Spectroscopic Studies of Solutes in Aqueous Solution

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Received: October 17, 2007; In Final Form: November 29, 2007

Absorption and fluorescence characteristics of aqueous solutions of salts, sugars, and amino acids were studied using UV−vis spectroscopy and spectrofluorometry. Motivation stemmed from unanticipated absorption spectral and fluorescence features of the "exclusion zone" seen adjacent to various hydrophilic surfaces. Those features implied a structure distinct from that of bulk water (*Adv. Colloid Interface Sci.* **2006**, *127*, 19). Absorption peaks at ∼270 nm similar to those observed in the exclusion zone were seen in solutions of the following substances: salts, Nafion 117 solution/film, L-lysine, D-alanine, D-glucose and sucrose. To determine the fate of the absorbed energy, we studied the fluorescence properties of these solutions. The salts showed fluorescence emission around 480−490 nm under different excitation wavelengths. The fluorescence intensity of LiCl was higher than NaCl, which was in turn higher than KCl—the same ordering as the absorption intensities. Fluorescence of Nafion 117 solution/film, L-lysine, D-alanine, D-glucose and sucrose were observed as well, with multiple excitation wavelengths. Hence, at least some of the absorbed energy is released as fluorescence. The results show features closely similar to those observed in the exclusion zone, implying that the aqueous region around the solutes resembles the aqueous zone adjacent to hydrophilic surfaces. Both may be more extensively ordered than previously thought.

1. Introduction

Interfacial water structure affects physical, chemical, and biological processes.[2−7] These effects are mediated by the ordering of water that generally occurs next to solid surfaces, where layers of ordered water are found with properties different from bulk water.

Among the features of interfacial water documented to differ from bulk water are viscosity, density, freezing temperature, and relative permittivity.[8−16] Despite these known physical differences between ordered and bulk water, however, the structure of interfacial water is not well understood. For example, the existence of long-range repulsions between surfaces in water due to ordering of water molecules has long been recognized, but there has been much disagreement on whether the effective range of this modified structure is small (a few angstroms) or large (a few thousand angstroms).[17,18]

Following the findings of long-range interactions between substrate and solvent by Drost-Hansen and colleagues,[19−22] Zheng and Pollack[1,23] suggested recently that water ordering next to hydrophilic surfaces might be more extensive than generally thought. They reported that colloidal and molecular solutes suspended in aqueous solution were profoundly and extensively excluded from the vicinity of various hydrophilic surfaces. The width of the solute-free zone ("exclusion zone") was typically several hundred microns, equivalent to many molecular layers of water. Such extensive zones were observed with a variety of solutes in the vicinity of many types of surface including artificial and natural hydrogels, biological tissues, hydrophilic polymers, monolayers, and ion-exchange beads. An example similar to what has been reported is shown in Figure 1.

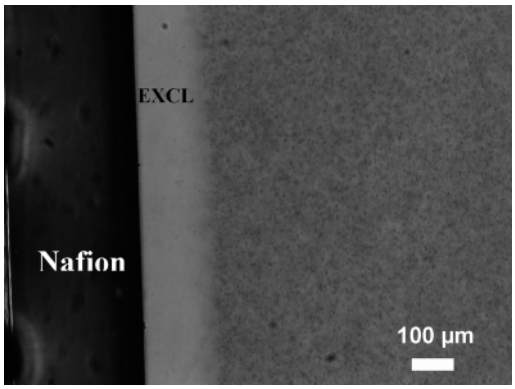

Figure 1. Solute-free aqueous zone ("EXCL") adjacent to the Nafion surface. The zone to the right of the exclusion zone (darker) contains 1 μm carboxylate microspheres.[24] The width of the exclusion zone, extending from the Nafion surface to the boundary of the microsphere zone, is approximately 150 μm.

According to such microscopic observations, as well as electrical potential measurements, UV−vis absorption spectra, infrared imaging, and NMR imaging, these solute-free zones are a physically distinct and less mobile phase of water that can coexist indefinitely with the contiguous solute-containing phase. Thus, the water appears to be extensively impacted by the hydrophilic surface.

In this report we focus on the absorption spectra in this zone, and their implication. Previous experiments showed that the UV−vis absorption spectrum was flat in regions distant from the Nafion surface; however, as the incident beam came closer to the Nafion, an absorption peak began to appear at approximately ∼270 nm. The peak grew with increasing proximity to Nafion and eventually dominated the spectrum. The result published earlier is reproduced in Figure 2, for reference.

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10.1021/jp710105n CCC: $40.75
Published on Web 02/26/2008

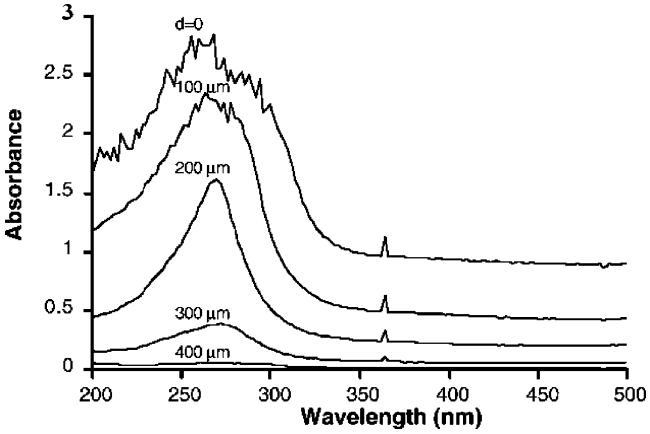

Figure 2. Spectra measured at varying distances (indicated on figure) from the Nafion surface.

Because the exclusion zone appears to represent a region of unexpectedly extensive ordering, and because this zone shows a characteristic ~270 nm absorption peak, we wondered whether such an absorption peak might be characteristic of other situations in which water ordering is expected. This was the primary question. A secondary objective was to determine the fate of the absorbed energy. Does this energy raise electrons to a higher energy state and release the energy as fluorescence?

2. Materials and Methods

2.1. Sample Preparation. ***Chemicals.*** Experimental substances include sodium chloride (EMD Chemical), potassium chloride (ACS reagent 99.0−100.5%, Sigma-Aldrich), lithium chloride (J. T. Baker), L-lysine monohydrochloride (Fluka), D-Alanine (Sigma), Nafion 117 solution (Fluka)/Nafion 117 perfluorinated membrane (0.007 in. thick, Aldrich),[18] D-glucose (called "dextrose", Sigma) and sucrose (Sigma). Samples were made in deionized water with pH 7.0. All experiments were carried out at 22−23 °C.

Deionized Water. All deionized water used in these experiments was obtained from the NANOpure Diamond ultrapure water system. The purity is certified to have a resistivity value up to 18.2 mΩ cm. An automatic sanitization cycle helps keep the system clean. In addition, a 0.2 μm hollow fiber filter is used for maintaining bacterial and particle-free water.

Baking Salts. In some experiments the NaCl, KCl, and LiCl were baked using the Furnace 1300 (Barnstead International) by setting the temperature at 600 °C to eliminate impurities. The temperature was set lower than the melting points of NaCl (801 °C), KCl (776 °C), and LiCl (605 °C).

Fluorescence Samples. Fluorescence is extremely susceptible to contamination by ubiquitous trace levels of organic chemicals. These include aromatic organic compounds, such as oils secreted by the experimentalist. In addition, suspended particulates such as dust and fibers will float in and out of the cuvette's sampling area via convection currents and cause fluorescence artifacts. Hence, great care was taken to avoid contamination during handling, and all cuvettes were kept covered during the experiments.

Fluorescence Measurements. The setup for measuring fluorescence spectra from the exclusion zone is shown in Figure 3. A sheet of Nafion 117 perfluorinated membrane was glued onto a 1 mm thick glass sheet, which was positioned onto one internal face of the cuvette. The cuvette was then filled with pure deionized water. A 150 μm slit oriented parallel to the face was used to create a narrow zone of excitation light. A small micrometer controlled the position of the slit relative to the Nafion surface.

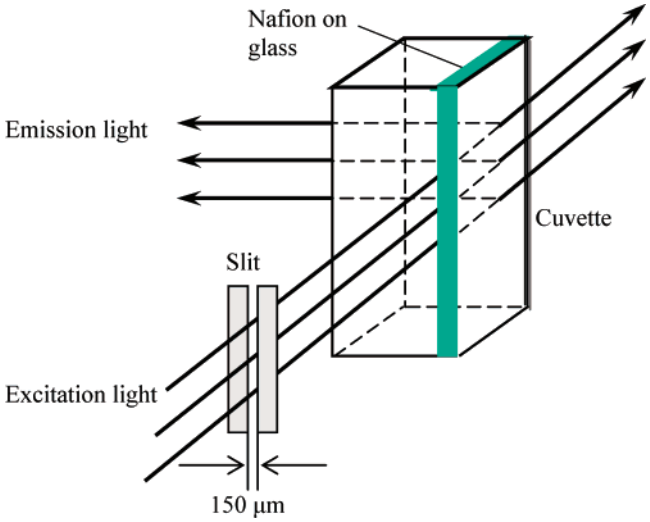

Figure 3. Optical system used for measuring fluorescence spectra as a function of distance from the Nafion surface.

2.2. UV−Vis Spectroscopy. Absorption-spectral measurements were performed on a single beam Hewlett-Packard (Model 8452A) diode-array spectrophotometer. A UV quartz micro-rectangular cuvette (Sigma Aldrich) was used, with inside dimensions 12.5 mm length, 2 mm width, and 45 mm height. The transmitting range of the cuvette is from 170 nm to 2.7 μm. The light-path length in the cuvette is 2 mm. Data were collected and plotted using the OLIS Globalworks program and computer data station supplied by the manufacturer. The displayed spectra are averages of at least ten scans.

2.3. Spectrofluorometry. All fluorescence experiments were performed on a Perkin-Elmer Luminescence Spectrometer (Model LS50B). The standard rectangular quartz cuvette with 10 mm light-path length (12.5 mm length, 12.5 mm width, 45 mm height) was used for holding samples. The incident light excites the sample over the entire path length and detects the light emitted at right angles to the excitation beam. There are two slits on both excitation and emission monochromators. Slit widths (bandwidth) could be adjusted in 0.1 nm increments according to experimental requirements. Data were collected using the FL WINLab program, which is supplied by the manufacturer.

2.4. Proton Nuclear Magnetic Resonance (^{1}H NMR). The ^{1}H NMR experiments were used to check for organic contaminants in samples. NMR experiments were carried using a Bruker Spectrometer AV-300 system, which is primarily used for ^{1}H nuclei observation. It hosts a BBI inverse probe for proton observations and multinuclear decoupling experiments. Here, we mainly examined chemical shifts to evaluate the type of proton environment.

3. Results

3.1. UV−Vis Spectral Absorption. Figure 4 shows the absorption spectra of saturated solutions of sodium chloride, potassium chloride, and lithium chloride, at pH 7.0. NaCl and KCl show subtle peaks near 270 nm, and LiCl shows a prominent peak at a similar wavelength. A question is the lowest concentrations at which such 270 nm absorption peaks can be detected. For example, a saturated NaCl solution is 5.3 M, and pure water is 55.35 M. Hence, roughly 10 water molecules surround each salt molecule. As the salt concentration was

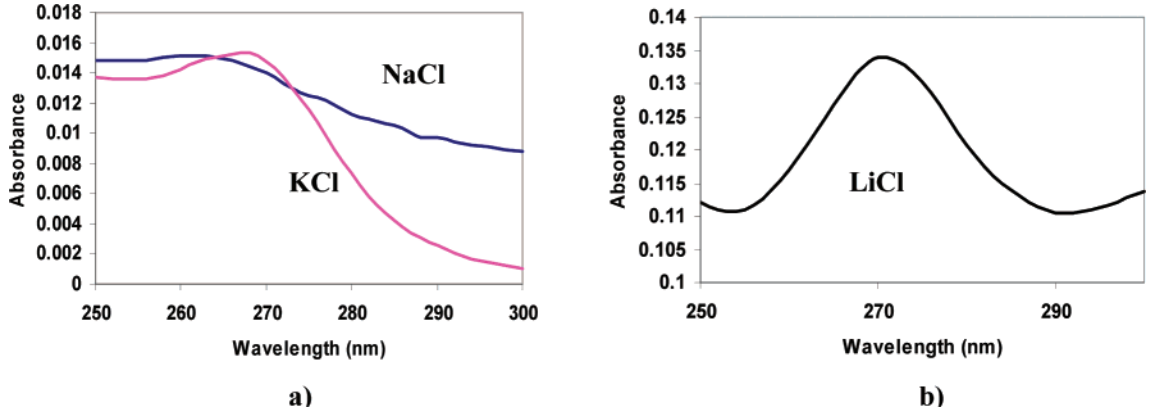

Figure 4. Absorption spectra of saturated solutions of (a) NaCl and KCl and (b) LiCl. Note difference of absorption intensities.

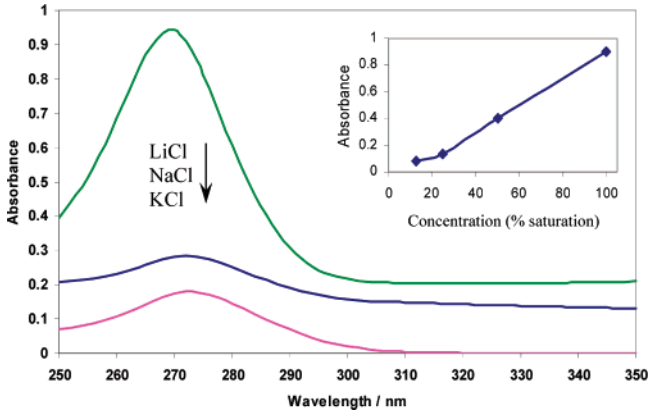

Figure 5. Absorption spectra of NaCl, KCl and LiCl solutions after purification by baking. Inset: amplitude of ~270 nm absorbance of LiCl solution as a function of salt concentration.

reduced, we found that the 270 nm absorbance peak size decreased concomitantly. The point at which it could no longer be detected was at a concentration of approximately 1 M, or roughly 50−60 water molecules per salt molecule. Lower salt concentrations might still show evidence of this peak, but detectability depends ultimately on the spectrometer's level of sensitivity.

Because some organic groups show peaks in this spectral region, the possibility exists that the observed peaks could have arisen spuriously, from aromatic impurities in the salts. Hence, the respective salts were baked to volatilize these organic components, and the resulting absorption spectra are shown in Figure 5. The ~270 nm peaks became more intense. The effect was especially dramatic for LiCl. Thus, the hypothesis that organic contaminants might have created spurious ~270 nm peaks is not supported.

To test further for the presence of organic contaminants, ^{1}H NMR experiments were carried out for salts after baking. Peaks at different chemical shifts represent protons in different chemical environments. For example, the chemical shifts of protons in H_2O and CH_2 are different. Only one peak at 4.7 ppm, representing the chemical shift position of the water signal, was seen for NaCl, KCl, and LiCl. Hence, NMR spectroscopy showed no sign of organic contaminants.

The same test was carried out for salts before baking. NMR spectroscopy again showed no sign of organic contaminants. Hence, any contaminants that may have been present prior to baking were probably in concentration low enough to lie beyond the sensitivity of the instrument.

Besides the salts described above, several other highly soluble or hydrophilic moieties were studied. Nafion 117 solution,

TABLE 1: UV Absorption Properties of 1 M Solutions of Various Solutes

	peak position	peak intensity
Nafion 117	~270 nm	0.18
L-lysine	~270 nm	0.34
D-alanine	~270 nm	0.12
D-glucose	~264 nm	0.06
sucrose	~268 nm	0.11

L-lysine monohydrochloride, D-alanine, D-glucose, and sucrose all showed absorption peaks near 270 nm. Absorption characteristics at 1 M concentration are shown in Table 1. The peak locations were indistinguishable from 270 nm for some solutes, and for others, the peak was shifted slightly toward blue by several nanometers. At concentrations lower than 1 M, peak intensities decreased but their positions remained unchanged. For D-alanine for example, the absorption intensities were respectively 0.121, 0.062, 0.032, 0.026, and 0.019, for concentrations of 1, 0.5, 0.3, 0.2, and 0.1 M, whereas peak positions determined by a Gaussian fitting algorithm were situated at ~268, ~266, ~266, ~267, and ~269 nm.

3.2. Fluorescence. When absorbed UV−visible photons cause electrons to transition to high-energy states, the return to the initial state, which generally occurs in less than 10^{-9} s, emits visible photons. This emission is well-known as fluorescence. A question that arose is whether substances that showed the ~270 nm absorption peaks might therefore fluoresce. This question has practical value, for fluorescence has higher information content than absorption; because two wavelengths are used, and because emitted light is read at right angles to excitation light, the range of linearity is large and the sensitivity is approximately 1000 times greater than that of absorption-spectrophotometric methods.[25] Hence, any observation of fluorescence would confer added utility.

Initial measurements were carried out in the exclusion zone next to a Nafion sheet, using the method shown in Figure 3. Figure 6 shows the fluorescence spectra obtained with the excitation light at various distances from the Nafion surface. Depending on distance, the emission-peak wavelength varied somewhat, but it remained consistently between 400 and 500 nm. Intensity generally although not always increased with proximity to the Nafion surface. Intensity overall was fairly low; this is anticipated because the slit used to restrict the excitation-wavelength window severely limited excitation intensity. Nevertheless, exclusion-zone fluorescence was apparent.

Fluorescence of NaCl, KCl, and LiCl solutions were checked as well. Fluorescence was confirmed in all three cases, although intensities were, again, relatively small—corresponding to the relatively small ~270 nm absorption. After baking, however,

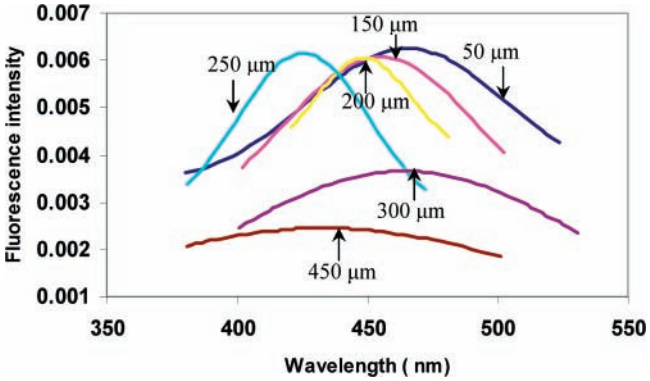

Figure 6. Emission spectra from exclusion zone adjacent to the Nafion sheet with 270 nm excitation, at different distances from the Nafion surface. Respective distances are indicated. Excitation bandwidth = 10 nm; emission bandwidth = 10 nm.

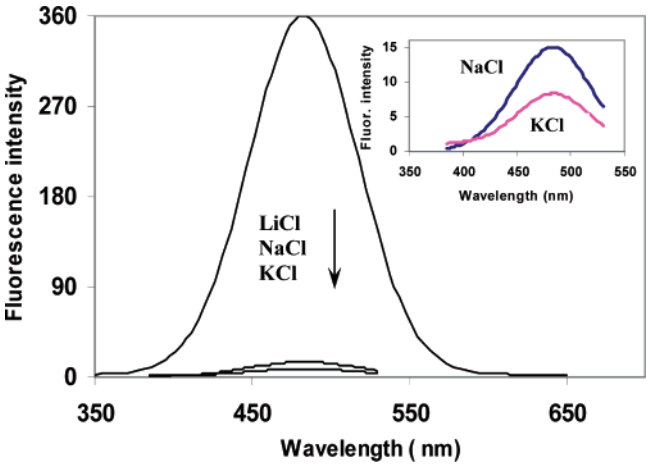

Figure 7. Emission spectra of LiCl, NaCl, and KCl after baking (λ_{exc} = 270 nm). Excitation bandwidth = 5 nm, emission bandwidth = 2.5 nm. Inset: magnified curves of emission intensity of NaCl and KCl.

the fluorescence became stronger, corresponding to the stronger absorption. As shown in Figure 7, LiCl showed a very large fluorescence peak centered around 480−490 nm. NaCl and KCl also gave peaks around 480−490 nm, but the intensities were weaker, just as the ~270 nm absorption peak was weaker. Thus, the ordering of fluorescence intensity was consistent with the ordering of ~270 nm absorption intensity, including the relative ranking LiCl ≫ NaCl > KCl. Given the consistency between UV-absorption and fluorescence-emission features, it does appear that at least some of the energy absorbed at ~270 nm goes into producing fluorescence.

Additional experiments were carried out to determine the dependence of fluorescence on excitation wavelength. Figure 8 shows the emission spectra of saturated solution of pre-baked LiCl, with excitation set at a series of wavelengths (slit width 5 nm). With ~310 nm excitation, emission was only faintly visible. However, an almost 50-fold increase in peak intensity was observed when exciting at 250 or 270 nm. Under these conditions, blue fluorescence (λ_{max} = ~483 nm) was clearly visible to the naked eye.

Results obtained using several other solutes are summarized in Table 2. All emissions lay in the range 300−500 nm. In general, the emission-peak wavelength shifted as the excitation wavelength shifted, although exceptions seem to be Nafion 117 solution and L-lysine solution. For excitations of 350 and 400 nm, the emissions generally remained within the range 420−440 nm. For excitations below 310 nm, the emission range was

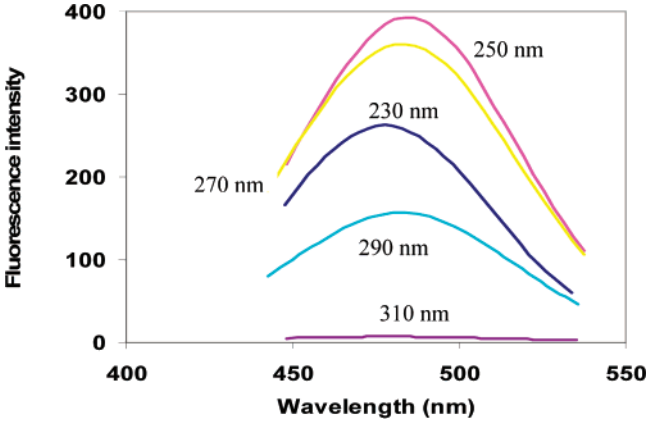

Figure 8. Emission spectra of LiCl at different excitation wavelengths, the latter indicated on the respective curves.

TABLE 2: Fluorescence Properties of Nafion 117 Solution, L-Lysine, D-Alanine, D-Glucose, and Sucrose[a]

	Nafion 117 solution		L-lysine		D-alanine		D-glucose		sucrose	
λ_{exc} (nm)	λ_{emi} (nm)	I	λ_{emi} (nm)	I	λ_{emi} (nm)	I	λ_{emi} (nm)	I	λ_{emi} (nm)	I
250	~459	8.7	~426	6.0	~385	1.3	~367	1.2	~351	3.1
270	~458	5.0	~423	4.1	~376	1.1	~347	1.2	~325	6.7
290	~445	3.7	~420	5.5	~398	1.2	~353	0.9	~322	3.1
310	~431	4.0	~393	6.2	~408	1.4	~393	0.5	~348	1.6
350	~440	4.7	~435	8.0	~425	1.1	~418	0.4	~417	0.9
400	~463	4.2	~470	6.6	~467	0.3	~459	0.2	~460	0.3

[a] λ_{exc}: wavelength of excitation. λ_{emi}: wavelength of emission peak. I: emission intensity.

slightly wider. With 310 nm excitation, the emission wavelengths of L-lysine and D-glucose were very close.

4. Discussion

Many substances are profoundly excluded from the zone adjacent to various hydrophilic surfaces including artificial and natural hydrogels, biological tissues, hydrophilic polymers such as Nafion, charged monolayers, and ion-exchange beads.[1,23] The substances include various colloidal particles, bacteria, proteins, and low-molecular-weight dyes. Surprisingly, the "exclusion zone" can extend to several hundred micrometers from the respective surface.

Although the genesis of this unexpectedly extensive zone is not yet settled, an increasing number of experiments have demonstrated that exclusion-zone water differs physically from bulk water.[1] These differences include the presence of steep electrical gradients, shorter NMR-relaxation times, reduced infrared emission, and distinctive UV−vis absorption spectra. These and other features have been suggestive of water ordering, and though such long-range ordering would seem unprecedented, theoretical arguments put forth by others imply that water ordering could extend infinitely under certain ideal conditions,[26] an assertion supported by precedent in the materials-science field.[27] Hence, an extant hypothesis is that the observed extensive exclusion may be a result of long-range water ordering.

The current work was motivated by one of the distinctive features of exclusion-zone water: the anomalous UV−vis absorption peak at ~270 nm. If exclusion-zone water is ordered, then the ~270 nm absorption peak may be an ordered water "signature." Indeed, we explored a variety of situations in which ordered water was anticipated, and the ~270 nm absorption was

consistently confirmed. These included saturated salt solutions, Nafion 117 solution/film, as well as concentrated solutions of sucrose, D-glucose, L-lysine, and D-alanine.

The possibility that these spectral features arose artifactually from the presence of water-soluble organic impurities was extensively checked. Eliminating volatile hydrocarbons by baking the salts failed to eliminate the 270 nm absorption peak. In fact, the peaks grew stronger. The absence of substantial contaminants was also confirmed using proton nuclear magnetic resonance (^{1}H NMR) experiments. Only one signal/peak at 4.7 ppm was found for NaCl, KCl, and LiCl, which is consistent with the chemical shift position of the water signal. On the basis of these controls, it appears that this anomalous absorption peak is indeed intrinsic to the salt solutions, and may well reflect extensive water structuring.

Another consideration is that the 270 nm peak might somehow be associated with the Cl^- ion, as most of the substances tested contained this ion. To check, we explored saturated solutions of NaI and KI. Along with peaks at 220 nm, these salts showed clear peaks at 270 nm similar to those seen with the Cl^- ion. Hence, the 270 nm peak was not a reflection of any particular anion in the salt.

Absorption at 270 nm is generally thought to arise from aromatic moieties. It is well-known for example that the side chains of tyrosine and tryptophan, the amino acids with aromatic rings, play a dominant role in near-ultraviolet absorption and subsequent fluorescence of proteins.[28−32] Thus, a useful method for determining protein concentration has been the measurement of the UV absorbance at ∼280 nm.[33,34] Dividing the measured absorbance by the protein's molar extinction coefficient yields the molar concentration. But lysine and alanine lack such aromatic moieties; yet, ∼270 nm absorption (together with fluorescence) was observed, implying that aromatic moieties alone are not fully responsible for absorption.

That L-lysine displayed such unexpected absorption and fluorescence characteristics at high concentrations (∼0.5 and 1 M) in aqueous media had been previously shown by Homchauddhuri and Swaminathan.[35,36] They speculated that the likely reason was the aggregation of L-lysine: this might arise from the presence of a bridging water molecule between each of N−H groups of the lysine−lysine pair. However, any such bridging would likely also impact that water's structure. Thus, it may be the buildup of such water structures that confers on L-lysine the observed absorption and fluorescence properties.

It appears that the ∼270 nm absorption is a unique characteristic of water in contact with various charged molecules. To understand more about this type of water, fluorescence measurements were carried out, motivated by the principal advantage of fluorescence over absorption spectroscopy: the ability to separate compounds on the basis of both their excitation and emission spectra, as opposed to the single spectrum obtainable with absorption.

The fluorescence results lead to two conclusions. First, for inorganic salts, the emission spectra were concentrated around 480−490 nm, whatever was the excitation wavelength. This implies that in salt solutions only one phase of ordered water may exist. Second, in the case of Nafion, L-lysine, D-alanine, D-glucose and sucrose, each excitation-spectral band had a characteristic emission band. The pairing of emission bands with corresponding excitation bands implies a structural polymorphism of water in solutions of these substances. In other words, these substances may exhibit a variety of quasi-crystalline structures, the nature of which remains to be determined.

It appears then, that the absorption peak at ∼270 nm and the corresponding fluorescence may be characteristic attributes of water structure. Both of these features are found in the exclusion zone, where water structuring is the hypothesized basis of exclusion, and both are found in multiple situations in which water structuring is anticipated as well. The ∼270 nm absorption can thus be interpreted as a reflection of the overall quasi-crystalline nature of water in the vicinity of charged or hydrophilic entities, whereas the fluorescence may indicate more subtle features of that structure. Indeed, it is possible that such measurements, long carried out in protein solutions, do not reflect the presence of aromatic moieties at all but reflect the water that is ordered around these proteins. The results obtained here imply such an interpretation.

Acknowledgment. We thank Dr. David Anick for his constructive comments on the manuscript. This study was supported by NIH Grants AT-002362 and AR-44813, and ONR Grant N00014-05-1-0773.

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(24) Nafion tubing is a registered trademark of PERMA Pure LLC. We used tubing TT-060, which has the inside diameter 0.052 in. and outside diameter 0.063 in. The Caboxylate Microspheres are purchased from Polysciences Inc., which are monodisperse polystyrene microspheres that contain surface carboxyl groups. Nafion 117 solution and Nafion 117 membranes are registered trades of E. I. du Pont de Nemours & Co., Inc. The solution's concentration is ∼5% in a mixture of lower aliphatic alcohols and water. The membrane is a per- fluorinated ion-exchange membrane which has wide variety of commercial uses. The acidic (hydrogen ion) form and derivatives are solid, superacid catalysts useful in a wide variety of synthetic applications.
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