

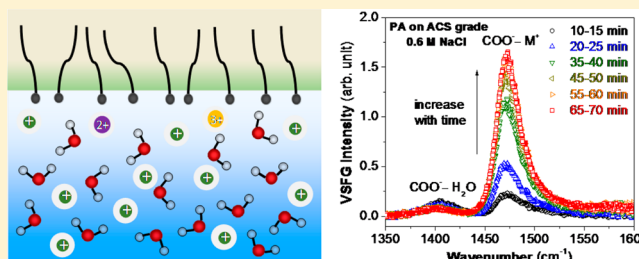
Influence of Salt Purity on Na⁺ and Palmitic Acid Interactions

Zishuai Huang, Wei Hua, Dominique Verreault, and Heather C. Allen*

Department of Chemistry & Biochemistry, The Ohio State University, 100 West 18th Avenue, Columbus, Ohio 43210, United States

S Supporting Information

ABSTRACT: The influence of salt purity on the interactions between Na⁺ ions and the carboxylate (COO⁻) head group of palmitic acid (PA) monolayers is studied in the COO⁻ and OH stretching regions using broad-band vibrational sum frequency generation (VSFG) spectroscopy. Ultrapure (UP) and ACS grade NaCl salts are used for aqueous solution preparation after proper pretreatment. The time evolution of VSFG spectra of PA monolayers on solutions made from these two grades of salts is different, which reveals that the salt purity has a significant impact on the interactions between Na⁺ ions and the COO⁻ group of PA. The trace metal impurities in ACS grade salt, which are more abundant than those in UP grade salt, are responsible for this difference due to their stronger affinity for the carboxylate group relative to Na⁺ and further affects the interfacial water structure. These results suggest that the alkali salt grade even after pretreatment is critical in the studies of alkali cation–carboxylate interactions and comparison of relative binding affinities of different cations.



INTRODUCTION

Cation-specific effects in aqueous solutions are essential to many chemical, biochemical, and atmospheric processes.^{1–8} Particularly, Na⁺ and K⁺, as the two most abundant alkali cations in intra- and extracellular fluids, play a critical role in cell signaling.^{9–11} While both are alkali cations, they exhibit notable chemical differences, for example, their binding affinities to ion pumps produce different intra- and extracellular Na⁺/K⁺ ratios, which, in turn, creates an ionic gradient across cell membranes.^{12–15} Computational studies by Jungwirth and co-workers have shown that the preferred interactions of Na⁺ and K⁺ with proteins can be attributed to the stronger affinity of Na⁺ for the carboxylate (COO⁻) groups within amino acid side chains.^{16,17} Other simulations of the cation-specific interactions with the carboxylate group and acetate ion have also shown stronger preference of Na⁺ to COO⁻ relative to K⁺.^{18–21} This analysis was also extended to other anionic groups in discriminating Na⁺ and K⁺ affinities in biological environments.²² In general, all of these results have been in agreement with the “law of matching water affinities” for which anions and cations with similar size and valence preferentially form stable ion pairs.²³

In addition to these theoretical studies, experimental work has been undertaken to elucidate the cation binding to the carboxylate group. For instance, Uejio et al. and Aziz et al. examined the cation-specific interactions with COO⁻ in amino acids as well as in formate and acetate aqueous solutions using X-ray absorption spectroscopy.^{21,24} More recently, Murda-chaw et al. investigated the alkali cation interactions with dicarboxylate dianions in the gas phase by photoelectron spectroscopy.¹⁸ The binding affinity of cations to the carboxyl head group of fatty acid monolayers at air/aqueous interfaces has also been of much interest. For example, the effect of

divalent cations on the structure and dissociation of long-chain fatty acid monolayers has been studied using infrared reflection–absorption spectroscopy (IRRAS),^{25,26} whereas Cremer and co-workers utilized surface-specific vibrational sum frequency generation (VSFG) spectroscopy to understand the cation-specific interactions with the carboxylate groups of fatty acid and acidic polypeptide monolayers and their influence on water organization at air/aqueous interfaces.^{5,27}

Most of the results obtained so far with Na⁺ and K⁺ ions have suggested that Na⁺ has a stronger binding affinity to COO⁻ relative to K⁺.^{5,22–24} However, a VSFG spectroscopy study by Tang and Allen pointed out a greater affinity of K⁺ than Na⁺ for the COO⁻ group of palmitic acid (PA) monolayers, in contradiction with the law of matching water affinities.⁸ This controversial finding initiated further experiments to assess the effect of salt purity and pretreatments on such studies.²⁸ Aside from instrumental and methodological factors, the purity of the salts utilized in the current experimental work was evaluated for their spectroscopic impact. As shown in Table 1, salts with different purities and pretreatments have been used among research groups. Note that there is typically a 1–2% contamination level in the ACS grade salts, part of which are polyvalent cations such as Mg²⁺, Ca²⁺, Fe³⁺, Pb²⁺, and Ba²⁺. The presence of such polyvalent cations, even in trace amounts, could significantly alter the binding affinities of monovalent cations and perturb the interfacial water organization through binding to the carboxylate group.²⁷

Special Issue: Terry A. Miller Festschrift

Received: July 7, 2013

Revised: September 15, 2013

Published: September 16, 2013

Table 1. Salts with Different Purities and Pretreatments Used Among Research Groups in Studies of Alkali Cation–Carboxylate Interactions

salt/carboxylate source	supplier	grade and purity	salt baking	solution filtration	ref
NaCl, KCl/polypeptide	Sigma-Aldrich	NaCl: 99.999%; KCl: 99.999%	no	no	5
NaCl, KCl/PA	Fisher Scientific	NaCl: ACS certified, 99%; KCl: EP/BP/USP/FCC, 99%	no	yes	8
NaCl, KCl/protein	Lachema	p. a. grade	not specified	not specified	16
NaCl, KCl/amino acid	Sigma-Aldrich	highest purity commercially available	no	no	21
NaOH, KOH/dicarboxylate acid	n/a	n/a	not specified	not specified	18
HCOONa, HCOOK, CH ₃ COONa, CH ₃ COOK	Sigma-Aldrich	98% or higher	no	no	24
NaCl/PA (UP grade)	Acros Organics	trace metals basis, 99.999%; polyvalent cations: ≤10 ppm	yes	yes	this work
NaCl/PA (ACS grade)	Fisher Scientific	ACS certified ACS, 99%; polyvalent cations: ~40 ppm	yes	yes	this work

In fact, salt purity in the aforementioned and other relevant studies can be critical and may have a significant impact on the experimental results. Very recently, Hua et al. reported on the effects of salt purity on the interfacial water organization of various bare aqueous salt solutions using conventional and heterodyne-detected (HD-) VSG spectroscopy.²⁸ Their results showed no appreciable difference in the water OH stretching region of spectra obtained from those salt solutions prepared from filtered ultrapure (UP) and ACS grade salts, thus indicating the negligible impact introduced by the presence of trace amount polyvalent cations. Incidentally, much less work has been done to address the impact of the trace amount of polyvalent cations at air/surfactant interfaces.²⁷

In this work, we clarify the impact of salt purity on the study of binding between one alkali cation (Na⁺) and the carboxylate group of PA monolayers at air/aqueous interfaces by means of VSG spectroscopy. Our results reveal the significantly different binding affinity of Na⁺ cations with the head group of PA by using filtered UP versus ACS grade salt solutions as the subphase. During the spectral monitoring, a small intensity decrease of the COO⁻ stretching peak is observed for the UP salt solution, whereas a pronounced intensity increase is seen for the ACS salt solution. For the whole OH stretching region, the intensity remains low and constant for the UP salt solutions but undergoes a gradual decrease over time for ACS grade salt solutions. The different spectral behavior is likely due to the stronger affinity of polyvalent metal ions present in the ACS grade salt.

EXPERIMENTAL DETAILS

Materials. PA (CH₃(CH₂)₁₄COOH, ≥99%) and acyl chain deuterated *d*₃₁-PA (CD₃(CD₂)₁₄COOH, 98%) were purchased from Sigma-Aldrich and Cambridge Isotopes, respectively. ACS grade (≥99%) and UP grade (trace metals basis, 99.999%) NaCl salts were purchased from Fisher Scientific and Acros Organics, respectively. Ultrapure water with a resistivity of 18.2–18.3 MΩ·cm and a measured pH of 5.6 (not purged of CO₂) was obtained from a Barnstead Nanopure system (model D4741, Thermolyne Corporation) equipped with additional organic-removing cartridges (D5026 Type I ORGANICfree Cartridge Kit; pretreat feed). All containers and glassware were carefully cleaned using concentrated sulfuric acid with the addition of a strong oxidizer, ammonium peroxydisulfate, and then thoroughly rinsed with UP water.

Preparation of Lipid and Aqueous Salt Solutions. PA and *d*₃₁-PA solutions were prepared at a 1.5 mM concentration

by dissolution in spectroscopic grade chloroform (Sigma-Aldrich). Both UP and ACS grade NaCl salts were baked at ~600 °C for 10 h in a muffle furnace (Isotemp model 550-14, Fisher Scientific). Stock salt solutions were prepared by dissolving ultrapure and ACS grade salts in ultrapure water and then filtering them twice using activated carbon filters (Whatman Carbon Cap 75, Fisher Scientific) to remove organic impurities. Their concentrations were then standardized by Mohr titration.²⁹ Both salt solutions were shown to be free of organic impurities, as revealed by their VSG spectra taken in the surfactant CH stretching region (2800–3000 cm⁻¹) (Supporting Information). All solutions were thermally equilibrated to room temperature (23 ± 1 °C) over 24 h before use.

Equilibrium Spreading of PA Monolayers. PA monolayers were overspread (~10 μL) on water and salt solutions in Petri dishes (5 cm diameter). The quantity of spread PA was equivalent to a mean molecular area of ~21 Å²/molecule,³⁰ which corresponds to a 2D-ordered (tilted liquid-condensed) phase.³¹ Following spreading, 10 min was allowed for solvent evaporation, after which, VSG spectra were collected for ~1 h. The acquisition time for each spectrum was 5 min.

Vibrational Sum Frequency Generation Spectroscopy. The VSG spectra were obtained on a broad-bandwidth VSG spectroscopy setup that has been described in detail elsewhere.^{8,32–34} Briefly, a Ti:sapphire oscillator (Tsunami, Spectra-Physics) with a center wavelength at 800 nm was used to seed two 1 kHz regenerative amplifiers (Spitfire femtosecond (fs) and picosecond (ps) versions, Spectra-Physics) that are pumped by a solid-state Nd:YLF laser (Evolution 30, Spectra-Physics) at 527 nm. The fs and ps amplifiers generate broad-band (~85 fs, ~345 cm⁻¹ bandwidth, ~1 W) and narrow-band (2 ps, ~17 cm⁻¹ bandwidth, >0.9 W) laser pulses at 800 nm, respectively.³⁴ The narrow-bandwidth beam was used as the visible (vis) beam, while the broad-bandwidth beam was further directed to an optical parametric amplifier (TOPAS-C, Light Conversion) coupled to a narrow-difference frequency generation system (NDFG, Light Conversion) that generated a tunable mid-infrared (IR) beam. The full spectral bandwidth of the generated broad-band IR beam was ~250 cm⁻¹ in the COO⁻ stretching region (1350–1600 cm⁻¹) and ~800 cm⁻¹ in the OH stretching region (3000–3800 cm⁻¹). The spectral resolution was ~8 cm⁻¹.³⁰ The average energies of the IR beam for the COO⁻ and OH stretching regions were ~6 and 10 μJ, respectively, while that of the vis beam was ~300 μJ. The copropagating vis and IR

beams impinge on the aqueous surface at incident angles of 53 and 68°, respectively, and are overlapped spatially and temporally to generate a sum frequency (SF) beam in the reflection direction. The ssp beam polarization combination (s-SF, s-vis, and p-IR) was used. Furthermore, most of the IR beamline was purged with dry nitrogen to minimize the absorption of the IR beam by water vapor in the air in both spectral regions.

RESULTS AND DISCUSSION

In the following discussion, VSGF spectra of PA monolayers in the COO⁻ and OH stretching regions are presented. The COO⁻ peak directly reveals the binding of the Na⁺ cation to the head group of PA, while the OH stretching band relates to the interfacial water structure that is affected by the surfactant,^{27,35–39} thus providing a complementary view of the binding event. Tang and Allen reported the VSGF spectra of PA on non-UP (e.g., ACS grade) salt solutions ~10–15 min after spreading and found a stronger affinity of K⁺ for the carboxylate group over Na⁺.⁸ It is also known that the intensity of the spectra may change with time due to monolayer relaxation⁴⁰ that can affect the comparison of the binding affinity of cations to carboxylate groups. Therefore, a time study was performed to see how the spectra change for aqueous solutions made from UP and ACS grade salts.

Direct Evidence of Na⁺ Interactions with the Carboxylate Group from the COO⁻ Stretching Region.

Figure 1 shows the time evolution of VSGF spectra in the COO⁻ stretching region of *d*₃₁-PA monolayers on 0.6 M NaCl solutions up to about 1 h after spreading. Two peaks at ~1405 and ~1470 cm⁻¹ are observed for both UP grade and ACS grade salts. The *d*₃₁-PA was chosen here to avoid the interference from CH₂ bending modes (~1465 cm⁻¹). The contribution to the signal in this region is assigned to the different species (complexed and hydrated) of the COO⁻ groups (see below). Note that the pK_a value of the carboxyl group is ~4.6–4.9 for short-chain fatty acids in the bulk, but it increases to ~7–10 for medium- and long-chain fatty acids at the air/water interface due to the closer packing occurring with increased chain length.^{41,42} For example, using different experimental techniques, the apparent surface pK_a of PA was found to lie in the range of 8–10.^{40,41,43,44} Hence, given a neat water subphase with pH 5.6, according to the calculations reported by Shen and co-workers, ~99.9% of the PA head groups are protonated prior to binding,⁴⁵ which is supported by the lack of a SFG COO⁻ signal from a *d*₃₁-PA monolayer on neat water (Figure 1a). The presence of the COO⁻ stretching signal observed in Figure 1b,c can be attributed to the deprotonation caused by the metal cations in the subphase,⁸ which will be discussed below. The peak at 1405 cm⁻¹ is assigned to the hydrated COO⁻ species,^{8,40,45} whereas the one at 1470 cm⁻¹ is indicative of ionic complexes of COO⁻ groups with cations.^{8,40} This ionic complex has been previously identified by IRRAS.^{25,26,46} The intensity of the high-frequency peak at ~1470 cm⁻¹ is much higher than that of the low-frequency peak at ~1405 cm⁻¹, suggesting that most COO⁻ groups form complexes with the metal cations (M⁺) in the subphase. In the following discussion, the emphasis will be put on the 1470 cm⁻¹ peak as it is directly related to the binding event between COO⁻ and Na⁺.

Figure 1b depicts the VSGF spectra of a *d*₃₁-PA monolayer on the solution prepared with the UP grade NaCl salt. After spreading, the 1470 cm⁻¹ peak displays a gradual but small

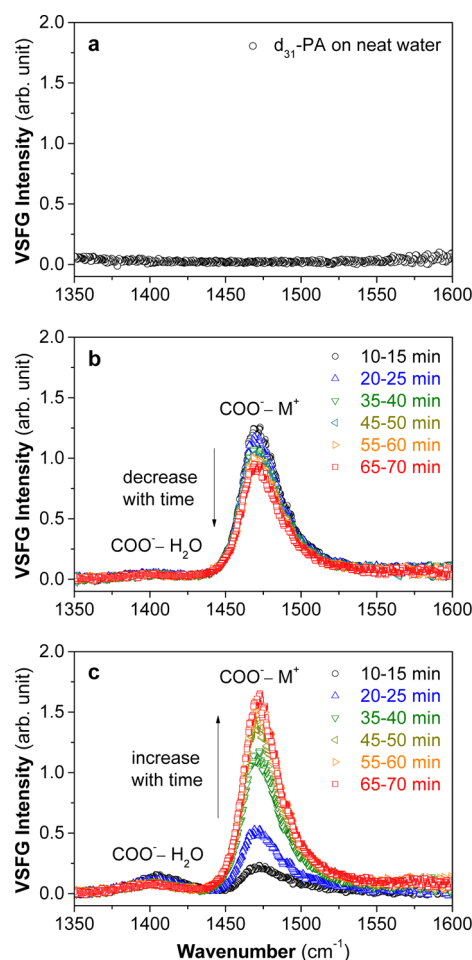


Figure 1. VSGF spectra of *d*₃₁-PA monolayers on neat water and NaCl salt solutions in the COO⁻ stretching region (1350–1600 cm⁻¹) up to about 1 h after spreading: (a) neat water, (b) UP grade 0.6 M NaCl solution, and (c) ACS grade 0.6 M NaCl solution. Spectra were taken ~10 min after spreading, as shown in the figure.

decrease in intensity (up to ~20%) and becomes relatively constant after about 1 h. Because the VSGF signal is responsive to the local ordering (anisotropy) at the air/aqueous interface,^{47,48} the intensity decrease observed here can be attributed to the PA monolayer relaxation; PA molecules become more disordered as no counteracting surface pressure is applied. Sakai and Umemura also observed surface pressure relaxation in Langmuir monolayers of zinc stearate by infrared external reflection spectroscopy, which was linked to a change in the orientation angle of the hydrocarbon chain relative to the surface normal.⁴⁹ In addition, Ma and Allen's VSGF study on phospholipid monolayers on water showed a significantly lower intensity of the symmetric phosphate (POO⁻) group at lower surface pressure (~12 mN/m),⁵⁰ which is similar to the COO⁻ intensity decrease observed here.

In the case of ACS grade NaCl salt, as seen in Figure 1c, the general features of the VSGF spectra remain nearly identical to those found in Figure 1b. The 1470 cm⁻¹ peak in the initial spectra taken ~10 min after spreading is much lower in intensity than that of UP grade salt (compare the black spectra in Figure 1b and c). Despite the different peak intensity ratio, the presence of two peaks at 1405 and 1470 cm⁻¹ in Figure 1c agrees with Tang and Allen's spectra (after ~10 min spreading).⁸ However, unlike the intensity decrease of the

1470 cm^{-1} peak observed in the spectra from the UP grade NaCl solution, there is a remarkable intensity increase for the ACS grade NaCl solution, indicating that more cation– COO^- surface complexes are present and, thus, stronger cation binding to COO^- .^{8,40} The intensity about 1 h after spreading becomes constant and almost 6 times higher than the initial intensity, although varying to some extent on different days (4–6 times higher; see the Supporting Information). This trend is significantly different from that observed in the UP grade NaCl solution spectra. Because both ACS and UP grade NaCl salts were pretreated following the same procedure and the experiments were performed under the same conditions, the difference in intensity change can be primarily attributed to the purity of the salts, that is, the presence of trace amounts of di- and trivalent cations, which have stronger binding affinities with the head group of PA.^{5,25–27,40} In particular, as shown in Table 1, the ACS grade NaCl contains more polyvalent metal cations (~ 40 ppm) than UP grade NaCl (≤ 10 ppm). It is expected that the quite small ($<0.01\%$) but non-negligible amount of polyvalent cations in the ACS grade salt bind to the COO^- groups due to their stronger binding affinity relative to Na^+ ,^{5,26} which largely accounts for the dramatic intensity increase of the 1470 cm^{-1} peak with elapsed time. Even though the ACS grade NaCl also contains some trace amounts of anions (e.g., Br^- , I^- , NO_3^- , ClO_4^- , etc., for a total amount of ≤ 200 ppm), the cation effect is dominant in the ion–monolayer electrostatic interactions. Not surprisingly, because the carboxyl head group of PA becomes negatively charged upon the ion-induced deprotonation, it is more likely to attract oppositely charged cations.^{8,25,40} The binding event is also believed to be cation-specific and to follow a Hofmeister series.^{51,52} However, whether the coanion has an influence or not on these interactions still needs further investigation.

Indirect Evidence of Na^+ Interactions with the Carboxylate Group from the OH Stretching Region.

The organization of water molecules in the OH stretching region is highly sensitive to its environment.^{53–58} For instance, the influence of trace amounts of organics on the interfacial water structure has been extensively studied.^{27,35–39} Because all of the salts used here are free of organics after pretreatment, the spectral change observed here can only be attributed to the cation–carboxylate binding.

Figure 2a presents the VSGF spectrum in the OH stretching region of a PA monolayer on neat water. There are four bands located at ~ 2940 , ~ 3200 , ~ 3450 , and ~ 3600 cm^{-1} . The 2940 cm^{-1} peak is assigned to the Fermi resonance of the CH_3 symmetric stretch with the overtone of the bending mode.^{8,45} The broad band from 3200 to 3450 cm^{-1} is a signature of collective OH stretching modes of hydrogen-bonded water molecules that display complex coordination and cooperativity in the interfacial region.^{56,59,60} The 3600 cm^{-1} peak results from weakly hydrogen-bonded OH groups of both fatty acid and water. These features are consistent with the VSGF spectra reported by Miranda et al.⁴⁵ and Tang and Allen.^{8,35} Although, as mentioned earlier, most of the PA molecules are intact at the water surface, a small amount of deprotonated carboxylic groups still exists, and, in turn, the presence of an electric field induced by this deprotonation can change the interfacial water structure,³⁵ as shown by the higher intensity relative to that of neat water in Figure 2a. Note that the spectra in Figure 2a are slightly different from previously reported ones, especially in the region from ~ 3600 to 3700 cm^{-1} . This was probably

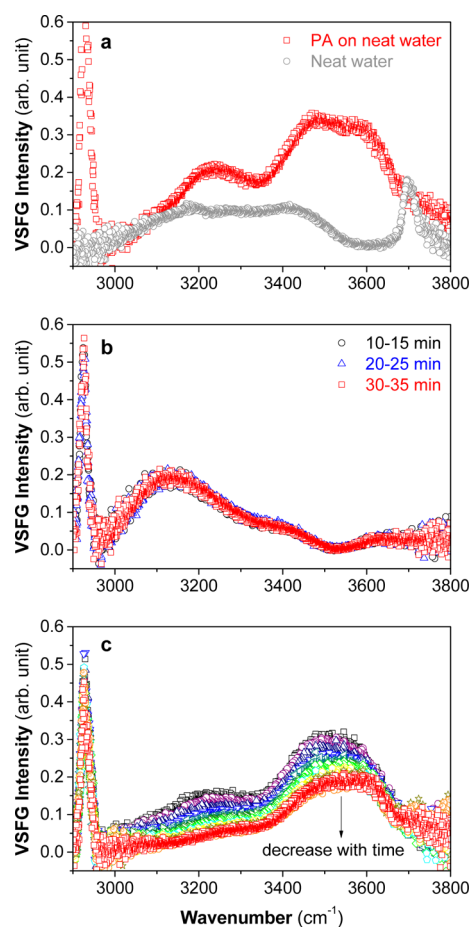


Figure 2. VSGF spectra of PA monolayers on neat water and NaCl salt solutions in the OH stretching region ($3000\text{--}3800$ cm^{-1}) up to about 1 h after spreading. (a) PA on neat water, (b) UP grade 0.6 M NaCl solution, and (c) ACS grade 0.6 M NaCl solution. Spectra were taken every 7 min on average, ~ 10 min after spreading.

caused by the lower energy on the high-frequency side of our previous incident IR profile that was not easily normalized out.

In the presence of NaCl in the subphase, the VSGF spectra of the PA monolayers have significantly changed (Figure 2b,c). The intensity of the OH stretching bands on UP grade NaCl solution (Figure 2b) changes dramatically relative to that of PA on neat water. The lower intensity in the high-frequency region in Figure 2b relative to Figure 2a indicates a loss of local ordering between water molecules just below the PA monolayer due to charge screening. In other words, the interaction of Na^+ with the COO^- head groups gives rise to a less charged interface that weakly aligns the water molecules. This explanation for the spectral intensity attenuation relative to PA on neat water is in accordance with previous VSGF studies of surfactant monolayers on salt solutions.^{27,35,37,61} In addition, the intensity remains constant even 30 min after spreading the PA monolayer on the UP grade NaCl solution, suggesting that a stable monolayer is formed. It could serve as evidence that the trace amounts of polyvalent metal ions in the UP grade NaCl salt did not result in a significant perturbation of the interfacial water organization.

In contrast, the VSGF spectra of a PA monolayer on ACS grade NaCl solution resemble that on neat water (compare Figure 2a and c). The three broad bands in the region from 3000 to 3800 cm^{-1} are easily discernible, and the peak

assignment is similar to that on the neat water subphase, although the peak intensity decreases, especially at $\sim 3200\text{ cm}^{-1}$. The spectral intensity decrease provides evidence of cations binding to COO^- as the water molecules become less aligned by the weaker interfacial electric field and the head group becomes less ordered with respect to orientation.^{27,35,36,61} Despite the overall lower intensity relative to that of PA on neat water, the spectral intensity on the ACS grade subphase is much higher at 3500 cm^{-1} than that for PA on the UP grade NaCl solution. As shown in Table 1, the concentrations of polyvalent cations in UP and ACS grade NaCl salts are ≤ 10 and ~ 40 ppm, respectively. Gurau et al. have reported the marked effect of trace amounts of divalent cations (1 and $10\text{ }\mu\text{M}$) on an arachidic acid ($\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$) monolayer in the OH stretching region relative to that on neat water.²⁷ It is expected that the impurity level of polyvalent cations between the UP and ACS grade salts in our experiment is sufficient and leads to the significant difference observed between Figure 2b and c.

The most significant difference between the UP and ACS grade salt solutions is the intensity changes with elapsed time. Unlike the VSG spectra given in Figure 2b, those in Figure 2c decreased in intensity and then became unchanged after about 1 h. As more polyvalent metal cations present in the ACS grade salt, which have stronger binding affinity, bind to COO^- groups, the water hydrogen bonding structure changes. Accordingly, the ionic complex between the cation and COO^- becomes predominant and leads to an increasing peak intensity at $\sim 1470\text{ cm}^{-1}$, as shown in Figure 1c. The two complementary views of the binding mechanism revealed by the VSG spectra from the COO^- and OH stretching regions are consistent with each other.

On the basis of the spectral changes of PA monolayers on UP and ACS grade NaCl solutions in both the COO^- and OH stretching regions, the purity of alkali salts is found to have a great impact on the resulting VSG spectra of fatty acids spread on their solutions (even after pretreatment). This may further cause difficulty in determining the relative binding affinity of cations, for example, Na^+ versus K^+ . Therefore, UP alkali salts are highly recommended for studies of cation–carboxylate binding. Note that the impact of organic contaminants with the UP grade salt on the cation–carboxylate binding has not yet been studied.^{5,6} Furthermore, to gain a better understanding of the cation–surfactant interaction, a complete study of other salts and surfactants (e.g., phospholipids) as well as of pretreatment effects is needed.

CONCLUSIONS

PA monolayers at the air/NaCl solution interface were monitored over time by VSG spectroscopy in the COO^- and OH stretching regions after spreading on the pretreated solutions prepared from UP and ACS grade NaCl salts. Significant differences were observed between the UP and ACS grade salt solution spectra. In the COO^- stretching region, the 1470 cm^{-1} peak, which is a signature of the ionic complex formed between the COO^- group and cations, undergoes a small intensity decrease for the UP salt solution, whereas for the ACS salt subphase, a drastic intensity increase is observed. In the OH stretching region, the intensity of the broad band from 3000 to 3800 cm^{-1} remains unchanged for the UP salt solution but gradually decreases for the ACS one.

The information conveyed by the VSG spectra of PA monolayers in the COO^- and OH stretching regions are

consistent with each other. Both results demonstrate the impact of trace metal contaminants on the cation–carboxylate binding, and these impurities largely affect the spectra of PA in both spectral regions. The changes in the spectra for UP salt solution are likely related to monolayer relaxation, but for the ACS salt subphase, the changes are largely due to the trace metal impurities that strengthen the cation–carboxylate binding. The grade of alkali salts therefore proves to be critical in exploring the cation–carboxylate binding and comparing relative binding affinities of different cations. The use of UP grade salt is strongly recommended especially in the studies related to alkali–carboxylate interactions. However, whether the salt purity effect applies to the interactions between other monovalent cations and negatively charged moieties such as the phosphate group in phospholipids is still unknown. More generally, special care should be taken in the selection of chemicals when investigating air/aqueous surfactant interfaces with surface-sensitive techniques.

ASSOCIATED CONTENT

Supporting Information

VSG spectra of NaCl salt solutions examined in the CH stretching region showing the absence of organic contamination after pretreatment. VSG spectra of neat water in the OH stretching region obtained during the entire experimental period, demonstrating system stability. Day-to-day comparison of VSG spectra in the COO^- stretching region, showing reproducibility. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*E-mail: allen@chemistry.ohio-state.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge the NSF-CHE (Grant No. 0749807) and the DOE-BES (Grant No. DE-FG02-04ER15495) for funding this work. W.H. acknowledges a Presidential Fellowship from The Ohio State University.

REFERENCES

- (1) Melander, W.; Horvath, C. Salt Effects on Hydrophobic Interactions in Precipitation and Chromatography of Proteins — Interpretation of Lyotropic Series. *Arch. Biochem. Biophys.* **1977**, *183*, 200–215.
- (2) Collins, K. D. Sticky Ions in Biological Systems. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5553–5557.
- (3) Baldwin, R. L. How Hofmeister Ion Interactions Affect Protein Stability. *Biophys. J.* **1996**, *71*, 2056–2063.
- (4) Collins, K. D. Ion Hydration: Implications for Cellular Function, Polyelectrolytes, and Protein Crystallization. *Biophys. Chem.* **2006**, *119*, 271–281.
- (5) Kherb, J.; Flores, S. C.; Cremer, P. S. Role of Carboxylate Side Chains in the Cation Hofmeister Series. *J. Phys. Chem. B* **2012**, *116*, 7389–7397.
- (6) Okur, H. I.; Kherb, J.; Cremer, P. S. Cations Bind Only Weakly to Amides in Aqueous Solutions. *J. Am. Chem. Soc.* **2013**, *135*, 5062–5067.
- (7) Donaldson, D. J.; Vaida, V. The Influence of Organic Films at the Air–Aqueous Boundary on Atmospheric Processes. *Chem. Rev.* **2006**, *106*, 1445–1461.

- (8) Tang, C. Y.; Allen, H. C. Ionic Binding of Na⁺ versus K⁺ to the Carboxylic Acid Headgroup of Palmitic Acid Monolayers Studied by Vibrational Sum Frequency Generation Spectroscopy. *J. Phys. Chem. A* **2009**, *113*, 7383–7393.
- (9) Bullock, T. H.; Orkand, R.; Grinnell, A. *Introduction to Nervous Systems*; W.H. Freeman: San Francisco, CA, 1977.
- (10) Chung, S.-H.; Andersen, O. S.; Krishnamurthy, V. *Biological Membrane Ion Channels Dynamics, Structure, and Applications*; Springer: New York, 2007.
- (11) Clausen, T. Role of Na⁺,K⁺-Pumps and Transmembrane Na⁺,K⁺-Distribution in Muscle Function. *Acta Physiol.* **2008**, *192*, 339–349.
- (12) Jorgensen, P. L. Sodium and Potassium-Ion Pump in Kidney Tubules. *Physiol. Rev.* **1980**, *60*, 864–917.
- (13) Spanswick, R. M. Electrogenic Ion Pumps. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1981**, *32*, 267–289.
- (14) Scheiner-Bobis, G. The Sodium Pump — Its Molecular Properties and Mechanics of Ion Transport. *Eur. J. Biochem.* **2002**, *269*, 2424–2433.
- (15) Lodish, H. F.; Berk, A.; Kaiser, C. A.; Krieger, M.; Bretscher, A.; Ploegh, H.; Amon, A.; Scott, M. P. *Molecular Cell Biology*; W.H. Freeman and Co.: New York, 2012.
- (16) Vrbka, L.; Vondrasek, J.; Jagoda-Cwiklik, B.; Vácha, R.; Jungwirth, P. Quantification and Rationalization of the Higher Affinity of Sodium over Potassium to Protein Surfaces. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15440–15444.
- (17) Heyda, J.; Pokorna, J.; Vrbka, L.; Vácha, R.; Jagoda-Cwiklik, B.; Konvalinka, J.; Jungwirth, P.; Vondrasek, J. Ion Specific Effects of Sodium and Potassium on the Catalytic Activity of HIV-1 Protease. *Phys. Chem. Chem. Phys.* **2009**, *11*, 7599–7604.
- (18) Murdachaew, G.; Valiev, M.; Kathmann, S. M.; Wang, X. B. Study of Ion Specific Interactions of Alkali Cations with Dicarboxylate Dianions. *J. Phys. Chem. A* **2012**, *116*, 2055–2061.
- (19) Hess, B.; van der Vegt, N. F. A. Cation Specific Binding with Protein Surface Charges. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 13296–13300.
- (20) Hajari, T.; Ganguly, P.; van der Vegt, N. F. A. Enthalpy–Entropy of Cation Association with the Acetate Anion in Water. *J. Chem. Theory Comput.* **2012**, *8*, 3804–3809.
- (21) Aziz, E. F.; Ottosson, N.; Eisebitt, S.; Eberhardt, W.; Jagoda-Cwiklik, B.; Vácha, R.; Jungwirth, P.; Winter, B. Cation-Specific Interactions with Carboxylate in Amino Acid and Acetate Aqueous Solutions: X-ray Absorption and Ab Initio Calculations. *J. Phys. Chem. B* **2008**, *112*, 12567–12570.
- (22) Jagoda-Cwiklik, B.; Vácha, R.; Lund, M.; Srebro, M.; Jungwirth, P. Ion Pairing as a Possible Clue for Discriminating between Sodium and Potassium in Biological and Other Complex Environments. *J. Phys. Chem. B* **2007**, *111*, 14077–14079.
- (23) Collins, K. D.; Neilson, G. W.; Enderby, J. E. Ions in Water: Characterizing the Forces That Control Chemical Processes and Biological Structure. *Biophys. Chem.* **2007**, *128*, 95–104.
- (24) Uejio, J. S.; Schwartz, C. P.; Duffin, A. M.; Drisdell, W. S.; Cohen, R. C.; Saykally, R. J. Characterization of Selective Binding of Alkali Cations with Carboxylate by X-ray Absorption Spectroscopy of Liquid Microjets. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6809–6812.
- (25) Simon-Kutscher, J.; Gericke, A.; Hühnerfuss, H. Effect of Bivalent Ba, Cu, Ni, and Zn Cations on the Structure of Octadecanoic Acid Monolayers at the Air–Water Interface as Determined by External Infrared Reflection–Absorption Spectroscopy. *Langmuir* **1996**, *12*, 1027–1034.
- (26) Le Calvez, E.; Blaudez, D.; Buffeteau, T.; Desbat, B. Effect of Cations on the Dissociation of Arachidic Acid Monolayers on Water Studied by Polarization-Modulated Infrared Reflection–Absorption Spectroscopy. *Langmuir* **2001**, *17*, 670–674.
- (27) Gurau, M. C.; Kim, G.; Lim, S. M.; Albertorio, F.; Fleisher, H. C.; Cremer, P. S. Organization of Water Layers at Hydrophilic Interfaces. *ChemPhysChem* **2003**, *4*, 1231–1233.
- (28) Hua, W.; Verreault, D.; Adams, E. M.; Huang, Z.; Allen, H. C. Impact of Salt Purity on Interfacial Water Organization Revealed by Conventional and Heterodyne-Detected Vibrational Sum Frequency Generation Spectroscopy. *J. Phys. Chem. C* **2013**, *117*, 19577–19585.
- (29) Finlayson, A. C. The pH Range of the Mohr Titration for Chloride-Ion Can Be Usefully Extended to 4–10.5. *J. Chem. Educ.* **1992**, *69*, 559–559.
- (30) Ma, G.; Allen, H. C. Condensing Effect of Palmitic Acid on DPPC in Mixed Langmuir Monolayers. *Langmuir* **2007**, *23*, 589–597.
- (31) Kaganer, V. M.; Möhwald, H.; Dutta, P. Structure and Phase Transitions in Langmuir Monolayers. *Rev. Mod. Phys.* **1999**, *71*, 779–819.
- (32) Allen, H. C.; Casillas-Ituarte, N. N.; Sierra-Hernandez, M. R.; Chen, X. K.; Tang, C. Y. Shedding Light on Water Structure at Air–Aqueous Interfaces: Ions, Lipids, and Hydration. *Phys. Chem. Chem. Phys.* **2009**, *11*, 5538–5549.
- (33) Hommel, E. L.; Allen, H. C. Broadband Sum Frequency Generation with Two Regenerative Amplifiers: Temporal Overlap of Femtosecond and Picosecond Light Pulses. *Anal. Sci.* **2001**, *17*, 137–139.
- (34) Ma, G.; Allen, H. C. Surface Studies of Aqueous Methanol Solutions by Vibrational Broad Bandwidth Sum Frequency Generation Spectroscopy. *J. Phys. Chem. B* **2003**, *107*, 6343–6349.
- (35) Tang, C. Y.; Huang, Z. S.; Allen, H. C. Interfacial Water Structure and Effects of Mg²⁺ and Ca²⁺ Binding to the COOH Headgroup of a Palmitic Acid Monolayer Studied by Sum Frequency Spectroscopy. *J. Phys. Chem. B* **2011**, *115*, 34–40.
- (36) Chen, X. K.; Hua, W.; Huang, Z. S.; Allen, H. C. Interfacial Water Structure Associated with Phospholipid Membranes Studied by Phase-Sensitive Vibrational Sum Frequency Generation Spectroscopy. *J. Am. Chem. Soc.* **2010**, *132*, 11336–11342.
- (37) Sovago, M.; Wurpel, G. W. H.; Smits, M.; Müller, M.; Bonn, M. Calcium-Induced Phospholipid Ordering Depends on Surface Pressure. *J. Am. Chem. Soc.* **2007**, *129*, 11079–11084.
- (38) Nihonyanagi, S.; Yamaguchi, S.; Tahara, T. Direct Evidence for Orientational Flip–Flop of Water Molecules at Charged Interfaces: A Heterodyne-Detected Vibrational Sum Frequency Generation Study. *J. Chem. Phys.* **2009**, *130*, 204704.
- (39) Sovago, M.; Vartiainen, E.; Bonn, M. Observation of Buried Water Molecules in Phospholipid Membranes by Surface Sum-Frequency Generation Spectroscopy. *J. Chem. Phys.* **2009**, *131*, 161107.
- (40) Tang, C. Y.; Huang, Z. S. A.; Allen, H. C. Binding of Mg²⁺ and Ca²⁺ to Palmitic Acid and Deprotonation of the COOH Headgroup Studied by Vibrational Sum Frequency Generation Spectroscopy. *J. Phys. Chem. B* **2010**, *114*, 17068–17076.
- (41) Kanicky, J. R.; Poniatowski, A. F.; Mehta, N. R.; Shah, D. O. Cooperativity among Molecules at Interfaces in Relation to Various Technological Processes: Effect of Chain Length on the pK_a of Fatty Acid Salt Solutions. *Langmuir* **2000**, *16*, 172–177.
- (42) Kanicky, J. R.; Shah, D. O. Effect of Degree, Type, and Position of Unsaturation on the pK_a of Long-Chain Fatty Acids. *J. Colloid Interface Sci.* **2002**, *256*, 201–207.
- (43) Gomez-Fernandez, J. C.; Villalain, J. The Use of FT-IR for Quantitative Studies of the Apparent pK_a of Lipid Carboxyl Groups and the Dehydration Degree of the Phosphate Group of Phospholipids. *Chem. Phys. Lipids* **1998**, *96*, 41–52.
- (44) Heikkilä, R. E.; Deamer, D. W.; Cornwell, D. G. Solution of Fatty Acids from Monolayers Spread at Air–Water Interface — Identification of Phase Transformations and Estimation of Surface Charge. *J. Lipid Res.* **1970**, *11*, 195–200.
- (45) Miranda, P. B.; Du, Q.; Shen, Y. R. Interaction of Water with a Fatty Acid Langmuir Film. *Chem. Phys. Lett.* **1998**, *286*, 1–8.
- (46) Gericke, A.; Hühnerfuss, H. The Effect of Cations on the Order of Saturated Fatty Acid Monolayers at the Air–Water Interface as Determined by Infrared Reflection–Absorption Spectrometry. *Thin Solid Films* **1994**, *245*, 74–82.
- (47) Shen, Y. R. *The Principles of Nonlinear Optics*; J. Wiley: New York, 1984.
- (48) Shen, Y. R. Surface Properties Probed by 2nd Harmonic and Sum-Frequency Generation. *Nature* **1989**, *337*, 519–525.

(49) Sakai, H.; Umemura, J. Evaluation of Structural Change During Surface Pressure Relaxation in Langmuir Monolayer of Zinc Stearate by Infrared External Reflection Spectroscopy. *Colloid Polym. Sci.* **2008**, *286*, 1637–1641.

(50) Ma, G.; Allen, H. C. DPPC Langmuir Monolayer at the Air–Water Interface: Probing the Tail and Head Groups by Vibrational Sum Frequency Generation Spectroscopy. *Langmuir* **2006**, *22*, 5341–5349.

(51) Vlachy, N.; Jagoda-Cwiklik, B.; Vácha, R.; Touraud, D.; Jungwirth, P.; Kunz, W. Hofmeister Series and Specific Interactions of Charged Headgroups with Aqueous Ions. *Adv. Colloid Interface Sci.* **2009**, *146*, 42–47.

(52) Kunz, W. Specific Ion Effects in Colloidal and Biological Systems. *Curr. Opin. Colloid Interface Sci.* **2010**, *15*, 34–39.

(53) Ji, N.; Ostroverkhov, V.; Tian, C. S.; Shen, Y. R. Characterization of Vibrational Resonances of Water–Vapor Interfaces by Phase-Sensitive Sum-Frequency Spectroscopy. *Phys. Rev. Lett.* **2008**, *100*, 096102.

(54) Shen, Y. R.; Ostroverkhov, V. Sum-Frequency Vibrational Spectroscopy on Water Interfaces: Polar Orientation of Water Molecules at Interfaces. *Chem. Rev.* **2006**, *106*, 1140–1154.

(55) Pieniazek, P. A.; Tainter, C. J.; Skinner, J. L. Surface of Liquid Water: Three-Body Interactions and Vibrational Sum-Frequency Spectroscopy. *J. Am. Chem. Soc.* **2011**, *133*, 10360–10363.

(56) Verreault, D.; Hua, W.; Allen, H. C. From Conventional to Phase-Sensitive Vibrational Sum Frequency Generation Spectroscopy: Probing Water Organization at Aqueous Interfaces. *J. Phys. Chem. Lett.* **2012**, *3*, 3012–3028.

(57) Shultz, M. J.; Baldelli, S.; Schnitzer, C.; Simonelli, D. Aqueous Solution/Air Interfaces Probed with Sum Frequency Generation Spectroscopy. *J. Phys. Chem. B* **2002**, *106*, 5313–5324.

(58) Richmond, G. L. Molecular Bonding and Interactions at Aqueous Surfaces as Probed by Vibrational Sum Frequency Spectroscopy. *Chem. Rev.* **2002**, *102*, 2693–2724.

(59) Jubb, A. M.; Hua, W.; Allen, H. C. Environmental Chemistry at Vapor/Water Interfaces: Insights from Vibrational Sum Frequency Generation Spectroscopy. *Annu. Rev. Phys. Chem.* **2012**, *63*, 107–130.

(60) Bonn, M.; Bakker, H. J.; Tong, Y. J.; Backus, E. H. G. No Ice-Like Water at Aqueous Biological Interfaces. *Biointerphases* **2012**, *7*.

(61) Nihonyanagi, S.; Yamaguchi, S.; Tahara, T. Water Hydrogen Bond Structure near Highly Charged Interfaces Is Not Like Ice. *J. Am. Chem. Soc.* **2010**, *132*, 6867–6869.