Aqueous Solvation of Polyalanine α-Helices with Specific Water Molecules and with the CPCM and SM5.2 Aqueous Continuum Models Using Density Functional Theory

Mateusz Marianski and J. J. Dannenberg*

Department of Chemistry, City University of New York—Hunter College and the Graduate School, 695 Park Avenue, New York, New York 10065, United States

S *Supporting Information*

ABSTRACT: We present density functional theory (DFT) calculations at the X3LYP/D95(d,p) level on the solvation of polyalanine α-helices in water. The study includes the effects of discrete water molecules and the CPCM and AMSOL SM5.2 solvent continuum model both separately and in combination. We find that individual water molecules cooperatively hydrogen-bond to both the C- and N-termini of the helix, which results in increases in the dipole moment of the helix/water complex to more than the vector sum of their individual dipole moments. These waters are found to be more stable than in bulk solvent. On the other hand, individual water molecules that interact with the backbone lower the dipole moment of the helix/water complex to below that of the helix itself. Small clusters of waters at the termini increase the dipole moments of the helix/water aggregates, but the effect diminishes as more waters are added. We discuss the somewhat complex behavior of the helix with the discrete waters in the continuum models.

Water and the solvation environment clearly influence the structures and energies of globular proteins. Crystals of such proteins generally contain significant numbers of water molecules. While some of these waters can be localized in the crystal structures, others cannot as their positions must vary from one unit cell to another. These latter waters are considered "disordered". Those waters whose oxygen positions (hydrogens are not evident in most X-ray structures) can be seen in the crystal structures (ordered waters) may have strong specific interactions with particular entities in the protein. These entities may often be polar or charged groups on the amino acid side chains, but waters are often found near the termini of α-helices, as discussed in more detail below. In 1989, Baldwin[1] first reported polyalanine α-helices (solubilized with lysines or glutamic acids) to be stable in aqueous solution. They later used glutamine to solubilze the helices.[2] Since that time, his group[3] and that of Kemp[4,5] have reported more quantitative data on the stabilities of α-helical polyalanine as a function of peptide length. Arora et al. have stabilized α-helices by including a covalent link in lieu of one of the helical H-bonds.[6−8] Our group[9−13] and others[14−17] have reported density functional theory (DFT) calculations on the stabilities of these helices without solvation, and solvated using the SM5.2 aqueous solvation model.[18] Kubelka has reported the effects of explicit waters on vibrational spectra of helices,[19] Ermakova has reported other studies of α-helices,[20,21] and Ireta has reported studies of β-sheets[22] interacting with individual water molecules

Over 15 years ago, Karplus demonstrated the existence of a cooperative hydration effect for amidic H-bonds, such as those found in peptides.[23] If such cooperativity is applied to α-helical peptides, one might expect individual water molecules that hydrate the ends of these helices to H-bond cooperatively with the other H-bonds within each of the three peptide chains within the α-helix. If this be the case, these waters would align with the dipole moment of the helix, causing the hydrated helix to have a larger dipole moment than the unhydrated form. This specific solvation effect would be different from the expectation that solvation of a dipolar molecule in a medium with a high dielectric constant would induce an ordering of this medium that would create an opposing dipole moment, as originally suggested by Onsager.[24] Do both kinds of solvation of α-helices occur? In this paper, we investigate the effects upon the energies and structures of capped polyalanine α-helices using hydration by specific water molecules as calculated by DFT, the CPCM,[25,26] and AMSOL SM5.2[27−29] water continuum model and combinations of both.

Another approach to aqueous solvation of the helices might involve molecular dynamics (MD) simulations. We have chosen not to use these methods as empirical force fields, which are parametrized to reproduce experimental properties of bulk water and do not properly include all physical forces nor do the most popular ones calculate the interactions between waters in a physically correct manner. For example, the commonly used TIP3P potential defines a water molecule with Coulombic interactions involving three point charges per water an r^{-6} attraction and an r^{-12} repulsion where each contribution is parametrized to reasonable structural and energetic results for gas-phase complexes of water and alcohols and liquid water.[30] A very recent paper using this approach has found differences in the

water structure for conserved and variable helices.[31] However, the lack of the capability for mutual polarization at a theoretical (rather than computational simulation) level would make these observations hard to interpret. While both continuum solvation and simple MD methods have their advantages, we use the (DFT) explicit waters and the continuum models as these allow us to examine the mutual effects of the solvent and the helix at a suitable (DFT) level of theory. Of course, the ideal solution would be to do MD using a Car–Parrinello approach,[32] and accurate quantum mechanical methods. However using such methods on so large a system is not currently practicable.

■ METHODS

Density functional theory (DFT) calculations were performed using the Gaussian 09[33] suite of computer programs. All calculations used the D95(d,p) basis set and the X3LYP functional.[34] The geometries of all the species were completely optimized starting from those previously published.[10] The structures were reoptimized after waters were added at the positions indicated and the lowest energy conformations chosen. The vibrational frequencies were calculated for all structures, using the normal harmonic approximations employed in the Gaussian 09 program,[33] to verify the stationary points and to calculate enthalpies at 298 K. All frequencies were real for the structures discussed, indicating that they are local minima on the potential energy surfaces. The counterpoise correction for basis set superposition error was incorporated using the single point a posteriori method,[35−38] rather than via optimization on the CP-corrected potential energy surfaces (CP-OPT)[39] in which up to 18 fragments were considered (the helix and each of the waters, except that the ensemble of all the backbone waters were considered one fragment) due to the excessive CPU time required.

We have chosen X3LYP due to its superior performance for neutral water clusters in general[40] and its excellent performance with d95(d,p) for water dimer.[41] This functional also gives folding energies from extended strand polyalanines to α-helices that are comparable to those obtained using ONIOM[42] with B3LYP/d95(d,p):AM1, whose incremental folding enthalpies[12] (upon extending the peptide by one residue) agree with experimental observations.[5] We used two different methods to calculate the solvation in bulk water: (1) the CPCM polarizable conductor-like solvent continuum model[25,26] with the (default) UFF option for the atomic radii used for the cavity as the model for the aqueous bulk solvent, and (2) the SM5.2 version of the AMSOL[29] solvation model as applied to the single-point DFT-optimized CPCM geometry using the AM1 semiempirical Hamiltonian as coded in the AMPAC.8.15 program[43] We have used a similar method in previous studies.[18,44]

■ RESULTS

We organize our results by first discussing solvation by a single water molecule in various positions on the α-helical form of acetyl(Ala)$_{15}$NHCH$_3$, followed by solvation by finite numbers of water molecules with different spatial distributions. Finally, we present the effect of the CPCM solvation model on the helix and the various specifically solvated structures. For the purpose of discussion, we divide the helix into three regions: (1) C-terminus; (2) N-terminus; and (3) backbone (the region between the termini).

Solvation by Discrete Water Molecules. We break the results on solvation by several water molecules into four parts: (1) solvation by several individual waters; (2) solvation by groups of interacting water molecules or water clusters; (3) solvation by the continuum model; and (4) combination of discrete and continuum solvation.

Solvation by One Water. Table 1 and Figures 1 and 2 present the data for solvation by individual water molecules. Table 1 includes the total interaction energies and enthalpies between the geometrically optimized helix, water, and hydrated complex after a posteriori CP correction. We have also broken down the contributions to the energy into the pure interaction between the species held in the structures of the optimized hydrated complex and the distortion energies of each component in a manner we have used previously for interactions in β-sheets.[45] We divide individual water molecules placed around the helix into three types that solvate (a) the C═O's near the C-terminus (C), (b) the N–H's near the N-terminus (N), and (c) the helical backbone between the termini (B).

Earlier studies have shown the interaction between water (as H-donor) and the C═O (as acceptor) to be the single strongest H-bond that a water can make with an amide.[23,46,47] The hydration of the three-terminal C═O's of the α-helix by an individual water follows this pattern. However, the water can form more than one H-bond due to the proximity of several C═O's. The most favorable positions for individual water molecules are illustrated in Figure 1. Since the strongest interaction of water molecules with small amides that can serve as models for peptides occur when the water acts as the H-bond donor and the carbonyl as the acceptor, that the strongest interactions of individual waters with the helix occur at the three C═O's with unsatisfied H-bonding positions near the C-terminus brings no surprise. The strongest single interaction with one water involves three H-bonds between the water and the C-terminus of the helix. The overall interaction energy of −11.1 kcal/mol results from H-bonding interactions of 15.1 and helix distortion of 3.9 kcal/mol. The corresponding enthalpy of interaction is −9.1 kcal/mol. The distortion energies of the water molecules were minimal (≤0.1 kcal/mol). Upon vibrational correction, these three single water hydrations have ΔH's of −9.1, −8.1, and −7.4 kcal/mol, which are all more than that reported for single hydrations of N-methylacetamide (NMA) as calculated using B3LYP/D95(d,p) of −5.6 kcal/mol.[46]

Hydration by single waters at two different positions at the N-terminus stabilize by 9.4 and 7.4 kcal/mol including distortion of 0.2 and 1.0 kcal/mol, respectively, and ΔH's of −7.7 and −5.8 kcal/mol, again greater than that reported for N-hydration of NMA (ΔH = −2.9 kcal/mol).[46]

Single waters interact with the backbone of the helix in three different conformations (see Table 3). In the preferred conformation (see Figure 2A), they stabilize by 4.3 to 6.7 kcal/mol with distortion energies that vary from 0.6 to 0.9 kcal/mol for the helix and 0.1 kcal/mol for the water and ΔH's that vary from 2.8 to 5.5 kcal/mol (see Table 1). These values are less than the 5.8 kcal/mol estimated for a single water–water H-bond in bulk water,[48] which should be expected for a hydrophobic surface. The stabilization energies increase as the backbone water moves from the N- to the C-terminus (see Table 1) as might be expected if one considers the length of the H-bonding chain between the N-terminus and the hydration position and the presumed increasing dipole moment of the helix fragment between the N-terminus and the hydration position. This behavior reflects

1438

dx.doi.org/10.1021/jp209177u |*J. Phys. Chem. B* 2012, 116, 1437–1445

Table 1. Counterpoise-Corrected Energies of Discrete Water Hydration (in kcal/mol) and Dipole Moments (D)

	position	opt (1 water)	opt (clusters)	frozen	dist (helix)	water	cluster	ΔH_{inter}	μ
				ΔE					
water (gas phase)									2.11
water (CPCM)									2.36
helix (gas phase)									69.8
helix (CPCM) see Figure 1									84.9
C	A	−9.3	−9.3	−11.6	2.2	0.1	0.1	−7.4	74.6
	B	−11.1	−11.1	−15.1	3.9	0.1	0.1	−9.1	70.0
	C	−10.1	−10.1	−10.8	0.6	0.1	0.1	−8.0	72.9
N	D	−9.4	−9.4	−9.6	0.2	0.0	0.0	−7.7	73.5
	E	−7.4	−7.4	−8.5	1.0	0.0	0.0	−5.8	73.0
	H-bond								
B	1	−4.3	−4.3	−5.2	0.8	0.1	0.1	−2.8	68.5
	2	−5.8	−5.8	−6.6	0.7	0.1	0.1	−4.3	68.1
	3	−6.5	−6.5	−7.3	0.7	0.1	0.1	−4.9	68.0
	4			complex converges to 3−10 helix at N-termini					
	5	−6.3	−6.3	−7.0	0.6	0.1	0.1	−4.7	68.2
	6	−6.5	−6.5	−7.1	0.5	0.1	0.1	−4.7	68.2
	7	−6.4	−6.4	−7.2	0.6	0.1	0.1	−4.7	68.5
	8	−6.6	−6.6	−7.3	0.6	0.1	0.1	−4.9	68.5
	9	−6.7	−6.7	−7.4	0.5	0.1	0.1	−4.9	68.5
	10	−6.9	−6.9	−7.8	0.7	0.1	0.1	−5.1	68.5
	11	−7.4	−7.4	−8.3	0.7	0.1	0.1	−5.5	69.1
	12	−6.7	−6.7	−7.7	0.9	0.1	0.1	−4.9	68.9
capping waters									
	4C	−47.2	−15.7	−21.0	2.7	−28.8	2.7	−38.8	79.2
	6C	−64.2	−7.8	−40.3	6.0	−29.9	26.5	−41.7	86.6
	11C	−131.2	−7.3	−58.8	6.7	−79.2	44.7	−108.5	85.4
	3N	−30.9	−11.8	−20.4	1.3	−11.9	7.3	−25.7	80.1
	6N	−64.3	−7.8	−25.1	3.1	−42.3	14.1	−38.8	72.7
	4C+3N	−78.4	−27.7	−40.8	3.1	−40.6	10.1	−64.8	89.5
	6C+3N	−95.8	−20.1	−61.0	7.3	−42.1	33.6	−77.7	97.0
	11C+6N	−196.8	−16.4	−85.4	9.8	−121.2	59.1	−163.1	88.9
	11B			helix hydrated in geometry of 4C+3N+11B hydrated helix					56.8
	4C+3N+11B	−144.4	−93.7	−112.8	8.7	−40.3	10.4	−112.7	73.0

what would be expected from Gilli's dual H-bond theory,[49−51] which compares the acidities and basicities of the H-bond donors and acceptors, taken together with our previous calculations that show the proton affinities of the terminal C=O's of chains of H-bonded formamides[52] and α-helices increase with the size of the helix.[11]

As can be seen in Table 1, the solvation by single waters, especially when placed at the helix termini, increases the dipole moment of the solvated complex over that of the vector sum of the dipoles of the water and the isolated helix. Thus, these individual waters tend to increase the cooperativity of the H-bonding in the system in the manner originally suggested by Karplus,[23] rather than interact as a dielectric would to counter the dipole moment of the solute.

Solvation by Clusters of Discrete Water Molecules. We also considered solvation by discrete waters in clusters of several sizes that are placed at the termini and at the backbone, as done for individual water molecules (see Table 1 and Figures 3−5). When small clusters of waters interact with the helix, each individual water molecule can interact with the helix directly or with other

water molecules (which may put them in a second or higher solvation shell). The lowest energy structure that contains a helix and *N* water molecules will involve a balance of the two types of interactions. To calculate the interaction energy between the helix solvated by a cluster of *N* waters, we need to consider the energy minimum for the equivalent *N*-water cluster in the absence of the helix. These were calculated starting from the geometries of clusters taken from a report by Shields,[53] which were extended to 11 waters and reoptimized using X3LYP using a posteriori CP corrections. Upon interaction with the helix, this *N*-water cluster will trade newly formed H-bonds to the helix for some of the water−water H-bonds found in the *N*-water clusters which generally become stronger as the quantity of water molecules grows.

The energies of interaction of the C-terminus with clusters of 4, 6, and 11 waters and the N-terminus with three and six waters are found in Table 1 and illustrated in Figure 3. The effect of adding 11 more waters at the backbone positions are also included in Table 1. The largest solvation cluster of specific waters considered contains 18 waters (4 at the C-terminus, 3 at
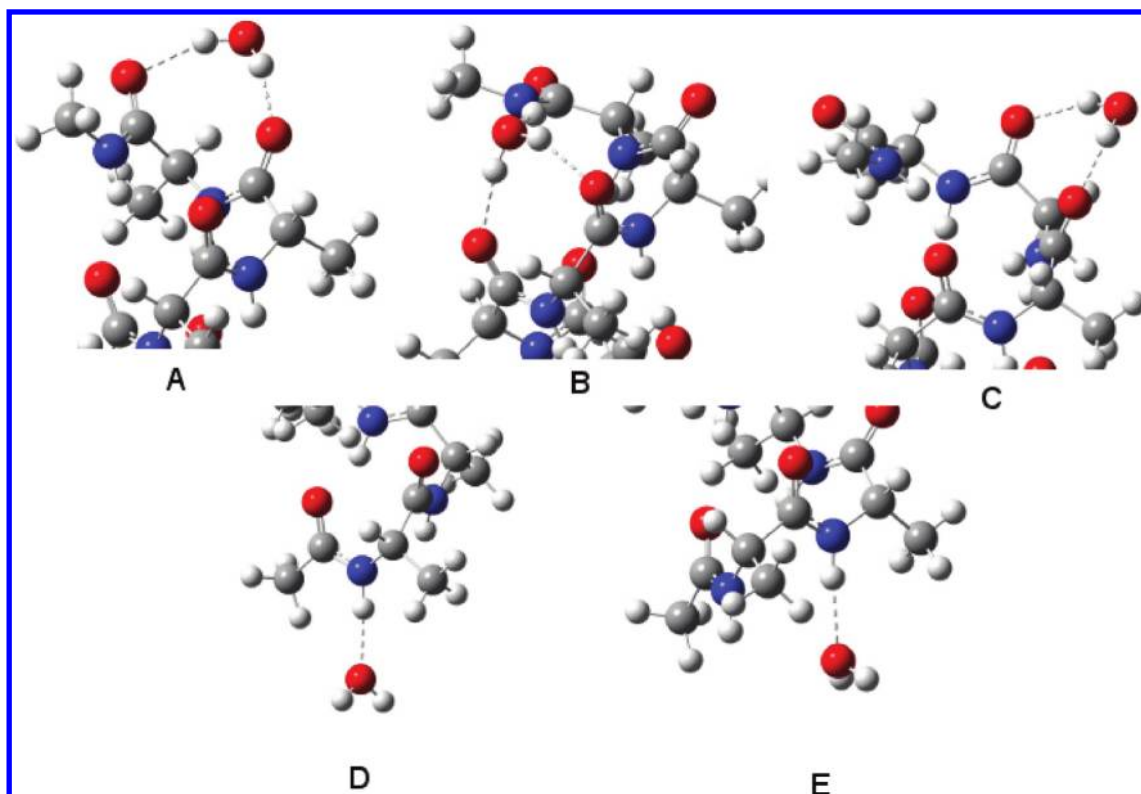
Figure 1. Optimized singly hydrated structures at the C (A, B, and C) and N (D and E) of a capped 15-alanine α-helix.
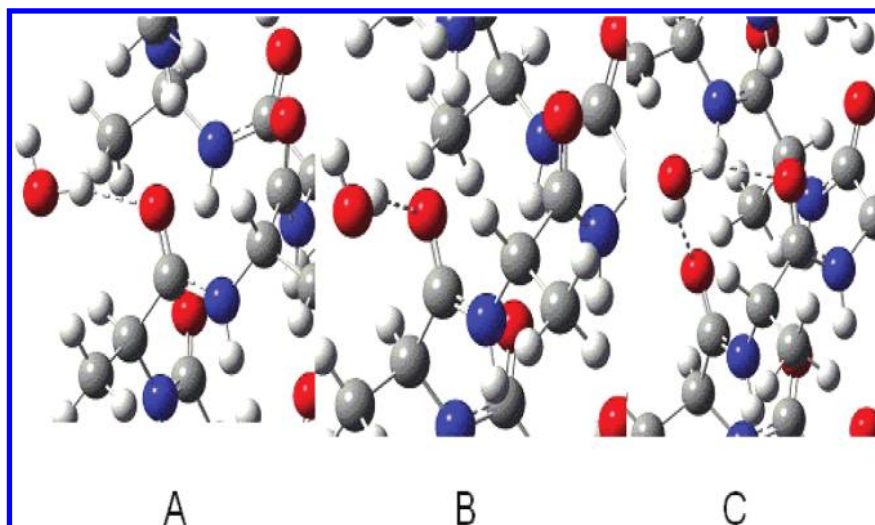

Figure 2. Typical optimized structures for waters interacting with the backbone of the α-helix: planar (A), perpendicular (B), and bridged (C).

the N-terminus, and 11 on the backbone). We note that the helix dipole moment increases from 69.8 to 79.2 or 9.4 D upon hydration by four waters at the C-terminus, which is 1.0 D more than 4 times the water dipole moment that would be expected from vector addition of perfectly aligned parallel dipole moments of the helix and the four waters. A similar result occurs when three waters are added to the N-terminus. In this case, the dipole moment increases to 80.2 or by 10.4 D (3.8 more than the parallel vector addition of the dipoles). When the helix is hydrated by both clusters (four at the C- and three at the N-termini) the dipole moment increases to 89.5 or by 19.7 D, which is essentially the sum of the dipole increase caused by the two clusters individually. Adding 11 waters to the helical backbone that do not interact with each other lowers the dipole moment by 13.0 to 56.8 D. Thus, the small water clusters align with the dipole of the helix with cooperatively enhanced H-bonding, but the backbone waters align opposite the helical dipole as would be expected for a dielectric medium. The cooperative H-bonding between the waters at each end does not extend through the helix when both small clusters are added.
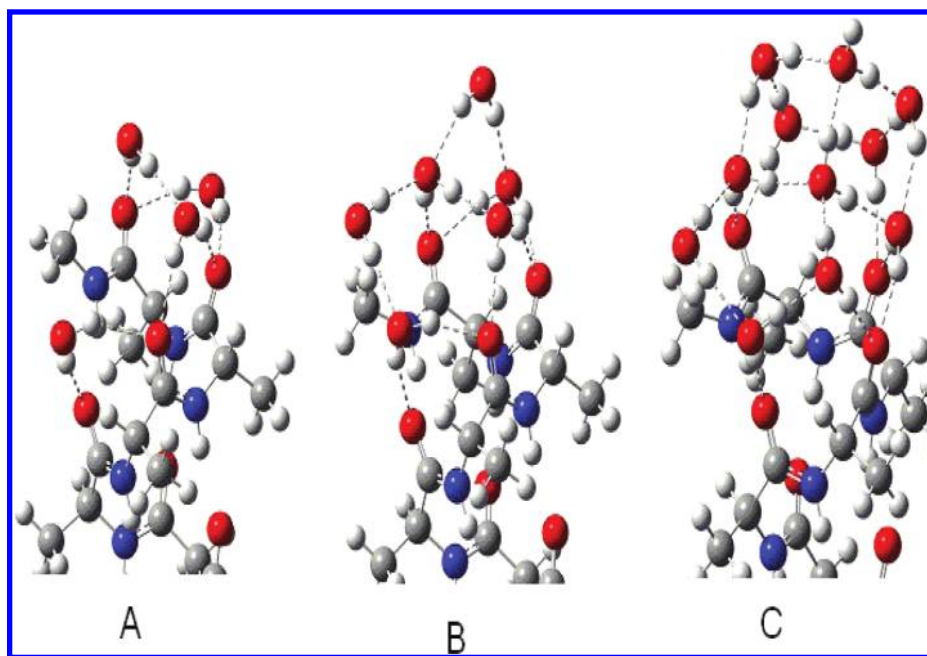
Figure 3. Optimized structures for the C-terminus of the helix solvated by 4 (A), 6 (B), and 11 (C) waters. Notice that as more waters are added their dipoles align less with the that of helix.
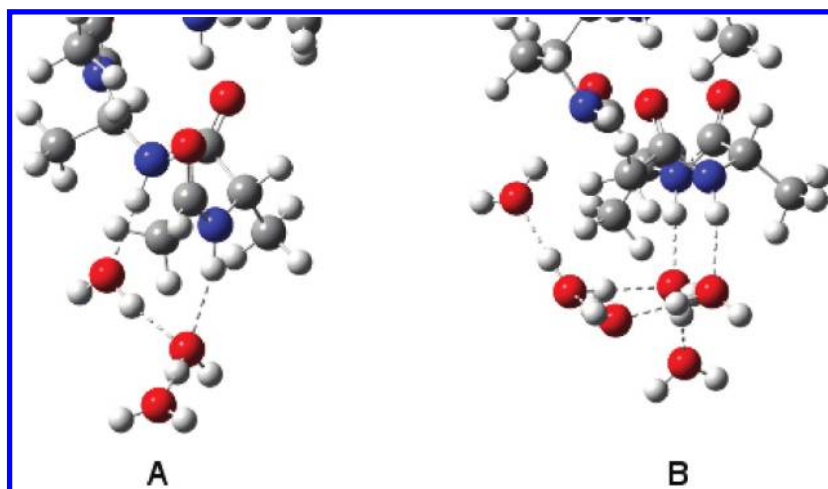

Figure 4. Optimized structures of the α-helix solvated by 3 (A) and 6 (B) waters at the N-terminus.

Increasing the sizes of the water clusters at each of the termini lowers the dipole moments, suggesting that additional waters at the termini now behave more as expected for a dielectric medium.

Solvation Using the Continuum Models. In this section, we present the data for the solvation of the helices with and without different amounts of discrete water molecules using both the CPCM and SM5.2 continuum water solvation models.

We used these models by themselves and in conjunction with individual discrete waters and various sized clusters of waters. Both models, like most other continuum models, estimate the free energy of solvation, ΔG_{solv}, rather than the enthalpy ΔH_{solv} or energy ΔE_{solv}, which we calculate using DFT and specific waters. Thus, any combination of the specific and continuum solvation energies must be understood with this qualification. As can be seen from the results that follow, these models give quite different pictures of the solvation free energies. A useful

comment on such models has recently appeared.[54] While many reports refer to the ΔE's for the gas phase, we prefer to use ΔH's as ΔE's do not refer to thermodynamic state functions.

Optimizing the helix in the CPCM continuum model for hydration stabilizes it by 55.5 kcal/mol and increases its dipole moment from 69.8 to 84.9 D. The ΔG_{solv} in CPCM is significantly less than the ΔH_{solv} calculated for either of the combinations of discrete water clusters placed at both ends of the helix.

In addition, while the energy or enthalpy of a specific water in the gas phase is clear, in a continuum solvent, we must also consider the ΔG of a discrete water molecule in the continuum model. We calculated the "solvation" free energy of one water molecule in the CPCM water model to be −4.8 kcal/mol (somewhat less than the experimental value for the free energy of 6.3 kcal/mol using the standard state for the gas phase suggested
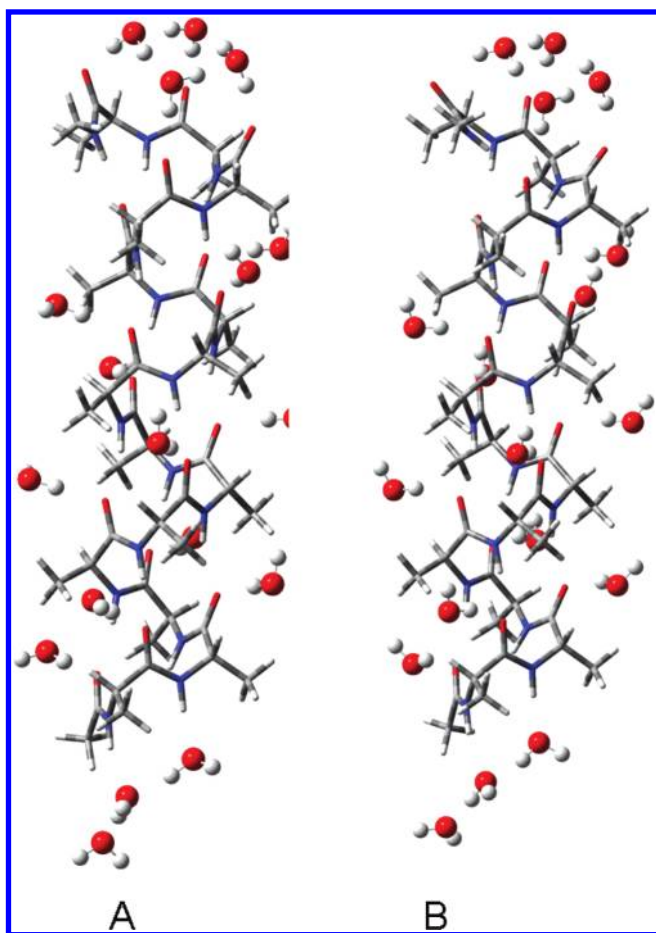
1441

dx.doi.org/10.1021/jp209177u |*J. Phys. Chem. B* 2012, 116, 1437–1445

Figure 5. Optimized helix with 18 waters: (A) CPCM aqueous phase; (B) gas phase.

Figure 6. Thermodynamic properties.

by Ben-Naim[55−57]), while the SM5.2 model reproduces the experimental value correctly. This value refers to a gas-phase standard state of 1 mol/L and differs from the value for a gas-phase standard state of 1 atm (−4.4 kcal/mol) by 1.9 kcal/mol at 298 K. We have previously estimated enthalpy of solvating a free water molecule in liquid water as −5.8 kcal/mol based upon the heat of vaporization at 298 K and an assumed average of 3.6 H-bonds per liquid water molecule.[48] However, the standard state used for the gas-phase water does not change the energy for the transfer of a water molecule from one part of the solution (e.g., near the solute) to another (e.g., in bulk water). If one considers the *difference* between the transfer energies from the gas-phase standard state, the energy of the standard state would cancel. The calculations for the solvation of the helix using a combination of discrete water molecules in the two continuum methods use the appropriate number of discrete waters immersed in the continuum solvent (N times −4.8 or −6.3 kcal/mol) as a reference point. If the continuum models alone (without discrete waters) properly solvate both the helix and the discrete water molecules, no energetic advantage or penalty would be associated with moving the discrete water molecules away from the helix and into the continuum. We note that this energy does not correspond to the transfer energy of water vapor to liquid water which requires attention to the standard states used.[57]

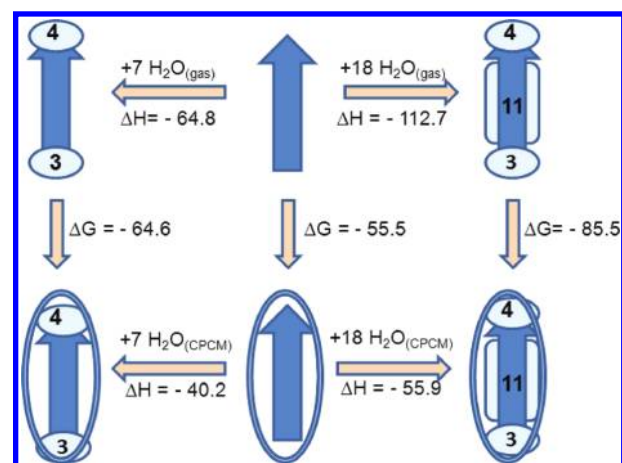*CPCM.* The CPCM model predicts the free energy of the (fully optimized) helix to be −55.5 kcal/mol, with an increase in the

dipole moment of 15.1 D from 69.8 to 84.9 D. We also considered the CPCM solvation energies of the helix/water clusters (again fully optimized) previously discussed (see Tables 1 and 2). As can be seen from Figure 6 and Tables 1 and 2, the overall CPCM solvation free energies become greater for the clusters containing waters. If N discrete water molecules were placed into the CPCM continuum, one would expect $N \times$ (−4.8) kcal/mol solvation energy. However, the results indicate the differential CPCM solvation energy between the naked helix and the various end-solvated helix/water clusters to be considerably more negative than −4.8 kcal/mol/water, which indicates that the waters favor specific interactions which stabilize the helix already solvated at the ends by 15.7 (−55.9 − (40.2)) kcal/mol (1.4/water). Thus, the calculations predict that the waters hydrating the ends of the helix are in more energetically favored position than those of bulk water, while those solvating the backbone provide significantly less stabilization.

SM5.2. The solvation energies calculated by SM5.2 using the AM1/AMSOL procedure differ considerably from those obtained from CPCM. First of all, the solvation energy of water in water is 6.3 kcal/mol, which agrees with the experimental value, although specific parametrization for water was used to achieve this.[27,28,56] The solvation energy of the bare helix calculated using SM5.2 is −91.7 kcal/mol significantly greater in magnitude than that predicted by CPCM. However, water clusters, when put into the SM5.2/AMSOL continuum model, are quite unstable. Water dimer is unfavorably solvated by 0.7 kcal/mol, and a 50-water cluster that we optimized for another study is destabilized by 2206 kcal/mol or 44 kcal/mol/water. While some destabilization might be expected from the entropy penalty required to hold the 50-water cluster intact within the bulk solvent, these values are much too large to be considered reasonable. The required ΔS would be −148 cal/deg/water at 298 K. For this reason, we do not trust the calculations of the helix solvated by water clusters using this model that appear in Table 2. On the other hand, the solvation of the helix with a single water at either the C- or N-terminus or backbone seems to give plausible results. The C-hydrated helix is more stabilized by 2.7 and the N-hydrated by 4.1 kcal/mol more, while the helix with a single water at a backbone position is hydrated by 3.5 kcal, less than expected from the sum of the individual hydration enthalpies of the helix and a single water.

Table 2. Enthalpies of Hydration by Single Water in Gas and Solvent Model, Free Energies of Transfer of Different Species (Values in Parentheses Correspond to SM 5.2 Solvent Model), and Dipole Moments for Polyalanine α-Helix and Its Hydrated Complexes (kcal/mol and D)

species	ΔH_{inter}		$\Sigma\Delta G_{solv}$	ΔG_{solv} (complex)	μ (CPCM)
	gas	aqueous			
helix			−55.5 (−91.7)		
water			−4.8 (−6.3)		
water dimer	−4.3	−3.3 (+9.1)	−9.6 (−12.6)	−7.8 (+0.7)	
50 water cluster			−240.0 (−315.0)	−43.3 (+2206.2)	
1 water@C	−8.0	−4.6 (−2.7)	−60.3 (−98.0)	−56.8 (−92.7)	88.1
1 water @N	−7.7	−3.9 (−4.1)	−60.3 (−98.0)	−56.4 (−94.4)	87.5
1 water@B	−4.7	−2.2 (+3.5)	−60.3 (−98.0)	−57.7 (−89.8)	84.5
4C+3N	−64.8	−40.2 (+131.5)	−89.2 (−136.0)	−64.6 (+60.4)	104.8
4C+3N+11B	−112.7	−55.9 (+207.2)	−142.3 (−205.5)	−85.5(+114.4)	105.2
6C+3N	−77.7	−48.5 (+147.3)	−98.8 (−148.6)	−69.7 (+76.4)	111.9
11C+6N	−163.2	−100.8 (+381.5)	−137.4 (−199.2)	−75.1 (+345.4)	103.6

Table 3. Hydration of Carbonyl Group in the Backbone (in kcal/mol), and μ (debye)

position	ΔE_{inter}		ΔE_{dist}		ΔH_{inter}	μ
	optimized	frozen	helix	water		
planar	−6.4	−7.2	0.6	0.1	−4.7	68.5
perpendicular	−4.6	−5.1	0.4	0.0	−3.2	67.9
bridged	−2.5	−7.4	4.7	0.1	−0.4	69.7

■ DISCUSSION

As can be seen from Table 1, the dipole moment of the helix increases upon being solvated by individual waters at the ends over that calculated for the gas phase. Furthermore, the dipoles of these monohydrates increase to more than the vector sum of the dipoles of the gas-phase helix and water (except in the case where the water forms three H-bonds) which indicates a cooperative H-bonding interaction between these waters and the helix in a manner similar to that originally suggested by Karplus several years ago.[23] On the other hand, hydration by individual waters at the backbone C═O's lowers the dipole by from 0.7 to 1.9 D, consistent with the behavior of a bulk dielectric medium to oppose the dipole moment of the solute.

Continuum hydration (CPCM) or by water clusters at either the C- or N-terminus or both together causes increases in the dipole moment of the aggregate over that of the gas phase helix. Figure 5 shows how the discrete waters reorient in CPCM from their gas-phase optimized positions. However, increasing the size of the water cluster has diminishing effects. For example, while going from four to six waters at the C-terminus significantly increases the dipole moment, going from 6 to 11 waters decreases it. Clearly, the waters in the cluster of 11 form a structure that retains features from the smaller hydration complexes for the inner solvation shell, but begins to incorporate the structural features one might expect from bulk solvent, particularly for the waters farther from the helix. This can be seen from the increasing number of H-bonds between the waters at the termini in Figures 3 and 4. Combining four waters at the C-terminus with three at the N-terminus increases the dipole moment to 89.5 D, which is the same as the sum of the individual increases for the hydrations at either end; however, combinations of the larger

clusters at each end result in lower increases in the dipoles that the sums of the individual effects, which indicates that there is no cooperativity between the solvation shells at each of the termini. Hydration by 11 waters at the backbone C═O's significantly reduces the dipole moment (by 10.8 D) over that of the gas-phase helix; however, backbone hydration of the helix already hydrated with four waters at the C-end and three at the N-end lowers the dipole moment by significantly more (about 16.5 D). When placed in the CPCM continuum solvent, the dipole moment of the helix solvated at the ends (4C−3N) increases by 15.3 D (from 89.5 to 104.8 D), while adding the 11 backbone waters increases the dipole slightly by 0.4 D (from 104.8 to 105.2 D). The foregoing behavior seems reasonable as the inner-shell end hydration is stabilized by H-bond cooperativity, while the backbone hydration creates an opposing dipole in the gas phase as would be expected for bulk water. In CPCM, the dipole moment increases for the end-hydrated helix as the counter dipole of the continuum would lower the energetic penalty for increasing the helical dipole. However, the effect of the backbone waters becomes attenuated from that in the gas phase, as the continuum bulk solvent provides a significant opposing dipole so that the contribution from the backbone waters becomes less energetically necessary.

As seen from Table 4, the H-bonds within the helix shorten considerably upon hydration by discrete waters at the two termini, especially those nearest the termini. Upon further hydration with discrete waters on the backbone, the H-bond lengths increase, especially those near the middle of the helix some of which become slightly longer than in the unsolvated helix in the gas phase. Placing the helix in the continuum CPCM model also causes the helical H-bonds to shorten. Adding the 18 discrete waters within the CPCM continuum results in a general lengthening of the H-bonds, especially near the center of the helix. The foregoing suggests that aqueous solvation of poly-alanine α-helices strengthens the H-bonds within the helix as the H-bonds generally shorten when capped with waters at the ends or when placed in the CPCM continuum solvent. The increases in the dipole moment under similar conditions are in accord with this assessment. While this may seem counterintuitive to some, it follows the example of water dimer whose O···O distance decreases and ΔH_{inter} becomes more negative upon going from the gas phase[58] to bulk water.[59] The observations that the ΔH's

Table 4. Length of Helical Hydrogen Bonds (Å) in Hydrated Helices (Numbering from the N-End)

H-bond	gas phase			CPCM		
	helix	4C+3N	4C+3N+11B	helix	4C+3N	4C+3N+11B
1	2.498	2.142	2.106	2.044	1.965	1.964
2	2.057	1.965	1.988	1.926	1.906	1.990
3	2.083	2.066	2.089	2.021	1.999	2.039
4	2.045	1.994	2.040	1.943	1.934	2.018
5	2.026	1.998	2.033	1.947	1.937	1.982
6	2.017	1.986	2.019	1.936	1.931	2.032
7	2.026	1.987	2.032	1.954	1.935	2.004
8	2.021	1.969	2.030	1.934	1.920	2.001
9	2.024	1.998	2.051	1.944	1.923	2.035
10	2.051	1.972	2.031	1.945	1.924	2.075
11	2.178	2.098	2.382	2.007	1.922	2.099
12	2.007	1.927	1.962	1.937	2.000	2.013
13	2.221	2.048	2.085	1.961		

for individual water hydrations at both the C and N termini are more negative than those for the hydrations of similar positions on NMA[46] confirm this suggestion.

Since the waters that solvate the ends of the helix play very different roles in stabilizing the structure from those near the backbone, one might expect that the different positions in the helix would be stabilized by different kinds of hydration. Crystal structures of proteins that contain α-helices commonly contain ordered waters near the ends of the helices. Some examples are the following pdb structures: 1AKI (hen egg-white lysozyme),[60] 3G4P (OXA-24 β-lactamase),[61] and 1Z98 (Aquaporin),[62] the last of which is a transmembrane protein. While transmembrane proteins need to have hydrophobic residues that interact with the lipids in the membrane, favorable interaction with aqueous phases on either side of the membrane must also be important. One such protein, the transmembrane domain of virus protein "u" (Vpu) from HIV-1, has been studied by NMR in a lipid bilayer,[63] and reported to have its hydrophobic backbones traversing the lipid and its hydrophilic termini in the aqueous medium on either side of the bilayer, consistent with the present report of solvation by discrete waters. The results for hydration by waters at the termini in both CPCM and SM5.2, support this suggestion. However, CPCM and SM5.2 differ with respect to the waters at the backbone. CPCM suggests that the backbone positions might be slightly hydrophilic (waters there are stabilized by −2.2 kcal/mol vs bulk water), but SM5.2 predicts such positions to be hydrophobic (destabilized by 3.5 kcal/mol vs bulk water).

The two continuum models used clearly differ in several aspects. However, they both suffer from the lack of data on ΔG_{solv} for amides. Only four experimental data points for very similar amides are known.[64] Furthermore, there is reason to believe that the ΔH_{solv} for polyamides (i.e., peptides) cannot be extrapolated from the ΔH_{solv} of simple amides.[65,66] Hence, the ΔG_{solv} should be similarly affected. Unfortunately, the continuum models, as well as MD models such as TIP3P, do not break the solvation free energy into enthalpic and entropic terms. We have recently noted[67] that the work done by making a hole in water is not taken into account in the early work[68] on hydrophobic solvation, resulting in the overestimation of the entropic

effect. Nevertheless, we have included the data for the continuum solvent models, as the data will be of general interest.

■ CONCLUSIONS

The aqueous solvation of α-helices, especially those that do not contain hydrophilic residues, occurs via two very different mechanisms. Water hydrates the termini through cooperative H-bonding, as suggested by Karplus, then hydrates the helix/water aggregate (partially hydrated at the termini) as would normally be expected from a solvent reaction field such as that incorporated in the CPCM method.

The hydration at the termini by individual waters and small water clusters increases the overall dipole moments of the water/helix aggregates. Individual waters at the termini cause the overall dipole moment to increase to more than the vector sums of the helix and the water. Individual waters that hydrate the helix backbone reduce the overall dipole moment (as would be expected for a solvent reaction field). We find the individual waters that solvate the termini to be more stable than a water placed in bulk solvent, and waters that solvate the backbone to be less stable than those in the bulk solvent. The latter result is consistent with the known hydrophobicity of the alanine residues used in the model.

The general shortening of the helical H-bonds upon hydration by specific waters at the termini and by hydration using the CPCM aqueous solvation model and the increases in the dipole moments under the same conditions suggests that the helical H-bonds become stronger upon aqueous solvation.

■ ASSOCIATED CONTENT

S **Supporting Information.** Cartesian coordinates and the complete reference for Gaussian 09. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: jdannenberg@gc.cuny.edu.

■ ACKNOWLEDGMENT

■ REFERENCES

(1) Marqusee, S.; Robbins, V. H.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5286.

(2) Scholtz, J. M.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *J. Am. Chem. Soc.* **1991**, *113*, 5102.

(3) Lopez, M. M.; Chin, D.-H.; Baldwin, R. L.; Makhatadze, G. I. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1298.

(4) Miller, J. S.; Kennedy, R. J.; Kemp, D. S. *J. Am. Chem. Soc.* **2002**, *124*, 945.

(5) Job, G. E.; Kennedy, R. J.; Heitmann, B.; Miller, J. S.; Walker, S. M.; Kemp, D. S. *J. Am. Chem. Soc.* **2006**, *128*, 8227.

(6) Chapman, R. N.; Dimartino, G.; Arora, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 12253.

(7) Liu, J.; Wang, D.; Zheng, Q.; Lu, M.; Arora, P. S. *J. Am. Chem. Soc.* **2008**, *130*, 4334.

(8) Wang, D.; Chen, K.; Kulp, J. L.; Arora, P. S. *J. Am. Chem. Soc.* **2006**, *128*, 9248.

(9) Wieczorek, R.; Dannenberg, J. J. *J. Am. Chem. Soc.* **2003**, *125*, 8124.

(10) Wieczorek, R.; Dannenberg, J. J. *J. Am. Chem. Soc.* **2004**, *126*, 14198.

(11) Wieczorek, R.; Dannenberg, J. J. *J. Am. Chem. Soc.* **2004**, *126*, 12278.

(12) Wieczorek, R.; Dannenberg, J. J. *J. Am. Chem. Soc.* **2005**, *127*, 14534.

(13) Wieczorek, R.; Dannenberg, J. J. *J. Am. Chem. Soc.* **2005**, *127*, 17216.

(14) Elstner, M.; Jalkanen, K. J.; Knapp-Mohammady, M.; Frauenheim, T.; Suhai, S. *Chem. Phys.* **2000**, *256*, 15.

(15) Bour, P.; Kubelka, J.; Keiderling, T. A. *Biopolymers* **2002**, *65*, 45.

(16) Ireta, J.; Neugebauer, J.; Scheffler, M.; Rojo, A.; Galvan, M. *J. Phys. Chem. B* **2003**, *107*, 1432.

(17) Vener, M. V.; Egorova, A. N.; Fomin, D. P.; Tsirelson, V. G. *J. Phys. Org. Chem.* **2009**, *22*, 177.

(18) Salvador, P.; Asensio, A.; Dannenberg, J. J. *J. Phys. Chem. B* **2007**, *111*, 7462.

(19) Turner, D. R.; Kubelka, J. *J. Phys. Chem. B* **2007**, *111*, 1834.

(20) Makshakova, O.; Ermakova, E. *J. Mol. Struct.: THEOCHEM* **2010**, *942*, 7.

(21) Makshakova, O.; Chachkov, D.; Ermakova, E. *Int. J. Quantum Chem.* **2011**, *111*, 2525.

(22) Ireta, J. *J. Chem. Theory Comput.* **2011**, *7*, 2630.

(23) Guo, H.; Karplus, M. *J. Phys. Chem.* **1994**, *98*, 7104.

(24) Onsager, L. *J. Am. Chem. Soc.* **1936**, *58*, 1486.

(25) Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995.

(26) Cossi, M.; Rega, N.; Scalmani, G.; Barone, V. *J. Comput. Chem.* **2003**, *24*, 669.

(27) Hawkins, G. D.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* **1998**, *102*, 3257.

(28) Hawkins, G. D.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem.* **1996**, *100*, 19824.

(29) Cramer, C. J.; Truhlar, D. G. *Chem. Rev.* **1999**, *99*, 2161.

(30) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926.

(31) Bhattacharjee, N.; Biswas, P. *J. Phys. Chem. B* **2011**, *115*, 12257.

(32) Car, R.; Parrinello, M. *Phys. Rev. Lett.* **1985**, *55*, 2471 LP.

(33) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A. et al. *Gaussian 09, Revision A.2*; Gaussian, Inc.: Wallingford, CT, 2009.

(34) Xu, X.; Goddard, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2673.

(35) Jansen, H. B.; Ros, P. *Chem. Phys. Lett.* **1969**, *3*, 140.

(36) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553.

(37) van Duijneveldt, F. B.; van Duijneveldt-van de, R. *Chem. Rev.* **1994**, *94*, 1873.

(38) Van Duijneveldt, F. B. Basis set superposition error. In *Molecular Interactions*; Scheiner, S., Ed.; Wiley: Chichester, UK, 1997; p 81.

(39) Simon, S.; Duran, M.; Dannenberg, J. J. *J. Chem. Phys.* **1996**, *105*, 11024.

(40) Bryantsev, V. S.; Diallo, M. S.; van Duin, A. C. T.; Goddard, W. A. *J. Chem. Theory Comput.* **2009**, *5*, 1016.

(41) Plumley, J. A.; Dannenberg, J. J. *J. Comput. Chem.* **2011**, *32*, 1519.

(42) Morokuma, K. *Philos. Trans. R. Soci. London, Ser. A: Math., Phys. Eng. Sci.* **2002**, *360*, 1149.

(43) *AMPAC, 8.16 ed.*; Semichem, Inc.: Shawnee, KS.

(44) Tsai, M. I.-H.; Xu, Y.; Dannenberg, J. J. *J. Phys. Chem. B* **2009**, *113*, 309.

(45) Plumley, J. A.; Tsai, M. I.-H.; Dannenberg, J. J. *J. Phys. Chem. B* **2011**, *115*, 1562.

(46) Dannenberg, J. J. *J. Phys. Chem. A* **2006**, *110*, 5798.

(47) Rablen, P. R.; Lockman, J. W.; Jorgensen, W. L. *J. Phys. Chem. A* **1998**, *102*, 3782.

(48) Dannenberg, J. J. *Adv. Protein Chem.* **2006**, *72*, 227.

(49) Gilli, P.; Pretto, L.; Bertolasi, V.; Gilli, G. *Acc. Chem. Res.* **2008**, *42*, 33.

(50) Gilli, P.; Gilli, G. J. Mol. Struct. Horizons in hydrogen bond research 2009—A Collection of Papers from the XVIIIth International Conference "Horizons in Hydrogen Bond Research", Paris, France, 14−18 September 2009; 2010; Vol. *972*, p 2.

(51) Gilli, G.; Gilli, P. *The Nature of the Hydrogen Bond*; Oxford University Press: Oxford, UK, 2009.

(52) Moisan, S.; Dannenberg, J. J. *J. Phys. Chem. B* **2003**, *107*, 12842.

(53) Shields, R. M.; Temelso, B.; Archer, K. A.; Morrell, T. E.; Shields, G. C. *J. Phys. Chem. A* **2010**, *114*, 11725.

(54) Ho, J.; Klamt, A.; Coote, M. L. *J. Phys. Chem. A* **2010**, *114*, 13442.

(55) Liptak, M. D.; Shields, G. C. *Int. J. Quantum Chem.* **2001**, *85*, 727–741.

(56) Kelly, C. P.; Cramer, C. J.; Truhlar, D. G. *J. Chem. Theory Comput.* **2005**, *1*, 1133.

(57) Ben-Naim, A. *J. Phys. Chem.* **1978**, *82*, 792.

(58) Goldman, N.; Fellers, R. S.; Brown, M. G.; Braly, L. B.; Keoshian, C. J.; Leforestier, C.; Saykally, R. J. *J. Chem. Phys.* **2002**, *116*, 10148.

(59) Bergmann, U.; Cicco, A. D.; Wernet, P.; Principi, E.; Glatzel, P.; Nilsson, A. *J. Chem. Phys.* **2007**, *127*, 174504.

(60) Artymiuk, P. J.; Blake, C. C. F.; Rice, D. W.; Wilson, K. S. *Acta Crystallogr., Sect. B* **1982**, *38*, 778.

(61) Garnham, C. P.; Campbell, R. L.; Davies, P. L. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 7363.

(62) Tornroth-Horsefield, S.; Wang, Y.; Hedfalk, K.; Johanson, U.; Karlsson, M.; Tajkhorshid, E.; Neutze, R.; Kjellbom, P. *Science* **2006**, *439*, 688.

(63) Park, S. H.; Mrse, A. A.; Nevzorov, A. A.; Mesleh, M. F.; Oblatt-Montal, M.; Montal, M.; Opella, S. J. *J. Mol. Biol.* **2003**, *333*, 409.

(64) Wolfenden, R. *Biochemistry* **1978**, *17*, 201.

(65) Avbelj, F.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 3137.

(66) Hayik, S. A.; Liao, N.; Merz, K. M. *J. Chem. Theory Comput.* **2008**, *4*, 1200.

(67) Kobko, N.; Marianski, M.; Asensio, A.; Wieczorek, R.; Dannenberg, J. J. *Comput. Theor. Chem.* **2011**, DOI: http://dx.doi.org/10.1016/j.comptc.2011.11.022.

(68) Frank, H. S.; Evans, M. W. *J. Chem. Phys.* **1945**, *13*, 507.

1445

dx.doi.org/10.1021/jp209177u |*J. Phys. Chem. B* 2012, 116, 1437–1445