HHS Public Access

Author manuscript

J Am Chem Soc. Author manuscript; available in PMC 2019 June 07.

Published in final edited form as:

J Am Chem Soc. 2015 March 25; 137(11): 3859–3866. doi:10.1021/jacs.5b00187.

Interactions between Hofmeister Anions and the Binding Pocket of a Protein

Jerome M. Fox[†], **Kyungtae Kang**[†], **Woody Sherman**[‖], **Annie Héroux**[⊥], **G. Madhavi Sastry**[¶], **Mostafa Baghbanzadeh**[†], **Matthew R. Lockett**[†,▽], and **George M. Whitesides**[*,†,‡,§]

[†]Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, United States

[‡]Wyss Institute for Biologically Inspired Engineering, Harvard University, 60 Oxford Street, Cambridge, Massachusetts 02138, United States

[§]The Kavli Institute for Bionano Science and Technology, Harvard University, 29 Oxford Street, Cambridge, Massachusetts 02138, United States

[‖]Schrödinger, 120 West 45th Street, New York, New York 10036, United States

[⊥]Photon Science Division, Energy Sciences Directorate, Brookhaven National Laboratory, Building 745, Upton, New York 11937, United States

[¶]Schrödinger, Sanali Infopark, 8-2-120/113 Banjara Hills, Hyderabad 11937, Andhra Pradesh, India

Abstract

This paper uses the binding pocket of human carbonic anhydrase II (HCAII, EC 4.2.1.1) as a tool to examine the properties of Hofmeister anions that determine (i) where, and how strongly, they associate with concavities on the surfaces of proteins and (ii) how, upon binding, they alter the structure of water within those concavities. Results from X-ray crystallography and isothermal titration calorimetry show that most anions associate with the binding pocket of HCAII by forming inner-sphere ion pairs with the Zn^{2+} cofactor. In these ion pairs, the free energy of anion–Zn^{2+} association is inversely proportional to the free energetic cost of anion dehydration; this relationship is consistent with the mechanism of ion pair formation suggested by the “law of matching water affinities”. Iodide and bromide anions also associate with a hydrophobic declivity in the wall of the binding pocket. Molecular dynamics simulations suggest that anions, upon associating with Zn^{2+}, trigger rearrangements of water that extend up to 8 Å away from their surfaces. These findings expand the range of interactions previously thought to occur between ions and proteins by suggesting that (i) weakly hydrated anions can bind complementarily shaped

[*]**Corresponding Author**, gwhitesides@gmwgroup.harvard.edu.

[▽]**Present Address** M.R.L.: Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

The authors declare no competing financial interest.

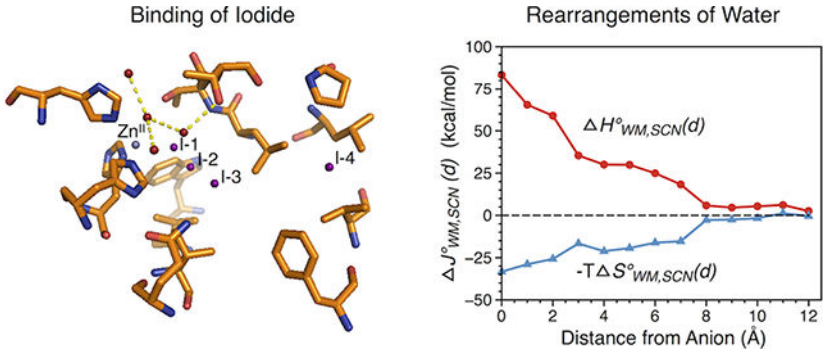
ASSOCIATED CONTENT

Supporting Information

Experimental methods, appendices, and supporting figures, tables, and references. This material is available free of charge via the Internet at http://pubs.acs.org. Structure factors and coordinates of anion-HCAII complexes are available in the Protein Data Bank (www.rcsb.org) with reference codes 4YGK, 4YGL, 4YGN, and 4YGJ.

hydrophobic declivities, and that (ii) ion-induced rearrangements of water within protein concavities can (in contrast with similar rearrangements in bulk water) extend well beyond the first hydration shells of the ions that trigger them. This study paints a picture of Hofmeister anions as a set of structurally varied ligands that differ in size, shape, and affinity for water and, thus, in their ability to bind to—and to alter the charge and hydration structure of—polar, nonpolar, and topographically complex concavities on the surfaces of proteins.

Graphical Abstract



INTRODUCTION

The non-covalent association of simple ions and proteins in aqueous solution plays a central role in many of the biochemical processes that constitute “life”. By binding and transporting Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, HCO_3^-, and Cl^-, ion channels in cell membranes regulate intracellular volume and pH,[1,2] control the uptake of nutrients and the release of metabolites,[3–5] engage in signal transduction,[6,7] and mediate action potentials;[8,9] by associating with—and subsequently oxidizing—I^-, thyroid peroxidases enable the production of essential iodine-containing hormones;[10] and by binding inorganic phosphate (and longer chain phosphate esters), kinases and phosphatases regulate the activity of enzymes and receptors throughout the cell.[11] Despite their importance in a range of biochemical phenomena, however, ion–protein interactions in aqueous environments remain incompletely understood at the molecular level.[12–17]

Two questions summarize existing uncertainty concerning the mechanisms by which ions and proteins interact in aqueous systems: (i) What attributes of ions and the surfaces of proteins determine where, and how strongly, they associate with one another? (ii) How do ions alter the structure of water solvating those surfaces (which differ in charge, topography, and organic functionality)? Answering the first question would explain why proteins exhibit different affinities for ions of the same charge (e.g., Na^+ vs K^+).[18–20] Answering the second question would explain how ions, by reorganizing the water solvating protein substructures (e.g., declivities, charged elements, polar and nonpolar surfaces), alter the interactions in which those substructures participate.[21–24]

This study addresses these two questions by examining ion− protein interactions in an experimentally well-defined model system: the binding pocket of human carbonic anhydrase

II (HCAII, EC 4.2.1.1).[25,26] Using isothermal titration calorimetry (ITC), X-ray crystallography, and molecular dynamics simulations, we examined the association of Hofmeister anions with the binding pocket of HCAII, and with the molecules of water filling that pocket. This binding pocket is a good model system for studying non-covalent interactions between ions and proteins for two reasons: (i) It has both a polar surface (Asn62, His-64, Asn-67, Gln-92)[27] and a nonpolar surface (Phe-131, Val-135, Leu-198, Pro-201, Pro-202, Leu-204).[28] (ii) It contains a positively charged metal cofactor (Zn^{2+}) that can associate with anions that occupy different positions in the Hofmeister series (e.g., SO_4^{2-}, CH_3COO^-, Cl^-, Br^-, NO_3^-, I^-, SCN^-).[29–33]

The Hofmeister series ranks the influence of ions on a wide variety of physical processes, most notably, their tendency to precipitate proteins from aqueous solution (Figure 1A; see also Appendix 1 of the Supporting Information (SI)).[16,34] We reasoned that anions with different positions in this series might exhibit different propensities to (i) partition into the binding pocket of HCAII (by interacting with the Zn^{2+} cofactor and, perhaps, polar and nonpolar residues) and (ii) reorganize molecules of water filling that pocket. By examining the association of Hofmeister anions with the binding pocket of HCAII, we thus hoped to identify attributes of ions that influence (i) where, and how strongly, they bind concavities on the surfaces of proteins and (ii) how, upon binding, they perturb the local structure of water.

Background: Key Terms and Concepts

Figure 1A shows the Hofmeister series of anions. Anions to the left of chloride, termed “kosmotropes”, tend to stabilize folded proteins (relative to unfolded proteins), and cause proteins to precipitate from aqueous solution.[16,34] Anions to the right of chloride, termed “chaotropes”, tend to promote denaturation, and enhance the solubility of proteins in solution. Kosmotropes are generally small (e.g., radius <1.8 Å for monovalent anions)[35] and strongly hydrated; chaotropes are generally large (e.g., radius >1.8 Å for monovalent anions) and weakly hydrated.[18]

We use the terms “strongly hydrated” or “weakly hydrated” to refer to the free energies of hydration of various anions ($\Delta G^\circ_{\text{hydration}}$, the free energy change associated with the transfer of one mole of ion from the gas phase to water at standard state).[36] For strongly hydrated anions, values of $\Delta G^\circ_{\text{hydration}}$ are more negative (e.g., $\Delta G^\circ_{\text{hydration}} \approx -90$ kcal/mol for CH_3COO^-);[36] for weakly hydrated anions, values of $\Delta G^\circ_{\text{hydration}}$ are *less* negative (e.g., $\Delta G^\circ_{\text{hydration}} \approx -50$ kcal/ mol for ClO_4^-).

RESULTS AND DISCUSSION

Ion Pairs and the “Law of Matching Water Affinities”

Several studies have proposed that ions associate with the surfaces of proteins by forming ion pairs in accordance with the so-called “law of matching water affinities” (Appendix 2 of the SI).[18,37–41] This qualitative “law” (or, perhaps, more appropriately, “empirically based hypothesis”) suggests that innersphere ion pairs form preferentially between oppositely charged ions with similar free energies of hydration. Two implications follow: (i) Small,

strongly hydrated ions—ions for which ion− water interactions are more free energetically favorable than water–water interactions—will associate with one another because the free energetic cost of partially desolvating those ions is more than compensated by the free energetic benefit of forming ion pairs. (ii) Large, weakly hydrated ions—ions for which ion–water interactions are less free energetically favorable than water–water interactions—will associate with one another because the free energetic cost of partially desolvating those ions is more than compensated by the free energetic benefit of forming additional water–water interactions.

Empirical support for the law of matching water affinities (as it pertains to ion–protein interactions) is based, in part, on observations that ions and/or surface charges with similar levels of hydration tend to associate with one another.[14,39,41] For example, weakly hydrated anions (e.g., SCN^-) tend to associate with the weakly hydrated side chains of lysine and arginine; strongly hydrated anions (e.g., HPO_4^{2-}) tend to associate with strongly hydrated cations (e.g., Ca^{2+}) present at low concentrations (10^{-7} M) within the cell.[18,42] (Spectroscopic examination of the association of divalent cations with carboxylate side chains of polypeptides indicate that this rule of thumb might not hold for multivalent ions.[43]) The absence of corroborating thermodynamic investigations, however, has left the mechanism of ion-pair formation implied by this theory both (i) incompletely validated and (ii) without a predictive quantitative extension (i.e., a simple rule, grounded in thermodynamics, capable of predicting the relative affinities of two ions for a particular charged group).[44] We tested the law of matching water affinities—and evaluated a possible quantitative extension of this theory—by examining the correlation between $\Delta G^\circ_{\text{hydration}}$ for Hofmeister anions and their affinity for a single charged element: the Zn^{2+} cofactor of HCAII.

Two States

We discuss the non-covalent association of anions and proteins by comparing two states: an initial state, which consists of anions and proteins—not interacting with each other—in aqueous solution, and a final state, which consists of anion–protein complexes in aqueous solution (Figure 1B). Changes in thermodynamic properties resulting from anion–protein association ($\Delta J^\circ_{\text{bind}}$, where J = G, H, or TS), thus, reflect a difference in thermodynamic properties between the initial state and the final state ($\Delta J^\circ_{\text{bind}} = J_{\text{final}} - J_{\text{initial}}$).

Thermodynamic Basis of Association between Anions and the Zn^{2+} Cofactor

Hofmeister anions bind Zn^{2+} too weakly (i.e., the free energy of binding is too small) to permit the direct examination of anion–Zn^{2+} interactions with ITC. To obtain values of $\Delta J^\circ_{\text{bind}}$ (where J = G, H, or TS) for the association of anions and Zn^{2+} (Figure 1B), we thus employed a competition assay (SI Methods and Figure S1) similar to that employed by Zhang et al. to study the binding of low-affinity ligands to the protein tyrosine phosphatase 1B (EC 3.1.3.48).[45] Briefly, using ITC, we measured the dissociation constant and enthalpy of binding for the association of HCAII and benzo[d]thiazole-2-sulfonamide (BTA)—a high-affinity ligand ($K_{d,BTA}$ = 60 ± 30 nM) that binds the Zn^{2+} cofactor of HCAII[46]—in the presence (and absence) of sodium salts of ten different Hofmeister anions (100 mM, Figure 1A). (We note: in this discussion, values of $K_{d,BTA}$ represent the pK_a-corrected values

corresponding to the association of the deprotonated form of BTA with the water-bound form of HCAII. This correction is detailed in the SI). In the presence of sodium salts, BTA displaces Zn^{2+}-bound anions, and the observed values of the dissociation constant $\left(K_{\mathrm{d,BTA}}^{\mathrm{obs}}\right)$ and enthalpy of binding $(\Delta H^{\circ\,\mathrm{obs}}_{\mathrm{bind,BTA}})$—that is, values estimated under the assumption that no ions are present—differ from values of the dissociation constant ($K_{\mathrm{d,BTA}}$) and enthalpy of binding ($\Delta H^{\circ}_{\mathrm{bind,BTA}}$) determined in the absence of ions in accordance with eqs 1 and 2,

$$K_{\mathrm{d,BTA}}^{\mathrm{obs}} = K_{\mathrm{d,BTA}} + \frac{K_{\mathrm{d,BTA}}}{K_{\mathrm{d,anion}}}[\mathrm{A_{tot}}] \quad (1)$$

$$\Delta H_{\mathrm{bind,BTA}}^{\mathrm{obs}} = \Delta H_{\mathrm{bind,BTA}}^{\circ} - \frac{\Delta H^{\circ}{}_{\mathrm{bind,anion}}}{1 + K_{\mathrm{d,anion}}/[\mathrm{A_{tot}}]} \quad (2)$$

where Kd,anion and ΔH°bind,anion are the dissociation constant and enthalpy of binding, respectively, for a specific anion, and $[\mathrm{A_{tot}}]$ is the total concentration of that anion. For each anion, we used eqs 1 and 2 to determine Kd,anion and ΔH°bind,anion; from these values, we estimated ΔG°bind,anion and $-T\Delta S^{\circ}$bind,anion (Figure 2A; see also SI).

Our results indicate that the chloride and the chaotropes engage in enthalpically favorable ($\Delta H^{\circ}_{\mathrm{bind,anion}} < 0$), entropically unfavorable ($-T\Delta S^{\circ}_{\mathrm{bind,anion}} > 0$) interactions with the Zn^{2+} cofactor (Figure 2A). (We note: although anions may have additional binding sites in the region of the binding pocket occupied by BTA, and associated binding events would be reflected in the thermodynamic parameters measured with competition experiments, there is no crystallographic evidence for such sites.[21–24]) Interestingly, from left to right across the Hofmeister series (i.e., with increasing chaotropicity of the anions), values of $\Delta H^{\circ}_{\mathrm{bind,anion}}$ decrease, and values of $-T\Delta S^{\circ}_{\mathrm{bind,anion}}$ increase with almost complete compensation, and values of $\Delta G^{\circ}_{\mathrm{bind,anion}}$ decrease only slightly (from −2.3 ± 0.1 kcal/mol for Cl^{-} to −3.2 ± 0.1 kcal/mol for SCN^{-}). This type of enthalpy/entropy (H/S) compensation is believed to arise, in many bimolecular interactions, from rearrangements in the molecules of water that solvate interacting species,[47,48] and, thus, suggests that anion–Zn^{2+} association is strongly influenced by thermodynamic contributions from desolvation of the anion and/or Zn^{2+} cofactor. (We note: with calorimetry—although less with ITC than with experimental methods that rely on Van't Hoff analysis—errors in measured values of $\Delta H^{\circ}_{\mathrm{bind}}$ translate to errors in estimates of $\Delta S^{\circ}_{\mathrm{bind}}$, and can cause H/S compensation to be perceived where it does not occur.[49] We used a number of precautions, and carried out statistical checks, to reduce such errors; see SI Methods).

Kosmotropes, in contrast with chaotropes, bind weakly to the Zn^{2+} cofactor (Figure 2A) or, in the case of SO_4^{2-} and HPO_3^{2-}, not at all (i.e., too weakly to be detected under the conditions of our experiments). For HCO_3^{-} and CH_3COO^{-}, values of ΔH°bind,anion and $-T\Delta S^{\circ}$bind,anion again nearly compensate one another, but not in a manner consistent with the trend exhibited by chaotropes. This inconsistency likely arises from different

mechanisms of binding. HCO_3^- is a substrate of HCAII; CH_3COO^- is a substrate analogue. Unlike values of $\Delta J°_{bind,anion}$ for chaotropes, values of $\Delta J°_{bind,anion}$ for HCO3− and CH_3COO^- involve contributions from hydrogen bonds between the bound anions and amino acids near the Zn^{2+} cofactor.[29,50]

To examine the relationship between the affinity of specific anions for the Zn^{2+} cofactor and the free energetic cost of anion desolvation, we plotted $\Delta G°_{bind,anion}$ for each anion (chaotropes and komostropes) against literature values[36] of their free energies of hydration ($\Delta G°_{hydration}$; Figure 2B). Values of $\Delta G°_{bind,anion}$ decrease linearly with $\Delta G°_{hydration}$, and indicate that anions most capable of shedding their first hydration shells bind most tightly to Zn^{2+}. This linear relationship, which suggests that the affinity of anions for the Zn^{2+} cofactor correlates inversely with their affinity for water, is consistent with the mechanism of ion pair formation implied by the law of matching water affinities.[38]

Evidence of Hydrophobic Interactions between Anions and HCAII

Modeling studies by several groups have suggested that large, poorly hydrated anions can associate with nonpolar regions on the surfaces of proteins.[19,51–54] Experimental studies have substantiated these predictions by demonstrating that weakly hydrated anions can associate with nonpolar concavities in synthetic host systems;[55,56] hydrophobic interactions between anions and the surfaces of proteins, however, have proven difficult to examine experimentally, and the role of hydrophobicity in ion–protein association in aqueous environments remains controversial.[37,57] We used X-ray crystallography to search for hydrophobic binding sites for ClO_4^-, SCN^-, I^-, and Br^- in the binding pocket of HCAII. These anions are four of the most poorly hydrated included in the present study (i.e., they have smaller values of $\Delta G°_{hydration}$ than the other anions examined, SI Table S6); thiocyanate, iodide, and bromide have the added advantage that they exhibit anomalous scattering (due to S, I, and Br atoms)—an attribute that makes them useful tools for the detection of secondary, low-occupancy binding sites.[58,59]

Structures of HCAII complexed with ClO_4^- and SCN^- reveal a single ion in the binding pocket—bound, in each case, to the Zn^{2+} cofactor. Both anions displace H_2O-338, shift the position of H_2O-263, and leave Zn^{2+} in a pentacoordinated geometry (Figure 3A, and SI Figure S3). By contrast, the structures of HCAII complexed with iodide and bromide show four binding sites (Figures 3B–D and S5C,D; Appendix 3 of the SI). Here, for simplicity of discussion, we discuss the binding sites of iodide, which are identical to those of bromide, by referring to them in order of their proximity to the Zn^{2+} cofactor (I-1 through I-4, closest to farthest away). I-1 and I-2 denote alternative binding sites for ion–Zn^{2+} complexation and are not occupied simultaneously; these likely permit the formation of an inner-sphere ion pair (one that involves ion− ion contact) and an outer-sphere ion pair (one that involves a shared solvating water), respectively. I-3 denotes a binding site at the border of the hydrophilic and hydrophobic surfaces; it sits in close proximity (3.5 Å) to the amine of Gln-92 (Figure 3B,C). I-4 denotes a binding site within a small hydrophobic declivity formed by five nonpolar side chains near the mouth of the binding pocket (Figure 3B,D). As there is no positive charge proximal to the I-4 site, and as analysis of the surface charge within this site (an analysis carried out with the Adaptive Poisson–Boltzmann Solver[60]

package for PyMOL, Appendix 4 of the SI) shows little excess positive charge, Coulombic attraction is not the primary driving force for the association of iodide (or bromide) with this site.

The absence of secondary binding sites for the thiocyanate anion, which has a volume, free energy of hydration, and polarizability nearly indistinguishable from those of the iodide anion (Table S6),[36] suggests that ion shape (a parameter rarely mentioned in discussions of ion—protein association) may influence the ability of ions to engage in hydrophobic interactions. The I-4 binding site, in particular, has a hemisphere-like shape that can easily accommodate spherical iodide and bromide anions, but not a linear anion such as SCN^- (Figures 3D and S5).

The I-4 binding site provides direct evidence that poorly hydrated anions (i.e., iodide and bromide) can associate with complementarily shaped hydrophobic declivities on the surfaces of proteins. Previous molecular dynamics simulations provide evidence of an attraction between chaotropic anions and nonpolar regions on protein-like polymers;[51,52] here, crystallographic evidence indicates that two chaotropes can associate directly with a binding site formed by five nonpolar side chains. The existence of such a binding site suggests that theories of ion–protein interactions focused exclusively on the formation of ion pairs may oversimplify the variety of these interactions.

Anion-Induced Perturbations of the Structure of Water within the Binding Pocket

Many studies have suggested that Hofmeister ions reduce or enhance the solubility of proteins—a process termed “salting out” or “salting in”, respectively—by reducing or enhancing hydration of solventexposed residues.[23,24,61,62] The mechanisms and thermodynamic implications of such adjustments to hydration, however, remain poorly understood. We examined ion-induced perturbations of water structure inside the binding pocket of HCAII by using the WaterMap method (Schrödinger Inc.;[63–65] see SI Methods). WaterMap uses explicit-solvent molecular dynamics simulations, and inhomogeneous solvation theory, to calculate the enthalpy, entropy, and free energy of hydration sites within solvated proteins, relative to bulk water.[66,67] Unlike X-ray crystal structures, which reveal only the positions of wellordered, highly localized (i.e., enthalpically stable) waters, WaterMap predicts the positions and thermodynamic properties of all waters—well-ordered or otherwise—in a structure. 2+

The association of anions with the Zn cofactor of HCAII (the process depicted in Figure 1B) is coincident with rearrangements in the molecules of water filling the binding pocket. To evaluate the thermodynamic contribution of these rearrangements to anion–Zn^{2+} association, we summed the thermodynamic properties (enthalpy, entropy, and free energy) of hydration sites located in the binding pockets of anionbound ($\Delta J°_{WM,HCA\text{-}anion}$) and native ($\Delta J°_{WM,HCA}$) HCAII complexes, and we calculated the difference of these sums (e.g., $\Delta J°_{WM,anion} = \Delta J°_{WM,HCA\text{-}anion} - \Delta J°_{WM,HCA}$, where WM denotes values calculated from WaterMap, and J = G, H, or TS; see SI Methods). Crystal structures of HCAII containing a variety of Zn^{2+}-bound anions (collected here and else-where)[21–24] allowed us to perform these calculations for anions spanning the Hofmeister series (SI Methods). Results from our calculations suggest that anions, upon forming ion pairs with Zn^{2+}, bring

about entropically favorable ($-T\Delta S°_{WM,anion} < 0$) and enthalpically unfavorable ($\Delta H°_{WM,anion} > 0$) rearrangements of water inside the binding pocket (Figure 4A). Interestingly, values of $\Delta J°_{WM,anion}$ (where J = G, H, or TS) are similar across the Hofmeister series (Figure 4A). This result, in light of the linear relationship between the free energy of anion–Zn^{2+} association and the free energy of anion hydration (Figure 2B), suggests that differences in the affinity of Hofmeister anions for the Zn^{2+} cofactor are not the result of differences in anion-induced rearrangements of water inside the binding pocket, but rather from differences in (i) the free energetic cost of anion desolvation and (ii) the free energetic benefit of forming an anion–Zn^{2+} pair.

Length Scale of Anion-Induced Perturbations of the Structure of Water within the Binding Pocket

The results of several spectroscopy studies of ions in bulk water suggest that the effect of ions on the structure of water is limited to their first hydration shells.[68–70] Complementary experimental examinations of ions adsorbed at interfaces, however, have remained difficult, and the length scale over which ions perturb interfacial water remains unclear.[71–73] Using results from WaterMap calculations, we estimated the distance over which Zn^{2+}-bound anions trigger rearrangements of water within the binding pocket of HCAII by examining $\Delta H°_{WM,anion}(d)$ and $-T\Delta S°_{WM,anion}(d)$, the changes in enthalpy and entropy, respectively, that result from binding-induced rearrangements of water that occur beyond a distance d Å from the surface of the Zn^{2+}-bound anion (Figure 4B). Figure 4C shows the representative case of thiocyanate (other ions show similar trends; Figure S6); this figure indicates that rearrangements of water coincident with the binding of SCN^- persist well beyond the first hydration shell (—2.5 Å) of this anion, and extend up to 8 Å away from its surface (beyond d = 8 Å, values of $\Delta J°_{WM,anion}(d)$ decrease to less than 10% of $\Delta J°_{WM,anion}$). This distance suggests that the influence of anions on the structure of water at protein/water interfaces—or, at least, within the declivities of proteins—can extend well beyond the single hydration shells that demark the limit of their influence on the structure of bulk water. This result is consistent with previous molecular dynamics simulations suggesting that water within confined regions (e.g., the binding pockets of proteins) exhibits long-range structure;[63] alterations to the charge/structure of such regions are, thus, likely to have long-range consequences (such as those depicted in Figure 4C).

Influence of Rearrangements of Water on the Binding of Anions to the I-4 Site

Hydrophobic interactions between ligands and proteins often involve the free energetically favorable release of water from hydrophobic binding pockets.[46,74,75] To examine the role of displaced water in the association of iodide or bromide with the hydrophobic I-4 site, we used WaterMap to estimate the thermodynamic properties of molecules of water filling the binding pocket of HCAII in the presence and absence of bound iodide or bromide anions. Results suggest that the binding of iodide and bromide (separately) is coincident with the displacement of two molecules of water that are enthalpically and entropically unstable (relative to bulk water; Figures 5 and S7); the association of iodide and bromide with the I-4 hydrophobic declivity, thus, resembles the interaction of nonpolar ligands with hydrophobic binding pockets.[46,75,76]

CONCLUSION

This study uses the binding pocket of HCAII as a tool to identify the properties of Hofmeister anions that determine (i) where, and how strongly, they associate with concavities on the surfaces of proteins and (ii) how, upon binding, they alter the structure of water within those concavities. We find that anions can associate with the binding pocket of HCAII by forming inner-sphere ion pairs with the Zn^{2+} cofactor, and, in the case of iodide and bromide, by associating directly with a hydrophobic declivity.

For anion–Zn^{2+} association, calorimetry and X-ray crystallography suggest that the free energy of anion binding is inversely proportional to the free energetic cost of anion dehydration; this relationship is consistent with the mechanism of ion pair formation suggested by the law of matching water affinities and, thus, suggests that this theory may explain, in some biophysical contexts, the relative affinity of anions for positive charges on the surfaces of proteins. The formal extension of the law of matching water affinities to positive charges present in specific environments (e.g., charges within specific classes of concavities) will require calorimetric and crystallographic studies of anion binding to pockets that differ in charge, topography, organic functionality, and water structure.

The association of iodide and bromide with a complementary shaped hydrophobic binding site suggests that the topography of protein surfaces (i.e., the shape of bumps, declivities, or, perhaps, ion-binding motifs) may influence where, and how strongly, weakly hydrated ions bind those surfaces, and highlights the inadequacy of continuum electrostatics models for predicting ion–protein interactions. As with hydrophobic ligand-protein association, where rearrangements of water and/ or van der Waals interactions can contribute significantly to the overall free energy of binding,[26,48,74,76] anion association with the I-4 site is likely sensitive to the local dielectric environment, which can differ significantly between (and within) binding pockets.[77,78] Accurate assessment of the prevalence and mechanistic basis of hydrophobic anion–protein interactions will, thus, require additional crystallographic studies and thermodynamic analyses of anion binding to different proteins.

Molecular dynamics simulations summarized in this work suggest an important unanticipated effect of ions on the structure of water within concavities on protein surfaces. Anions, upon associating with the Zn^{2+} cofactor, trigger rearrangements of water that extend well beyond their first hydration shells (up to ~8 Å). This result suggests that concavities on surfaces may amplify the distance over which ion-induced perturbations of water structure extend, to distances well beyond that which characterizes the limit of their influence on the structure of water in homogeneous solution. This amplification is consistent with the notion that water within concavities on proteins exhibits long-range structure[63]—and, thus, long-range sensitivity to perturbations—and suggests that ions bound to topographically complex surfaces may alter the hydration state of residues beyond those immediately adjacent to their binding sites. We note, however, that like the binding events themselves, ioninduced perturbations of water structure are likely to be sensitive to local environmental influences (e.g., electrostatics, protein topography) and, thus, may differ significantly between concavities on the surfaces of proteins (and surfaces).

The results of this study suggest that the “Hofmeister series” describes what can be considered to be—in the context of anion–protein association—a series of ligands. Even when these ligands are identical in charge, they differ in their volume, shape, and affinity for water, three attributes that strongly influence their ability to bind to—and to alter the charge and hydration structure of—polar, nonpolar, and topographically complex concavities on the surfaces of proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank Serena Bai and Phil Snyder (Harvard University) for initial X-ray crystallography studies of the association of iodide and the binding pocket of HCAII, and Benjamin Breiten (Harvard University) for helpful discussions. This work was supported by the National Science Foundation under Award No. 1152196. A.H. was additionally supported by NIH/ NIGMS grant 8P41GM103473–16 and DOE/BER grant BO70. Structural data were collected at beamline X25 of the National Synchrotron Light Source under support of DOE/ BES Contract No. DE-AC02–98CH10886.

REFERENCES

(1). Eveloff JL; Warnock DG Am. J. Physiol 1987, 252, F1. [PubMed: 3544865]

(2). Sterling D; Casey JR Biochem. Cell Biol 2002, 80, 483. [PubMed: 12440690]

(3). Desai SA; Bezrukov SM; Zimmerberg J Nature 2000, 406, 1001. [PubMed: 10984055]

(4). Hedrich R; Neher E Nature 1987, 329, 833.

(5). Takahashi H; Yamazaki M; Sasakura N; Watanabe A; Leustek T; Engler JA; Engler G; Van Montagu M; Saito K Proc. Natl. Acad. Sci. U.S.A 1997, 94, 11102. [PubMed: 9380766]

(6). Clapham DE Cell 2007, 131, 1047. [PubMed: 18083096]

(7). Jasti J; Furukawa H; Gonzales EB; Gouaux E Nature 2007, 449, 316. [PubMed: 17882215]

(8). Catterall WA Science 1988, 242, 50. [PubMed: 2459775]

(9). Sanguinetti MC; Tristani-Firouzi M Nature 2006, 440, 463. [PubMed: 16554806]

(10). McLachlan SM; Rapoport B Endocr. Rev 2013, 13, 192.

(11). Hunter T Cell 1995, 80, 225. [PubMed: 7834742]

(12). Tobias DJ; Hemminger JC Science 2008, 319, 1197. [PubMed: 18309069]

(13). Jungwirth P; Cremer PS Nat. Chem 2014, 6, 261. [PubMed: 24651180]

(14). Vrbka L; Vondrásek J; Jagoda-Cwiklik B; Vácha R; Jungwirth P Proc. Natl. Acad. Sci. U.S.A 2006, 103, 15440. [PubMed: 17032760]

(15). Collins KD Biophys. Chem 2012, 167, 33.

(16). Nostro P. Lo; Ninham B. Chem. Rev 2012, 112, 2286. [PubMed: 22251403]

(17). Moreira LA; Boström M; Ninham BW; Biscaia EC; Tavares FW Colloids Surf. A: Physicochem. Eng. Asp 2006, 282–283, 457.

(18). Collins KD Biophys. Chem 2006, 119, 271. [PubMed: 16213082]

(19). Heyda J; Hrobarik T; Jungwirth P J. Phys. Chem. A 2009, 113, 1969. [PubMed: 19053553]

(20). Vrbka L; Jungwirth P; Bauduin P; Touraud D; Kunz W J. Phys. Chem. B 2006, 110, 7036. [PubMed: 16571019]

(21). Zhang Y; Furyk S; Bergbreiter DE; Cremer PS J. Am. Chem. Soc 2005, 127, 14505. [PubMed: 16218647]

(22). Chen X; Yang T; Kataoka S; Cremer PS J. Am. Chem. Soc 2007, 129, 12272. [PubMed: 17880076]

(23). Kalra A; Tugcu N; Cramer SM; Garde SJ Phys. Chem. B 2001, 105, 6380.

(24). Zhang Y; Cremer PS Curr. Opin. Chem. Biol 2006, 10, 658. [PubMed: 17035073]
(25). Krishnamurthy VM; Kaufman GK; Urbach AR; Gitlin I; Gudiksen KL; Weibel DB; Whitesides GM Chem. Rev 2008, 108, 946. [PubMed: 18335973]
(26). Snyder PW; Lockett MR; Moustakas DT; Whitesides GM Eur. Phys. J. Spec. Top 2014, 223, 853.
(27). Eriksson AE; Kylsten PM; Jones TA; Liljas A Proteins 1988, 4, 283. [PubMed: 3151020]
(28). Christianson DW; Fierke CA Acc. Chem. Res 1996, 29, 331.
(29). Mazumdar PA; Kumaran D; Swaminathan S; Das AK Acta Crystallogr. Sect. F: Struct. Biol. Cryst. Commun 2008, 64, 163.
(30). Jonsson BM; Hakansson K; Liljas A FEBS Lett. 1993, 322, 186. [PubMed: 8482389]
(31). Mangani S; Hakansson K Eur. J. Biochem 1992, 210, 867. [PubMed: 1336460]
(32). Kannan KK; Ramanadham M; Jones TA Ann. N.Y. Acad. Sci 1984, 429, 49. [PubMed: 6430186]
(33). Fisher Z; Boone CD; Biswas SM; Venkatakrishnan B; Aggarwal M; Tu C; Agbandje-Mckenna M; Silverman D; McKenna R Protein Eng. Des. Sel 2012, 25, 347. [PubMed: 22691706]
(34). Zhang Y; Cremer PS Annu. Rev. Phys. Chem 2010, 61, 63. [PubMed: 20055667]
(35). Collins KD Proc. Natl. Acad. Sci. U.S.A 1995, 92, 5553. [PubMed: 7539920]
(36). Marcus Y Ion Properties; Marcel Dekker: New York, 1997.
(37). Jungwirth P; Winter B Annu. Rev. Phys. Chem 2008, 59, 343. [PubMed: 18031215]
(38). Collins KD Biophys. J 1997, 72, 65. [PubMed: 8994593]
(39). Hess B; van der Vegt NF A. Proc. Natl. Acad. Sci. U.S.A 2009, 106, 13296. [PubMed: 19666545]
(40). Collins KD Methods 2004, 34, 300. [PubMed: 15325648]
(41). Uejio JS; Schwartz CP; Duffin AM; Drisdell WS; Cohen RC; Saykally RJ Proc. Natl. Acad. Sci. U.S.A 2008, 105, 6809. [PubMed: 18463292]
(42). Ries-Kautt M; Ducruix A Methods Enzymol. 1997, 276, 23. [PubMed: 27799097]
(43). Kherb J; Flores SC; Cremer PS J. Phys. Chem. B 2012, 116, 7389. [PubMed: 22697172]
(44). Collins KD; Neilson GW; Enderby JE Biophys. Chem 2007, 128, 95. [PubMed: 17418479]
(45). Zhang YL; Zhang ZY Anal. Biochem 1998, 261, 139. [PubMed: 9716416]
(46). Snyder PW; Mecinovic J; Moustakas DT; Thomas SW; Harder M; Mack ET; Lockett MR; Heroux A; Sherman W; Whitesides GM Proc. Natl. Acad. Sci. U.S.A 2011, 108, 17889. [PubMed: 22011572]
(47). Dunitz JD Chem. Biol 1995, 2, 709. [PubMed: 9383477]
(48). Breiten B; Lockett MR; Sherman W; Fujita S; Al-Sayah M; Lange H; Bowers CM; Heroux A; Krilov G; Whitesides GM J. Am. Chem. Soc 2013, 135, 15579. [PubMed: 24044696]
(49). Chodera JD; Mobley DL Annu. Rev. Biophys 2013, 42, 121. [PubMed: 23654303]
(50). Sjöblom B; Polentarutti M; Djinovic-Carugo K Proc. Natl. Acad. Sci. U.S.A 2009, 106, 10609. [PubMed: 19520834]
(51). Lund M; Vácha R; Jongwirth P Langmuir 2008, 24, 3387. [PubMed: 18294017]
(52). Lund M; Vrbka L; Jungwirth PJ Am. Chem. Soc 2008, 130, 11582.
(53). Huang DM; Cottin-Bizonne C; Ybert C; Bocquet L Phys. Rev. Lett 2007, 98, 177801. [PubMed: 17501535]
(54). Horinek D; Netz RR Phys. Rev. Lett 2007, 99, 226104. [PubMed: 18233302]
(55). Gibb CLD; Gibb BC J. Am. Chem. Soc 2011, 133, 7344. [PubMed: 21524086]
(56). Carnegie RS; Gibb CLD; Gibb BC Angew. Chem., Int. Ed 2014, 53, 11498.
(57). Pegram LM; Wendorff T; Erdmann R; Shkel I; Bellissimo D; Felitsky DJ; Record MT Proc. Natl. Acad. Sci. U.S.A 2010, 107, 7716. [PubMed: 20385834]
(58). Dauter Z; Dauter M; de La Fortelle E; Bricogne G; Sheldrick GM J. Mol. Biol 1999, 289, 83. [PubMed: 10339407]
(59). Dauter Z; Dauter M; Rajashankar KR Acta Crystallogr. Sect. D: Biol. Crystallogr 2000, 56, 232. [PubMed: 10666615]
(60). Baker NA; Sept D; Joseph S; Holst MJ; McCammon JA Proc. Natl. Acad. Sci. U.S.A 2001, 98, 10037. [PubMed: 11517324]

(61). Pegram LM; Record MT J. Phys. Chem. B 2008, 112, 9428. [PubMed: 18630860]
(62). Pegram LM; Record MT J. Phys. Chem. B 2007, 111, 5411. [PubMed: 17432897]
(63). Young T; Abel R; Kim B; Berne BJ; Friesner RA Proc. Natl. Acad. Sci. U.S.A 2007, 104, 808. [PubMed: 17204562]
(64). Abel R; Young T; Farid R; Berne BJ; Friesner RA J. Am. Chem. Soc 2008, 130, 2817. [PubMed: 18266362]
(65). Beuming T; Farid R; Sherman W Protein Sci. 2009, 18, 1609. [PubMed: 19569188]
(66). Lazaridis TJ Phys. Chem. B 1998, 102, 3531.
(67). Lazaridis TJ Phys. Chem. B 1998, 102, 3542.
(68). Omta AW; Kropman MF; Woutersen S; Bakker HJ Science 2003, 301, 347. [PubMed: 12869755]
(69). Wachter W; Kunz W; Buchner R; Hefter GJ Phys. Chem. A 2005, 109, 8675.
(70). Buchner R; Hefter GT; May PM J. Phys. Chem. A 1999, 103, 1.
(71). Boström M; Williams DRM; Ninham BW Langmuir 2001, 17, 4475.
(72). Otten DE; Shaffer PR; Geissler PL; Saykally RJ Proc. Natl. Acad. Sci. U.S.A 2012, 109, 3190.
(73). Caleman C; Hub JS; van Maaren PJ; van der Spoel D Proc. Natl. Acad. Sci. U.S.A 2011, 108, 6838.
(74). Barratt E; Bingham RJ; Warner DJ; Laughton CA; Phillips SEV; Homans SW J. Am. Chem. Soc 2005, 127, 11827. [PubMed: 16104761]
(75). Homans SW Drug Discovery Today 2007, 12, 534. [PubMed: 17631247]
(76). Bingham RJ; Findlay JBC; Hsieh S-Y; Kalverda AP; Kjellberg A; Perazzolo C; Phillips SEV; Seshadri K; Trinh CH; Turnbull WB; Bodenhausen G; Homans SW J. Am. Chem. Soc 2004, 126, 1675. [PubMed: 14871097]
(77). Li L; Li C; Zhang Z; Alexov EJ Chem. Theory Comput 2013, 9, 2126.
(78). Dwyer JJ; Gittis AG; Karp DA; Lattman EE; Spencer DS; Stites WE; García-Moreno EB Biophys. J 2000, 79, 1610. [PubMed: 10969021]

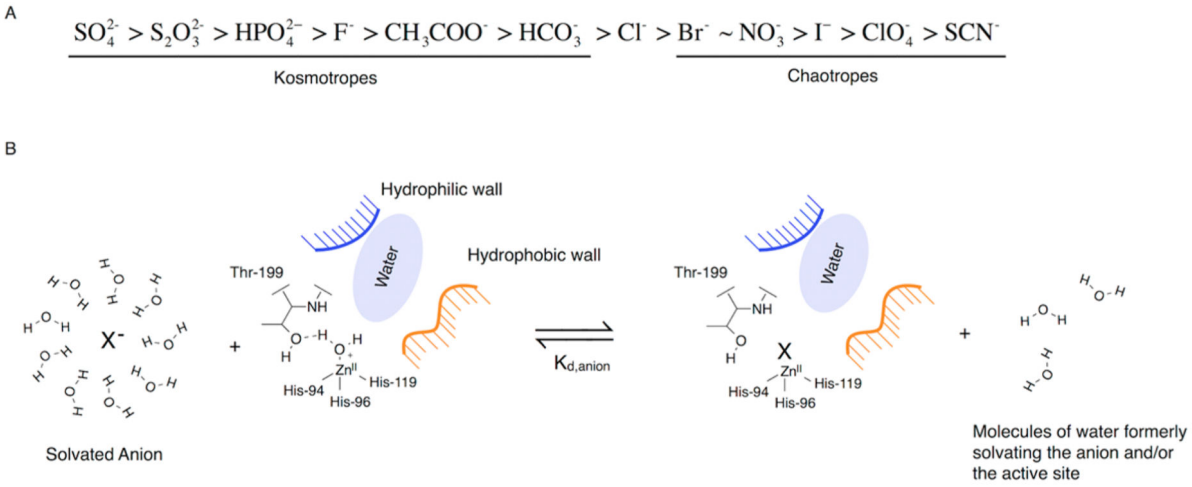


Figure 1.

The model system. (A) The Hofmeister series: anions ranked according to their propensity to precipitate proteins from aqueous solution. In this study, we examined the following anions: SO_4^{2-}, HPO_4^{2-}, CH_3COO^X, HCO_3^-, Cl^-, Br^-, NO_3^-, I^-, ClO_4^-, and SCN^-. (B) The association of anions with the Zn^{2+} cofactor involves two states: an initial state (left) with the anion and protein in aqueous solution, and a final state (right) with the anion–protein complex in aqueous solution. Thermodynamic parameters measured with ITC ($\Delta \mathcal{J}_{bind}$, where $J = H, TS$, or G) represent a difference between the initial and final states.

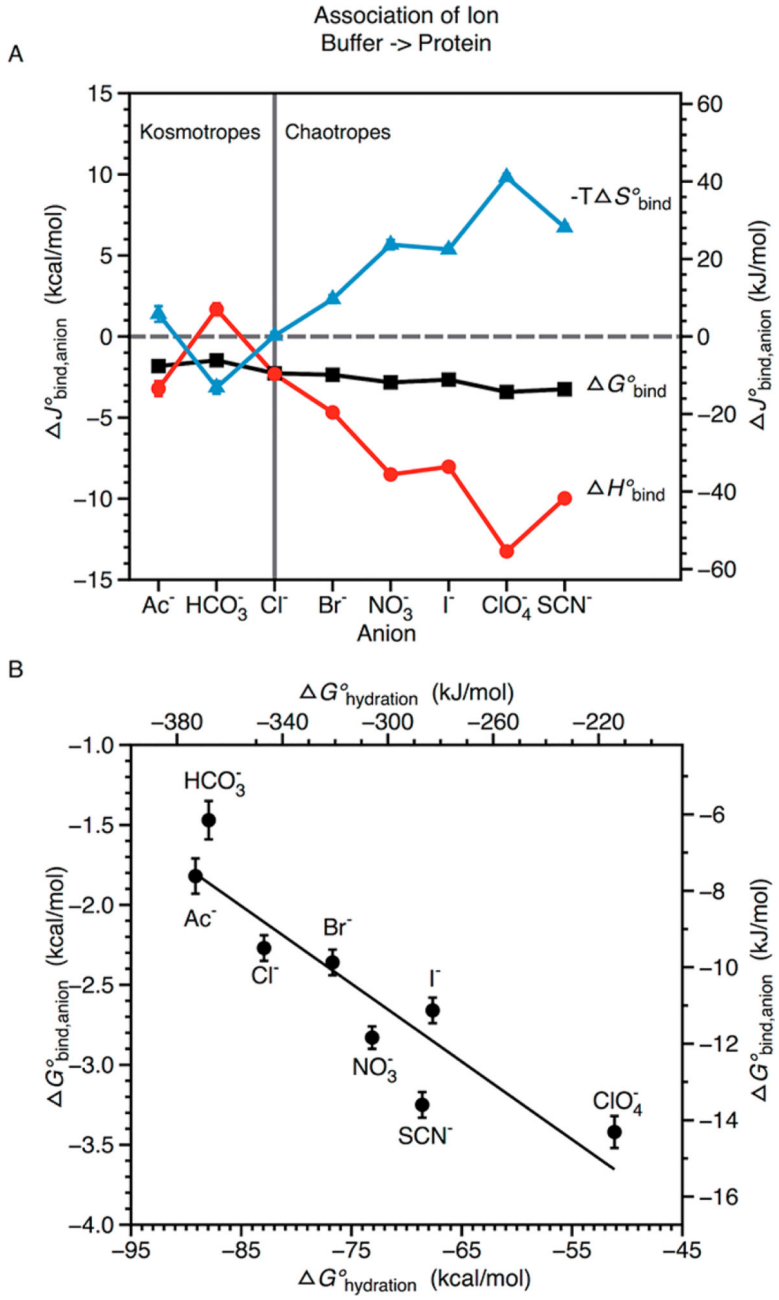


Figure 2.

Thermodynamics of anion binding. (A) A plot showing thermodynamic parameters for the association of anions and HCAII (298.15 K, pH = 7.6, 10 mM sodium phsophate buffer; the process depicted in Figure 1B). H/S compensation, revealed by the plot, often arises from rearrangements in the organization of waters that solvate interacting species. (B) A comparison of free energies of hydration ($\Delta G°_{hydration}$) with free energies of binding ($\Delta G°_{bind,anion}$). Values of $\Delta G°_{bind,anion}$ decrease linearly with $\Delta G°_{hydration}$ ($R^2 = 0.83$), suggesting that anions with a lower free energetic cost of dehydration bind more tightly to the Zn^{2+} cofactor. Values of $\Delta G°_{hydration}$ are taken from Marcus.[36] Error bars represent

standard error (n = 23 for the association of HCAII and BTA in the absence of anions, and n ≥ 7 for the association of HCAII and BTA in the presence of each anion; see SI Methods).

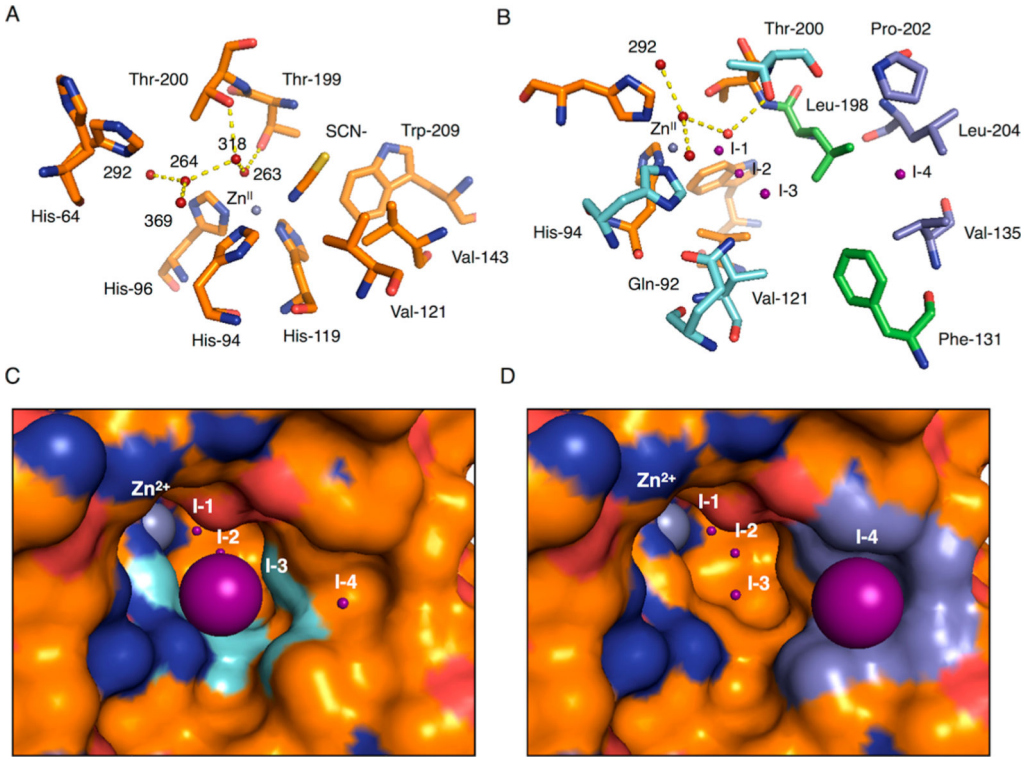


Figure 3.

Structural basis of anion binding. (A) X-ray crystal structure of the active site of HCAII complexed with SCN^- (PDB entry 4YGK). Both ClO_4^- and SCN^- displace H_2O-338 (the "so-called" deep water, displayed in SI Figure S3) and shift the position of H_2O-263 (the catalytically important Zn^{2+}-bound water). (B) X-ray crystal structure of the active site of HCAII complexed with iodide (PDB entry 4YGN) sites are further elaborated in Appendix 3 of the SI). Iodide sites are numbered in order of their proximity of the Zn^{2+}-bound cofactor. I-1 and I-2 denote alternative binding sites for the Zn^{2+}-bound iodide (an inner-sphere ion pair and an outer-sphere ion pair, respectively). I-3 denotes a binding site at the border of the hydrophobic and hydrophilic surfaces. I-4 denotes a binding site in the hydrophobic wall. Colors represent amino acids as follows: cyan (within 5 Å of I-3), light purple (within 5 Å of I-4), green (within 5 Å of both I-3 and I-4). (C) A detail of the I-3 binding site. Carbon atoms within 5 Å of the iodide are colored cyan. (D) A detail of the I-4 binding site. Carbon atoms within 5 Å of the iodide are colored light purple. In both (C) and (D), the iodide anions in the I-3 and I-4 positions, respectively, and the Zn^{2+} cofactor are shown as spheres that indicate their solvent-accessible surface area (i.e., the ion/water contact surface); the surface of the protein is also represented in this way.

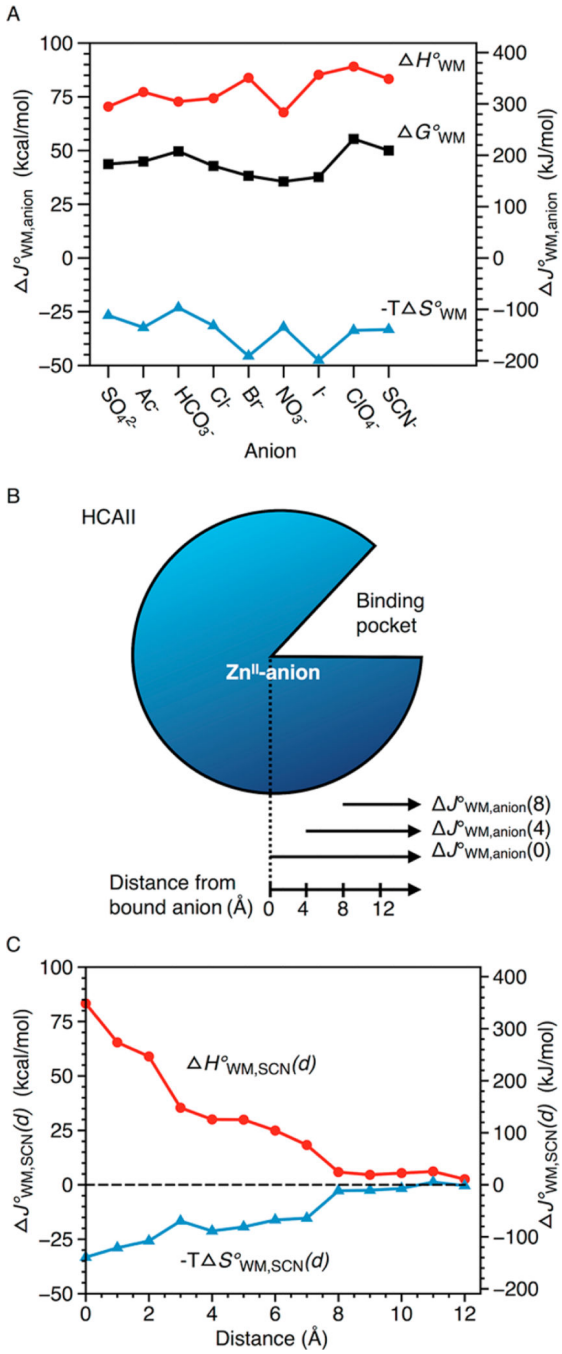


Figure 4.

Results from WaterMap calculations. (A) A plot showing the contribution of anion-induced rearrangements of water inside the binding pocket of HCAII to the thermodynamics of anion–Zn^{2+} association. Values of $\Delta J°_{WM,\ anion}$ represent the total difference of thermodynamic properties (enthalpies, entropies, and free energies) of waters in anion-bound and anion-free binding pockets ($\Delta J°_{WM,anion} = \Delta J°_{WM,HCA\text{-}anion} - \Delta J°_{WM,HCA}$, where J = H, TS, or G). (B) A schematic defining regions for calculating $\Delta H°_{WM,anion}(d)$ and $-T\Delta S°_{WM,anion}(d)$, the enthalpy and entropy, respectively, associated with rearrangements of

water (resulting from anion–Zn^{2+} association) occurring beyond d Å from the surface of the Zn^{2+}-bound anion (i.e., waters located between a distance of d Å from the Zn^{2+}-bound anion and the edge of the binding pocket). Calculations are based on crystal structures of anion-HCAII complexes. (C) A plot showing values of $\Delta H°_{WM,anion}(d)$ and $-T\Delta S°_{WM,anion}(d)$ for the binding of SCN^- to Zn^{2+}. This plot suggests that SCN^- triggers rearrangements of water that extend up to 8 Å from its surface.

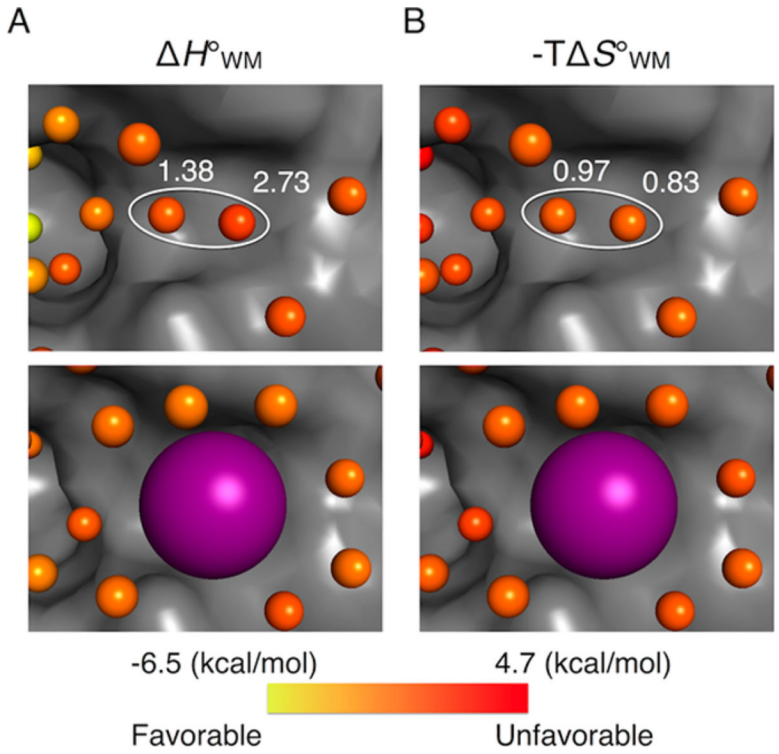


Figure 5.

Rearrangements of water in the I-4 binding pocket. (A-B) WaterMap results for the I-4 binding pocket shown in Figure 3D: (top) without iodide bound and (bottom) with iodide bound. Waters are colored according to (A) their enthalpies ($\Delta H°_{WM}$) and (B) their entropies ($-T\Delta S°_{WM}$), relative to bulk water. Results suggest that the binding of iodide to the I-4 binding pocket causes displacement of two enthalpically and entropically unstable (relative to bulk water) molecules of water (circled and labeled with their corresponding thermodynamic quantities). In all images, the surfaces of the protein (gray) and iodide anion (purple) represent the protein/water and ion/ water contact surfaces, respectively.