# Direct and Reverse Hofmeister Effects on Interfacial Water Structure

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ABSTRACT: The adsorption of Hofmeister anions to negatively charged hydrophilic, negatively charged hydrophobic, and positively charged hydrophilic aqueous/oxide interfaces was studied by analyzing changes in the adjacent interfacial water structure using vibrational sum frequency spectroscopy (VSFS). At the negatively charged quartz/water interface at pH 10.0, it was found that the introduction of sodium salts of weakly hydrated monovalent anions (i.e., SCN^- and ClO_4^-) led to less attenuation of the water structure than when NaCl was added to solution. Addition of $NaBr$ and $NaNO_3$ gave rise to intermediate behavior. These results are consistent with less exclusion of the more weakly hydrated anions from the quartz substrate and the counterion double layer. As such, the monovalent anions followed a direct Hofmeister series. When the quartz surface was modified with an octadecyltrichlorosilane (OTS) monolayer, the ordering of the anions remained the same. Again, this is consistent with preferential exclusion of Cl^- over SCN^- and ClO_4^- in accordance with a direct Hofmeister series. The quartz surfaces were also modified with a thin layer of TiO_2. At low pH, this created a positively charged hydrophilic interface. Under these conditions, SCN^- and ClO_4^- attenuated the VSFS water peaks to a greater extent than Cl^-. This apparent monovalent anion series reversal, however, was also caused by the preferential adsorption of the more weakly hydrated anions in comparison to the more strongly hydrated anions. In fact, the reversal was the result of preferential anion adsorption and double layer formation at the positively charged oxide/aqueous interface as opposed to preferential Na^+ partitioning at the negatively charged oxide/aqueous interfaces.
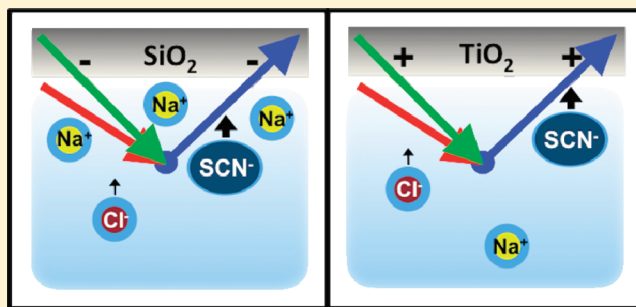
INTRODUCTION

The Hofmeister series is a rank ordering of ions that originally described the propensity of salts to precipitate proteins from aqueous solution.[1-3] Thermodynamic models propose that ions which favorably partition to protein surfaces lead to salting-in effects, while ions that are depleted from the protein/water interface cause salting-out.[4] These interactions can be directly probed using model systems.[5,6] The order in which monovalent anions influence the solubility of proteins in the direct Hofmeister series is as follows

$$Cl^- > NO_3^- > Br^- > ClO_4^- > SCN^-$$
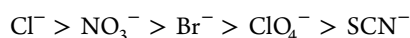
The anions further to the left are more strongly hydrated than those to the right. Weakly hydrated anions are known to decrease protein stability and increase their solubility. In addition, they decrease the surface tension of the protein/water interface[7] and increase protein denaturation.[8] By contrast, more strongly hydrated anions generally have the opposite effect and salt proteins out of solution.

A variety of spectroscopic, thermodynamic, and computational investigations have been conducted to study specific anion effects at charged and uncharged interfaces.[5,6,9-12] A direct Hofmeister series is observed for phenomena ranging from protein stability and phase behavior to the precipitation of inorganic nanoparticles and enzyme activity.[8,13,14] Molecular dynamics simulations have corroborated the idea that larger and more polarizable anions interact more favorably with

protein surfaces.[15-17] Moreover, calculations employing a salt ion-partitioning model (SPM) have been used to interpret and predict Hofmeister ion effects at the air/water and protein/water interfaces.[18,19] These studies also showed that more weakly hydrated anions usually partition to the interface to a greater extent than other anions. Similar results have also been found for Langmuir monolayers of octadecylamine (ODA).[20]

By contrast with the studies noted above, there are numerous systems and conditions for which a reversed or partially reversed anion series is observed.[21-23] In macromolecular and colloidal samples, such behavior often occurs when the organic molecules are below their isoelectric point and, as such, positively charged.[24,25] This is the case for lysozyme, which aggregates at low salt concentrations (<200-300 mM) according to a reverse Hofmeister series. As the salt concentration is increased (>300 mM), however, the anion ordering for the liquid-liquid phase transition of lysozyme reverts to a direct series.[25] In a recent study, the effects of anions on water structure at the bovine serum albumin (BSA)/aqueous interface were investigated. The results demonstrated that a reversal in Hofmeister ordering for water structure occurred when the monolayer charge was changed from negative to positive as the sample pH was lowered below the

isoelectric point.[24] A reversed Hofmeister series for anions was also observed for a positively charged surfactant film whose charge was independent of the subphase pH. This result helped confirm the generality of the reversal in anion series ordering when a macromolecule, thin film, or colloidal system is positively charged, independent of the specific pH.

Vibrational sum frequency spectroscopy is an important tool for monitoring the liquid/solid interface (VSFS).[26−30] It can be specifically utilized to monitor interfacial water structure in the presence of polymers, proteins, and surfactant layers with numerous different salts present in the adjacent aqueous solution.[6,20,24] The extent of salt interactions at these surfaces can be quantified by monitoring changes in the VSFS signal derived from the alignment of water directly adjacent to interfacial macromolecules. Since the VSFS water signal is sensitive to the surface potential, changes in the OH stretch band intensity can be caused by anion adsorption, which modulates the surface charge. In some cases, changes in the oscillator strength of the VSFS OH stretch are linearly proportional to interfacial potential and can be exploited to obtain equilibrium dissociation constant data for the anions by invoking a Gouy−Chapman−Stern model.[6,31]

Recently, we have used quartz and TiO_2 substrates to study the behavior of cations at the liquid/solid interface by using their chloride salts.[26] The advantage of these inorganic substrates is that they are chemically simple compared with peptides or proteins while still allowing key properties like charge and degree of hydrophobicity to be varied. Herein, we extend this idea to investigate the effects of six monovalent Hofmeister anions on water structure adjacent to quartz and TiO_2 substrates by using the respective sodium salts. Both negatively and positively charged substrates were employed. Moreover, we also investigated the effects of anions on water adjacent to octadecyltrichlorosilane (OTS) covered quartz, which is representative of a more hydrophobic surface. From the results, it was evident that the rank ordering of the interactions of monovalent anions with surfaces of varying charge sign and degree of hydrophobicity consistently followed a direct Hofmeister series. This was the case whether the interfacial water ordering appeared to follow a direct or reversed series. Specifically, this means that weakly hydrated anions such as SCN^- partitioned to the interface more effectively than strongly hydrated anions like Cl^-. These results are summarized in Figure 1.

■ EXPERIMENTAL SECTION

Preparation of Negatively Charged Surfaces. IR grade quartz discs (1″ diameter by 1/8″ thick; Quartz Plus Inc., Brookline, NH) were cleaned in piranha solution according to previously described procedures.[32] For studies of the quartz/water interface, these substrates were used directly after cleaning. The cleaned quartz discs were also used as substrates for the preparation of negatively charged hydrophobic surfaces. This involved the formation of an octadecyltrichlorosilane (OTS, 95%, Acros Organics) monolayer.[33,34] Prior to monolayer preparation, the cleaned quartz discs were immersed in a 2 M NaOH solution for 15 min, rinsed with copious amounts of deionized water, and dried completely at 130 °C for 30 min to ensure a water-free surface. A 0.1% OTS solution was prepared in hexanes, and the dry quartz pieces were immersed in this solution for 2 h to form the OTS monolayer. After this, the samples were rinsed with purified water and sonicated in a
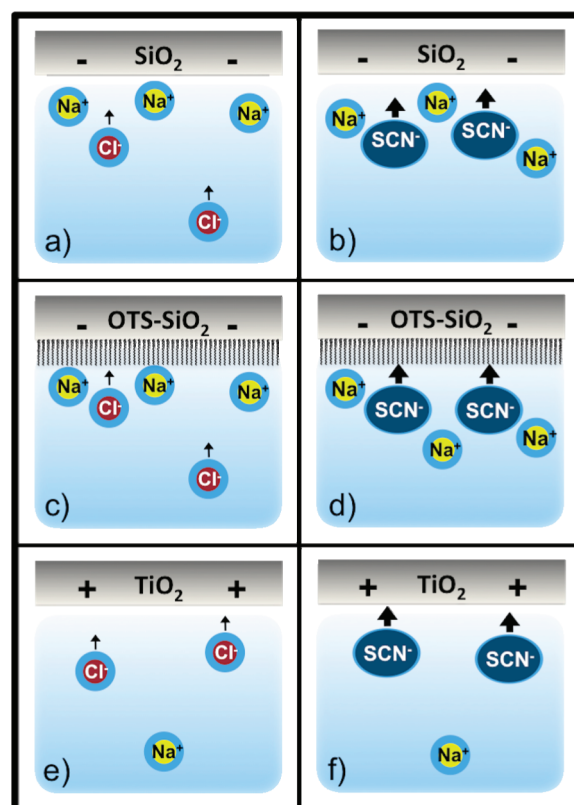
Figure 1. (a) Na^+ partitions more effectively than Cl^- to the negatively charged quartz/water interface. (b) Larger anions like SCN^- are less excluded from the negatively charged quartz/water interface than Cl^-. This same trend holds for (c) Cl^- and (d) SCN^- at the quartz/water interface even if the surface is silanized with an OTS monolayer. (e) Cl^- shows only minimal preference over Na^+ at the positively charged TiO_2/water interface. (f) SCN^- shows strong preferential partitioning compared with Na^+ at the positively charged TiO_2/water interface.

50/50 volume mixture of ethanol and water to ensure the removal of residual OTS.

Preparation of Titania Surfaces. TiO_2 thin films were prepared by evaporating titanium(IV) isopropoxide (97% Ti($OiPr)_4$; Aldrich, Milwaukee, WI) onto cleaned quartz discs, followed by oven calcination to oxidize the organic precursor into a thin TiO_2 film suitable for VSFS studies.[32] The films were characterized by X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and VSFS to elucidate their elemental composition, roughness, and surface charge. The thickness of the films was ∼1.0 nm. Such layers were sufficiently thin to avoid the larger nonresonant sum frequency generation background that would have been present when using thicker TiO_2 films.

Preparation of Salt Solutions. Salt solutions were prepared in purified water (18 MΩ·cm; NANOpure Ultrapure Water System, Barnstead, Dubuque, IA). High purity inorganic salts were used to make 0.10 M stock solutions of NaSCN (≥99.999%), $NaClO_4$ (≥99.99%), $NaNO_3$ (≥99.995%), NaBr (≥99.99%), and NaCl (≥99.999%). All of these salts were purchased from Sigma-Aldrich, Co. The lower concentration salt solutions used in the experiments described below were prepared by further diluting the stock solutions in purified water to between 100 μM and 10.00 mM. These concentrations were optimal for observing ion-specific effects yet low enough to avoid significant variations in the pH of the bulk solutions.

The pH of the aqueous phase was adjusted by using NaOH or HCl. It should be noted that pH 10.0, which was used with quartz substrates, is well above the isoelectric point for quartz.[35] This value was chosen to maximize the number of deprotonated OH surface groups while limiting the total ionic strength. The concentration of excess Na^+ counterions was, therefore, 100 μM from pH adjustment. Additionally, pH 2.0 was used with TiO_2 substrates. Under these acidic conditions, the liquid/solid interface had a positive charge. Moreover, there was a background of 10 mM Cl^- from pH adjustment. The effects of these added counterions, of course, contributed to all VSFS spectra that were obtained. However, the differences between VSFS water spectra with different salts were due to specific anion effects, as the effects of the added ions for pH adjustment were held constant for each system.

VSFS Experiments. VSFS is based on sum frequency generation (SFG), a surface-specific nonlinear optical technique. In this spectroscopy, visible photons are mixed at the surface with infrared photons to generate radiation at the sum frequency in a coherent process.[36] The probability of obtaining a sum frequency response is significantly enhanced when the frequency of the infrared beam is on resonance with molecular vibrations from the sample. The SFG response is linearly proportional to the intensity of the visible and infrared beams as well as to the square of the second-order nonlinear susceptibility constant, which is only observed in materials without a center of inversion symmetry. A more detailed explanation of the theoretical background can be found elsewhere.[37,38]

The VSFS experimental setup consisted of a 1064 nm Nd:YAG laser (17 ps pulsed; 50 mJ; rate 20 Hz; Continuum, Santa Clara, CA), which pumped an optical parametric generation/amplification (OPG/OPA) stage (LaserVision, Bellevue, WA). The OPG/OPA generated a tunable infrared beam (2000 and 4000 cm^{-1}) and a fixed frequency visible beam (532 cm^{-1}). The visible beam was 1 mJ/pulse and the infrared beam was ~0.6 mJ/pulse at 3200 cm^{-1}. The two beams were spatially and temporally aligned at an interface of interest and the sum frequency response was recorded with a photomultiplier tube (Hamamatsu, Japan). For VSFS experiments at the solid/liquid interface, the solid surfaces were placed inside a homemade Teflon flow cell, which has been previously described in detail.[39] Sodium salts were sequentially introduced from most strongly hydrated (Cl^-) to most weakly hydrated (SCN^-) for a particular system. The flow cells were thoroughly flushed under all conditions.

■ RESULTS

Interactions of Hofmeister Anions with Negatively Charged Surfaces. VSFS water spectra for quartz surfaces in contact with pH 10.0 sodium salt solutions are shown in Figure 2. The spectra are dominated by the presence of two OH stretch features at 3200 and 3450 cm^{-1}. These are usually assigned to water molecules with tetrahedrally coordinated water structure and less ordered water, respectively.[38] The pH 10.0 solution without added salt gave rise to the highest VSFS intensity. Addition of 0.100 mM sodium salts reduced the intensity of the VSFS signal. As can be seen, the amount of the intensity decrease for the water peaks followed a Hofmeister series for these monovalent anions. Specifically, the most weakly hydrated monovalent anions (SCN^- and ClO_4^-) led to the least attenuation in OH stretch intensity, while the signal was decreased to the greatest extent in the presence of Cl^-.
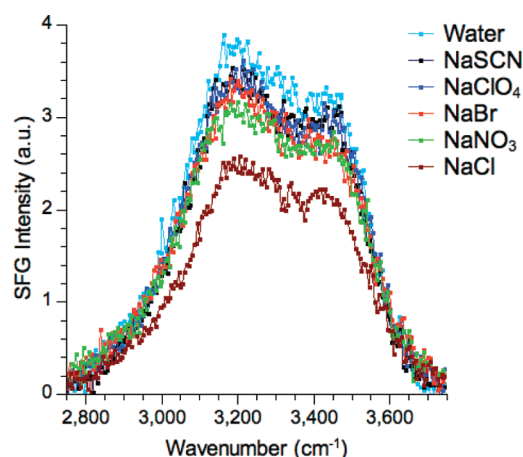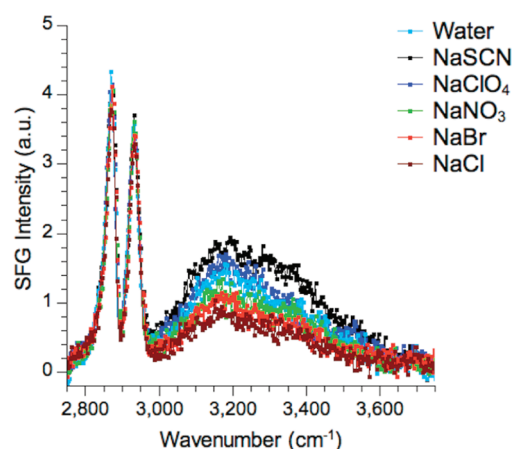

Figure 2. Quartz/water interfaces at pH 10.0 in contact with 0.10 mM sodium salt solutions display a direct Hofmeister series.

The attenuation in OH stretch signal should largely be caused by cation adsorption and the creation of a diffuse Na^+ double layer.[27] The double layer leads to a screening effect and can be characterized in a rudimentary fashion by Gouy–Chapman theory,[6] which is not ion specific. Previous experiments clearly showed that the VSFS water signal is cation specific under the conditions of these experiments when the counterion is Cl^-.[26] The data in Figure 2 reveal that there are also specific anion effects. These arise from the degree to which the anion is excluded from the double layer and the charged quartz surface. Since Cl^- shows the greatest attenuation of the OH stretch signal, it should be more extensively excluded from the interface than SCN^- or ClO_4^-. In fact, as anions partition to the interface, they help maintain the surface potential at a more negative value, leading to greater water alignment and a more prominent OH stretch signal. Of course, anions in the double layer also contribute to screening, just like Na^+. The data for the interfacial exclusion of monovalent anions roughly follow a direct Hofmeister series: $Cl^- > NO_3^- > Br^- > ClO_4 \approx SCN^-$. It should be noted that increasing the salt concentration of the solutions beyond 0.100 mM led to greater signal attenuation of the water peaks and the ion-specific differences were more difficult to discern. Conversely, the addition of less than 0.100 mM salt led to less signal attenuation, which also made anion-specific differences difficult to observe. In fact, ~0.100 mM salt concentrations represented nearly the optimal conditions for observing specific anion effects in this system.

In a next set of experiments, we wished to compare the relative partitioning of anions to a hydrophobic interface. To do this, experiments were conducted with the same quartz substrates in the presence of an OTS monolayer. In this case, the substrate still bore a small negative charge, as the surface was not completely silanized even at full monolayer coverage.[40] The presence of an OH stretch signal between 3000 and 3600 cm^{-1} showed that some water was aligned even in the absence of added salts at pH 10.0 (Figure 3, light blue data), although less OH stretch intensity was observed than in the analogous salt-free case in Figure 2, where the surface was unsilanized. There also was a very small peak near 3700 cm^{-1} from the free OH stretch. Another important difference was the presence of two strong CH stretch peaks at 2875 and 2940 cm^{-1}. These corresponded to the CH_3 symmetric stretch and Fermi resonance, respectively, from the hydrophobic self-assembled

Figure 3. OTS-covered quartz/water interfaces at pH 10.0 in contact with 0.10 mM sodium salt solutions display a direct Hofmeister effect.

monolayer.[40] The destructive interference between the CH and OH stretch peaks resulted in a signal minimum near 2980 cm^{-1}.[41] Such interference is consistent with a net alignment of interfacial water molecules with their OH groups pointing toward the interface, analogous to the bare quartz interface. Further control experiments demonstrated that decreasing the pH to 3.0 eliminated most of the water peak signal, while raising it to 11.0 increased it further (data not shown). As such, much of the OH stretch intensity in the absence of salt is likely associated with the surface charge. Nevertheless, it should be cautioned that the molecular level details of the interfacial water structure are complex at hydrophobic/aqueous interfaces.

Once again, the same five sodium salts were added to the solution, which led to changes in the OH stretch intensity (Figure 3). Among the monovalent salts, the addition of 0.100 mM NaSCN led to a very small increase in the water signal, while the three more hydrated monovalent salts led to a decrease. The addition of NaClO$_4$ gave rise to almost no change. Thus, the order of signal attenuation was Cl$^-$ > Br$^-$ > NO$_3^-$ > ClO$_4^-$ > SCN$^-$, which again corresponded to a direct Hofmeister series. The small signal increase in the case of the SCN$^-$ indicated that this anion actually partitioned to the quartz/OTS/water interface better than Na$^+$. As such, it made the surface potential more negative. Indeed, this effect overcame any interfacial partitioning of Na$^+$ as well as the screening effect of the salt. Such a result is in line with the idea that the most weakly hydrated anions can decrease the surface tension of the oil/water interface.[25,42−44] Moreover, this is an excellent demonstration of the fact that Na$^+$ is strongly partitioned away from a hydrophobic interface even if the surface bares a net negative charge. The net decrease in signal observed when 0.100 mM NaCl, NaBr, and NaNO$_3$ were added to solution indicated that these three anions did not partition to the hydrophobic interface strongly enough to overcome cancelation effects of interfacial Na$^+$ partitioning or simple charge screening. In fact, Cl$^-$ may actually show some exclusion from the hydrophobic interface as it increases the surface tension of the oil/water interface.[43]

Taken together, the results in Figures 2 and 3 are clear. More strongly hydrated monovalent anions are partitioned away from negatively charged surfaces to a greater extent than more weakly hydrated monovalent anions. This idea is shown schematically in Figure 1a−1d. It holds for both relatively hydrophilic interfaces (e.g., quartz) as well as hydrophobic

interfaces (e.g., OTS). The degree of difference between the monovalent anions may, however, be greater at hydrophobic substrates where SCN$^-$ showed enrichment rather than depletion. Such a result is in broad agreement with the idea that weakly hydrated anions have a higher propensity for interfacial partitioning.[16]

Interactions of Hofmeister Anions with Positively Charged Surfaces. In a next set of studies, we wished to determine the effect of switching the interfacial charge from negative to positive. A positively charged hydrophilic interface can be made on a quartz substrate by coating it with a thin TiO$_2$ layer.[32] VSFS spectra in the OH stretch range at the TiO$_2$/water interface showed an intensity minimum at pH 5.5 (Figure 4a). The water structure gave rise to the least intense
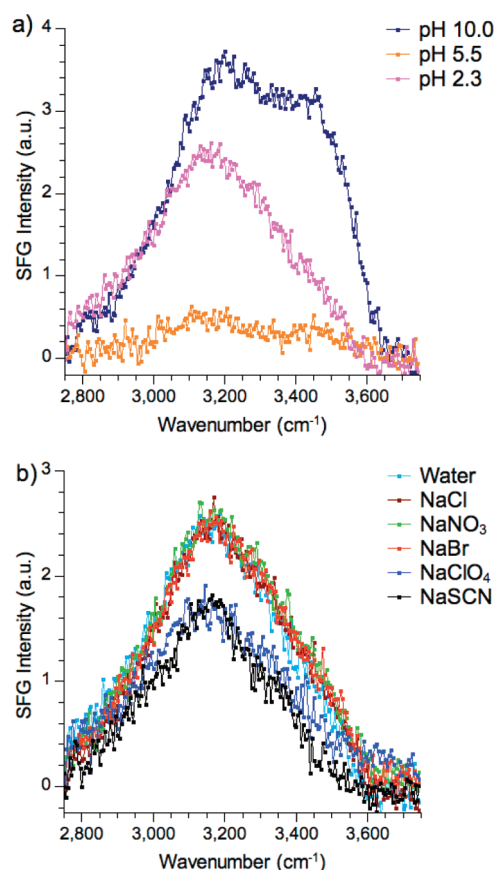
Figure 4. (a) VSFS spectra for the TiO$_2$/water interface at various pH values. (b) VSFS spectra of the same interface at pH 2.3 in contact with 0.10 mM sodium salt solutions.

features under these conditions because the sample was near its isoelectric point. At pH 10.0, the water molecules oriented with their hydrogen atoms facing toward the negatively charged surface. By contrast, the water orientation flipped when the pH was lowered to 2.3 because the surface possessed a net positive charge due to the protonation of the TiOH surface moieties.[32]

Experiments were performed at pH 2.3 with the same five sodium salts used above at 0.1 mM concentrations. The corresponding VSFS spectra are shown in Figure 4b. The most weakly hydrated anions, SCN$^-$ and ClO$_4^-$, caused the OH stretch signal to attenuate significantly on the positively charged TiO$_2$ surface. On the other hand, the intensity of the OH stretch peaks was almost unchanged with 0.100 mM NaBr, NaNO$_3$, or NaCl compared to conditions without added salt.

This indicated that very little of these latter three anions adsorbed under the conditions of these experiments. The spectroscopic data are a reverse Hofmeister series since the magnitude of OH attenuation occurred in the following order: $SCN^- > ClO_4^- > Br^- \approx NO_3^- \approx Cl^-$. The weakly hydrated anions adsorbed to a greater extent than strongly hydrated anions (Figure 1e–1f). There was, of course, a key difference between the data with TiO_2 and the data with quartz. Namely, the Na^+ concentration should be depleted from the TiO_2 interface on electrostatic grounds. The degree of Na^+–anion interactions in solution may play a role in the extent to which the various anions could adsorb, although the differences in the activity coefficients for all five salts are rather small.[45]

DISCUSSION

The macroscopic reversal of the monovalent anion Hofmeister series observed for the positively charged aqueous/solid interface demonstrated herein is reminiscent of the reversed Hofmeister series seen in protein aggregation when a biomacromolecule bares a net positive charge.[21−25] In that case, more aggregation (or salting-out) is observed when sodium salts of the most weakly hydrated anions are added to solution. This occurs because anion adsorption to the macromolecule/aqueous interface attenuates the repulsion between positively charged polymer molecules and leads to salting out. By contrast, the most weakly hydrated anions lead to the salting-in of macromolecules that are initially uncharged or negatively charged in the absence of salt.[14,24] This type of apparent Hofmeister reversal is, therefore, merely a macroscopic phenomenon as the microscopic behavior is the same. Namely, weakly hydrated anions adsorb preferentially compared with strongly hydrated anions.

Neutral and negatively charged polypeptides and proteins typically exhibit a direct Hofmeister series for anions of sodium salts.[6,24] This is the case even when divalent anions are considered, as these species cause greater salting-out behavior than monovalent anions because of their greater interfacial depletion. It should be possible to achieve microscopic reversal for anion Hofmeister ordering. As shown herein, this did not occur upon flipping the charge at the oxide/water interface or modulating its hydrophobicity. However, when explicit sites of positive charge such as arginine or lysine are added to a protein, then at least a partial reversal of the anion Hofmeister series may occur in the presence of divalent anions. This takes place because anions such as SO_4^{2-} interact more strongly with the sites of positive charge compared with monovalent anions.[5,24] In such cases, both macroscopic and microscopic reversal can occur because the ordering of the adsorption series itself is at least partially reserved. Such a reversal with divalent anions is fundamentally different than the one explored herein at the TiO_2/water vs quartz/water interface with monovalent anions. Namely, the one in the present work was caused by a switch from preferential Na^+ to preferential anion adsorption rather than a reversal in the ordering of the interactions of the anions with the surface.

An additional mechanism for partial or full reversal of the monovalent Hofmeister series involving differential ion pairing may also be possible. It has been postulated that ion interactions in aqueous solution should follow the law of matching water affinity (LMWA).[46] This model suggests that ions of similar size and hydration form more favorable anion–cation pairs compared with the pairing of a large poorly hydrated ion with a smaller better hydrated ion of opposite charge. Therefore, partial or full Hofmeister series reversal might be achievable by incorporating strongly hydrated positively charged groups into a soluble polymer chain. In such cases, more strongly hydrated monovalent anions would preferentially pair with the polymer and cause salting-out behavior. As such, this type of reversal would be both macroscopic and microscopic.

In summary, we have shown that the sodium salts of more weakly hydrated monovalent anions preferentially adsorb at the oxide/water interface whether the negatively charged surface is hydrophobic or hydrophilic. The same anion absorption ordering was also found when the surface charge was positive. In this latter case, the macroscopic behavior showed an anion series reversal in terms of water structure. This apparent reversal, however, was due to the predominance of anions in the double layer for positively charged surfaces vs the preferential adsorption of Na^+ with negatively charged substrates. Indeed, the preferential ordering of monovalent anion adsorption remained unchanged.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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