THE JOURNAL OF PHYSICAL CHEMISTRY B LETTERS

2008, *112,* 12567–12570
Published on Web 08/16/2008

Cation-Specific Interactions with Carboxylate in Amino Acid and Acetate Aqueous Solutions: X-ray Absorption and *ab initio* Calculations

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Received: June 12, 2008; Revised Manuscript Received: July 16, 2008

Relative interaction strengths between cations ($X = Li^+$, Na^+, K^+, NH_4^+) and anionic carboxylate groups of acetate and glycine in aqueous solution are determined. These model systems mimic ion pairing of biologically relevant cations with negatively charged groups at protein surfaces. With oxygen 1s X-ray absorption spectroscopy, we can distinguish between spectral contributions from H_2O and carboxylate, which allows us to probe the electronic structure changes of the atomic site of the carboxylate group being closest to the countercation. From the intensity variations of the COO^-_{aq} O 1s X-ray absorption peak, which quantitatively correlate with the change in the local partial density of states from the carboxylic site, interactions are found to decrease in the sequence $Na^+ > Li^+ > K^+ > NH_4^+$. This ordering, as well as the observed bidental nature of the $-COO^-_{aq}$ and X^+_{aq} interaction, is supported by combined *ab initio* and molecular dynamics calculations.

1. Introduction

Ion-selective interactions play an important role in many chemical, environmental, and biological processes occurring in aqueous solution. The cationic interaction with the protein's carboxylate groups is of special interest due to its effect on protein association and enzymatic activity,[1] yet the details and consequences of ion pairing on the molecular level are far from being fully understood. A useful macroscopic measure, though, in characterizing ion-specific interactions is the hydration energy. It has been established empirically that the tendency for contact ion-pair formation correlates with the match between the hydration enthalpies, i.e., in the present case the cation and anionic carboxylate group.[2−4] For instance, the hydration enthalpy of sodium matches those of major intracellular anions or anionic groups, such as carboxylate, better than potassium. Also, the effects ions have on water are specific, although the existence of long-range solvation effects (structure-making/breaking) is currently debated.[5,6]

Observations of ion-specific effects on proteins date back as early as 1888, when Hofmeister recognized[7] that inorganic salts can be ranked by their ability to "salt out" hen egg white protein in aqueous solution. Hofmeister series have since been observed for numerous phenomena in aqueous ion solutions, including their interfaces.[8] It applies to many different proteins as well as to polymers.[9,10] In a 1985 review on the subject,[11] no less than 38 macroscopic effects following the Hofmeister series of inorganic solutes were identified in aqueous solutions, indicating its universal importance. Understanding the microscopic origin of this remarkable phenomenon, which is at the heart of aqueous chemistry, calls for a combined experimental and theoretical effort.

On the theoretical side, a recent combined molecular dynamics and quantum chemical study has revealed that sodium interacts more strongly with protein surfaces than potassium,[12] and that the ion-specific interaction is mediated by weak ion pairs $(COO^-{:}X^+)_{aq}$. This finding and interpretation thereof is awaiting firm experimental confirmation. To that end, spectroscopic measurements with high sensitivity to the local electronic structure, applicable to the aqueous phase, are desirable. Among the most powerful techniques are electron spectroscopy techniques, ideally in conjunction with high-intensity and tunable synchrotron radiation from third-generation synchrotron light facilities. Such experiments, including X-ray absorption (XAS) and emission (XES), or photoelectron (PES) spectroscopy became feasible only recently for aqueous solutions.[13−17] XAS, used in this work, is highly sensitive to the empty valence states of a given atomic site, and hence, the technique is most suitable for probing orbital changes mediated by ion−ion interactions. This was previously exploited for the study of ion pairing in salt[18] and amino acid aqueous solutions.[19] Here, we report oxygen K-edge XAS measurements from aqueous solutions of X-acetate ($X = Li^+$, Na^+, K^+, NH_4^+), and of glycine aqueous solutions, with additions of either NaCl or KCl. Recently, an

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10.1021/jp805177v CCC: $40.75

XA study focused on the carbon signal of the carboxylate in the presence of alkali cations in water.[20] The O1s XA transition investigated here, as opposed to C1s XAS,[20] directly probes the atomic site of the $-COO^-_{aq}$ group closest to the cation, and has thus superior sensitivity to differences in counterion interactions. Another important advantage of the present study is that the bonding geometry can be determined, which is information not accessible otherwise. Additionally, certain differences in the O1s XA spectra of neat water and solutions can be assigned to the distortion of the water network. Both acetate and glycine solutions were studied as to ensure that even the small acetate is a sufficiently good model system for ion−ion interactions at the protein surface in water.

2. Methods

Experimental. XAS measurements at the oxygen K-edge of acetate and glycine/salt aqueous solutions were performed at the U41-PGM undulator beamline, BESSY, Berlin. The experimental setup has been described in detail previously.[21] Briefly, the aqueous solution is circulated (1 L/min) within a stainless steel closed tubing system, inside an UHV chamber, as to warrant continuous renewal of the irradiated liquid sample. In the interaction region, the X-ray radiation hits the sample flowing behind a 150 nm thick Si_3N_4 membrane. X-ray absorption is recorded by total fluorescence yield (FY) measurements using a 5×5 mm^2 GaAsP photodiode. Due to the long attenuation lengths of X-rays, on the order of a few micrometers, the method is primarily bulk sensitive. The setup is readily applicable to more complex molecules than studied here, and for FY measurements, the use of an oxygen-free membrane is equally well suited as a free-vacuum jet experimental setup. However, when detecting the total electron yield (TEY), a windowless setup is required;[20,22] TEY, moreover, preferentially probes the solution interface, determined by the electron mean free path in aqueous solutions.

The aqueous solutions were prepared freshly before each measurement, and highly demineralized water, and salts of the highest purity commercially available (Sigma Aldrich) were used. The pH of the solution was measured within ±0.1 accuracy (pH meter 766, Knick), and no further pH adjustment was necessary. At the pH values of the as-prepared aqueous solutions, pH 8.6 (1 M Na-acetate), pH 8.7 (1 M Li-acetate), pH 8.1 (1 M K-acetate), pH 7.0 (1 M NH_4-acetate), pH 7.4 (1 M glycine in 1 M NaCl), and pH 7.4 (1 M glycine in 1 M KCl), in all cases, >99.9% of the carboxylate groups are deprotonated, as calculated from the law of mass action.

Computational. The combined *ab initio* and molecular dynamics approach is analogous to that applied in our previous study.[23] First, MD simulations of each of the investigated ion pairs in water (800 SPCE water molecules in a periodic cubic cell) were performed employing a nonpolarizable forcefield.[24,25] Each of the systems contained 800 water molecules in a cubic periodic box with up to six cation/anion pairs. After nanosecond equilibration, several nanoseconds of production runs were performed at 300 K and 1 atm with a 2 fs time step with the program Gromacs 3.3.1.[26] Cation−anion radial distribution functions were then extracted from the simulations.

In the next step, calculations were carried out using a polarizable continuum solvent model. Geometries of the contact ion pairs were obtained from gas phase *ab initio* optimizations, except that in order to get relevant aqueous phase anion−cation distances we took these values from the previous MD simulations. Namely, for each ion pair, the distance corresponded to the position of the principal cation−anion oxygen peak of the radial distribution function. The geometries of all investigated contact ion pairs (highlighting the employed cation−anion oxygen distances) are depicted in Figure 1. Note the symmetric bidentate structure of each of the alkali cation−acetate pairs. For ammonium cation, the optimal structure of the ion pair is slightly asymmentric, however, with a negligible (below 0.5 kcal/mol) barrier toward symmetrization.

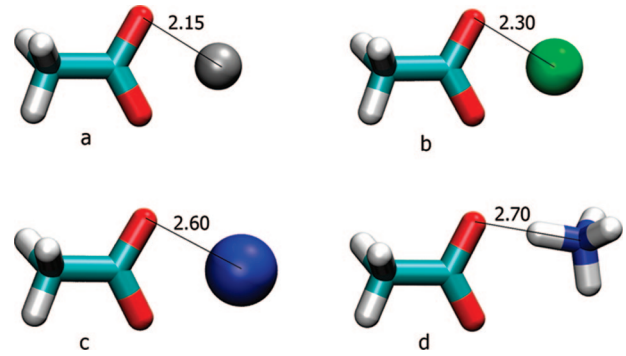

Figure 1. Calculated geometries of contact ion pairs of (a) lithium acetate, (b) sodium acetate, (c) potassium acetate, and (d) ammonium acetate. All distances are given in Ångstrom.

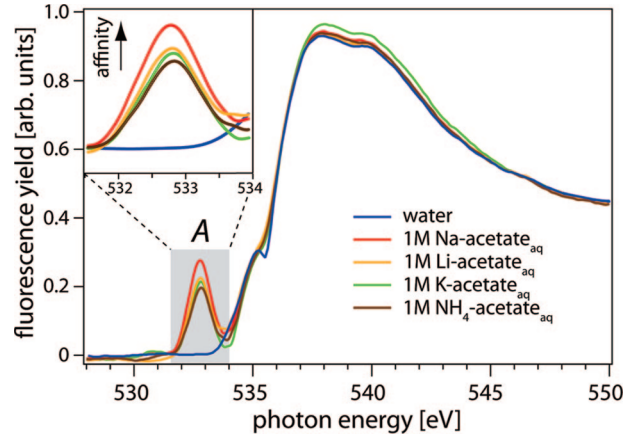

Figure 2. Oxygen 1s XA spectra for different X-acetate (X = Li^+, Na^+, K^+, NH_4^+) solutions. Enlargement of the region between 531.5 and 534.5 eV is presented on the top left and correlated with the cation binding affinity.

In each case, the free energy of ion pairing was evaluated as the difference between the energy of the solvated contact ion pair and the energies of the separately solvated cation and anion in water. *Ab initio* calculations were performed at the second-order Møller−Plesset perturbation theory level (MP2), employing the aug-cc-pVTZ basis sets for acetate with additional core-valence basis functions (cc-pCVTZ) added for C and O and the cc-pVDZ set for the cations.[27] Water was described within a polarizable continuum solvent using the COSMO model.[28,29] All COSMO parameters were taken as the default ones except for the ionic radius of sodium, which was reduced by 1.3% to match exactly the experimental difference between hydration free energies of Na^+ and K^+ amounting to 17.5 kcal/mol.[30] The present *ab initio* calculations were performed using the Gaussian 03 program package.[31]

3. Results and Discussion

Figure 2 contrasts the oxygen K-edge X-ray absorption (XA) spectra of a series of 1 M X-acetate aqueous solutions (X = Li^+, Na^+, K^+, NH_4^+) as well as of pure liquid water, and Figure 3 shows the analogous data for 1 M glycine in 1 M NaCl and

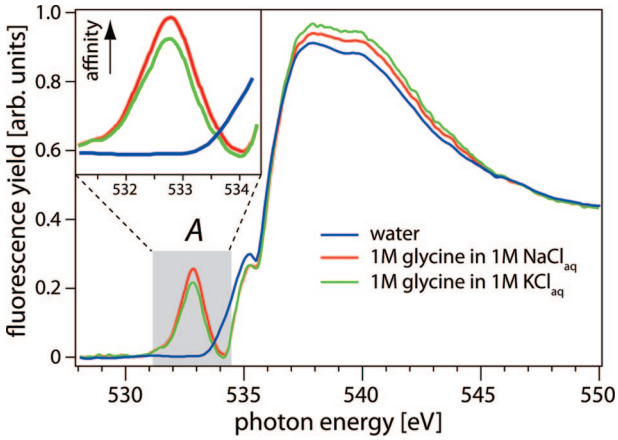

Figure 3. Oxygen 1s XA spectra of 1 M solution of glycine in 1 M NaCl and 1 M glycine in 1 M KCl. Enlargement of the region between 531 and 534.5 eV is presented on the top left and correlated with the cation binding affinity.

1 M glycine in 1 M KCl aqueous solutions. All spectra were measured under the same experimental conditions, and slight changes in the photon flux were accounted for by normalizing the spectral intensities. This leads to identical intensities in the (background) region >545 eV photon energy, i.e., sufficiently far away from the absorption edges. The O1s XA spectra of the solutions are dominated by the characteristic water features. These are the pre-edge at 535 eV and the main- and post-edges at 538 and 540 eV, respectively, in agreement with the reported values.[32,33] All XA solution spectra of Figure 2 almost fully overlap with the neat water spectrum, the main differences being the occurrence of a new peak (labeled *A*) at 532.8 eV photon energy, with a constant width of 1.2 eV (fwhm) for each X, and some systematic intensity changes in the main- and post-edge regions. Peak *A* is a sole spectral signature of the $-COO^-_{aq}$ group, and arises from the promotion of a O1s core-level electron to the lowest unoccupied molecular orbital (LUMO), of π^* character. The peak position and width of *A* are the same for all acetate solutions and also for the glycine solutions. Intensities of *A*, however, change considerably. For acetate solutions (Figure 2), the intensities of *A* decrease in the sequence $Na^+ > Li^+ > K^+ > NH_4^+$, and for the two glycine solutions (Figure 3), *A* is more intense for NaCl than for KCl.

The observed *A*-peak intensity changes (Figures 2 and 3) can be attributed to the change of the local density of unoccupied states at the oxygen site of $-COO^-_{aq}$, induced by interaction with the cations. Since the measurements were performed at identical concentrations (1 M), and also the stoechiometric anion-to-cation ratios are always the same, the observed intensity changes quantitatively correlate with the strength of ion pairing. Intensities of the acetate-specific O1s absorption signal are hence proportional to the total number of empty states of p symmetry in the integration interval. Higher *A*-peak intensities result from electron withdrawal from the carboxyl group by the cations, which is directly connected with an increase of the empty p DOS from the carboxyl group. The effect scales with the strength of the COO^-_{aq} to cation(aq) interaction. A similar observation was recently reported for ion pairing in alkali-halide salt aqueous solutions between the Na^+ and OH^-,[18] where also different pairing situations, contact pairs vs shared solvent, have been considered explicitly. Note that electron correlation contributions to peak *A*, beyond a density of states approximation, can be neglected because the associated redistribution of spectral intensity occurs typically within 10 eV of the absorption edge.[34−36]

TABLE 1: Free Energy Change upon Replacing Sodium with Lithium, Potassium, or Ammonium in a Contact Ion Pair with Acetate in Water

	ΔG (sodium → other cation) (kcal/mol)
lithium	+0.3
potassium	+2.5
ammonium	+3.4

Although the solute concentration in the present study is not higher than 1 M (compared to 55 M of water), changes of the water hydrogen-bonding network can be identified, which is manifested by intensity variations in the main/post-edge region of the sodium and potassium acetate solution spectra (Figure 2). Contrary to the *A*-peak intensity changes, an increase of the main/post-edge intensity correlates with the reverse order of X, i.e., $Na^+ < Li^+ < K^+ < NH_4^+$. Consistent with this behavior, higher main/post-edge intensities are observed for glycine/KCl than for gylcine/NaCl aqueous solutions (Figure 3). A similar intensity increase of the main-edge was observed when adding NaCl to the water,[37] and has been attributed to strong perturbation of the electronic structure of water molecules within the anion hydration shell. An experimental detail, deserving further consideration, is the observation that the *A*-peak intensity increases from K^+ to Na^+ and is slightly larger for acetate than for glycine, indicating a somewhat different chemical environment of the carboxylate group within these two species.

In discussing the XAS intensity changes of Figures 2 and 3 in terms of $-COO^-$-ion interaction strengths, we have not yet explicitly addressed the molecular interaction geometry. The important issue is whether or not the cation binds equally to the two oxygen atoms of the $-COO^-_{aq}$ group. Our results show that the width of peak *A* is independent of the cation used. Hence, for each cation studied, the interaction geometry is essentially of the same bidental nature, in agreement with the calculated structures presented in Figure 1. All alkali cations are observed to take middle positions between the two carboxylate oxygens. In the case of calculations of the NH_4^+, there is a tendency of a weakly preferred interaction with one of the oxygens of the carboxylate. However, this preference for an asymmetric structure is very weak (below 0.5 kcal/mol) and will be smeared out by vibrational motions; therefore, it is not observed in the experiment. Note also that, in contrast to cations studied here, in $-COOH_{aq}$, the two oxygens are clearly distinguishable, giving two distinct peaks in the photoelectron spectrum.[38]

We now compare the observed ion pairing ordering with results from calculation, and we also discuss the reason why Li^+ departs from the Hofmeister series. Computational results of the relative strength (with respect to Na^+) of cation pairing with acetate in water are summarized in Table 1. We see that sodium forms the most stable ion pair with aqueous acetate, followed by lithium which is only marginally less stable. The tendency for ion pairing with potassium and particularly with the ammonium cation is weaker, the former result being observed and discussed already in our previous studies.[12,23] The fact that lithium binds slightly less strongly than sodium to acetate is in accord with the empirical law of matching water affinities.[39] It states that ions prefer to pair with counterions or ionic groups which have comparable hydration enthalpies which can be translated in a simple Born solvation picture to surface charge densities.[40] From this point of view, Na^+ matches most closely the COO^- group, followed by Li^+, K^+, and NH_4^+.

Conclusions. By means of X-ray absorption and combined *ab initio* and molecular dynamics simulations, we have deter-

mined the ordering of a series of cations ($X = Li^+$, Na^+, K^+, NH_4^+) in terms of the strength of interaction with anionic carboxylate groups of acetate and glycine in aqueous solutions. The strength of ion pairing with the COO^-_{aq} decreases in the sequence $Na^+ > Li^+ > K^+ > NH_4^+$ both in the experiment and calculation. The cations thus follow, with the exception of lithium, the Hofmeister series. The observed cationic ordering can be qualitatively rationalized in terms of the empirical law of matching water affinities within which Na^+ matches best the hydration enthalpy of $-COO^-_{aq}$, followed by Li^+, K^+, and NH_4^+. The structure of all the alkali cation−carboxylate ion pairs is bidentate. Calculations present the ammoinum−carboxylate ion pair as slightly asymmetric with a marginal barrier toward symmetrization, which explains why asymmetric structure is not observed in the experiment. The present systems mimic the process of ion pairing between cations of biological relevance with charged basic groups at protein surfaces.

Acknowledgment. We are grateful to the Czech Ministry of Education (grant LC512) and the Czech Science Foundation (grant 203/08/0114) for support. Part of the work in Prague was supported via Project Z40550506.

References and Notes

(1) Waigh, T. A. *Applied Biophysics*; Wiley & Sons: Chichester, 2007.
(2) Collins, K. D. *Biophys. J.* **1997**, *72*, 65.
(3) Collins, K. D. *Methods* **2004**, *34*, 300.
(4) Collins, K. D. *Biophys. Chem.* **2006**, *119*, 271.
(5) Omta, A. W.; Kropman, M. F.; Woutersen, S.; Bakker, H. J. *Science* **2003**, *301*, 347.
(6) Laage, D.; Hynes, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 11167.
(7) Lewith, S. *Arch. Exp. Pathol. Pharmakol.* **1888**, *23*, 1.
(8) Cacace, M. G.; Landau, E. M.; Ramsden, J. J. *Q. Rev. Biophys.* **1997**, *30*, 241.
(9) Baldwin, R. L. *Biophys. J.* **1996**, *71*, 2056.
(10) Inouye, K.; Kuzuya, K.; Tonomura, B. *J. Biochem.* **1998**, *123*, 847.
(11) Collins, K. D.; Washabaugh, M. W. *Q. Rev. Biophys.* **1985**, *18*, 323.
(12) Vrbka, L.; Vondrasek, J.; Jagoda-Cwiklik, B.; Vacha, R.; Jungwirth, P. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15440.
(13) Aziz, E. F.; Eisebitt, S.; de Groot, F.; Chiou, J.; Dong, C.; Guo, J.; Eberhardt, W. *J. Phys. Chem. B* **2007**, *111*, 4440.
(14) Aziz, E. F.; Zimina, A.; Freiwald, M.; Eisebitt, S.; Eberhardt, W. *J. Chem. Phys.* **2006**, *124*, 114502.
(15) Nolting, D.; Aziz, E. F.; Ottosson, N.; Faubel, M.; Hertel, I. V.; Winter, B. *J. Am. Chem. Soc.* **2007**, *129*, 14068.
(16) Guo, J. H.; Augustsson, A.; Kashtanov, S.; Spångberg, D.; Nordgren, J.; Hermansson, K.; Luo, Y.; Augustsson, A. *J. Electron Spectrosc. Relat. Phenom.* **2005**, *144*−*147*, 287.
(17) Guo, J. H.; Luo, Y.; Augustsson, A.; Rubensson, J. E.; Sathe, C.; Ågren, H.; Siegbahn, H.; Nordgren, J. *Phys. Rev. Lett.* **2002**, *89*, 137402.
(18) Aziz, E. F.; Eisebitt, S.; Eberhardt, W.; Cwiklik, L.; Jungwirth, P. *J. Phys. Chem. B* **2008**, *112*, 1262–1266.
(19) Aziz, E. F.; Eberhardt, W.; Eisebitt, S. *Z. Phys. Chem.* **2008**, *222*, 727–738.
(20) Uejio, J. S.; Schwartz, C. P.; Duffin, A. M.; Drisdell, W. S.; Cohen, R. C.; Saykally, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6809.
(21) Aziz, E. F.; Freiwald, M.; Eisebitt, S.; Eberhardt, W. *Phys. Rev. B* **2006**, *73*, 75120.
(22) Winter, B.; Faubel, M. *Chem. Rev.* **2006**, *106*, 1176.
(23) Jagoda-Cwiklik, B.; Vacha, R.; Lund, M.; Srebro, M.; Jungwirth, P. *J. Phys. Chem. B* **2007**, *111*, 14077.
(24) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. *J. Phys. Chem.* **1987**, *91*, 6269.
(25) Jungwirth, P.; Finlayson-Pitts, B. J.; Tobias, D. J. *Chem. Rev.* **2006**, *106*, 1137.
(26) Lindahl, E.; Hess, B.; van der Spoel, D. *J. Mol. Model.* **2001**, *7*, 306.
(27) Dunning, T. H. *J. Chem. Phys.* **1989**, *90*, 1007.
(28) Klamt, A.; Schuurmann, G. *J. Chem. Soc., Perkin Trans. 2* **1993**, 799.
(29) Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995.
(30) Schmid, R.; Miah, A. M.; Sapunov, V. N. *Phys. Chem. Chem. Phys.* **2000**, *2*, 97.
(31) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 03*, Windows 03 ed.; Gaussian, Inc.: Pittsburgh PA, 2003.
(32) Wernet, P.; Nordlund, D.; Bergmann, U.; Cavalleri, M.; Odelius, M.; Ogasawara, H.; Naslund, L. A.; Hirsch, T. K.; Ojamae, L.; Glatzel, P.; Pettersson, L. G. M.; Nilsson, A. *Science* **2004**, *304*, 995.
(33) Smith, J. D.; Cappa, C. D.; Wilson, K. R.; Messer, B. M.; Cohen, R. C.; Saykally, R. J. *Science* **2004**, *306*, 851.
(34) Hawlicka, E.; Swiatla-Wojcik, D. *J. Phys. Chem. A* **2002**, *106*, 1336.
(35) Zaanen, J.; Sawatzky, G. A.; Allen, J. W. *Phys. Rev. Lett.* **1985**, *55*, 418.
(36) Zaanen, J.; Sawatzky, G. A.; Fink, J.; Speier, W.; Fuggle, J. C. *Phys. Rev. B* **1985**, *32*, 4905.
(37) Cappa, C. D.; Smith, J. D.; Wilson, K. R.; Messer, B. M.; Gilles, M. K.; Cohen, R. C.; Saykally, R. J. *J. Phys. Chem. B* **2005**, *109*, 7046.
(38) Jungwirth, P.; Winter, B. *Annu. Rev. Phys. Chem.* **2008**, *59*, 343.
(39) Collins, K. D.; Neilson, G. W.; Enderby, J. E. *Biophys. Chem.* **2007**, *128*, 95.
(40) Born, M. *Z. Phys.* **1920**, *1*, 45.

JP805177V