroup and thus deprotonation capability of the cation, ranked in the order of $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Na}^+ > \text{K}^+$, is found to be well explained by the law of matching water affinities. Nucleation of surface 3D lipid structures is observed from Ca^{2+} , Mg^{2+} , and Na^+ , but not from K^+ , consistent with the lowest binding affinity of K^+ . Unraveling cation and pH effects on DPPA monolayers is useful in further understanding the surface properties of complex systems such as organic-coated marine aerosols where organic films are directly influenced by the pH and ionic composition of the underlying aqueous phase.

Keywords

DPPA, pKa, phosphatidic acid, ions, compression isotherms, Brewster angle microscopy

1. Introduction

Phosphatidic acid (PA) is crucial for the maintenance of cell membranes. Although it exists in small amounts (~1%) due to its high turnover rate (Munnik, 2010), PA is a key metabolite in the synthesis and breakdown of larger membrane phospholipids in addition to participating in other important cellular functions, e.g., vesicle fission and fusion, cell signaling, and protein binding (Athenstaedt and Daum, 1999; Eaton et al., 2014; Kooijman et al., 2003; Kooijman and Testerink, 2010; Wang et al., 2006). Besides being a minor phospholipid in the cell membranes of mammals and plants, PA is also known to be widely distributed among different species of marine microorganisms (Ackman, 1989).

Phospholipids from the marine biota such as dipalmitoylphosphatidic acid (DPPA) and dipalmitoylphosphatidylcholine (DPPC) are believed to be important sources of saturated fatty acids found in the sea surface microlayer (SSML) and at the surface of marine aerosols (Lee et al., 2007; Marty et al., 1979; Tervahattu et al., 2002). In particular, saturated fatty acids with 16 (palmitic acid) and 18 (stearic acid) carbon alkyl chains are known to be the most abundant. These surface-active organic compounds ultimately form thin films that affect aerosol growth and volatility, radiative absorption and scattering, reactivity with atmospheric gases, and cloud condensation nuclei activity (Andrews and Larson, 1993; Beaver et al., 2010; Burrows et al., 2014; Clifford et al., 2007; Glass et al., 2006; Moroi et al., 2004; Reeser et al., 2013). In turn, the interfacial properties of these films are directly influenced by the pH and ionic composition of the aqueous phase.

Interestingly, such complexity is generally not modeled in current atmospheric simulations as primary organic aerosols are still treated as uniform, and differences in aerosol size and hygroscopicity due to organic matter are typically neglected (Burrows et al., 2014). As marine

aerosols have regional distributions of ions and organic matter that can be distinctly different (Ault et al., 2013; Millero et al., 2008), they will have, as stated by Elliott et al.: “[...] *different critical organic parameters not limited to interfacial coverage, the two dimensional phase state, chemically dependent hygroscopicities, mass to carbon ratio and even density*” (Elliott et al., 2014). Thus understanding the surface properties of model organic films with environmentally-relevant ions at air-aqueous interfaces will help to improve atmospheric aerosol climate modeling.

In that regard, DPPA monolayers can be useful and relevant proxy systems for the SSML and organic coatings on marine aerosol surfaces (Lin et al., 2015). Moreover, DPPA constitutes a simple enough lipid model (Scheme 1) for studying the effects of ions on lipid surface behavior and morphology including alkyl chain conformation, hydration state, and ion interactions with the phosphate headgroup at air-aqueous interfaces (Tang and Allen, 2009; Tang et al., 2011; Tang et al., 2010). Previous studies have shown that DPPA monolayers are impacted by the pH, ionic composition, and hydrogen bonding of the underlying aqueous solution, all of which affect the ionization state of DPPA’s phosphate headgroup (Kim et al., 1996; McNamee et al., 2011; Miñones Jr. et al., 2002; Papahadjopoulos, 1968; Patil et al., 1979). For example others have previously examined the surface phase behavior of DPPA monolayers on water and shown how it is affected by different pH and temperature conditions (Miñones Jr. et al., 2002). However, the influence of solution ionic composition and DPPA headgroup-ion complexation has yet to be fully elucidated.

In this work, we examine the effects of pH and marine-relevant cations (Na^+ , K^+ , Ca^{2+} , and Mg^{2+}) on DPPA’s phase behavior, stability, and surface morphology using surface pressure-area isotherms and Brewster angle microscopy. The investigation of these properties is particularly

applicable to marine aerosol surfaces, where enrichment of specific cations may impact the surface morphology and organization of organic films (Ault et al., 2013; Millero et al., 2008).

2. Materials and Methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphate (DPPA) monosodium salt (>99%, lyophilized powder) was purchased from Avanti Polar Lipids (Alabaster, AL; M_w 670.873 g/mol). The average molecular weight (M_w) is 670.455 g/mol due to the variability in the Na^+ content. NaCl, KCl, MgCl_2 , and CaCl_2 salts (>99%, ACS certified) were purchased from Fisher Scientific (Waltham, MA). Chloroform and methanol (HPLC grade) as well as trace metal grade HCl and NaOH were also from Fisher Scientific. Ultrapure water with a resistivity of $18.2 \text{ M}\Omega\cdot\text{cm}$ and a measured pH of 5–6 (the water pH is slightly acidic due to dissolved atmospheric CO_2) was obtained from a Barnstead Nanopure filtration system (D4741, Thermolyne Corporation, Dubuque, IA) equipped with additional organic removing cartridges (D5026 Type I ORGANIC-free Cartridge Kit; Pretreat Feed).

Scheme 1. Chemical structure of (singly protonated) DPPA.

2.2 Preparation of lipid and salt solutions

DPPA stock solutions were prepared by dissolution in a 7:3 (v/v) chloroform:methanol mixture. All aqueous salt solutions were prepared at 0.6 M by dissolution in pure water. Salts and

salt solutions were pretreated according to previously reported procedures to minimize the impact of organic contamination (Hua et al., 2013). NaCl and KCl salts were baked at 650 °C, whereas MgCl₂ and CaCl₂ solutions were filtered at least thrice using activated carbon filters (Whatman Carbon-Cap 75, Fisher Scientific) to remove organic contaminants. A separate set of carbon filters was used for each salt to avoid any ion cross-contamination. The significance of organic contamination and the need to treat salts cannot be understated and is discussed in the Supporting Information (see Fig. S1). The concentration of these purified stock solutions was determined by Mohr's chloride titration method (Finlayson, 1992). Each solution was prepared by serial dilutions of the purified stock solutions. The pH of the prepared solutions was adjusted by adding the required amounts of stock concentrated HCl and 10 M NaOH.

2.3 Compression isotherm measurements

Surface pressure-molecular area (Π -A) compression isotherms of DPPA monolayers on neat water and salt solutions were obtained using a computer-controlled Langmuir trough (Minitrough, Biolin Scientific USA, Paramus, NJ). The trough is made of Teflon and has dimensions of 168 mm $\times$ 85 mm. The monolayer is symmetrically compressed using two Delrin-coated barriers. Surface pressure was measured during compression by the Wilhelmy plate method. Filter paper (Ashless grade, Whatman) was used as Wilhelmy plates. Prior to each experiment, the presence of extraneous surface-active contaminants was checked by sweeping the surface of the aqueous solution with the barriers until no significant change in the surface pressure (<0.1 mN/m) was observed. A measured amount of DPPA was spread dropwise onto the aqueous surface using a glass microsyringe (1725RN, Hamilton, Reno, NV). After spreading, solvent evaporation was allowed for approximately 10 min. The barriers were compressed at a rate of 5 mm/min/barrier.

Isotherms were also tested at 10 and 15 mm/min/barrier compression rates with no observable differences found. All experiments were performed at room temperature (21 ± 1.5 °C) and atmospheric pressure and humidity. Isotherms were repeated in sets of triplicates, with a maximum allowed error of ± 0.25 A² (see Fig. S2 in the Supporting Information). A maximum difference of ± 0.5 A² exists between sets due to small daily fluctuations in temperature.

2.4 Brewster angle microscopy (BAM) imaging

BAM images were collected simultaneously with the compression isotherms on a custom-built BAM setup described previously (Adams and Allen, 2013; Griffith et al., 2012). The laser source (Research Electro-Optics, Boulder, CO) emits 5 mW *p*-polarized light at 543 nm. The output laser beam is first attenuated by a half-wave plate (Ekspla, Lithuania) then filtered by a Glan polarizer (Ekspla, diameter 14 mm) before reaching the aqueous surface at the Brewster angle (53.1° on water; this value was slightly adjusted for the various salt solutions). The reflected beam is collected by a 10 $\times$ infinity-corrected super-long working distance objective lens (CFI60 TU Plan EPI, Nikon Instruments, Melville, NY; NA 0.21) and collimated by a tube lens (MXA22018, Nikon Instruments; focal length 200 mm) onto a back-illuminated EM-CCD camera (iXon DV887-BV, Andor Technology USA, Concord, MA; 512×512 active pixels with $16 \mu\text{m} \times 16 \mu\text{m}$ pixel size). Because the inclined position of the imaging optics results in only a narrow region of the image being well focused, final images were cropped down from their full size ($800 \mu\text{m} \times 800 \mu\text{m}$) to show the most resolved region. The lateral resolution of the BAM images was $\sim 1.3 \mu\text{m}$. In the BAM images shown here, a blue color scale was chosen to enhance imaging contrast. Light and dark blue areas correspond to regions with high and low lipid coverage, respectively.

3. Results and Discussion

3.1. pH effects on DPPA monolayers

The Π -A compression isotherms of DPPA on pure water at different pHs are shown in Fig. 1. The region before lift-off as shown in the DPPA isotherm at pH 5.6 corresponds to the gaseous (G)-liquid-condensed (LC) coexistence phase of DPPA. Lift-off into a tilted-liquid condensed (TC) phase occurs at approximately $47.5 \text{ \AA}^2/\text{molecule}$. (For isotherms at $\text{pH} \leq 10$, the mean molecular area (MMA) at lift-off is determined by prolonging the tangent of the slope of the TC transition down to $\Pi = 0 \text{ mN/m}$.) With a high phase transition temperature of 67°C (Jacobson and Papahadjopoulos, 1975), DPPA does not exhibit a distinct liquid-expanded (LE) phase like DPPC, which indicates the presence of attractive van der Waals forces between the alkyl chains and hydrogen bonding between phosphate headgroups causing DPPA to exist in a highly ordered phase at room temperature (Grigoriev et al., 2003; Rudolphi-Skórska et al., 2014; Yun et al., 2003). DPPA has also been shown to form hexagonal packing suggestive of hydrogen bonding interactions (Boggs, 1986; de Meijere et al., 1997; Lin et al., 2015). A transition from the TC phase to the untilted-LC (UC) phase is indicated by a kink in the isotherm at about $43.5 \text{ \AA}^2/\text{molecule}$ and 20 mN/m . At about $42 \text{ \AA}^2/\text{molecule}$ and 53 mN/m , the monolayer reaches collapse. The limiting area of the DPPA isotherm at pH 5.6 corresponds approximately to the area of two compact alkyl chains ($2 \times 20 \text{ \AA}^2$) and is in relatively good agreement with values published in the literature, even though there is a notable variability depending on the experimental conditions (see Table S1 & S2 of the Supporting Information).

Fig. 1. Compression isotherms of DPPA monolayers on water at various pH. Inset: Expansion of mean molecular area relative to water at pH 5.6 in the TC (10 mN/m) and UC (40 mN/m) phases.

Depending on the pH but also on the lipid packing density, the phosphate moiety bears a charge that varies between 0 and $-2e$, where e is the elementary electronic charge. As pH increases, the DPPA monolayer progressively deprotonates and goes from neutral to increasingly negatively charged. However, a negatively charged monolayer attracts excess protons (H^+) to the lipid/aqueous interface similar to how a negatively charged surface attracts counter-ions for an electrical double-layer (Vega Mercado et al., 2011). The concentration of protons at the surface ($[\text{H}^+]_s$) being higher than in the bulk causes the pH in the local environment of the phosphate group to be less than the bulk pH (Wang et al., 2012). This decrease in surface pH can be quite significant depending on the lipid system and degree of ionization; for instance, it has been reported to be as much as 4 pH units lower for stearic acid monolayers (Vega Mercado et al., 2011). Even a

compression of the monolayer can result in a decrease of surface pH due to the higher charge density of the increasingly closely packed monolayer. Effectively, this means the surface pK_a of the monolayer is increased, and thus the bulk pH must then be higher to observe deprotonation. To examine the effect of the different DPPA species (charged and neutral), isotherms were obtained in a pH range (from 1.7 to 13.0) extending beyond the apparent surface pK_a values, which have been previously reported to be $pK_{a1,s} \approx 3.2-3.8$ and $pK_{a2,s} \approx 7.9-8.5$ in aqueous dispersions and monolayers (Abramson et al., 1964; Patil et al., 1979).

No significant changes in the monolayer MMA are observed below pH 10 (see inset of Fig. 1). This contradicts previous observations made by Miñones et al., that observed a small condensing effect upon lowering the pH from 6 to 3 (Miñones Jr. et al., 2002), but agrees with Luckham et al., who reported that DPPA isotherms at pH 1 and 5.6 are almost superimposable (Luckham et al., 1991). This suggests that DPPA exists predominantly as a neutral (0) species at pH ~ 10 . This is supported by a monolayer pH titration study by Patil et al. that observed a pK_a at only 8.7 (Patil et al., 1979). However, while a deprotonated phosphate headgroup has more repulsive electrostatic interactions than its neutral form, it also has more available attractive hydrogen(H)-bonding sites to bond with neighboring headgroups (Boggs, 1986). These two opposing effects could cancel each other, effectively leading to identical isotherms. As isotherms give a macroscopic picture and not molecular level information, differentiating the two species may not be possible. However, the appearance of further expanded isotherms at higher pHs indicate that there is significant electrostatic repulsion beyond the condensing effect of increased H-bonding, such that two species do not have identical isotherm behavior. The most likely explanation is that when both species exist, isotherms may not distinguish the two due to the

hydrogen bonding, but when the (-1) species starts to dominate over the (0) species as the pH increases, electrostatic repulsion becomes stronger and monolayer expansion is observed.

At pH 10.3 there is a noticeable difference in the isotherm attributed to the emergence and dominance of singly deprotonated (-1) DPPA species. The lift-off area expands from 47.5 to 51.5 Å²/molecule and the UC phase disappears (Fig. 1). The surface pressure as well as the collapse pressure increase due to the stronger electrostatic repulsion between singly protonated DPPA molecules. At pH 13, an even greater expansion is observed (Fig 1). Excess repulsion from the increased negative charges results in the immediate onset of a condensed phase, with disappearance of any unique phase behavior leading up to the first of two collapse phases. The first collapse onset begins around 53 mN/m just as seen in the isotherms at lower pH, while the second collapse starts around 64 mN/m. The presence of two collapse pressures is indicative of immiscibility of two different components (Dynarowicz-Łatka and Kita, 1999). This behavior can be explained by emergence of the (-2) species at pH 13. While the (-1) species experiences more repulsion than the (0) neutral species and is further expanded, both are still capable of hydrogen bonding and thus remain miscible and retain a common collapse pressure (Boggs, 1986). However, the (-2) species does not hydrogen bond well and is immiscible with the (0) and (-1) species, resulting in a unique collapse pressure. Therefore, the deprotonation of DPPA occurs at much higher pHs (10.3, 13) than expected from reported pK_a values (3.5, 8.3), an increase in pK_a of about 5-7. This phenomena of large pK_a shifts for charged monolayers has also been reported for other species such as fatty acids, and L-phenylalanine (Griffith and Vaida, 2013; Kanicky et al., 2000; Kanicky and Shah, 2002, 2003; Tang et al., 2010)

3.2. Cation effects on DPPA monolayers

Fig. 2 shows compression isotherms of DPPA monolayers on a series of 0.6 M chloride salt solutions at pH 5.6. All salts are seen to expand the monolayer, inducing the onset of the condensed phases much earlier and at larger MMAs than pure water. Thus at any given fixed MMA, the salt isotherms reveal higher surface pressures, whereas at any given fixed surface pressure, they show larger MMAs. By inspection, the expansion of the DPPA monolayer is cation-specific and follows the order $\text{Na}^+ > \text{K}^+ \approx \text{Mg}^{2+} > \text{Ca}^{2+}$, with respective lift-off areas of 51.5, 50.5, 50.5, and 48.5 $\text{\AA}^2/\text{molecule}$. This ordering appears to follow the trend of surface charge density. Due to the small and exposed phosphate headgroup of DPPA, many factors must be considered such as ion size, hydration, and surface charge density which all contribute to the ability of cations to complex with and disrupt the monolayer. The ionization state of the phosphate headgroup is also altered via pH to compare the relative binding affinities of each cation for DPPA.

Fig. 2. Compression isotherms of DPPA monolayers at pH 5.6 on different 0.6 M chloride salts aqueous solutions. The isotherm on water is also shown for comparison. Inset: Expansion of mean molecular area relative to water at pH 5.6 in the TC (10 mN/m) and UC (40 mN/m) phases.

3.2.1. Monovalent cation interactions with DPPA monolayers

A comparison of cation-headgroup interactions at the various ionization states of DPPA is first made between the monovalent cations. The effect of Cl^- co-ions can be neglected as studies have shown that chloride does not display an affinity for lipid headgroups (Vácha et al., 2009). Fig. 3 shows the Π -A isotherms of DPPA monolayers on 0.6 M NaCl and KCl solutions at pH 2.5, 5.6 and 10.1. One immediately observes unique isotherms for each pH, which greatly contrasts with those on pure water that are identical at pH 2.5 and 5.6. This is evidence of the presence of different DPPA species induced by the cation complexation. Thus, indicated here, in the presence of Na^+ and K^+ , the DPPA monolayer is populated predominantly by neutral (0) species at pH 2.5, singly protonated (-1) species at pH 5.6, and fully deprotonated (-2) species at pH 10.2.

The isotherm of DPPA on NaCl at pH 2.5 shows only a minor expansion of the monolayer compared to that on neat water at pH 5.6. The lift-off area is increased by $\sim 1 \text{ \AA}^2/\text{molecule}$ while the phase transition area and surface pressure remain the same. The isotherm of DPPA on KCl at nearly the same pH shows an even smaller expansion of $\sim 0.5 \text{ \AA}^2/\text{molecule}$. These very small area changes suggest that when the pH of the solution is low, H^+ inhibits cation-induced deprotonation. However, Na^+ and K^+ can still complex with the DPPA headgroup at very low pHs, albeit weakly. Although DPPA is predominantly neutral at pH 2.5, there could be a small fraction of the singly protonated (-1) species induced by cation complexation that contributes to the small expansion of the monolayer. At pH 5.6, the isotherm of DPPA on salts show similar expansion behavior compared to that on water at pH 10.3, providing further evidence that DPPA is (-1) from cation-

induced deprotonation. At pH 10.1, the isotherm on monovalent salts show more ordering of the condensed phase compared to that of water at pH 13.0, but are still similar as they share the same protonation state (-2) as well as disappearance of the TC-UC phase transition.

The speciation of DPPA with Na^+ and K^+ fits well with the apparent surface pK_a values reported in the literature. This can be explained by the fact that not only H^+ but also metal cations are attracted to negatively charged phosphate headgroups. The cations shield the phosphate charge as well as displacing H^+ at the surface, thus effectively decreasing the local $[\text{H}^+]_s$ concentration (Vaz et al., 1978; Wang et al., 2012). This has an effect of restoring the surface pH to a value more closely resembling that of the bulk. This is also in line with the “electrostatic/hydrogen-bond switch mechanism” of phosphatidic acid (Kooijman and Testerink, 2010) in that the H-bonding and protonation state of DPPA are affected by local ions or charged groups and consistent with observations made by Vaknin and co-workers (Wang et al., 2012). Similar results were also reported from sum frequency vibrational spectroscopy studies for palmitic acid by Allen and co-workers showing that cations including Na^+ and K^+ deprotonate the carboxylic acid headgroup at pH 5.6 where the headgroup is normally protonated on pure water. (Casillas-Ituarte et al., 2010; Tang and Allen, 2009; Tang et al., 2011; Tang et al., 2010)

Fig. 3. Compression isotherms of DPPA monolayers on 0.6 M monovalent (Na^+ , K^+) chloride salt solutions at various pHs. The isotherms on water are also shown for comparison. The DPPA isotherm on water at pH 2.6 is not shown as it is superimposable to the one at pH 5.6.

The relative binding affinity of Na^+ vs. K^+ is inferred from the differences in the compression isotherms for each ionization state. Na^+ and K^+ have the same charge and similar hydration radii (2.18 and 2.12 Å, respectively (Marcus, 1991)) and thus one might expect the monolayer to experience similar expansion. However when comparing Na^+ and K^+ , Na^+ is consistently further expanded by $\sim 1 \text{ Å}^2/\text{molecule}$ at all pHs (Fig. 4). This difference suggests K^+ interacts more weakly with the phosphate headgroup than Na^+ and, in turn, is unable to induce deprotonation as effectively. The collapse pressures of DPPA with Na^+ are also noticeably higher than that on water for the same protonation state, while the collapse pressures with K^+ by contrast display no increase. Na^+ complexation with the headgroup has a stabilizing effect on the monolayers allowing for

higher surface pressures before collapse. This is also supported by nucleation structures observed in BAM images discussed later in this paper. Mixed equimolar solutions of Na^+ and K^+ also reveal isotherms identical to those of Na^+ (data not shown). This all indicates that Na^+ outcompetes K^+ , forms a more stable complex with DPPA, and thus has a higher binding affinity for the DPPA headgroup.

One possible explanation for the higher affinity of Na^+ over K^+ is found in the empirical law of “matching water affinities” proposed by Collins, which states that ions of similar sizes form inner-sphere ion pairs due to their similar absolute free energies of hydration (Collins, 1997). Na^+ has a higher surface charge density than K^+ and is a better match with phosphate, allowing easier deprotonation and subsequent binding. MD simulations have also observed weaker binding of K^+ than Na^+ to the phosphate (Vácha et al., 2009); it is clear that Na^+ ions are attracted to the headgroup region (Lin et al., 2015).

An alternative explanation involves interaction of Na^+ with the carbonyl groups of the DPPA alkyl chains. MD simulations have also shown a greater preference of Na^+ for the carbonyl moieties than K^+ (Böckmann et al., 2003; Cordoní et al., 2009; Gurtovenko and Vattulainen, 2008; Mukhopadhyay et al., 2004). If Na^+ can penetrate into the headgroup region and interact with the carbonyl moiety of DPPA, steric effects may be responsible for the greater expansion of the DPPA monolayer compared to K^+ . Indeed both salt isotherms converge at higher surface pressures, which is due to squeezing out of some ions from the DPPA monolayer. This does not preclude the absence of Na^+ complexation with the carbonyl groups, but it is very weak as Na^+ ions are eventually expelled from the headgroup region. This agrees with MD simulations that show little impact on lipid conformations in the condensed phase due to the low degrees of freedom (Giner Casares et al., 2008). However, All of the Na^+ ions are not squeezed out and a portion remain complexed with

the headgroup as seen by its impact on the collapse pressure. This suggests that direct complexation with phosphate may be a factor.

Fig. 4. Mean molecular area expansion of DPPA monolayers on 0.6 M salt chloride solutions relative to water at various pH in the TC (10 mN/m) and UC (40 mN/m) phases.

3.2.2 Divalent cation interactions with DPPA monolayers

There is great interest in the effect of divalent cations for both biological and atmospheric studies and their increased binding affinity compared to monovalents (Bu et al., 2009). Divalent cations inherently have higher binding affinities relative to H^+ and monovalent cations due to their higher surface charge densities. The effects of divalent ions on other lipids have been well

documented in many other studies; however, no studies have shown the impact of Mg^{2+} vs. Ca^{2+} with pH effects for DPPA.

Ca^{2+} and Mg^{2+} are observed to affect the protonation of DPPA, similar to Na^+ and K^+ . At pH 10, the DPPA monolayer is predominantly singly protonated (-1) on pure water, but is fully deprotonated and bound by divalent cations. At pH 5.6, DPPA is mostly neutral on pure water but becomes singly protonated in the presence of cations. However, at a lower pH of 2.2, the DPPA isotherm on CaCl_2 remains the same as that of pH 5.6 (Fig. 5), which indicates that Ca^{2+} still binds to DPPA. DPPA is likely strongly bound in the presence of Ca^{2+} , existing as a 1:2 or 1:1 complex. This is further supported by expansion areas: when increasing the pH from 5.6 to 9.2, the expansion of DPPA area in the TC phase doubles from 1 to 2 $\text{\AA}^2/\text{molecule}$. This expansion could reflect a change in the binding ratio from a 1:2 to 1:1 Ca^{2+} :DPPA complexation. A similar change in the complexation ratio in this pH range was also reported by Wang et al. for Ca^{2+} on dimyristoylphosphatidic acid (DMPA), an analogue of DPPA with 14 carbon alkyl chains (Wang et al., 2012). Mg^{2+} also causes similar behavior, expanding the monolayer from 3 to 6 $\text{\AA}^2/\text{molecule}$ from pH 5.6 to 9.2. As only divalent and not monovalent cations display area doubling, this is attributed to their ability to complex neighboring DPPA molecules. Charge bridging by divalent cations can offset electrostatic repulsions and stabilize the monolayer. This explains why isotherms on divalent metal chloride solutions are more condensed than those on water for the same DPPA protonation state. Without the presence of cations at pH 5.6, DPPA would remain entirely protonated. However, adding Ca^{2+} or Mg^{2+} deprotonates the phosphate headgroup, causing the monolayer to expand; but cation lipid bridging prevents the monolayer from expanding as much as it would without ions.

Fig. 5. Compression isotherms of DPPA monolayers on 0.6 M divalent (Mg^{2+} , Ca^{2+}) chloride salt solutions at various pHs. The isotherms on water are also shown for comparison. The DPPA isotherm on water at pH 2.6 is not shown as it is superimposable to the one at pH 5.6.

In contrast to CaCl_2 , DPPA isotherms on MgCl_2 at pH 2.2 are slightly less expanded compared to pH 5.6 (Fig. 4). While this could indicate the presence of all three DPPA species, it is unlikely that there would be any neutral DPPA species without any bound Mg^{2+} , particularly as an isotherm expansion is observed. It is more likely that there is a lower fraction of Mg^{2+} bound to the monolayer, resulting in a lower degree of deprotonation and thus a reduced expansion compared to pH 5.6. Therefore, Ca^{2+} displays stronger interactions with the phosphate headgroup compared to Mg^{2+} . Ca^{2+} has also been found to bind strongly with phosphatidic acid even at low micromolar concentrations (Faraudo and Travesset, 2007). However, a larger expansion of the monolayer is observed for Mg^{2+} than Ca^{2+} at all pHs (Fig. 4). This can be reconciled and explained by Collins'

law of matching water affinities. Ca^{2+} should have a higher binding affinity to phosphate than Mg^{2+} by forming a tight inner-sphere complex with phosphate. Mg^{2+} has more size mismatch with phosphate, being a small, hard cation, and holding its solvation shell more strongly than Ca^{2+} (Marcus, 1991). Thus, Mg^{2+} likely forms an outer-sphere solvent-separated complex with the phosphate headgroup. As Ca^{2+} forms tight inner-sphere complexes, it loses some of its hydration shell, which brings the bridging DPPA molecules closer together. Yet, the isotherm differences between Mg^{2+} and Ca^{2+} cannot be accounted for based only on ionic and hydrated radii differences. Such a small DPPA monolayer expansion below Ca^{2+} cross-sectional area is surprising and suggests that DPPA-ion interactions have little to do with steric effects.

3.5 *Morphology of DPPA monolayers on water and salt solutions*

Fig. 6 shows the BAM images of DPPA monolayers on water before and after the onset of the condensed phase at pH 5.6. DPPA starts in the G-LC coexistence phase as evidenced by the large bright areas of condensed lipid islands separated by dark lipid-poor regions. The monolayer quickly forms a homogeneous layer that remains stable even beyond the point of collapse. This is in contrast with an earlier study that reported small bright spots attributed to nucleation and collapse structures with long white striated streaks (Miñones Jr. et al., 2002). However, with NaCl, CaCl_2 , and MgCl_2 solutions, these striated crystalline collapse structures are also observed (Fig. 7), indicating that cations are binding DPPA in a way to act as nucleation sites, instead of simply folding it into multilayered structures. These nucleation sites may also act to stabilize the monolayer explaining the higher collapse pressures. Interestingly, these striated collapse structures are not observed for DPPA on KCl solutions as the images are indistinguishable from that on pure water (Fig. 7b). This indicates that K^+ does not nucleate crystalline aggregates, but instead forms less stable amorphous structures. K^+ does not affect the collapse surface pressure compared to

Na⁺, Ca²⁺, and Mg²⁺ (Fig. 2), except at high pH. This is further evidence of the relatively weak binding affinity of K⁺. On the other hand, Na⁺, Ca²⁺, and Mg²⁺, have higher collapse pressures correlated with the presence of the crystalline striations in the BAM images (Fig 7c-e).

Fig. 6. BAM images of a DPPA monolayer on water (pH 5.6) (a) in the G-LC phase (50 MMA), (b) at the onset of the TC phase (38 MMA), and (c) in the UC phase just prior to collapse (<34 MMA). Scale bar is 100 μm. Circular interference patterns are due to dust particles.

Fig. 7. BAM images of DPPA monolayers in the collapse phase at pH 5.6 for (a) water, (b) NaCl, (c) KCl, (d) MgCl₂, and (e) CaCl₂ salt solutions. Scale bar is 100 μm.

4. Conclusions

DPPA molecules spread on neat water at $\text{pH} < 10$ are mainly comprised of neutral species and form closely packed monolayers. A change in the protonation state increases monolayer repulsion from the negative charges, resulting in weakening of the tight packing. However, we observe that DPPA monolayers require pHs higher than expected to deprotonate due to the negatively charged monolayers attracting H^+ to the surface. Cations reverse this increased pK_a trend by deprotonating the headgroup and shielding the charges. Divalent cations are also able to further mitigate monolayer disruption by bridging neighboring DPPA molecules. Based on the isotherm expansion, the following order of binding affinity can be deduced: $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Na}^+ > \text{K}^+$. This trend agrees well with that predicted from the law of matching water affinities, further supporting the empirically derived rule.

The unique surface behavior of DPPA is attributed to its small phosphate headgroup size and sterically open structure. As the headgroup is unencumbered by large moieties such as those found in other glycerophospholipids, the monolayer is able to pack tightly which inhibits steric insertion from ions. Thus it is electrostatics that primarily affects the protonation state and ultimately governs its monolayer behavior. DPPA headgroup interactions with cations and the resulting impact on the monolayer is extremely sensitive to environmental conditions of pH and relative abundance of ions. The extent of these interactions and their effect on DPPA surface speciation and surface pK_a will be investigated by surface-sensitive spectroscopy and discussed in a forthcoming publication.

DPPA-salt systems were chosen as proxies to further understand the surface properties of marine aerosols. DPPA monolayers on salt solutions serve as a model system of the organic layer on marine aerosols; it enables examination of surface processes such as film condensation and

expansion, changes in lipid domain morphology (including refractive index changes), and cation-phosphate complexation, all of which have the potential to influence the evaporative and radiative properties, as well as the surface reactivity of marine aerosols. It is expected that charge speciation of the organic layer induced by pH and ionic composition of the aqueous core will impact marine aerosol processing and ultimately have direct and indirect effects on climate changes. Thus a better understanding of marine aerosol surface properties can benefit and help improve existing atmospheric simulations and climate modeling.

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600 **Graphical Abstract**

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603 Isotherm experiments reveal the effects of ions on DPPA molecules. A speciation-dependent
604 expansion of the DPPA monolayer is observed.

Highlights

- Deprotonation of DPPA monolayer expands monolayer
- pK_a s of DPPA monolayer are higher than in solution
- Na^+ , K^+ , Mg^{2+} , Ca^{2+} change protonation state from that of water, reduce pK_a of DPPA monolayer
- Binding affinity w/ DPPA goes in order of $Ca^{2+} > Mg^{2+} > Na^+ > K^+$. K^+ has weakest affinity for DPPA
- Salt solutions must be carefully cleaned for organic contaminants for surface studies