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**Characterization of Selective Binding of Alkali Cations with Carboxylate by X-Ray
Absorption Spectroscopy of Liquid Microjets**

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Abbreviations: NEXAFS, Near Edge X-Ray Absorption Fine Structure

Abstract:

We describe a new approach for characterizing selective binding between oppositely charged ionic functional groups under biologically relevant conditions. Relative shifts in K-shell X-ray absorption spectra (XAS) of aqueous cations and carboxylate anions indicate the corresponding binding strengths via perturbations of carbonyl antibonding orbitals. XAS spectra measured for aqueous formate and acetate solutions containing lithium, sodium, and potassium cations reveal monotonically stronger binding of the lighter metals, supporting recent results from simulations and other experiments. The carbon K-edge spectra of the acetate carbonyl feature centered near 290 eV clearly indicate a preferential interaction of sodium versus potassium, which was less apparent with formate. These results are in accord with the “Law of Matching Water Affinities,” relating relative hydration strengths of ions to their respective tendencies to form contact ion pairs. Density functional theory calculations of K-shell spectra support the experimental findings.

Introduction:

The discovery of the selective interactions between simple ions and proteins dates back over a century to Hofmeister's studies with chicken egg protein; proteins could be selectively "salted in" or "salted out" by addition of various salts to the solution (1). This "Hofmeister effect" has subsequently been observed with more salts and many more proteins, with relative magnitudes following the "Hofmeister series" ordering, as do various related phenomena (2, 3). Nevertheless, it remains to understand the origin of "Hofmeister effects," despite enormous effort (4, 5).

To rationalize biological ion specificity, such as the physiologically important preferential binding of sodium versus potassium with proteins, the "Law of Matching Water Affinities" was proposed by Collins (6, 7). Based on charge densities and electrostatic arguments, this law holds that ions with similar hydration free energies form the most stable (insoluble) contact ion pairs. In the case of proteins, the carboxylate group is considered to have a hydration energy much closer to that of sodium than to that of potassium, which is manifested in the sodium binding free energy being larger by 2.22 kcal/mol, as determined by Vrbka et al. (8) via simulations and conductivity measurements. The simulations indicated that the interaction is localized on the carboxylate groups of the protein. Conductivity measurements were performed on protein solutions for experimental support, revealing a larger relative decrease upon addition of sodium chloride compared to potassium chloride, indicative of sodium being more efficiently removed from solution than potassium. The rationalization of the preferential interaction was again that there was a closer match of hydration energy, which was

reflected in more stable contact ion pairing between the protein carboxylate groups and sodium, versus potassium ions.

In this paper, we examine the selective interactions of alkali cations with the carboxylate groups of the model carboxylate anions formate and acetate, using novel liquid microjet technology to introduce the aqueous solutions into a soft X ray spectroscopy experiments and finding strong support for the above conclusions reached by Vrbka, et al. (8). The carboxylate K-shell spectra comprise transitions from the atomic 1s core levels to π^* carbonyl antibonding orbitals, the latter shifting to higher energies in response to stronger interaction with the cation. This experiment thus provides a direct probe of such cation-anion interactions in aqueous systems, which are difficult to access by other methods, e.g. mass spectroscopy. Similar experiments, but with quasi-static liquid cells, were recently reported for NaCl solutions by Aziz et al. (9), wherein X-ray “spectral fingerprints” of contact ion pairs were found. However, their approach is not likely to work with complicated solutes, as in the present study, due to the usage of a windowed coupling; the Si_3N_4 used would absorb in the biologically relevant energy ranges and drastically reduce photon flux.

Results:

We examined several carboxylate salts to study the trend of preferential interactions between alkali metal cations and carboxylate ions using the technique of near edge X-ray absorption fine structure (NEXAFS) spectroscopy, introducing the liquid solutions into the high vacuum X-ray experiment with a liquid microjet system. This novel approach, in conjunction with theory, has recently been used to reveal subtle

conformational and geometric interactions in aqueous systems, comprising both biological molecules and ionic solutions (10-14). Important for the current study, the rapid sample flow (ca. 50 m/s) minimizes X-ray photo damage, which we have found to be severe .

Simple carboxylate salts were employed due to their straightforward structure, high solubility, and easily distinguished $1s \rightarrow \pi^*_{C=O}$ feature. Formic acid and acetic acid are the simplest organic acids, minimizing possible alkane chain effects. Additionally, both formate and acetate anions have shown preferential interactions with sodium versus potassium in previous experimental and theoretical studies (8). The current experimental study was expanded to include the lithium ion as an additional test of the “Law of Matching Water Affinities,” although technical difficulties were encountered with the associated supporting calculations.

An overlay of the NEXAFS spectra of the three different aqueous acetate solutions is shown in Figure 1. This figure is an expansion of the carbon K-edge spectra displaying the peak that is the result of the $1s$ electron being promoted to the antibonding π orbital (15). There are small features on the high-energy edge of each of the peaks, most likely due to associated vibrational states of the carbonyl group. It was not possible to compare the σ^* region, above 290 eV, of the respective spectra due to potassium having transitions which obscure the underlying features at that energy range. Between each of the scans on the overlay, there is a spacing of about .05 eV on the leading edge of the feature; the largest blue shift is found in the comparison of lithium to the other two cations, noting that lithium has an ionic radius of 90 pm, compared with those of sodium and potassium, 116 and 152 pm, respectively (16).

The carbon K-edges of formate complexes exhibit a marked shift between lithium and the other two cations, as evident in Figure 2; lithium exhibits a blue shift of approximately 0.2 eV. However, in these scans, there is no discernable shift between the potassium and sodium complexes; hence, preferential interactions are difficult to assess in this case. The difference in sizes between the formate and the acetate anions affects their hydration and electrostatic properties in terms of the “Law of Matching Water Affinities,” thus determining the strength of the cation- anion interaction and the resulting shifts in their K-shell spectra.

Using previously calculated radial distribution functions (8) as a guideline for approximate equilibrium distances (2.5 Å and 2.9 Å, respectively), structures were optimized for the acetate and formate complexes of both sodium and potassium. Unfortunately, the StoBe deMon software (17), the program used for the NEXAFS calculations, does not include an effective core potential basis set for lithium. Calculations performed without this potential exhibited Mulliken populations that did not accurately reflect the charges of the system of interest, and thus could not produce comparable energy values, although they did reproduce the general shape of the spectra. This behavior was discovered for sodium, when comparing results with and without an effective core potential; the Mulliken population did not correspond with the charges of the system and it produced differences in calculated spectra. This is important in the current study, considering the small shifts that we are explicitly examining, whereas such a treatment is quite suitable for systems where these small shifts are inconsequential. The usage of Dolg pseudopotentials was also attempted and found did not accurately reflect the experimental spectra due to errors in the magnitude of the interaction of the carboxylate

with lithium; this is logical due to notoriously difficult nature of lithium, and the errors that are found in its total valence correlation energy (18). Figure 3 shows an expanded view of the $\pi^*_{C=O}$ feature from the calculation of the carbon K-edge of the isolated acetate ion pair with both potassium and sodium. The size of the relative spectral shift, (< 0.1 eV) is an expected result due to the different inter-ion distances of complexes with the respective cations. It should be noted that a calculation was done to verify that even when choosing equal distances, sodium transitions were blue shifted with respect to those of potassium.

As mentioned above, the magnitude of the experimental K-shell shifts differed between formate and acetate; this is confirmed by our theoretical calculations, and reflects the corresponding ion binding energies. Such differences in the strengths of the interactions for formate and acetate were also found in other studies (8). Shown in Figure 4 are the StoBe results for isolated formate ion pairs with sodium and potassium. The two spectra are very similar, but display a reversal of the shifts found for acetate. The magnitude of this small theoretical shift is beyond the precision of the current experiment (ca. 0.005 eV), but both the experimental and theoretical formate spectra clearly display smaller shifts than do the corresponding acetate spectra.

Figure 5 shows an enlarged section of theoretical spectra of hydrated acetate ion pairs, computed using equilibrated molecular dynamics snapshots. At a higher computational cost, the same result of a global shift between the sodium and potassium spectra was found. There is a blurring of some of the higher energy σ^* features (not shown in the figure). The distances between the cation and the acetate (sodium closer than potassium) vary among the different MD snapshots and corresponding calculations, which does affect the magnitude of the spectral shift from snapshot to snapshot. In previous

results, there was a ca. 2 kcal/mol difference in the association energy between the sodium and potassium acetate complexes (8); this would correspond to a difference of about 0.08 eV, which is similar to the spectral shifts observed in the present study.

The form of aqueous acetate and formate ions, viz. protonated or deprotonated, is governed by the acid/base equilibrium. Noting that pK_b for acetate is 9.243 and for formate 10.255,^{*} a pH of approximately 9.5 is obtained for the ca. 2 molar solutions used in this work, implying that the deprotonated forms dominate, as they would at biological pH. All of the solutions studied here were held at the same concentration. Therefore, we can discount variations in the composition of the solutions as influencing the above results.

Discussion and Conclusions:

The relative interaction strengths of the monovalent cations of sodium, potassium, and lithium with acetate and formate anions in aqueous solutions were examined using NEXAFS of liquid microjets. An unambiguous difference between the effects of lithium and those of the two other cations is evident on the carbon K-edge for both acetate and formate; a preferential interaction of sodium versus potassium is evidenced by a small but reproducible relative blue- shift in the carbonyl feature, reflecting a shift in the π^* orbital to higher energies. In the formate system, the corresponding sodium/potassium spectral shifts were barely discernable. Calculated K-shell spectra support the experimental findings, with a slight overestimation of the shifts, but unambiguously matching the observed trends. At the pH used in these experiments, the deprotonated forms of the anions are predominant, as they would be under biological conditions. Future experiments will include extending the study to other alkali ions, examining relevant divalent cations

^{*} (2007) *CRC Handbook of Chemistry and Physics* (Taylor and Francis, Boca Raton, FL).

(Mg²⁺, Ca²⁺), as well as concentration and pH dependent studies. In any case, the current results establish a new way of probing selective interactions of ions with biological molecules in aqueous environments under relevant conditions, and provides support for the “Law of Matching Water Affinities,” which invokes ion pairing to explain Hofmeister effects on proteins.

Materials and Methods:

Samples:

All samples were purchased from Sigma-Aldrich in a crystalline powder forms at a stated purity of 98% or higher and used without further purification. Solutions were prepared with 18 MΩ (Millipore) water to compose a solution of 2.0 M.

Experimental Details:

The NEXAFS spectra were obtained on the relevant edges at Beamline 8.0.1 of the Advanced Light Source (ALS), at Lawrence Berkeley National Lab. Carbon K-edge and oxygen K-edge spectra were collected both from the liquid jet as well as from the gas phase background. Detailed carbon spectra were obtained in the range of 285-289 eV, and were subsequently calibrated to HOPG peaks previously recorded by beamline scientists. A gold mesh was used to collect I₀ farther up the beamline, which was used to account for intensity fluctuations. The spectra were subsequently area normalized.

The experimental apparatus comprises: a main chamber where the x-ray beam intersects with the microjet stream, a section for trapping the microjet with a skimmer to reduce pressures, and a differential pump section to allow for a windowless coupling to the

beamline. The measurements in this paper were obtained using a chopped fused silica microjet of 30 μm attached to an HPLC pump with an average flow rate of .9 mL/min. The signal was collected by total electron yield (TEY) with a biased copper electrode of 1.8 kV; the usage of TEY has been shown to be a bulk technique due to the larger escape depths of electrons (19). A more detailed description of the experimental apparatus has been previously published by Wilson et al. (20).

The formate carbon K-edge spectra were treated due to a large signal to noise. The data was treated with a five point second order Savitzky- Golay smoothing scheme (21); it was empirically verified that this did not broaden rather reduced the noise in the signal.

Computational Details:

StoBe DeMon (17), a density functional theory based commercial package, for computing core-level spectra, was used to aid interpretation of the experimental spectra. The functionals developed by Perdew, Berke and Erzenhoff (22) were used in addition to the revised exchange functional of Hammer, Hansen and Norkov (23). The atom of interest was modeled using the IGLO basis set (24), the remaining non-hydrogen atoms were replaced with model core potentials provided in the program (17). The design of the calculation gives the energetic isolation of the 1s orbital of interest, which is then partially excited by replacing its electron with a half core hole. The use of a half core hole is a result of the sudden approximation, which has been shown when used in conjunction with the transition potential method to approximate the relaxation of the molecule under the influence of the core hole (19, 25, 26). A stick spectrum is produced and then uniformly broadened with Gaussians of width .5 eV. The spectra are not globally shifted, as would

be the normal procedure to compare with experimental spectra, so absolute transition values between theory and experiment cannot be compared; the magnitude of the shifts between theory and experiment are instead compared. StoBe has been shown to reproduce relative shifts well; errors of note have been found in the relative intensities rather than the relative energies (27).

Kohn-Sham corrections were calculated for the acetate gas phase system by calculating both the ground state and the excited state (28). These were found not to affect the relative shift between the potassium and sodium acetate systems; accordingly, these effects are not included in the figures given in this paper.

The structures noted as gas phase systems were taken after geometry optimization; for the solvated systems, molecular dynamics were performed to provide snapshots for subsequent calculations. An initial geometry of a single ion pair was built using a commercial software package, MacroModel from Schrodinger Inc., and was subsequently placed in an 18.62 Å cube with over 200 water molecules and employing periodic boundary conditions. A molecular dynamics calculation was performed at 298K for 20 nanoseconds. From the molecular dynamics trajectories, snapshots were taken over 70 picoseconds apart as to assure that the snapshots were uncorrelated. These snapshots were subsequently “ice cream scooped” to include the system of interest and the 20 closest waters, due to the need for a manageable sized cluster for the core level calculations.

StoBe is a cluster code and thus does not have the ability to use periodic boundary conditions. Thus, in a solvated system it is important to note these calculations are a guideline and a tool to help interpretations as opposed to a comprehensive predictive tool.

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Figure 1. An overlay of the experimental π^* feature in the K-shell spectrum of the acetate anion for 2 M solutions of potassium acetate, sodium acetate, and lithium acetate revealing distinct shifts between the cations, indicative of preferential interactions.

Figure 2. An overlay of the experimental carbon K-edge spectra of lithium formate, sodium formate, and potassium formate. The sodium and potassium spectra are nearly indistinguishable, whereas lithium is distinctly blue shifted. Clearly, the observed shifts between sodium and potassium formate are smaller than those found for acetate.

Figure 3. Theoretical calculations of the isolated ion pairs of acetate with alkali cations, produced with StoBe . The magnitude of the spectral shift is ca. 0.07 eV between sodium and potassium. Lithium was not calculated due to a lack of core potential basis sets.

Figure 4. Computed spectrum for isolated ion pairs of formate with sodium and potassium. The two are very similar, but show a slight reversal of the trend previously noted. Lithium was not calculated due to a lack of core potential basis sets.

Figure 5. Calculated K-edge spectrum for hydrated ion pairs of acetate with sodium and potassium. The coordinates were taken from molecular dynamics snapshots. The energy range is slightly altered from previous calculations. The relative shift between the cations is larger than 0.05 eV.









