Microsolvation of Cysteine: A Density Functional Theory Study

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Microsolvation of the neutral, zwitterion, and unconventional zwitterion (formed by the proton transfer from the thiol to the amine group) was performed using PBE1PBE/6-311+G(d,p) calculations. A large sampling of the configurations of the clusters involving one to six water molecules was created by analogy to glycine clusters and through analysis of hydrogen-bonding trends. Clusters of the neutral tautomer are lowest in energy with the inclusion up to five water molecules. With six water molecules the neutral and zwitterion are nearly isoenergetic. The unconventional zwitterion, while a stable structure when at least one water molecule is associated with it, remains energetically noncompetitive with the other two tautomers regardless of the degree of microsolvation.

1. Introduction

The structure of amino acids is sensitive to phase. In the gas phase, they adopt the neutral tautomer having an amine group and a carboxylic acid group. In aqueous solution, they are found as zwitterions, possessing ammonium and carboxylate groups. The zwitterion is typically not even a stable structure in the gas phase.[1] Jensen and Gordon[2] posed two questions concerning glycine: (1) How many water molecules are needed to stabilize the zwitterion tautomer, making it a stable structure (a local energy minimum)? (b) How many water molecules are needed to make the neutral and zwitterion tautomers isoenergetic. In this paper we ask these two questions of the amino acid cysteine.

Jensen and Gordon[2] answered the question of how many water molecules are needed to stabilize the glycine zwitterion with MP2/DZP++//HF/DZP calculations: two water molecules suffice. The recent laser ablation molecular beam Fourier transform microwave (LA-MB-FTMW) spectroscopy study of the glycine−one-water complex by Alonso found glycine to be in its neutral form.[3] Aikens and Gordon[4] later tackled the second question for glycine. They sequentially added water molecules to the glycine neutral and zwitterion tautomers, surveying the configuration space by a Monte Carlo simulation, and then optimizing the lowest energy structures at HF/6-31++G(d,p). Single-point energy calculations at MP2 allowed for a ranking of the structures. With seven water molecules, the two tautomers were of equivalent energy.

We took a different approach to generating the configurations of the glycine−water clusters.[5] Starting with the smaller water clusters, we optimized a number of configurations, identified hydrogen-bonding patterns, and used these patterns to suggest starting configurations for the larger clusters. We optimized the energy of the clusters at PBE1PBE/6-31+G(d,p) and corrected for zero-point vibrational energy obtained at B3LYP/6-31+G(d). We too found that the neutral and zwitterion tautomers are of equal energy with seven associated water molecules.

The gas-phase structure of cysteine has only recently been experimentally identified. Kleinermanns and co-workers observed the gas-phase Fourier transform infrared spectrum of cysteine and found it consistent with the neutral tautomer on the basis of comparison to B3LYP/6-31G(d,p) computed and scaled frequencies.[6] In particular, the O−H stretch at 3572 cm^{-1} (3576 cm^{-1} computed) and the O−H in-plane bend vibration at 1118 cm^{-1} (1110 cm^{-1} computed) indicate the neutral tautomer. The LA-MB-FTMW study of cysteine by Alonso identified six conformers of cysteine, all of which are the neutral tautomer.[7] Comparison of the observed rotational constants with those computed at MP2/6-311++G(d,p) aided in the determination of the six structures.

The conformers of neutral cysteine have been examined computationally a number of times. The first systematic examination using ab initio methods was by Gronert, who identified 42 conformers at HF/6-31G*.[8] The lowest energy conformer at MP2/6-31+G*//HF/6-31G* has the hydroxyl hydrogen *trans* to the carbonyl so that it can form a hydrogen bond to the amine lone pair (this conformation we designate **N0-a**; see below). Fernandez-Ramos and co-workers[9] reoptimized the five lowest energy conformers found by Gronert at B3LYP/6-31++G** and found little difference in their energies from the MP2 energies. Saldej optimized eight low-lying conformations of cysteine at B3LYP/aug-cc-pVTZ and also found **N0-a** to be the minimum energy structure.[10]

Because the cysteine zwitterion is unstable in the gas phase,[10] some treatment of solvent is needed. No systematic microsolvation study of cysteine, in either its neutral or zwitterion form, has been reported. Pecul optimized three configurations of the cysteine zwitterion with four water molecules at B3LYP/aug-cc-pVDZ but did not report any water clusters of the neutral tautomer.[11] The lowest energy cluster (called **Z4-a** below) has two waters accepting hydrogen bonds from the ammonium group and no hydrogen bond between the ammonium and carboxylate groups. The alternative approach to compute the zwitterion in water is to use a continuum method. Fernandez-Ramos and co-workers[9] identified one zwitterion conformation at PCM/B3LYP/6-31G*; it has a hydrogen bond between the ammonium proton and a carboxylate oxygen and weak interactions between a second ammonium proton and sulfur and between the thiol hydrogen and oxygen. This zwitterion is about 2 kcal mol^{-1} lower in energy than the most stable neutral conformer. Saldej optimized four conformations of the cysteine zwitterion at IEF-PCM/B3LYP/aug-cc-pVDZ.[10] All four possess a hydrogen bond between the ammonium and the carboxylate

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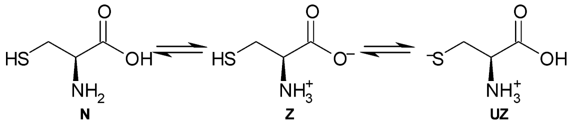
groups. However, these zwitterions are all predicted to be at least 1.2 kcal mol^{-1} higher in energy than the neutral tautomer, in conflict with the known predominance of the zwitterion in aqueous solution. Saldej suggested that the method is erroneous, requiring alternate parameters for the charged (zwitterionic) molecule.

Kass and co-workers have recently reported a combined gas-phase experiment and computational study of the acidity of cysteine.[12] Surprisingly, they find that the thiol proton is more acidic than the carboxylic acid proton. This opens the possibility of a second cysteine zwitterion, the unconventional zwitterion (**UZ**), whereby the thiol proton transfers to the amine. The conventional zwitterion (**Z**) has a proton transfer from the carboxylic acid to the amine. Fernandez-Ramos[9] did optimize a single conformer of this unconventional zwitterion with PCM. It possesses two internal hydrogen bonds to the thiolate, one from the ammonium and one from the hydroxyl group. This unconventional zwitterion is predicted to be nearly 8 kcal mol^{-1} higher in energy than the conventional zwitterion.

In this paper we systematically microsolvate the neutral (**N**) and conventional (**Z**) and unconventional (**UZ**) zwitterions of cysteine using density functional theory. In addition to glycine, the microsolvation technique has been applied to tryptophan and arginine. With tryptophan, even with six water molecules the neutral tautomer remains lower in energy than the zwitterion.[13,14] With arginine, a single associated water molecule makes the zwitterion more stable than the neutral.[15] Microsolvation studies of biologically important molecules have flourished lately, with both experimental and theoretical studies of such systems as amino acids[16−18] and nucleic acid bases[19,20] and base pairs.[21−23]



We will address the two questions previously mentioned: How many water molecules are needed to (a) stabilize the zwitterion and (b) make the neutral and zwitterion isoenergetic? In addition, we examine whether the unconventional zwitterion is energetically competitive with either of the other two tautomers.

2. Computational Methods

We examined the clusters formed from cysteine, as the neutral, zwitterion, or unconventional zwitterion, with one to six water molecules. The clusters are labeled as **N*x*-*y***, **Z*x*-*y*** or **UZ*x*-*y***, where **N**, **Z**, or **UZ** indicates the neutral, zwitterion, or unconventional zwitterion, respectively, ***x*** designates the number of water molecules in the cluster, and **y** indexes the different configurations.

Starting geometries of the clusters were constructed using GaussView 3.09,[24] building off of the glycine clusters[5] and recognizing hydrogen-bonding patterns in the clusters with fewer water molecules. Water molecules were placed to enable maximal hydrogen bonding between the water and cysteine and between water molecules. These trends are discussed below.

Computations were performed using the Gaussian-03 suite.[25] All configurations were optimized at B3LYP/6-31+G(d)[26] along with computation of their analytical vibrational frequencies to confirm all structures to be local energy minima. The structures were reoptimized at PBE1PBE/6-311+G(d,p).[27] This computational method performs well for hydrogen-bonded systems[28] and provided nice agreement with CCSD(T) relative energies for the smaller water clusters of glycine.[5] These PBE1PBE energies were then corrected for the zero-point vibrational energies (ZPVEs), using the unscaled values obtained at B3LYP/6-31+G(d). These energies are useful for comparison with low-temperature jet-expansion experiments that might be performed in the future. These energies are uncorrected for the effect of basis set superposition error because the standard corrections (counterpoise calculations) overestimate the error,[29] are difficult to apply to geometry optimization, and, given the large number of configurations addressed here, are prohibitively expensive to implement. Further justification of the computational method is provided below.

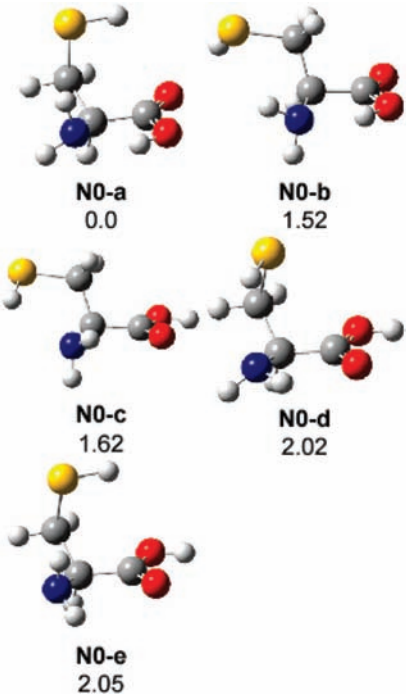


Figure 1. Structure and relative energy (kcal mol^{-1}) of the five lowest energy conformers of cysteine at PBE1PBE/6-311+G(d).

3. Results

3.1. Benchmarks. We utilized the PBE1PBE/6-311+G(d,p) method in our study of the microsolvation of glycine.[5] We justified use of this method by comparing the geometries and relative energies of glycine conformers and configurations of glycine with two water molecules obtained with PBE1PBE/6-311+G(d,p) and CCSD(T)/6-311+G(2d,p)//PBE1PBE/6-311+G(d,p). Though we believe this benchmark is sufficient to justify use of this method for the study of microsolvation of any amino acid, we describe below a benchmark study comparing energies of cysteine conformers and the cysteine−one-water and cysteine−two-water clusters.

3.1.1. Comparison of Cysteine Conformations. Alonso and co-workers identified 11 conformers of cysteine at MP4/6-311++G(d,p)//MP2/6-311+G(d,p).[7] We have reoptimized all of these conformers at PBE1PBE/6-311+G(d,p). Single-point energies were also obtained at CCSD(T)/6-311+G(d,p) using the PBE1PBE-optimized geometries, and these energies were corrected for zero-point vibrational energy, computed at PBE1PBE/6-311+G(d,p). The five lowest energy structures at PBE1PBE are shown in Figure 1, and all 11 are drawn in Figure S1 in the Supporting Information.

The relative energies of all 11 conformations of cysteine are listed in Table 1. All three methods identify the same lowest

TABLE 1: Relative Energy (kcal mol^{-1}) of the Cysteine Conformers N0

N0	PBE1PBE[a]	MP4[b]	CCSD(T)[c]
a	0.00	0.00	0.00
b	1.52	1.51	1.42
c	1.62	1.29	0.54
d	2.02	1.21	0.72
e	2.05	0.93	0.44
f	2.46	1.67	1.14
g	2.75	2.89	2.60
h	3.12	2.42	1.77
i	3.14	2.19	1.49
j	3.25	2.24	1.77
k	4.26	2.82	2.15

[a] PBE1PBE/6-311+G(d,p). [b] MP4/6-311++G(d,p)//MP2/6-311+G(d,p), ref 7. [c] CCSD(T)/6-311+G(d,p)//PBE1PBE/6-311+G(d,p).

TABLE 2: Relative Energies (kcal mol^{-1}) of Some of the Cysteine−One-Water and Cysteine−Two-Water Clusters

	PBE1PBE[a]	HF[b]	MP2[c]	CCSD(T)[d]
N1-a	0.0	0.0	0.0	0.0
N1-b	0.26	0.20	−0.27	−0.32
N1-c	0.49	0.35	0.13	−0.16
Z1-a	12.88	21.40	14.74	16.14
UZ1-a	17.10	29.50	24.38	24.41
N2-a	0.0	0.0	0.0	0.0
N2-b	0.19	0.06	−0.38	−0.44
N2-c	1.04	1.05	0.81	0.93
Z2-a	10.73	19.27	11.71	12.99
UZ2-a	14.73	23.88	19.24	19.38

[a] PBE1PBE/6-311+G(d,p). [b] HF/6-311+G(d,p). [c] MP2/6-311+G(d,p) + ZPE(HF). [d] CCSD(T)/6-311+G(d,p)//PBE1PBE/6-311+G(d,p) + ZPE(PBE1PBE).

energy conformer (**N0-a**). However, both MP4 and CCSD(T) predict that **N0-e** is the second lowest energy conformer, while PBE1PBE predicts it is **N0-b**. Apparently, PBE1PBE overemphasizes the strength of the intramolecular hydrogen bond to the amine of **N0-b**. MP4 and CCSD(T) disagree as to what is the third lowest energy conformer, though PBE1PBE and CCSD(T) do agree on this. Furthermore, the relative energies of the conformers differ by as much as 2.2 kcal mol^{-1}, but the difference is half that much for the five lowest energy conformations. Therefore, while the agreement between the energetic predictions from the DFT method and the ab initio methods is not as close as hoped for, PBE1PBE does properly identify the lowest energy conformer and the energy differences are similar to those observed at CCSD(T).

3.1.2. Comparison of Cysteine−Water Configurations. The second benchmark involves comparison of the relative energies of cysteine−one-water and cysteine−two-water clusters. We optimized a number of these clusters (see below) at PBE1PBE/6-311+G(d,p). We compare the relative energies of some of these clusters computed at this level and at CCSD(T)/6-311+G(d,p)//PBE1PBE/6-311+G(d,p) with ZPVE(PBE1PBE/6-311+G(d,p)) values in Table 2. Aikens and Gordon[4] have advocated for the use of MP2 for computing the relative energies of amino acid−water clusters, and indicated that even HF is reasonably capable of evaluating their energies. Energies obtained with these methods (HF/6-311+G(d,p) and MP2/6-311+G(d,p), both corrected using ZPVE computed at HF/6-311+G(d,p)) are listed in Table 2.

While HF and PBE1PBE provide very similar relative energies of the neutral configuration clusters, HF grossly overestimates the relative energies of the zwitterions. HF is simply unsuitable for the task at hand.

There is in general nice agreement between the CCSD(T) and MP2 relative energies. They agree on the relative energetic ordering of the different clusters, with energy differences no greater than a couple of kilocalories per mole.

CCSD(T) does not predict the same lowest energy cluster in either the one-water or two-water clusters as does PBE1PBE. This is due to, as we saw in the cysteine conformers, the overestimation of the strength of the SH···N hydrogen bond. However, the energy difference among the neutral configurations is small with both the PBE1PBE and CCSD(T) methods.

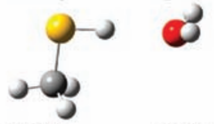
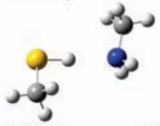
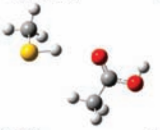
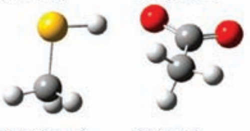
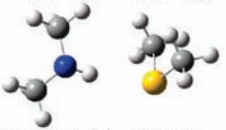
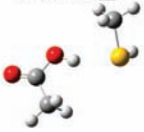
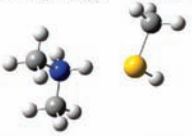
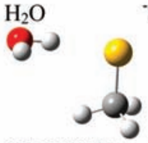
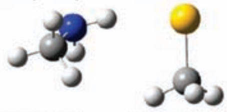
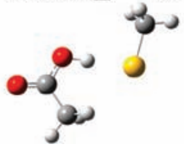
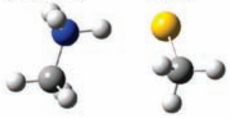
PBE1PBE, MP2, and CCSD(T) do predict the same energetic ordering of the cysteine tautomers with one or two waters, namely, $E(\mathbf{N}) < E(\mathbf{Z}) < E(\mathbf{UZ})$. Unfortunately, the energy differences are underestimated by PBE1PBE relative to CCSD(T) or MP2, by an amount that suggests that PBE1PBE may be in error by about 2−3 kcal mol^{-1} for the energy of the zwitterion and 4−6 kcal mol^{-1} for the unconventional zwitterion. These disparities are larger than what we observed in the glycine−one-water benchmark. Optimization of these clusters at CCSD(T) is unaffordable, and computation of the larger water clusters is prohibitively expensive with either MP2 or CCSD(T) as well. We must, therefore, use the PBE1PBE results with the caveat that they may be overestimating the stabilities of the zwitterion and unconventional zwitterion relative to the neutral structures.

3.1.3. Justification of Use of B3LYP/6-31+G(d) Zero-Point Vibrational Energies. To reduce the size of the computations, we employ B3LYP/6-31G+(d) ZPVEs. We have computed the ZPVEs for all of the one-, two-, and three-water clusters at B3LYP/6-31+G(d) and PBE1PBE/6-311+G(d,p) with geometries optimized at that same level. The correlation between these two sets of ZPVEs is excellent; the plot of the B3LYP vs PBE1PBE ZPVEs gives a linear fit with a slope of 1.0009 and a correlation coefficient of 0.9996. The intercept is 0.52 kcal mol^{-1}, indicating that B3LYP systematically underestimates the ZPVE. However, since we are using relative energies throughout, we can safely employ the B3LYP ZPVEs for the larger clusters.

3.1.4. Selection of Configurations. The number of configurations available to the cluster of cysteine and any number of water molecules is large, and especially large with five or six water molecules. One approach to sampling the configuration space is through a Monte Carlo search. This approach leads to many noncompetitive configurations. Instead we opt for a semirational approach, where we sample a number of configurations designed analogous to the glycine clusters we reported previously, and using the strength of the possible hydrogen bonds, we build up from smaller clusters involving cysteine.

In our study of the water clusters of glycine,[5] we discerned a number of trends in the structure of the clusters. For the water clusters of the neutral glycine, the first two waters associate with the carboxylic acid group. Additional waters then form water rings, and with six or more water molecules, a water will act as the donor in a hydrogen bond to the amine group. With the zwitterion, the ammonium group must participate as the donor in at least two hydrogen bonds, typically donating to two water molecules. Subsequent water molecules then interact with these first two waters and the carboxyl oxygens. For both neutral and zwitterion clusters, the best structures position the water molecules to participate in three (or four) hydrogen bonds. Typically, this means cube- or cagelike structures where the heavy-atom donor or acceptor occupies the corner of a cube (or a cage). These trends were understood in terms of the

TABLE 3: Hydrogen Bond Strengths (kcal mol^{-1}) Computed at PBE1PBE/6-311+G(d,p)

Donor	Acceptor	ΔE	Donor	Acceptor	ΔE
H_2O	H_2O	6.6[a]	H_2O	$CH_3C\underline{O}OH$	6.5[a]
$CH_3COO\underline{H}$	H_2O	6.5[a]	H_2O	CH_3COO^-	12.9[a]
H_2O	NH_2CH_3	6.5[a]	NH_2CH_3	H_2O	1.7[a]
$NH_3CH_3^+$	H_2O	18.7 (1 water)[a] 17.1 (2 water)[a] 15.8 (3 water)[a]			
$HSCH_3$	H_2O	2.1	$HSCH_3$	NH_2CH_3	3.4
					
$HSCH_3$	$CH_3COO\underline{H}$	2.3	$HSCH_3$	CH_3COO^-	2.0[b]
					
H_2O	$HSCH_3$	3.0	$HN(CH_3)_2$	$S(CH_3)_2$	1.9
					
$CH_3COO\underline{H}$	$HSCH_3$	5.5,[c] -0.2[d]	$H_2N(CH_3)_2^+$	$HSCH_3$	14.1
					
H_2O	$^-SCH_3$	15.3	$HN(CH_3)_2$	$^-SCH_3$	9.1
					
$CH_3COO\underline{H}$	$^-SCH_3$	31.9,[c] 26.2[d]	$CH_3NH_3^+$	$^-SCH_3$	11.2[b]
					

[a] Reference 5. [b] Computed with PCM (solvent is water). [c] Relative to *s-anti*-acetic acid. [d] Relative to *s-syn*-acetic acid.

hydrogen bond strength for a variety of relevant cases; these are summarized in Table 3.

We now add to this table the hydrogen bond strengths that involve sulfur. The thiol group can serve as either a proton donor or a proton acceptor with water, an amine, or a carboxylic acid group. It can also accept a proton from an ammonium group and donate to a carboxylate group. The thiolate group can accept a proton from water, an amine, an ammonium, or a carboxylic acid group.

The thiol group is a poor hydrogen bond donor, whether to water (ΔE = 2.1 kcal mol^{-1}), methylamine (ΔE = 3.4 kcal mol^{-1}), or acetic acid (ΔE = 2.3 kcal mol^{-1}). In the attempt to optimize the structure where methanethiol donates a proton to the carboxylate group, the proton transfers. To estimate the strength of this type of interaction, we optimized this hydrogen-bonded pair using PCM with water as the solvent. This leads to an aqueous-phase hydrogen bond energy of 2.0 kcal mol^{-1}.

The thiol group is also a poor hydrogen bond acceptor. The strength of the hydrogen bond between methanethiol (as the acceptor) and water is 3.0 kcal mol^{-1}, indicating a slight preference for hydrogen bonding in this orientation (the MeSH···OH_2 bond strength is 2.1 kcal mol^{-1}). To minimize the participation of donation to nitrogen, we evaluate the Me_2NH···SMe_2 pair to obtain the strength of the NH···S bond: 1.9 kcal mol^{-1}. For the acetic acid−methanethiol pair, we use the *s-anti* conformation of acetic acid, since this is the only conformation available to the carboxylic acid group in cysteine to donate a proton to the thiol group. This hydrogen bond strength is 5.5 kcal mol^{-1}, but is actually endothermic relative to that of the *s-syn* conformation of acetic acid. The hydrogen bond between the ammonium group and sulfur (as exemplified in the $Me_2NH_2^+$···SHMe pair) is quite strong, 14.1 kcal mol^{-1}, but thiol is a poorer acceptor than water toward the ammonium group.

The thiolate group is a strong hydrogen bond acceptor; the hydrogen bond strengths increase in the order amine < water < carboxylic acid (9.1, 15.3, and 31.9 kcal mol^{-1}, respectively). The donation of the proton from *s-anti*-acetic acid to methanethiolate results in a long O−H distance (1.093 Å), suggesting that proton transfer is likely. Proton transfer, in fact, occurs in the optimization of the $MeNH_3^+$···^-SMe pair. We estimate this hydrogen bond strength by optimizing this pair using PCM with water as the solvent; its strength is 11.2 kcal mol^{-1}.

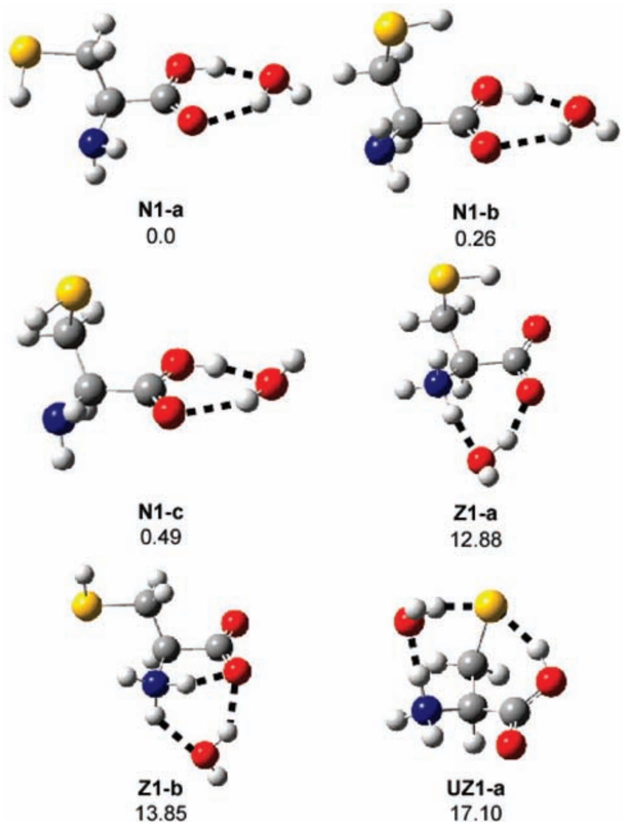


Figure 2. Structure and relative energy (kcal mol^{-1}) of the lowest energy configurations of the cysteine−one-water clusters.

These hydrogen bond strengths suggest certain configurations of the cysteine−water clusters will be more favorable than others. The hydrogen bonds involving the thiol group are uncompetitive with those involving the other functional groups, so we anticipate minimal involvement of the thiol group in the lowest energy configurations involving neutral and zwitterion cysteine. By this we mean that the thiol will interact through space with water molecules and other functional groups of cysteine, not by forming close interactions indicative of hydrogen bonding.

For the clusters involving the unconventional zwitterion, we anticipate that all of the competitive clusters will exhibit strong intramolecular hydrogen bonding between the thiolate and either the ammonium group or the carboxylic acid (in its *s-anti* conformation). Water molecules will hydrogen bond to the thiolate and bridge to the other functional groups.

3.2. Cysteine−One-Water Clusters. We identified fifteen different configurations containing one water and cysteine in its neutral form. These are shown in Figure S2 in the Supporting Information. In the three lowest energy configurations (Figure 2), the water bridges across the carboxylic acid group and the amine orients both of its hydrogens toward the carbonyl oxygen. The lowest energy structure **N1-a** has the thiol proton pointed toward the nitrogen lone pair, but this distance is too long (2.44 Å) to be a hydrogen bond. This conformation corresponds to **N0-c**, the third lowest energy conformation of cysteine. **N1-b** and **N1-c** differ by rotation about the $C_{sp^3}-C_{sp^3}$ bond. The lowest energy cluster having the conformation of **N0-a** is **N1-g**, and it is 3.06 kcal mol^{-1} higher in energy than **N1-a**. Preservation of the internal hydrogen bond to nitrogen requires the *s-anti* conformation of the carboxylic acid group, which is about 5 kcal mol^{-1} more energetic than the *c-syn* form. Already with one water molecule, the inherent benefit of the *c-syn* form allows for favorable hydrogen bonding to water, and results in much more favorable configurations.

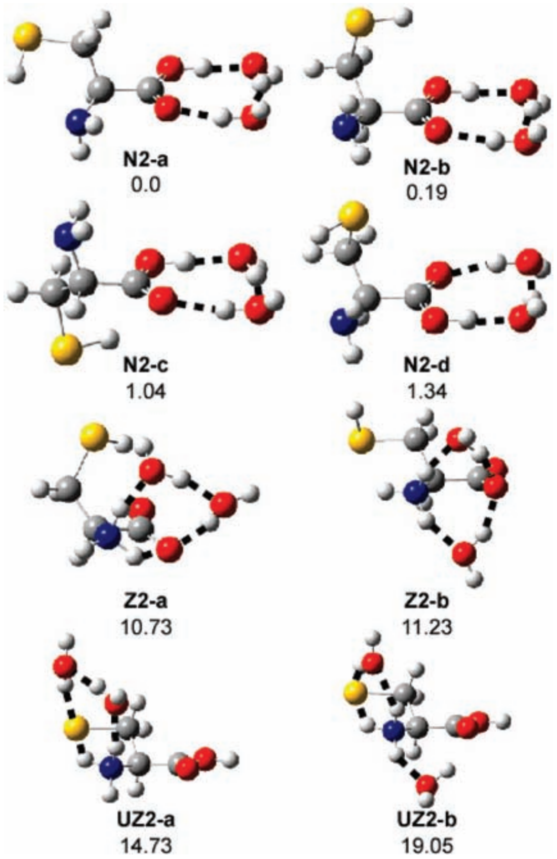


Figure 3. Structure and relative energy (kcal mol^{-1}) of the lowest energy configurations of the cysteine−two-water clusters.

Unlike with glycine, whose zwitterion requires at least two water molecules, there are two clusters involving the conventional cysteine zwitterion and one water molecule. As we saw with glycine, the zwitterion is unstable due to the ammonium group; one of its protons will transfer to the anionic atom unless it is stabilized. In **Z1-a**, the ammonium is stabilized by donating a proton to the water molecule and through a weaker interaction with the thiol group. In **Z1-b**, the ammonium is stabilized by donating two protons into hydrogen bonds, one with water and one to a carboxyl oxygen, along with a weaker interaction of the third proton with the thiol group. Though these two configurations are local energy minima, they are both much higher in energy (by more than 13 kcal mol^{-1}) than the neutral configuration.

Somewhat surprising is the fact that there is one stable configuration involving the unconventional zwitterion complexed with one water, **UZ1-a**. The sulfur anion accepts a proton from the carboxylic acid group (by necessity in its *s-anti* conformation) and from water. The water also stabilizes the ammonium by accepting one of its protons, but the ammonium cannot participate in a second hydrogen bond. Though **UZ1-a** possesses the strong hydrogen bond between the carboxylic acid group and the thiolate, this configuration is very energetic, lying 17.1 kcal mol^{-1} above **N1-a**.

3.3. Cysteine−Two-Water Clusters. We identified fifteen configurations of neutral cysteine with two water molecules, shown in Figure S3 in the Supporting Information. The four lowest energy configurations are drawn in Figure 3. The six lowest energy clusters all have the two water molecules bridging the carboxylic acid group via three hydrogen bonds. Three of

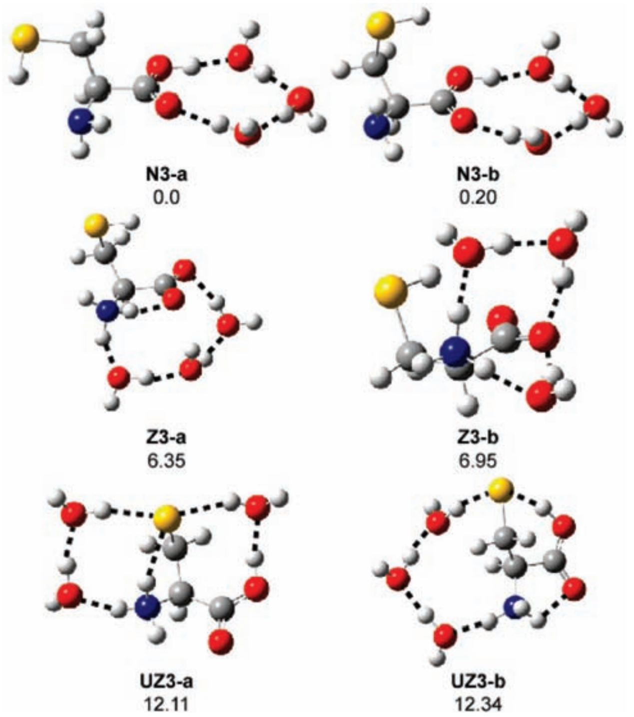


Figure 4. Structure and relative energy (kcal mol^{-1}) of the lowest energy configurations of the cysteine−three-water clusters.

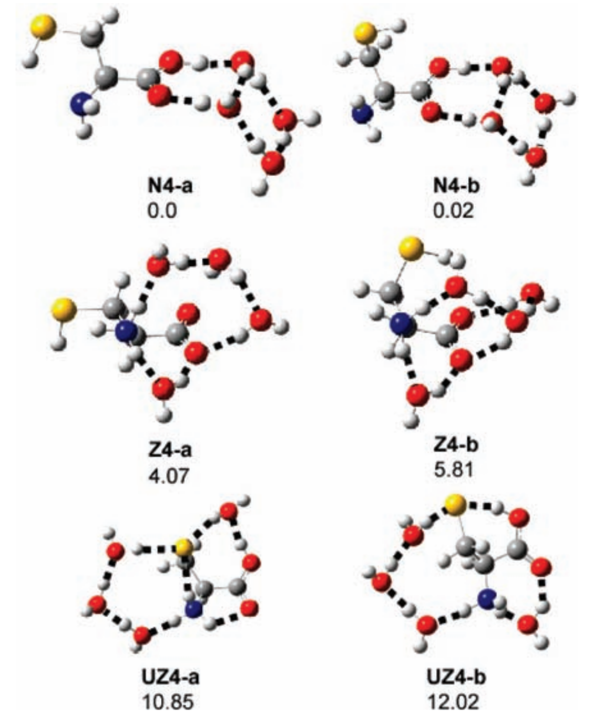


Figure 5. Structure and relative energy (kcal mol^{-1}) of the lowest energy configurations of the cysteine−four-water clusters.

the four lowest structures, **N2-a**, **N2-b**, and **N2-c**, are reminiscent of the three lowest energy structures of **N1**; they differ by the orientation of the thiol group about the $C_{sp^2}-C_{sp^2}$ bond. The lowest energy structure **N2-a** has the weak interaction of the thiol proton with the nitrogen lone pair.

Five configurations of the cysteine zwitterion with two waters were identified (Figure S3, Supporting Information), and the two lowest energy clusters are shown in Figure 3. In these two, the ammonium is stabilized by donating two protons. In **Z2-a**, a two-water bridge spans the ammonium and carboxylate group, while the second hydrogen bond is from the ammonium to the carboxylate oxygen. **Z2-b** has each water molecule bridging the ammonium and carboxylate groups. These zwitterion clusters remain very high in energy, more than 10 kcal mol^{-1} above **N2-a**.

There are three configurations of the cysteine unconventional zwitterion with two water molecules, and we show the lowest two in Figure 3. The most stable configuration **UZ2-a** has a two-water hydrogen-bonded bridge spanning the ammonium and thiolate groups. In addition, these two groups engage in their own direct hydrogen bond. It lies 14.7 kcal mol^{-1} higher in energy than **N2-a**. **UZ2-b** has one water bridging the ammonium and the thiolate, and the other water bridges the ammonium and carboxylic acid groups. **UZ2-c** involves a hydrogen bond between the *s-anti*-carboxylic acid group and the thiolate, with the two waters forming a bridge between the ammonium and thiolate groups. All other attempts to locate a cluster with the *s-anti*-carboxylic acid donating a proton to the thiolate led to proton transfer to the sulfur.

3.4. Cysteine−Three-Water Clusters. We identified sixteen configurations of neutral cysteine with three water molecules. These are shown in Figure S4 in the Supporting Information. The two lowest energy structures **N3-a** and **N3-b** (Figure 4) have the same cysteine conformation as in the lowest energy clusters with one or two water molecules (see above). Here the three water molecules form a chain that bridges the ends of the carboxylic acid group. They differ again by the position of the thiol group about the $C_{sp^3}-C_{sp^3}$, with the lower energy configuration allowing the thiol proton to point toward the nitrogen lone pair.

We located eight configurations of the cysteine zwitterion with three water molecules (shown in Figure S4 in the Supporting Information). The two lowest energy configurations are shown in Figure 4. The critical hydrogen bonds are those that stabilize the ammonium group. In **Z3-a**, one ammonium proton is hydrogen bonded to a carboxylate oxygen and a second proton is hydrogen bonded to a three-water chain that ends with a hydrogen bond to the other carboxylate oxygen. A one-water and a two-water bridge connect (via hydrogen bonds) two of the ammonium protons to one carboxylate oxygen in **Z3-b**. These zwitterion clusters remain far higher in energy than the neutral clusters: **Z3-a** is 6.35 kcal mol^{-1} above **N3-a**.

The nine clusters of the cysteine unconventional zwitterion with three water molecules are displayed in Figure S4 in the Supporting Information, and the two lowest energy clusters are shown in Figure 4. Both of these low-energy clusters have the *s-anti*-carboxylic acid configuration. In **UZ3-a**, a water molecule intervenes between this proton and the sulfur atom, while in **UZ3-b**, this proton directly hydrogen bonds to the thiolate. **UZ3-a** benefits by having three hydrogen bonds to sulfur. These clusters lie more than 12 kcal mol^{-1} in energy above **N3-a**.

3.5. Cysteine−Four-Water Clusters. We identified 22 clusters of neutral cysteine with four water molecules (see Figure S5 in the Supporting Information), and the two lowest energy clusters are shown in Figure 5. Neutral cysteine in these lowest energy clusters adopts the same conformation as in the lowest energy configurations with fewer water molecules. In both **N4-a** and **N4-b**, the four water molecules form a ring connected by hydrogen bonds, and this water ring makes two hydrogen bonds to the carboxylic acid group.

Ten configurations of the conventional zwitterion of cysteine with four waters were located, and the two lowest energy structures are shown in Figure 5. The lowest energy cluster **Z4-a**

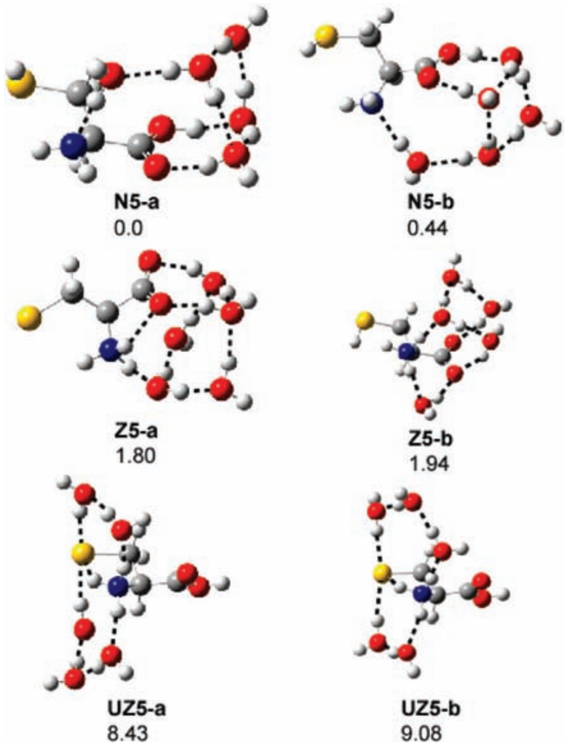


Figure 6. Structure and relative energy (kcal mol^{-1}) of the lowest energy configurations of the cysteine−five-water clusters.

has a one-water chain and a three-water chain that bridge the ammonium and one oxygen of the carboxylate. The middle water of the three-water chain interacts with the other oxygen, but the O···H distance is just beyond 2 Å. This structure corresponds with the lowest energy cluster previously identified by Pacul.[11] In **Z4-b**, a two-water chain bridges the carboxylate group. The third water molecule then bridges the ammonium group and the water chain, while the last water bridges the ammonium and carboxylate groups. Both of these clusters remain energetically well above the neutral clusters.

We found ten configurations of the cysteine unconventional zwitterion with four water molecules. The two lowest energy structures are shown in Figure 5. Both involve the *s-anti*-carboxylic acid group. In **UZ4-a**, this carboxylic acid proton donates to a water molecule which donates one of its protons in a hydrogen bond to sulfur, while in **UZ4-b**, the carboxylate proton is directly donated to sulfur. Both clusters have a three-water chain that bridges the ammonium group to the sulfur. **UZ4-a** is nearly 11 kcal mol^{-1} more energetic than **N4-a** and is 5 kcal mol^{-1} above **Z4-a**.

3.6. Cysteine−Five-Water Clusters. We located forty-four configurations formed of five water molecules and neutral cysteine. These structures are drawn in Figure S6 in the Supporting Information, and the two lowest energy clusters are drawn in Figure 6. These two structures, **N5-a** and **N5-b**, differ noticeably from the lowest energy clusters involving fewer water molecules. The amine group is oriented such that one hydrogen is directed toward the carbonyl oxygen and the other toward the sulfur. The lone pair is directed toward the hydrogen of a water, which then hydrogen bonds to a four-water ring (like in **N4a** and **N4b**) that hydrogen bonds to the carboxylic acid group. These clusters benefit from the four-water ring and a water that participates in three hydrogen bonds. The cluster having a five-water ring (**N5e**) lies 1.03 kcal mol^{-1} above **N5a**.

The seventeen configurations of the zwitterion of cysteine and five water molecules are drawn in Figure S6 in the Supporting Information, and the two lowest energy clusters are displayed in Figure 6. **Z5-a** has a hydrogen-bonded ring of five water molecules. This ring makes hydrogen bonds to the oxygens of the carboxylate group and accepts one proton from the ammonium group. A second ammonium proton forms a hydrogen bond to the carboxylate oxygen atom. The next lowest cluster **Z5-b** has a four-water ring that hydrogen bonds to the ammonium group and both oxygens. The fifth water bridges across a second ammonium hydrogen and a carboxyl oxygen. The energy gap between the lowest energy zwitterion cluster **Z5-a** and the neutral cluster **N5-a** is 1.80 kcal mol^{-1}.

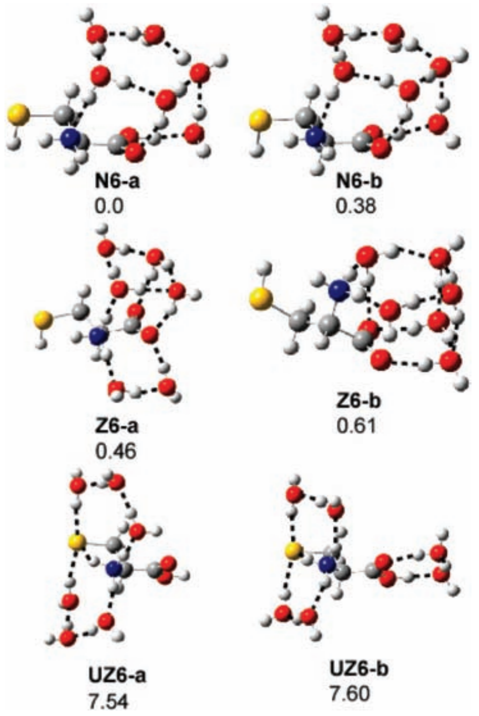


Figure 7. Structure and relative energy (kcal mol^{-1}) of the lowest energy configurations of the cysteine−six-water clusters.

The eighteen configurations of the unconventional zwitterion with five water molecules are drawn in Figure S6 in the Supporting Information. The two lowest energy configurations **UZ5-a** and **UZ5-b** are quite similar. They have identical cysteine conformations with an intramolecular hydrogen bond formed of an ammonium proton to the sulfur anion. Both have a two-water bridge and a three-water bridge that span an ammonium proton and the sulfur; the difference is whether the three-water bridge is on the side of the carbonyl oxygen or the hydroxyl oxygen. The lowest energy configuration possessing the *s-anti*-carboxylic acid arrangement is **UZ5-c**, 0.3 kcal mol^{-1} higher in energy than **UZ5-b**. The unconventional zwitterion remains much higher in energy than the neutral form: **UZ5-a** is 7.4 kcal mol^{-1} above **N5-a**.

3.7. Cysteine−Six-Water Clusters. We identified 50 different configurations of neutral cysteine with six water molecules. These are shown in Figure S7 in the Supporting Information, and the two lowest energy configurations are drawn in Figure 7. The cysteine conformation in both **N6-a** and **N6-b** is identical to that of the two lowest energy clusters involving five water molecules; one amine proton is oriented toward the carbonyl oxygen, and the other points toward the sulfur atom. In both **N6-a** and **N6-b**, a five-water ring hydrogen bonds to the amine lone pair and the carbonyl oxygen. The sixth water molecule links the water ring to the hydroxyl proton via two hydrogen bonds. The two structures differ in the orientation of the hydrogen bonds in the water ring; one is clockwise and the other is counterclockwise about the ring.

The sixty-seven identified clusters of the cysteine zwitterion with six water molecules are displayed in Figure S7 in the Supporting Information. The lowest energy cluster **Z6-a** (see Figure 7) has a four-water ring that hydrogen bonds to an ammonium proton and the carboxylate oxygens. The other two waters form a chain that spans the ammonium group and the proximate oxygen. **Z6-b** has a two-water hydrogen-bonded water bridge spanning the carboxyl oxygens and a four-water bridge that spans two of the ammonium hydrogens. These two water bridges are hydrogen bonded to each other. The thiol group is orientated to make a weak interaction with the third ammonium hydrogen in both clusters. **Z6-a** is less than 0.5 kcal mol^{-1} higher in energy than **N6-a**.

We located twenty-eight configurations of the cysteine unconventional zwitterion and six water molecules, shown in Figure S7 in the Supporting Information. The lowest energy cluster **UZ6-a** has two three-water hydrogen-bonded chains that bridge an ammonium hydrogen and sulfur. The third ammonium hydrogen is involved in a hydrogen bond to the sulfur. **UZ6-b** has three two-water chains; two of them bridge an ammonium hydrogen and sulfur, and the third bridges the ends of the carboxylic acid. It too has a hydrogen bond from an ammonium proton to sulfur. Even with the three hydrogen bonds to both the ammonium group and the thiolate, these unconventional zwitterion clusters remain noncompetitive with the neutral or conventional zwitterion clusters, lying more than 7 kcal mol^{-1} higher in energy than **N6-a**.

4. Discussion

The trends in the structure of the cysteine clusters are readily understood by examining the three different tautomers individually. The lowest energy conformation of cysteine **N0-a** has an internal hydrogen bond between the proton of the *anti*-carboxylic acid group and nitrogen. In all of the water clusters of neutral cysteine, this weak hydrogen bond is absent, replaced instead by the inherently more stable *syn*-carboxylic acid group. In the lowest energy clusters involving neutral cysteine and one to four water molecules, the cysteine fragment adopts the **N0-c** conformation. This conformation allows for waters to make strong hydrogen bonds to the carbonyl oxygen and the alcohol proton along with favorable water ring structures. The amine hydrogens point toward the carbonyl oxygen, and the thiol hydrogen points toward the amine lone pair, providing weaker dipole−dipole stabilization.

With five or six water molecules, the cysteine adopts a slightly modified conformation; the amine rotates, directing one hydrogen toward the carbonyl oxygen. This allows a water molecule to donate into the amine lone pair while also engaging in hydrogen bonds to the waters associated with the carboxylic acid group.

The structures of the water clusters of neutral cysteine are very similar to those we previously reported for neutral glycine, and they share common structural motifs. The first two water molecules associate with the carboxylic acid group. The next two waters create a water ring. With five or more water molecules, the amine group rotates so that a water molecule can bridge the amine and the water ring, forming a cage structure.

In our glycine microsolvation study we noted that the critical aspect of stabilizing the zwitterion structure was the need to form hydrogen bonds to the ammonium group. A single water was insufficient to stabilize the glycine zwitterion. However, there are two stable cysteine zwitterion clusters with one water. In both **Z1-a** and **Z1-b** the thiol group orients to interact with an ammonium hydrogen, providing additional stabilization. Nonetheless, all of the clusters of the zwitterion are marked by having two strong hydrogen bonds to the ammonium. With the cluster having three or fewer waters, one of the hydrogen bond acceptors is a carboxylate oxygen, which requires a near-eclipsing arrangement of the ammonium and carboxylate groups. With the bigger clusters, this eclipsing interaction can be avoided and larger water chains can bridge the two charged centers (the ammonium cation and the carboxylate anion). Once these charged groups are addressed, the waters prefer to form a maximum degree of hydrogen bonding among themselves, typically by creating rings or cagelike structures.

TABLE 4: Energy Differences (kcal mol^{-1}) between the Lowest Energy Neutral, Conventional, and Unconventional Cysteine Water Clusters

no. of water molecules	*E*(**Z** − **N**)	*E*(**UZ** − **N**)	*E*(**UZ** − **Z**)
1	12.88	17.10	4.22
2	10.73	14.73	4.00
3	6.35	12.11	5.76
4	4.07	10.85	6.78
5	1.80	8.43	6.63
6	0.46	7.54	7.08

Again, the structures of the water clusters of glycine and cysteine zwitterions are similar. The key difference is the weak association between an ammonium hydrogen and the sulfur atom. The stabilization energy afforded by this interaction (about 2 kcal mol^{-1} in its ideal case; see Table 1) allows for the zwitterion−one-water cluster to exist; glycine, lacking the thiol group, needs two water molecules to stabilize the zwitterion.

The unconventional zwitterion requires water to stabilize the charge buildup on the ammonium and the sulfur. One might imagine these two groups stabilizing each other, i.e., the ammonium group donating a hydrogen to the thiolate. In the gas phase, optimization of the $CH_3NH_3^+\cdots{}^-SCH_3$ ion pair led to proton transfer. In aqueous solution, the association of these two is very strong (11 kcal mol^{-1}; see Table 1). We found no unconventional zwitterion with just one water and an ammonium−thiolate hydrogen bond; the lone water is insufficient to stabilize this ion pair. However, with two or more water molecules, the low-energy clusters all contained this type of strong hydrogen bond. The next most favorable hydrogen bond is between a carboxylate proton and thiolate. This can only occur in the unconventional cysteine if the carboxylic acid adopts its less favorable *anti* arrangement. Nevertheless, this strong hydrogen bond (36 kcal mol^{-1}) is found in many of the smaller water clusters. It is not until the larger clusters where three hydrogen bonds to the sulfur are made (using water and ammonium) that the carboxylic acid−sulfur hydrogen bond is lost.

Returning now to Gordon's two questions concerning amino acid structure, we see that the cysteine zwitterion and the unconventional zwitterion are local energy minima with just one associated water molecule. The second question of how many water molecules are needed to bring the zwitterion and neutral tautomers to equal energy is addressed in Table 4.

Only the neutral tautomer exists in the gas phase. With one associated water molecule, both the conventional and unconventional tautomers are stable structures, but they are much higher in energy than the neutral tautomer. With each successive addition of a water molecule to the cluster, both zwitterions become more stable relative to the neutral form. With six water molecules, the largest clusters we examined, the neutral tautomer is only 0.46 kcal mol^{-1} lower in energy than the conventional zwitterion.

Given the uncertainties in the energy calculations, we believe that with six or seven water molecules the cysteine neutral and conventional zwitterions are essentially degenerate. This is similar to the situation with glycine. With six water molecules, the energy gap between the two tautomers of cysteine (0.46 kcal mol^{-1}) is smaller than the gap for glycine (1.7 kcal mol^{-1}). A seventh molecule results in the glycine neutral and zwitterion tautomers being equal in energy. The weak interaction of the thiol group with the ammonium does provide some real stabilization, reducing the number of water molecules (by one) needed to bring the cysteine zwitterion in energy parity with the neutral tautomer.

The energy gap between the novel unconventional zwitterion and the neutral tautomer is also reduced with increasing water association. The cluster of the unconventional zwitterion with one water is highly energetic, lying 17.1 kcal mol^{-1} above the neutral form. This gap is reduced to 7.5 kcal mol^{-1} with six waters. This reduction of 9.5 kcal mol^{-1} is less than the gap reduction for the conventional zwitterion (12.4 kcal mol^{-1}). The effect of each water addition to the unconventional zwitterion is diminishing, so we believe that it is unlikely that further microsolvation will ever result in the unconventional zwitterion becoming competitive with the neutral tautomer.

The last column of Table 4 relates the relative energies of the two zwitterions. For all degrees of water microsolvation, the conventional zwitterion is more stable than the unconventional zwitterion. Also, while both zwitterions become more stable relative to the neutral tautomer, they do so at different rates. With each successive addition of water, the conventional tautomer becomes ever more stable than the unconventional tautomer; with one water **Z1-a** is 4.22 kcal mol^{-1} lower in energy than **UZ1-a**, and with six waters, the gap grows to 7.08 kcal mol^{-1}. This trend reflects two phenomena. First, since both zwitterions have a permanent large dipole moment, they favorably interact with the dipole moment of each added water molecule to a much larger degree than does the neutral tautomer. Thus, sequential microsolvation will lower the energies of both zwitterions relative to the neutral tautomer. Second, the hydrogen bonds to the carboxylate oxygens are stronger than the hydrogen bonds to the sulfur anion. Microsolvation of water will thus preferentially favor the conventional zwitterion. Since there are more sites for hydrogen bonding to the carboxylate (which could accept six hydrogen bonds) than the thiolate (perhaps three weak hydrogen bonds), each addition of water will favor the zwitterion. This will continue until the first solvation shell is filled about both species, after which we anticipate an asymptotic stabilization due to long-range dielectric stabilization.

The two previously reported PCM computations involved optimizing the conformers of the cysteine tautomers, which led to structures with internal hydrogen bonds.[9,10] We find, however, that the larger water clusters of the zwitterion do not have any internal hydrogen bonds, and thus possess a greater dipole moment than the conformer used in these prior computations. The microsolvated unconventional zwitterion maintains an internal hydrogen bond between the ammonium and thiolate, but the previous optimized PCM structure has the unfavorable *anti* configuration of the hydroxyl group necessary to form a second internal hydrogen bond. It is quite possible that these "optimized" structures fail to properly balance internal vs external hydrogen bonds and thereby fail to properly assess their different solvation energies.

We have carried out two different PCM calculations. In the first, the free energy is obtained by a PCM calculation of **6N-a**, **6Z-a**, and **6UZ-a** with frozen geometry, including the six explicit water molecules. The relative free energies are (**6N-a**) 0.0, (**6Z-a**) −2.65, and (**6UZ-a**) 1.30 kcal mol^{-1}. In the second computation, we optimized the geometry of each of the three cysteine tautomers starting with its geometry in **6N-a**, **6Z-a**, and **6UZ-a**. This gives relative free energies of (**6N-a**) 0.0, (**6Z-a**) −1.36, and (**6UZ-a**) 1.28 kcal mol^{-1}. Both of these PCM results and the microsolvation data suggest that the unconventional cysteine zwitterion in aqueous solution appears to be at best a very minor component. Further, these computations consistently predict that the dominant species in solution is the conventional zwitterion.

5. Conclusions

The structure of cysteine is sensitive to its environment. Through successive addition of water molecules to the three tautomers, we can identify when the zwitterion tautomers become stable critical points on the potential energy surface and what degree of microsolvation is needed to bring the neutral and zwitterion to equal energy. Only the neutral tautomer exists in the gas phase. A single water molecule is sufficient to stabilize both the conventional and unconventional zwitterion. The neutral and zwitterion clusters containing six water molecules are nearly isoenergetic. The thiol group provides a weak internal stabilization of the charged groups of the zwitterion. This leads to the need for a reduction in the number of waters needed to bring the zwitterion into energetic parity with the neutral tautomer from seven with glycine to six with cysteine. The unconventional zwitterion, formed by proton transfer from the thiol to the amine, is a stable structure when associated with at least one water. It is, however, significantly higher in energy than both the neutral and conventional zwitterion and is therefore only a minor contributor to the aqueous population of cysteine.

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Supporting Information Available: Full citation for ref 25, coordinates of all clusters optimized at PBE1PBE/6-311+G(d,p), and Figures S1−7. This information is available free of charge via the Internet at http://pubs.acs.org.

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