The Effect of Solvation on Biomolecular Conformation: 2-Amino-1-phenylethanol

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Received: February 7, 2007; In Final Form: June 2, 2007

Small molecule neurotransmitters form one the most important classes of pharmaceutical molecules. While the behavior of these molecules in their neutral forms in the gas phase is well understood, their behavior in more biologically relevant scenarios (protonated and in aqueous solution) has received comparatively little attention. Here we address this problem by using molecular mechanics simulations to build up a detailed picture of the conformational behavior of 2-amino-1-phenylethanol, a noradrenaline analogue, in aqueous solution in both its neutral and protonated forms. For the sake of comparison, equivalent simulations are also performed on the gas-phase molecules and gas-phase hydrated clusters. These calculations reveal the important role that water has to play in determining the conformational preferences and dynamic behavior of the molecules. Water molecules are found to bridge between the various functional groups within the molecule, significantly affecting their relative stabilities in comparison to the gas-phase values. The reorganization of these solvation structures also provides a mechanism for conformational interconversion. The role of the solvent in mediating interactions between the various functional groups within the molecule suggests that in noradrenaline the catechol groups will be able to interact, albeit indirectly, with the other functional groups, thereby influencing the behavior of the molecule.

Introduction

Over the last 10 years, improvements in spectroscopic techniques and computational methodologies have rendered accessible the gas phase study of an ever increasing range of small molecules, both in isolation[1] and in complex with solvent clusters.[2] Of all the classes of molecule to benefit from these advances, none have received more attention that the neurotransmitters; their small size, biological significance, and pharmaceutical relevance have made them attractive targets. Foremost among these targets have been the catecholamines —adrenaline, noradrenaline and dopamine—and the ephedra—ephedrine (a compound found in plants that has a similar effect to adrenaline; it is used in the treatment of asthma[3]) and pseudoephedrine (Figure 1).

Much of the work performed on these molecules has been concerned with identifying their conformational preferences, and before such properties can be discussed in detail, it is necessary to establish a method for classifying the observed conformations. The nomenclature adopted in this study is that introduced by Graham et al.[4] in their original work on APE, and since included in many studies on small molecule neurotransmitters, including the work on APE by Miller and Clary.[5,6] It should also be noted that the stereochemistry employed in this work is that used by Miller and Clary[5,6] with an *R* configuration at the chiral center (Figure 2a). The molecule APE contains four rotatable bonds about which conformational change can occur (Figure 2). The conformation of the molecule is defined principally in terms of rotation about φ_1 using the descriptor XY, where X describes the arrangement about the $C_{ar}-C-C-N$ dihedral and Y the arrangement about the O−C−C−N dihedral. X and Y can be either A or G, corresponding to the anti and gauche conformations respectively. So the AG conformer would have an anti arrangement about $C_{ar}-C-C-N$ and a gauche arrangement about O−C−C−N. Some examples of this notation are given in Figure 2. The description is further refined with the inclusion of a number to describe the intramolecular hydrogen bonding, giving the descriptor XY*n*, where $n = 1$ denotes an OH−N hydrogen bond and $n = 2$ denotes an NH−O hydrogen bond. For molecules with greater complexity, such as catechol hydroxyl groups, further complexity can be introduced into this notation.

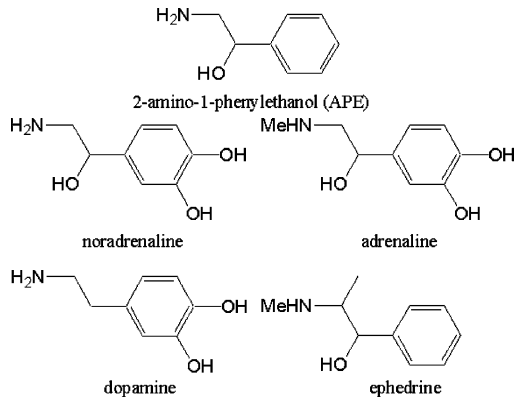

Figure 1. The structure of APE and some related neurotransmitters.

Early studies on APE[4] revealed that the molecular conformation is characterized by the formation of a single intramolecular OH−N hydrogen bond, and that there are two low energy conformations in which such an arrangement is possible: AG1 and GG1 (Figure 2). Ab initio calculations suggested that GG1

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10.1021/jp071059w CCC: $37.00
Published on Web 08/02/2007

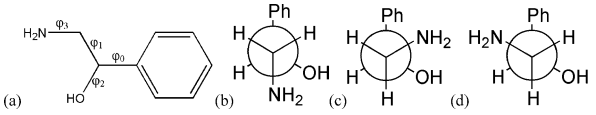

Figure 2. (a) Rotatable bonds in APE (b) AG conformation (c) GG conformation (d) GA conformation.

is lower in energy, but AG1 was found via spectroscopy to be the more highly populated. The same study also considered the effect of hydration with a single water molecule and found that, when the water molecule was present, only the GG conformer was observed. Macleod et al.[7] studied APE + nH$_2$O ($n = 1-4$) clusters and found that for $n > 1$ AG conformers were preferred and that water molecules tended to cluster around the polar groups, avoiding the "(hydrophobic) aromatic ring." APE has also been studied, via both circular dichroism[8] and NMR,[9] as a bidentate ligand in complex with cobalt, where the binding mode is found to be exclusively AG.

Theoretical studies by Miller and Clary using the torsional path integral Monte Carlo (TPIMC) method[10] to study both isolated APE[5] and APE hydrated by up to four water molecules,[6] have found that, in both cases, the AG conformer is preferred.

In the equivalent adrenaline analogue, 2-methylamino-1-phenylethanol[11] (MAPE), all of the observed structures displayed an intramolecular OH−N hydrogen bond, although NH-π and dispersion interactions were also deemed to be significant. As in APE, the GG conformer was found to be slightly lower in energy than two AG conformers, with all three being observed spectroscopically.

Noradrenaline differs from APE only in the addition of the two catechol hydroxyl groups, and Snoek et al.,[12] using ab initio calculation, found that the minimum energy conformers are very similar to those for APE, with the AG1 conformer lying around 1 kcal/mol^{-1} lower in energy than the GG1 conformer. Spectroscopic measurements, however, suggested that only the AG1a conformer is present in the gas phase (the "a" in AG1a denotes the formation of a hydrogen bond between the two catechol hydroxyl groups). This spectroscopic result seems all the more surprising when more recent calculations, performed at higher levels of ab initio theory and with larger basis sets (MP2 with basis sets up to aug-cc-pVQZ), suggest that the two conformers are actually isoenergetic in the gas phase.[13] Van Mourik and Früchtl[14] have also used ab initio calculations to demonstrate that the barriers to rotation about φ_1 are too high for any interconversion between AG and GG conformers to occur in the gas phase.

In work on noradrenaline hydrated by a small number of water molecules,[15] it has been shown that the water molecules prefer to bind to the solute via the catechol hydroxyl groups. In structures where the water does not bind via the catechol OH groups, the results are similar to those seen in APE: the molecule tends to remain in its gas-phase minimum energy conformation, with the water molecules binding in a way that causes relatively little perturbation. Alagona and Ghio[16] used ab initio methods to study noradrenaline in both its neutral and protonated forms, approximating solvent via the polarizable continuum method (PCM).[17] They concluded that in both cases the AG conformer is most stable.

Adrenaline, related to MAPE as noradrenaline is to APE, has been studied using the same combination of ab initio calculation and spectroscopy that has been seen before,[18,19] and only two populated conformers were identified, both AG1a. The addition of a single water molecule gives similar results to those found for noradrenaline with the solvent molecule binding to the catechol hydroxyl groups.[19]

The dopamine analogue 2-phenylethylamine is found to exist preferentially in conformations in which the amino group is gauche to the phenyl ring[20,21] with this conformation stabilized relative to the anti conformation by the formation of an NH-π interaction. Hydration of 2-phenylethylamine with up to two water molecules has also been considered.[22] A single water molecule prefers to bind so as to donate a hydrogen bond to the amino groups and accept a CH−O type hydrogen bond[23] from the aromatic ring. With two water molecules this arrangement is no longer seen, the solvent molecules instead preferring the formation of a cyclic structure around the amino group, similar to that seen in the water trimer.[24]

Urban et al.[25] used semiempirical methods to study dopamine in its neutral, protonated and unprotonated forms in both the gas phase and in aqueous solution. In the gas phase they found that, in the neutral molecule, all three staggered conformers were approximately isoenergetic, while in the charged forms the gauche conformers were preferred. In solution, they found that the neutral and anionic forms were unaffected by the solvent, whereas in the cationic form the anti conformer became lowest in energy. These calculations—along with other calculations on dopamine employing continuum solvation[26]—must, however, be viewed in the light of known deficiencies in semiempirical methods. It has been shown that continuum models are inadequate for modeling the conformational preferences of dopamine.[27] Studies on protonated dopamine have been performed using explicit solvation models[28] and have concluded that the molecule prefers to adopt a gauche arrangement. The same study also used ab initio calculation to investigate the structure of protonated dopamine in the gas phase, finding that a gauche arrangement is again preferred.

Ephedrine, which differs from APE via the addition of two methyl groups, and its diastereomer pseudoephedrine have also received considerable interest. A combined spectroscopic and ab initio study[3] revealed that in both cases the preferred gas phase conformation is AG, with the molecule forming an OH−N hydrogen bond. In an analogous study on the same molecules in complex with up to two water molecules,[29] two types of structure were observed: 'insertion' structures in which the water molecules insert into the hydrogen bond between the hydroxyl and amino groups, and "addition" structures in which the water molecules bind to the hydroxyl group without disrupting the intramolecular hydrogen bond. NMR experiments have been used to study the conformational preferences of ephedrine and pseudoephedrine in solution.[30] In water, ephedrine adopts a conformation in which the hydroxyl and amino groups are trans, possibly due to the increased steric bulk that comes with solvation. In pseudoephedrine, however, the preferred conformation is one in which the two polar groups adopt a gauche arrangement.

The preference for a gauche conformation of the two polar groups, which can be seen throughout this initial discussion, is termed the gauche effect,[31] and has been proposed as an empirical rule for predicting conformational preferences. Such a rule has been shown, however, to be overly simplistic when considering the hydration of biomolecules.[32]

From the above discussion, it is clear that the conformational preferences of many of these molecules, in their neutral forms and in the gas phase, or in clusters involving a small number of water molecules, are well understood. The studies discussed above have also undoubtedly played an important role in helping to construct a detailed understanding of the fundamental forces that govern conformational preference in small molecules, but there are still question marks over how well these results will

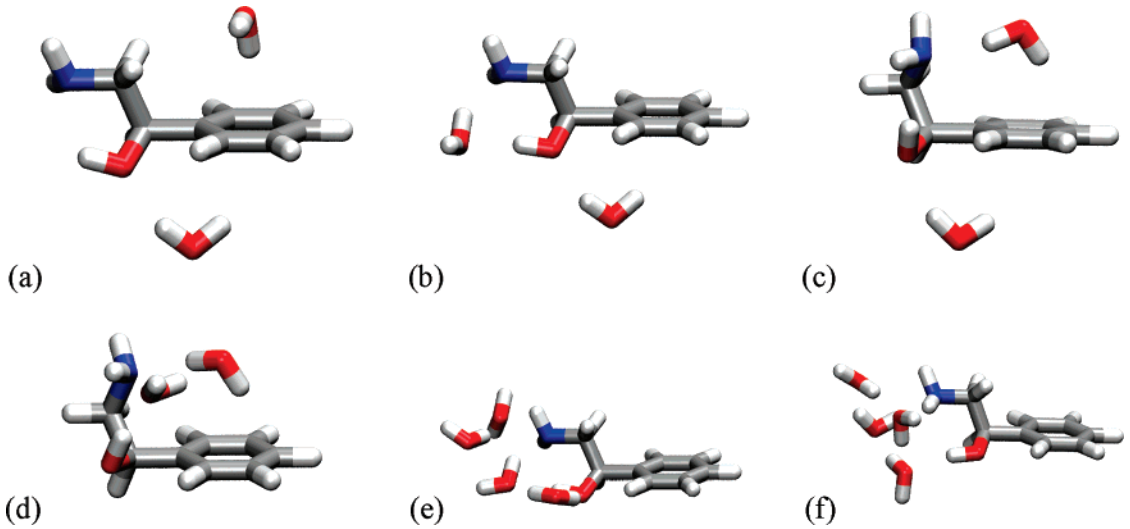

Figure 3. Hydrated cluster structures used as starting points for molecular dynamics simulations: (a) AG-add, (b) AG-ins, (c) GG′-add, (d) GG′-ins, (e) AG-droplet, and (f) AG′-droplet.

transfer to biology, where most molecules are found in aqueous solution, at a pH at which the neurotransmitters would be found in their protonated form. Noradrenaline, for example, is found to be 93% protonated at pH 7.3,[33] and it is widely accepted that a ligand binds to a receptor in the protonation state that it takes at pH 7.4.[33] The combined effect of solvation and protonation is well illustrated by a study of APE using NMR spectroscopy.[34] In this work it was shown that, in solution, the molecule (which exists almost entirely in its protonated form) exists as 81% AG conformer, 6% GG conformer and 10% GA conformer. That the AG conformer is most highly populated is unsurprising given the gas-phase results. Conversely, the presence of a significant fraction as the GA conformer would be most surprising given only the gas-phase results. Chemically this result is quite intuitive: without an intramolecular hydrogen bond, the GA conformer is very unstable in the gas phase and gains most from the introduction of solvent. It does, however, illustrate that gas-phase results cannot be relied on for a complete explanation of solution phase behavior, and there are two fundamental questions which remain largely unaddressed:

1. What is the role of bulk solvation in determining the conformational behavior of these molecules?
2. What is the effect of protonation on this conformational behavior?

One possible route to studying the behavior of small molecules in aqueous solution is offered by molecular mechanics (MM) simulation, and such methods have been successfully applied to these problems. In earlier work we have used molecular dynamics (MD) simulations to elucidate the effect of bulk solvation on electronic circular dichroism in 1-phenylethanol[35] and 1-phenylethylamine[36] as well as to elucidate a general mechanism for rotation of the protonated amino group.[37] Nagy and co-workers have used Monte Carlo methods to study the behavior of several protonated neurotransmitters in aqueous solution. In noradrenaline[33] they concluded that the AG conformer was most highly populated, and in dopamine[28] they found that a gauche conformation was preferred (although the preference was much greater than experiment suggests).

In this work, molecular mechanics methods will be employed to investigate the properties of APE, illustrating their ability to act as powerful tools for the elucidation of important chemical information, and giving new insights into the effect of bulk solvation and protonation on small biomolecules.

Methods

The conformational preferences and dynamic behavior of APE, in both its neutral and protonated forms, with an *R* configuration at the chiral center, have been investigated. To facilitate comparison with previous experimental and theoretical work, solution-phase studies have been complemented by calculations on the gas-phase molecules and their hydrated clusters. In the gas phase, three molecular dynamics simulations were run for each of APE and APE-H^+. For APE two simulations commenced from the coordinates of the AG1 and GG1 conformers determined as minima in the work of Miller and Clary,[5] and the third simulation started from the GA conformer, which has not previously been identified as an energy minimum (Figure 2). The analogous conformations for APE-H^+, being AG2, GG2 and GA, were also used as starting points for gas-phase simulations. In all cases the conformers were first subjected to a 5000 step minimization using the steepest descent algorithm,[38] followed by a 3 ns molecular dynamics simulation at 300 K, with atomic coordinates recorded every 0.1 ps.

Simulations on the hydrated clusters APE + $n$$H_2O$ were, for the unprotonated molecule, commenced from the coordinates of the six minimum energy conformations identified by Miller and Clary[6] and shown in Figure 3: AG-add (n = 2); AG-ins (n = 2); GG′-add (n = 2); GG′-ins (n = 2); AG-droplet (n = 4); and AG′-droplet (n = 4). For the protonated simulations, exactly the same starting conformations were adopted. The simulation protocol was identical to that employed in the gas-phase simulations.

In aqueous solution, three simulations were run for each of APE and APE-H^+, in this case commencing from the same conformations as were used in the gas-phase simulations. For each simulation a single molecule of APE was minimized for 5000 steps using the steepest descent algorithm before being solvated in a cubic water box of side 50 Å containing 3962 TIP3P[39] water molecules. A 25 Å sphere around the solute was then defined, and all water molecules outside this sphere were deleted. All water molecules overlapping with the solute were also deleted. The system was then partitioned into a 23 Å/25 Å reaction region/buffer region for stochastic boundary molecular dynamics. The solvent was first minimized using the steepest descent algorithm for 100 steps and then subjected to a 2.5 ps equilibration period at 1000 K, during which the solute was constrained. With the solute still constrained a further steepest

TABLE 1: Simulation Details for Molecular Dynamics Simulations of Solution-phase APE and APE-H$^+$

trajectory type	small molecule
method	molecular dynamics
time-step	1 fs
sampling frequency	100 fs for first 0.5 ns, 500 fs thereafter
total number of frames	1000
computational platform	linux cluster
software package	CHARMM
boundary condition	stochastic boundary
unit cell	sphere radius 25 Å
force field	CHARMM22
solvent	water
solvent force field	TIP3P
source	ref 5

descent minimization of 200 steps was performed, followed by 5 ps of equilibration at 300 K, 200 steps of steepest descent minimization and 15 ps of equilibration at 300 K. A 10 ps unconstrained equilibration was then performed, and the system was found to be well equilibrated in all cases (results not shown). This was then followed by a 3 ns unconstrained production simulation at 300 K, with atomic coordinates recorded every 0.1 ps for the first 1 ns and every 0.5 ps thereafter. The simulations were performed using a deformable boundary potential with a Langevin friction coefficient of 62.0 ps^{-1} applied to the water oxygen atoms.[40]

All molecular dynamics simulations were performed using the CHARMM[41] program with the CHARMM22[42] force field and using the SHAKE[43] algorithm to constrain bonds to hydrogen, allowing a time step of 1 fs. In accordance with the recent suggestions of Murdock et al.,[47] a summary of the simulation details is given in Table 1.

To assess the relative stability of the various conformers, Monte Carlo simulations were performed using version 4.2 of the BOSS program[44] with the OPLS all atom force field.[45] A single molecule of either APE or APE-H$^+$, with the dihedral angle φ_1 constrained to 0°, was placed in a box containing 267 TIP4P[39] water molecules in the NPT ensemble at a temperature of 298 K and a pressure of 1 atm. For APE the system was equilibrated for 1.6×10^8 steps before 4.0×10^8 steps of averaging, for APE-H$^+$ the system was equilibrated for 0.8×10^8 steps before 1.0×10^8 steps of averaging. φ_1 was then incremented by 2° and the whole process repeated until φ_1 had been rotated through 360°, with the use of double wide sampling[46] to give an effective increment of 1°. The process was repeated for both molecules in the gas phase at a temperature of 298 K, with the use of 10° increments and "single wide" sampling, with 2×10^5 steps of equilibration and 8×10^5 steps of averaging at each dihedral increment.

For all molecular mechanics calculations the partial charges used for each atom were the average charges for that atom in the AG1 and GG1 minimum energy conformations calculated (via Mulliken population analysis[48]) at the MP2/6-31+G* level of theory. All ab initio calculations were performed using the Gaussian98[49] program.

Results and Discussion

1. Gas-Phase Simulations. Although biologically irrelevant, gas-phase calculations can be used to provide insights into the behavior of APE and APE-H$^+$ that will be invaluable when it comes to assessing the effect of solvation on these molecules. Without a detailed understanding of the gas phase behavior of the molecules, it will be impossible to determine which of the observed effects are due to the solvent, and which are due to the underlying properties of the molecules. The Monte Carlo free energy profile calculated for rotation about φ_1 (Figure 4) identifies three minima, corresponding to the GA, AG, and GG conformations, at $\varphi_1 = 60°$, 180°, and 300°, respectively. The GA conformer is the least stable of the three minima, consistent with the fact that it has never previously been observed in any gas-phase studies. Of the two structures stabilized by the formation of an intramolecular hydrogen bond, both of which have been previously observed, GG is found to be the lower in energy. This result is in disagreement with the results obtained by Miller and Clary[5] who found, using an MM3 potential,[50] that the AG conformer is lower in energy. It is, however, in agreement with the MP2 calculations of Graham et al.[4] The greater stability of the GG conformer has been attributed to its ability to form a weak NH$-\pi$ or cation$-\pi$ interaction between the amino and aromatic groups.[25] The effect of protonation on the AG and GG conformers is small, but the GA conformer is destabilized significantly relative to the other conformations by the addition of the extra proton. This is due to the strengthening of the intramolecular hydrogen bond that results from the increased charge on the amino group.

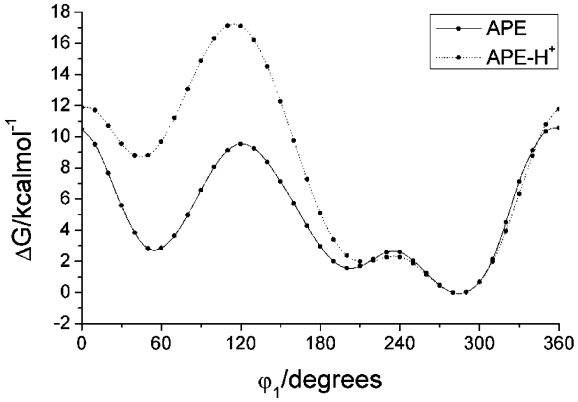

Figure 4. Gas-phase rotational energy profiles calculated from Monte Carlo simulations. $\varphi_1 = 60°$ corresponds to the GA conformation, $\varphi_1 = 180°$ corresponds to the AG conformation, and $\varphi_1 = 300°$ corresponds to the GG conformation.

1.1. APE. The results from the molecular dynamics simulations concur with previous gas-phase calculations; the AG and GG conformations are stable in the gas phase, whereas the GA conformation is not. Furthermore, no conformational interconversion between the GG and AG conformers is observed in the simulations (though it is important to be aware that this may be a function of simulation length). In both the AG1 and GG1 conformations, the molecules remain locked in their starting conformation throughout the whole simulation, the intramolecular hydrogen bond is maintained and all four rotatable bonds are fixed. Only in the simulation starting from the GA conformation is any real dynamic behavior observed (Figure 5). During the first 1.4 ns of the GA simulation, considerable movement about the dihedral angles φ_2 and φ_3 is observed; neither the OH nor NH_2 group is involved in any hydrogen bonding. After 1.4 ns, a rotation about the φ_1 dihedral angle moves the molecule into an AG type conformation, which allows for the formation of the intramolecular hydrogen bond and the dihedral angles involving the two hydrogen-bonding groups become much more stable.

1.2. APE-H$^+$. The results from the molecular dynamics simulations on gas-phase APE-H$^+$ agree with those from the APE simulations for the AG and GG conformers. Again, the starting conformation is retained throughout the simulations, and there is no evidence of any conformational change, where the difference lies is in the results for the simulation starting from

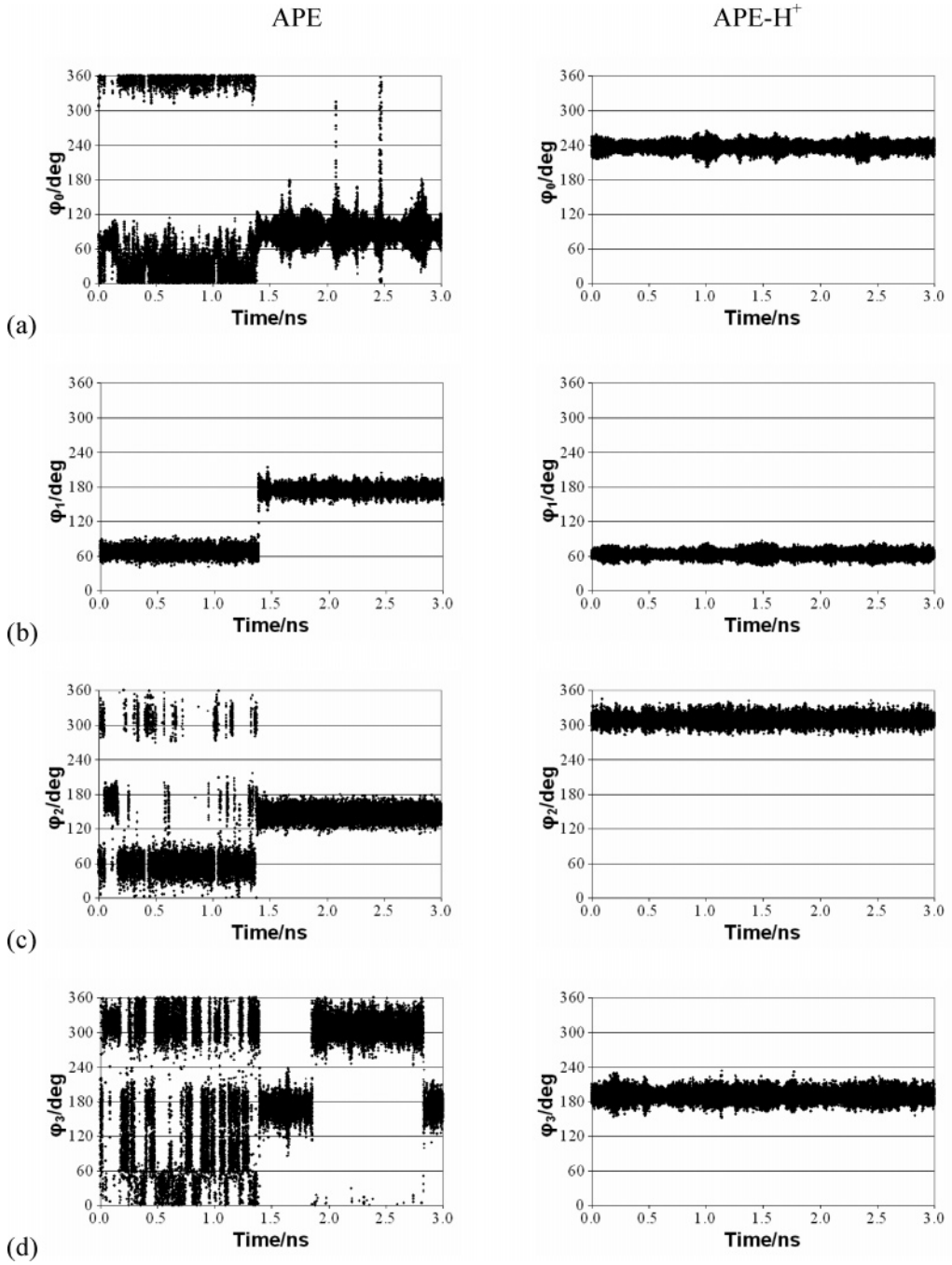

Figure 5. Dihedral angle monitors over 3 ns gas-phase MD simulations starting from the GA conformer for APE and APE-H^+: (a) φ_0, (b) φ_1, (c) φ_2, and (d) φ_3. $\varphi_1 = 60°$ corresponds to the GA conformation, $\varphi_1 = 180°$ corresponds to the AG conformation, and $\varphi_1 = 300°$ corresponds to the GG conformation.

the GA conformer (Figure 5). Now, the GA conformation persists throughout the simulation, and the flexibility that was seen in φ_2 and φ_3 when APE was in the GA conformer is no longer observed. The lack of rotation in φ_3 could be attributed to the formation of a cation$-\pi$ interaction, which would be much stronger than the analogous NH-π interaction in APE.[51,52] The additional stability of the hydroxyl group is harder to rationalize, although it is possible that the hydroxyl group is stabilized by the low fluctuation amplitude of the NH_3^+ group.

The calculations described in sections 1.1 and 1.2 provide an insight into the behavior of the molecules APE and APE-H^+ in the gas phase. It is, however, important to be aware of the limitations of these calculations. One such limitation is the lack of an explicit representation of polarization effects within the force field models used.[53] Polarization is known to play an important role in determining the gas phase interactions of many molecules,[54] and cation$-\pi$ interactions of the type mentioned in section 1.2 are known to be particularly sensitive to polarization effects.[55,56] Harder et al. have demonstrated the improvement that can be obtained for small molecule calculations through the inclusion of an explicit representation of polarization,[57] and it is wise to be mindful of these results when considering simulations employing fixed-charge models.

2. Hydrated Cluster Simulations. *2.1. APE.* The most obvious result from the simulations of the APE clusters is that the presence of just two water molecules destabilizes the system relative to the gas phase, and increased conformational interconversion is observed (Figure 6). A similar result has previously been observed by Zwier, who concluded that the formation of water bridges between functional groups increases the

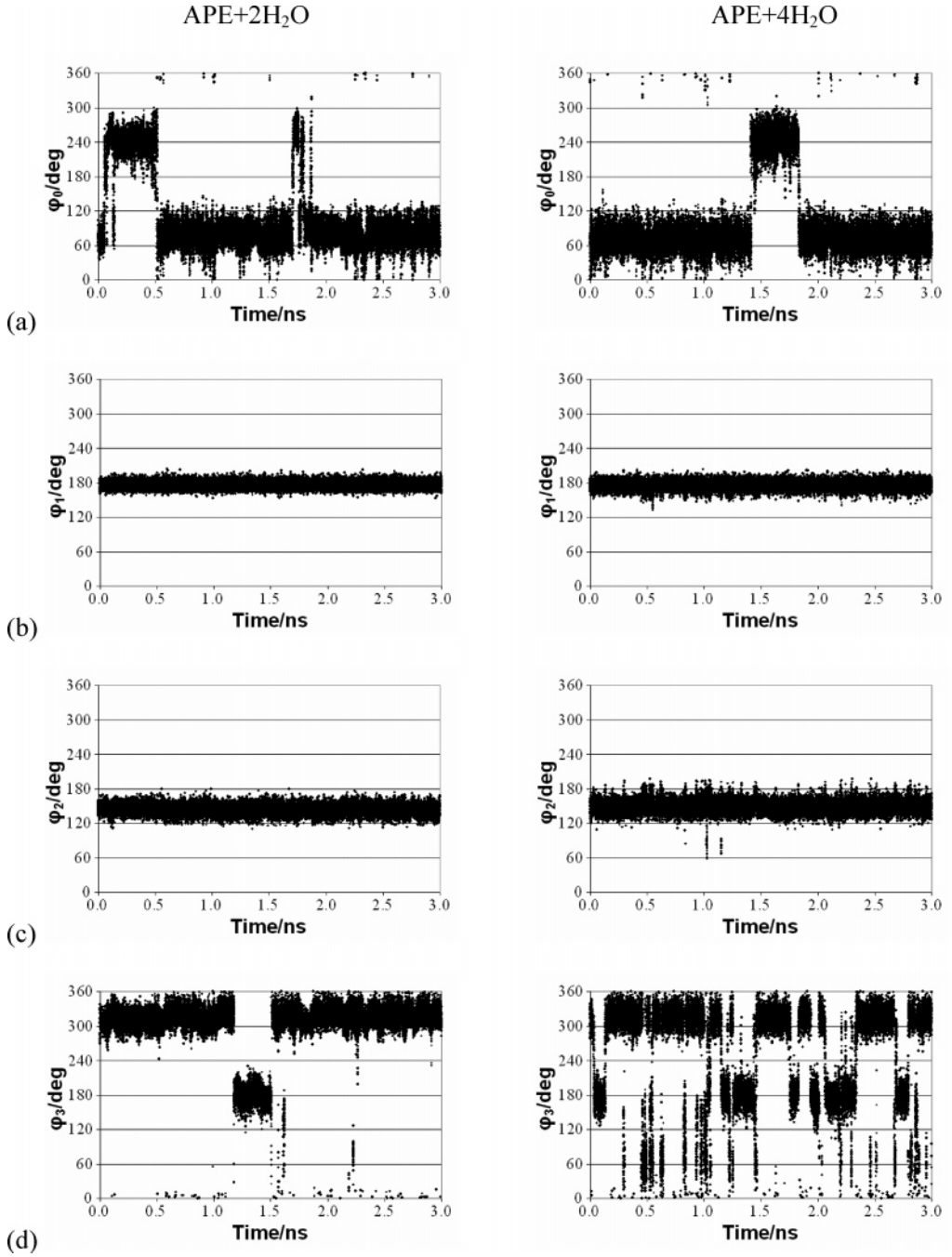

Figure 6. Dihedral angle monitors over 3 ns MD simulations for APE + $2H_2O$ commenced from AG-add conformation and APE + $4H_2O$ commenced from AG-droplet conformation: (a) φ_0, (b) φ_1, (c) φ_2, and (d) φ_3. $\varphi_1 = 60°$ corresponds to the GA conformation, $\varphi_1 = 180°$ corresponds to the AG conformation, and $\varphi_1 = 300°$ corresponds to the GG conformation.

conformational space accessible to a molecule.[58] In the simulation commencing from the AG-add structure (Figure 6), the AG conformation is preserved, together with the orientation of the hydroxyl group, but the presence of the water molecules has introduced flexibility in the other two dihedrals. This phenomenon is observed in all of the APE + $2H_2O$ simulations; the presence of the two water molecules introduces flexibility in φ_3, while the general conformation of the molecules remains fixed.

It is surprising that φ_3 is able to rotate without any concomitant rotation in φ_2. Rotation about φ_3 in the AG-add simulation would be expected to disrupt the intermolecular hydrogen bond within APE. This should, in turn, lead to movement in the φ_2 dihedral as the constraints on the hydroxyl group are removed. However, none is observed. To retain the hydrogen-bonded structure an inversion of the NH_2 group takes place, driven by the movement of the water molecules. This can be seen in the two different φ_3 dihedral angles that arise from each of the two NH_2 hydrogen atoms (Figure 7).

The simulation results can also be used to determine the mechanism by which this inversion takes place (Figure 8). In Figure 8a, an addition structure is present. As a water molecule moves in Figure 8b, it pulls NH_{green} with it, forcing a rotation. NH_{white} follows this rotation (Figure 8c). Repulsion between OH and NH_{green} forces the two groups apart (Figure 8d), and the NH_2 group is inverted (Figure 8e), allowing the original addition structure to reform.

Previous ab initio calculations by van Mourik[19] have shown that an umbrella inversion such as this is not possible for interconversion between adrenaline and pseudoadrenaline in the

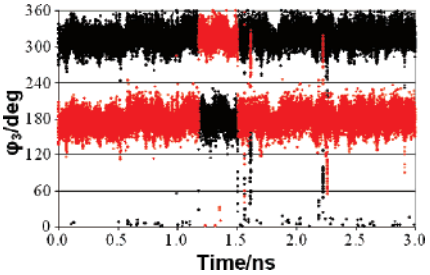

Figure 7. Monitoring of the two φ_3 dihedral angles during the 3 ns APE + $2H_2O$ simulation commencing from the AG-add conformation.

gas-phase due to a high energetic barrier. However, the replacement of the methyl group with a hydrogen atom would certainly lower that barrier. Ab initio calculations (results not shown) have been performed to assist in understanding the effect of the solvent on this process, but suggest that the mechanism shown above has an almost identical energetic pathway whether the water molecules are included or not. It follows that the role the water plays must be in allowing the two polar groups to move into a conformation in which they point toward each other, with the repulsion between these two groups providing the driving force for the actual inversion.

Simulations including APE and 4 water molecules were initiated from the AG-droplet and AG′-droplet conformations (Figure 3). In these simulations the conformational stability of the molecule is further disrupted relative to that of the gas-phase molecule (Figure 6). The φ_1 dihedral angle still remains fixed throughout the simulations but the φ_2 dihedral is starting to display some flexibility and all three staggered conformations about φ_3 are now accessible, with frequent interconversion between them.

As well as considering the behavior of the solute during these simulations, it is instructive to consider the behavior of the solvent molecules. To do this, the water density around APE has been calculated for each of the cluster simulations (Figure 9). It is clear from these water densities that there are preferred water binding sites around the molecules, and that these preferred binding sites vary according to the conformation of the solute.

Similar plots were produced by Miller and Clary[6] to analyze the results of their TPIMC calculations at 100 K. In the AG + $2H_2O$ conformations, Miller and Clary identified five hydration sites, four of which can be seen in Figure 9a, with the MD simulations used in this work seeming to offer an additional water site, possibly due to the higher temperature at which the simulations are performed. The discrepancies are indicated in Figure 9a, with the site not identified by Miller and Clary shown by an arrow, and the site identified by Miller and Clary but not identified in this work shown by a circle. In the GG + $2H_2O$ simulations, Miller and Clary observed only three hydration sites, two of which are seen in the MD simulations, with the third water position, located below the aromatic ring and stabilized by an OH-π interaction, not present in the MD simulations. Preferred instead are the water sites corresponding to hydrogen bonding to the hydroxyl group of APE. Energetic considerations would suggest that such an interaction is more likely than binding to the aromatic ring, with an OH−O hydrogen bond being considerably stronger than an OH-π hydrogen bond.[23]

Where the results from the MD simulations differ most significantly from those of Miller and Clary is in the clusters with 4 water molecules. Miller and Clary found, in agreement with the spectroscopic results of Macleod et al.,[7] that four water

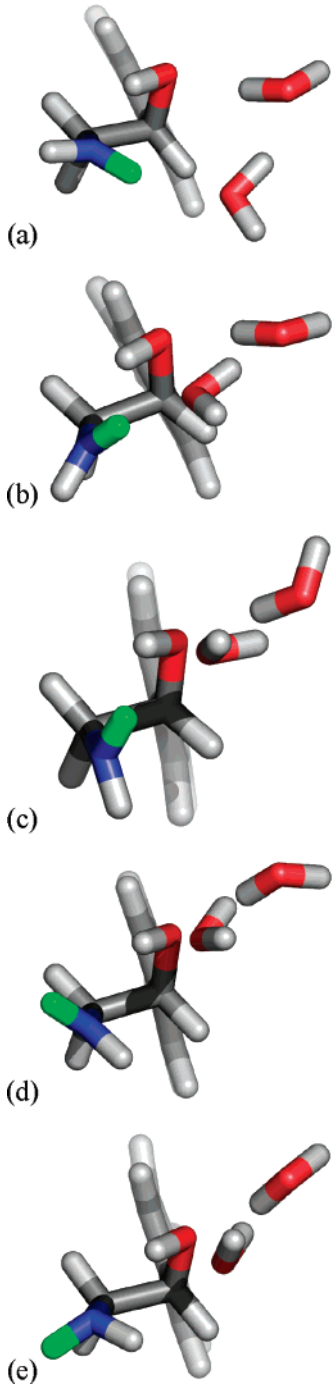

Figure 8. Mechanism for inversion of the NH_2 group, taken from the AG-add simulation.

molecules tended to form a droplet around the polar groups. The MD simulations, however, show a preference for structures in which the water molecules all bind to the solute (Figure 9c): a result that also disagrees with other published studies suggesting that water molecules prefer to form droplets in hydrated clusters.[59] Logically, it is surprising that the MD simulations should favor a structure in which all four water molecules bind to the solute. In the structures preferred by the MD simulations (Figure 9c), one of the molecules occupies a position below the aromatic ring, forming an OH-π type interaction with the ring. The OH-π interaction energy is around -3.17 kcal/mol^{-1},[60] considerably weaker than the water dimer OH−O hydrogen bond energy of -4.90 kcal/mol^{-1}.[61] Given this information, a structure in which a water molecule interacts with another water molecule rather than the aromatic ring would

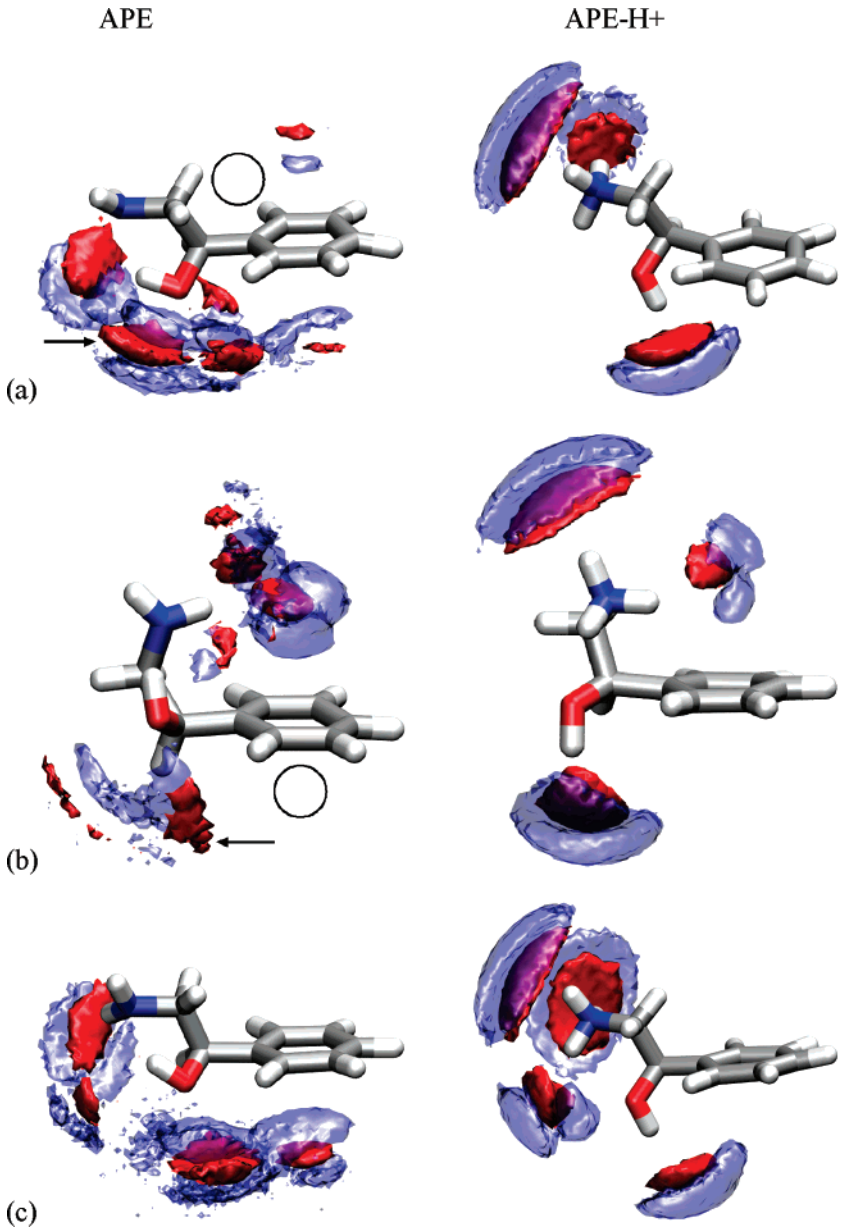

Figure 9. Water densities around APE-H^+ and APE obtained over 3 ns MD simulations for the conformations (a) AG + $2H_2O$, (b) GG′ + $2H_2O$, and (c) AG′ + $4H_2O$. Oxygen density is shown in red, hydrogen density in blue. In panels a and b, the arrow denotes a water binding site identified in this work but not in the work of Miller and Clary; the circle denotes a binding site identified by Miller and Clary that was not identified in this work.

seem preferable. Given that such a result has indeed been observed experimentally, it seems likely that the MD simulation results are in some way biased by the underlying force field. The most likely explanation is that the force field lacks the necessary complexity to accurately describe the complex, with the omission of polarization (as discussed above) no doubt an important consideration. A similar conclusion was also reached by Mobley et al., who attempted to optimize a fixed charge force field model and found that "with current charge models and force fields, hydration free energies cannot be computed to accuracies better than roughly 1 kcal/mol on average, suggesting that binding free energies probably also cannot reliably be computed with accuracies greater than 1 kcal/mol."[62]

2.2. APE-H^+. Simulations of APE-H^+ + $2H_2O$ again show far more dynamic flexibility than did the corresponding gas-phase simulations (Figure 10a). However, simulations including 4 water molecules show very little conformational interconversion (Figure 10b), as was the case in the gas-phase simulations. While these results may seem surprising, a possible explanation is offered by consideration of the water density around the solute in each of the cluster simulations (Figure 9). The water densities around APE-H^+ reveal that the hydration sites are much better defined than they were in APE. In the simulations involving two water molecules there are three hydration sites that are occupied during each simulation. Of these, one corresponds to an interaction with the hydroxyl group, and the other two involve interactions with the amino group. The hydroxyl group orientation is retained throughout the simulation, and so is the hydroxyl hydration. The conformational change that is observed arises from rotation of the amino group, which occurs with minimal disruption to the solvent structure. When four water molecules are present, the situation is different. Three water molecules bind tightly to the amino hydrogen atoms and the remaining water molecule binds to the hydroxyl group. Rotation of the amino group would involve breaking three hydrogen bonds, with a large associated energetic penalty, or rotating the whole

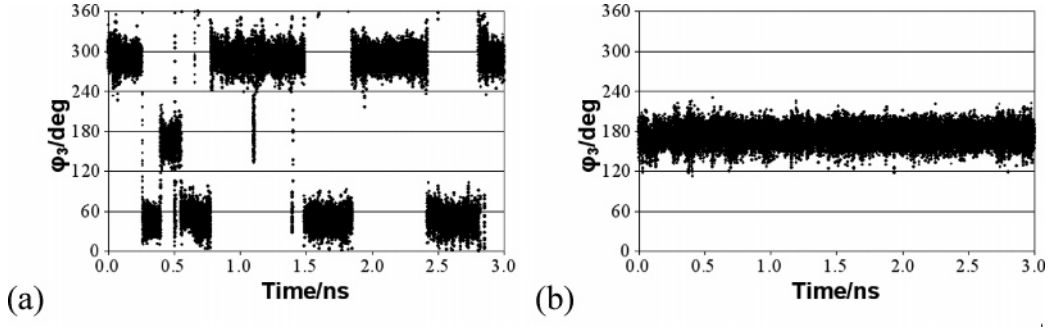

Figure 10. Dihedral angle monitors for φ_3 over 3ns MD simulation for (a) APE-H^+ + $2H_2O$ commenced from AG-ins conformation and (b) APE-H^+ + $4H_2O$ commenced from AG′-droplet conformation.

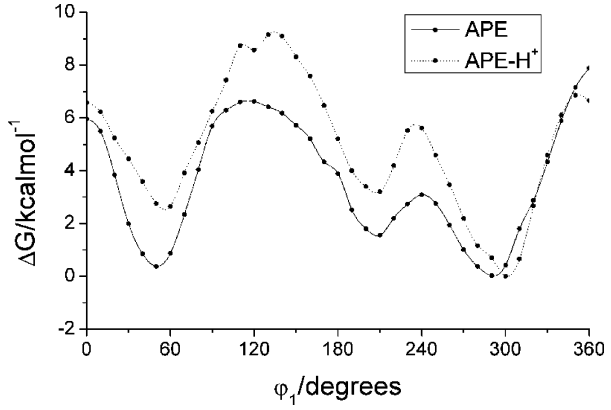

Figure 11. Monte Carlo calculated free energy profiles for rotation about φ_1 in APE and APE-H^+. $\varphi_1 = 60°$ corresponds to the GA conformation, $\varphi_1 = 180°$ corresponds to the AG conformation, and $\varphi_1 = 300°$ corresponds to the GG conformation.

solvation system, which would again be accompanied by a large energetic penalty. In an earlier paper,[37] it was shown that rotation of the protonated amino group in 1-(*R*)-phenylethylamine-H^+ required the presence of two solvation shells.

The other major difference between the APE and APE-H^+ cluster simulations is that the faces of the aromatic ring no longer appear as preferential sites for hydration as they did in APE. Presumably, the extra charge on the amino group in APE-H^+ increases the strength of the hydrogen bond to the point where an aromatic hydrogen bond can no longer compete; such an interaction is now only observed when it results from a bridging structure that links the ring to one of the polar groups, as in Figure 9c.

3. Solution-Phase Simulations. The Monte Carlo calculated free energy profiles for rotation about φ_1 in aqueous solution (Figure 11) are significantly different to the equivalent gas-phase rotational profiles (Figure 4). The greatest differences occur for the GA conformation; where previously this conformation was significantly higher in energy than either of the other minima, it is now lower in energy than the AG conformation for both APE and APE-H^+, and very close in energy to the GG conformation in APE. Previous studies have also found that solvation confers the greatest stabilizing effect on this conformation,[16] because the polar groups are able to form intermolecular hydrogen bonds, where intramolecular hydrogen bonds are inaccessible. In both molecules the GG conformation remains lower in energy than the AG conformation, which is in contrast to previous calculations on dopamine. Urban et al.[25] found that in the gas phase the gauche forms were preferred, but that preferential solvation of the anti form resulted in this becoming the preferred solution phase conformation. This result was attributed to the fact that the charged NH_3^+ group is more available for interactions with surrounding water molecules in the anti conformation; in the gauche conformation it is partially shielded by the aromatic ring.

Another important result of the addition of solvent is that the barriers to interconversion between the different minima have been considerably lowered. For example, in the gas phase the barrier to interconversion from AG to GA in APE-H^+ was 15.1 kcal/mol^{-1}, whereas in solution this has been reduced to 6.2 kcal/mol^{-1}. From these results, it is clear that both protonation and solvation have a significant effect on the biological behavior of APE.

3.1. APE. The most striking result from the solution-phase simulations of APE (Figure 12) is that the GA conformation of the molecule now appears stable. In the gas-phase this conformation was inherently unstable (Figure 5) but, as the Monte Carlo calculations indicated, the effect of solvation is to lower the energy of this conformation relative to the AG and GG conformations. Although the GA conformation is now stable, interconversion between the GA and AG conformations occurs quite frequently (Figure 12), demonstrating that the energetic barrier separating these two conformers is relatively small. The GG conformation, meanwhile, remains dynamically isolated from the other two conformers. This result seems to disagree with those obtained from the Monte Carlo simulations, where the barrier between the AG and GA conformations is larger than that between the AG and GG conformations. One important point to note here is that the relatively short simulation length employed, 3 ns, is not sufficient for complete sampling to have been obtained. For this reason, it is important that any conclusions about the relative energies of the conformations are based on the Monte Carlo calculations rather than the molecular dynamics simulations. Within the BOSS program,[44] the enthalpic and entropic contributions to the perturbation free energy are calculated using an umbrella sampling technique;[63] further analysis of these data reveals that the discrepancy arises as a result of a large entropic contribution to the Monte Carlo calculated free energy profile. If the enthalpic component alone is considered, then the barrier between the AG and GA conformations is found to be smaller than the barriers separating the GG conformer from either of the other conformations. Although this analysis provides an explanation for the difference between the results obtained from the Monte Carlo and molecular dynamics simulations, it is important to be aware of the limitations of this comparison. In this work two different force fields have been used: OPLS[45] for the Monte Carlo calculations and CHARMM[42] for the molecular dynamics. Although these two force fields are similar in terms of both their objectives and design,[54] they are not necessarily equivalent,[54] and any comparison of results obtained using the different methods should be treated with caution.[64] Mu et al. have, for

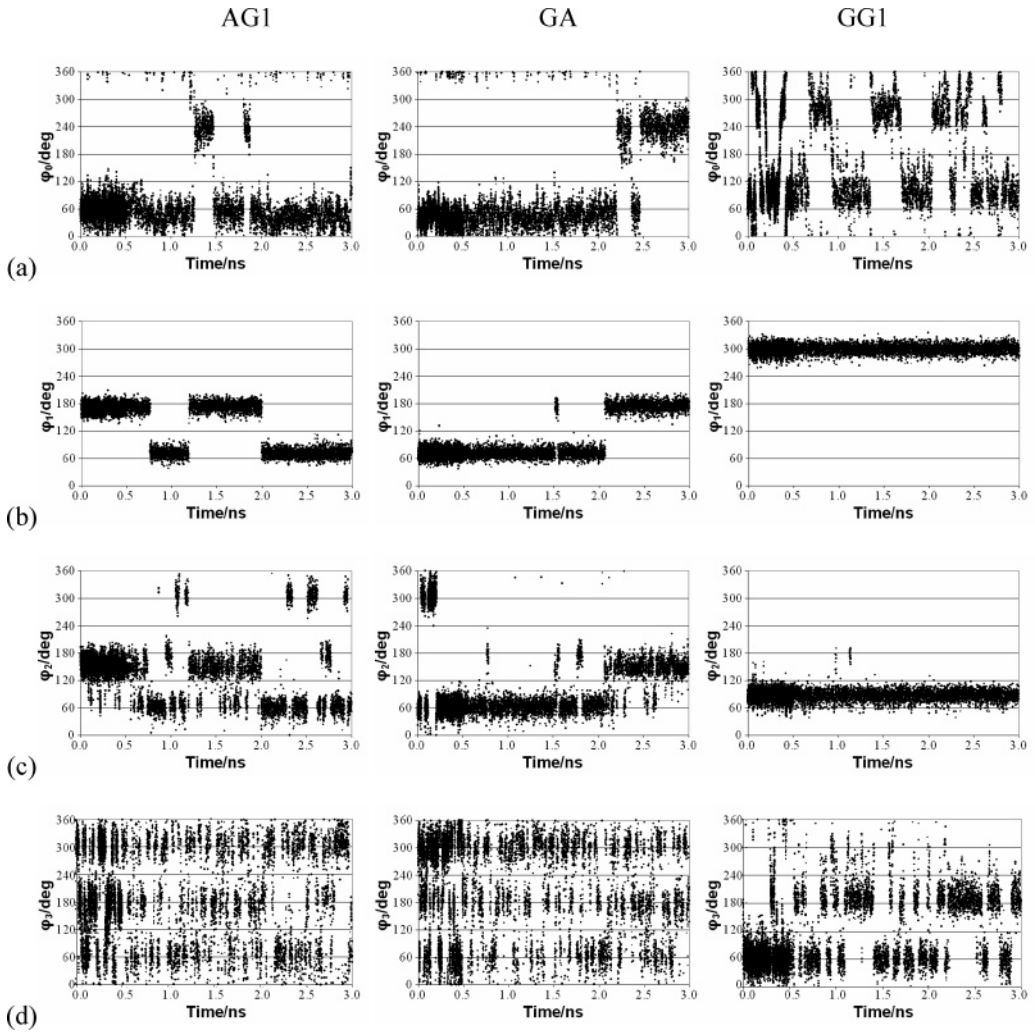

Figure 12. Dihedral angle monitors for 3 ns MD simulations of APE commencing from AG1, GA, and GG1 conformations (a) φ_0, (b) φ_1, (c) φ_2, and (d) φ_3. $\varphi_1 = 60°$ corresponds to the GA conformation, $\varphi_1 = 180°$ corresponds to the AG conformation, and $\varphi_1 = 300°$ corresponds to the GG conformation.

example, investigated the conformational dynamics of trialanine in water using a variety of force fields including both CHARMM and OPLS, observing considerable differences in the results obtained with the different models.[65]

Using the results from the molecular dynamics simulations, it is also possible to observe the mechanism for interconversion between the AG and GA conformations (Figure 13).

In Figure 13a, the hydroxyl group is effectively surrounded by two solvation spheres, the first consisting of H_2O_{orange} and H_2O_{cyan}, the second containing the H_2O_{black} molecules. The amino group, meanwhile, interacts with an H_2O_{blue} molecule that is involved in hydrogen bonding to the lower face of the aromatic ring.

As H_2O_{yellow} moves closer to the amino group (Figure 13a-b), competition begins for the amino hydrogen bond between H_2O_{yellow} and H_2O_{blue}.

H_2O_{yellow} becomes involved with the solvation structure around the hydroxyl group (Figure 13c), resulting in a bridging water network between the hydroxyl and amino groups. At the same time, the H_2O_{blue} molecules rearrange so as to retain the interaction between the amino group and the aromatic ring (Figure 13d), pushing the amino group further in the direction of the AG structure.

In Figure 13e, H_2O_{red} is pulled into the hydrogen-bonding network, further reinforcing the network to create an effective third solvation shell consisting of H_2O_{red} and H_2O_{yellow}, along with the amino group.

Single-point ab initio calculations, performed at the MP2/6-31+G* level of theory on the structures shown in Figure 13, have been used to evaluate the energetic profile for this conformational interconversion. Minimizations have been performed on the solute (with the key dihedral angles constrained) to remove the strain energy resulting from structural distortions introduced by the MD simulations, and the same procedure has been repeated on the bare solute molecule at each step in the reaction, to provide a comparison with the hypothetical gas-phase rotational profile. The resulting energy profile is shown in Figure 14.

The barrier to rotation in the gas-phase exceeds 5 kcal/mol^{-1}, which is well above the limit at which gas phase conformational change is feasible.[14] The presence of water molecules, however, serves to lower the energetic barrier to around 2 kcal/mol^{-1}, illustrating the effect that solvation can have on the energetics of the system. There are several other observations that should be made regarding this energy profile. In the gas-phase calculations, the AG conformation appears to be higher in energy than the GA conformation, contradicting previous results. This result occurs because the hydrated structure does not maintain the intramolecular hydrogen bond of the AG conformation. In the 'solution' calculation, the energy of the AG conformation

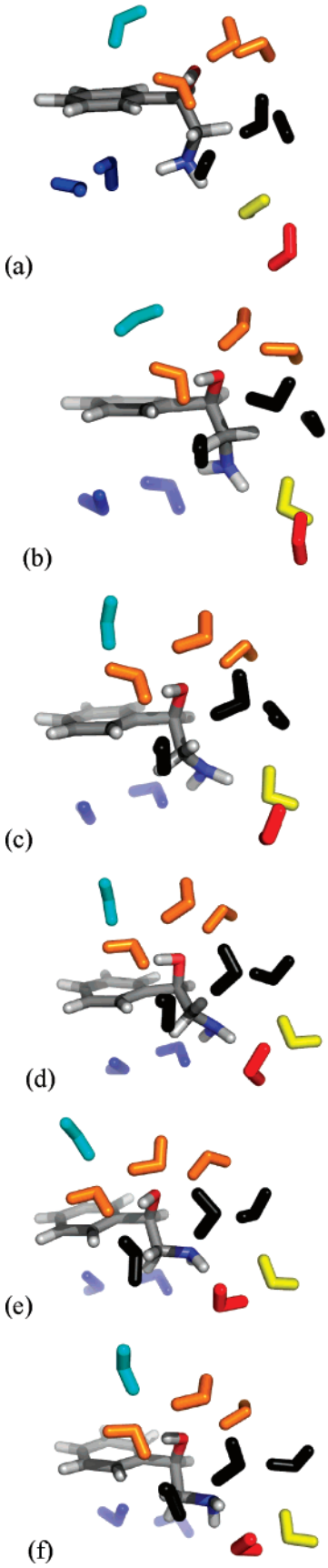

Figure 13. Mechanism for interconversion between GA and AG conformations in APE.

is extremely low, suggesting that movement back to the GA conformation would be impossible, when in fact the simulations show that such a process can occur (Figure 12). In reality the very low energy of the final structure is due to the incorporation of the extra water molecule (H_2O_{red} in Figure 13) into the solvation system, which allows for the formation of two additional hydrogen bonds. In bulk solution, this water molecule would be surrounded by other water molecules throughout this process, and the change in hydrogen-bonding energy would not be so extreme.

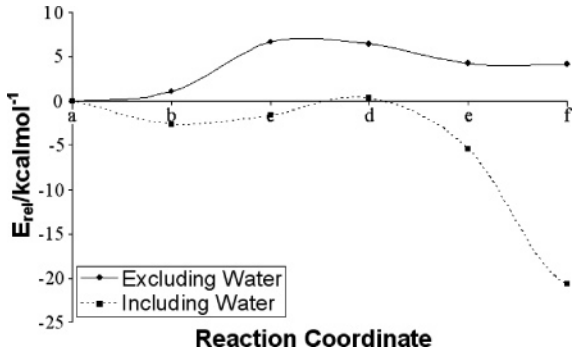

Figure 14. Energy profile for rotation about φ_1 mechanism, calculated at the MP2/6-31+G* level of theory. Lettering corresponds to that used in Figure 13.

Solvation also has a significant effect on the propensity for rotation about φ_2. In the gas phase, and in the hydrated clusters, no rotation about φ_2 was observed, except in the simulations commencing from the GA conformer, which does not contain an intramolecular hydrogen bond. In aqueous solution, the φ_2 dihedral remains fixed when the solute is in the GG conformation, but is able to rotate when the molecule is in the AG conformation.

These deviations in φ_2, and the concomitant variability in the φ_3 dihedral angle when the molecule is in either an AG or GG conformations, reveal that there is not a single hydrogen bond that persists throughout the entire simulation, as was the case in the gas phase. Rather, the nature of the hydrogen bonding between the groups is adapted according to the instantaneous solvation network around the molecule, and can be either direct or "bridged" by intermediate water molecules.

The situation is analogous in the molecule having a GG conformation, albeit with the complication that the NH_2 group is able to participate in the formation of a network of hydrogen bonds to the phenyl ring (Figure 15). This effect is also responsible for the slightly higher value of φ_0 that occurs in the GG simulation than in either of the other two simulations.

Only in the simulation commencing from the GA conformation is there noticeably different behavior. In this case, since there is no possibility for either direct or bridged hydrogen bonds between the two polar groups of APE, the same constraints on the geometry of the hydroxyl group are not present. In spite of this, only two of the three staggered conformations about φ_2 are readily accessible to the molecule, with the conformation having $\varphi_2 = 300°$ barely populated at all. It is also clear that each conformation has a 'preferred' orientation for φ_2. In the AG conformation, φ_2 is generally around 180°, and in the GA conformation, $\varphi_2 = 60°$ is preferred.

In the GA conformation, where any kind of direct interaction between the polar groups, or even any simple bridging interaction, is impossible, individual solvation shells are formed around each of the two polar groups. These solvation shells can then be weakly linked by intermediate water molecules. The result of this is a weak interaction between the two polar groups of the APE molecule, not sufficiently strong to lock the conformation of the molecule in place, but enough to dictate that if one

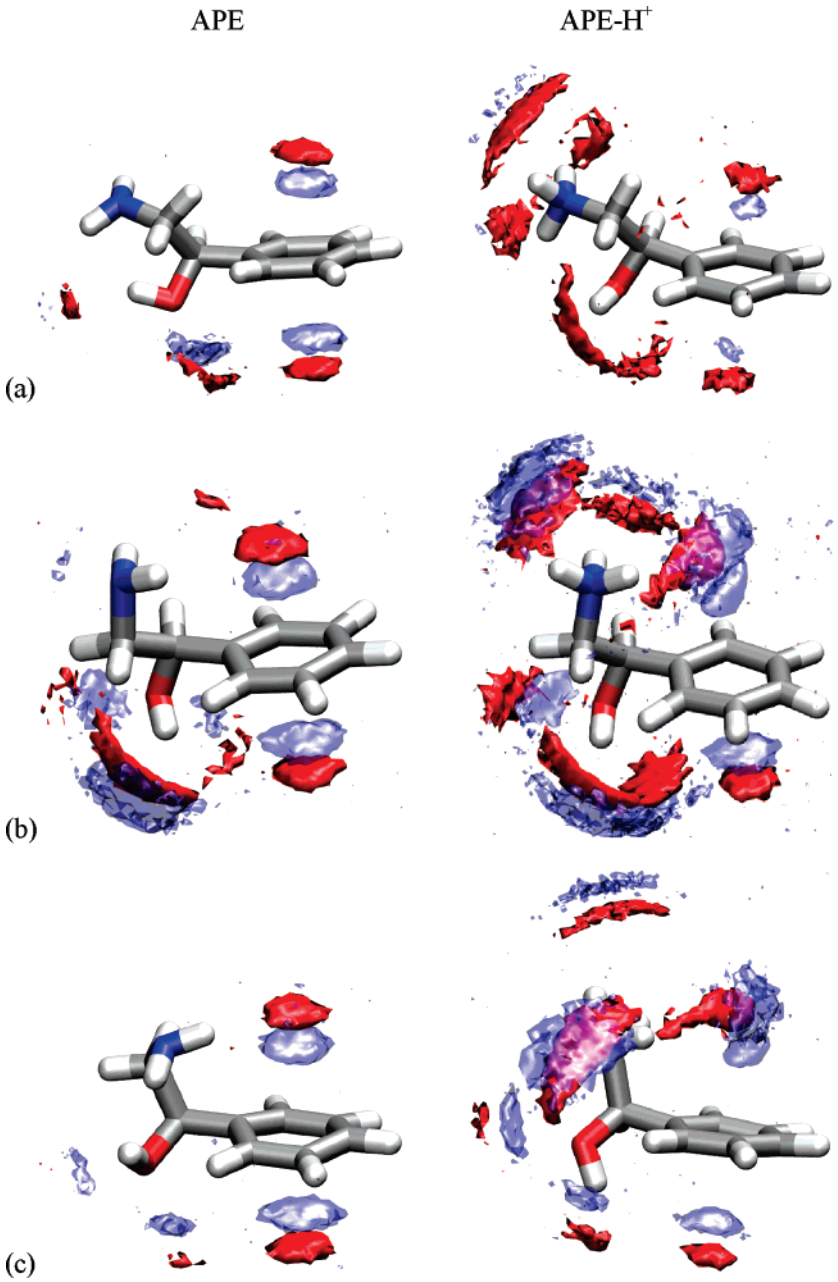

Figure 15. Water densities around APE and APE-H+ obtained over 3 ns MD simulations in bulk water for the conformations (a) AG, (b) GA, and (c) GG. Oxygen density shown in red, hydrogen density in blue.

of the solvation shells is disrupted, the other will move in response so as to regenerate the favorable interaction.

It is also instructive to compare these results with those from simulations of 1-(*R*)-phenylethylamine.[36] For PEA, in aqueous solution, free rotation of the amino group was observed. The fact that free rotation is not observed in the APE simulations is attributable to the interaction between the amino and hydroxyl groups, whether direct or mediated by bridging water molecules.

The water densities in Figure 15 offer an insight into why certain conformers are preferred over the others in solution. In APE the hydration pattern around each molecule is very similar. All three conformations have two hydration sites above and below the plane of the aromatic ring, as is commonly found for phenyl rings in water,[66] with a further two around the hydroxyl group. Only in the GA conformer is there any evidence for an additional hydration site around the hydroxyl group, in the GG and AG conformers the third hydrogen-bonding position around the hydroxyl group is occupied by the amino group. So, the formation of one additional hydrogen bond means that GA is stabilized more than either of the other two conformations, which are both stabilized approximately equally.

Throughout all of these conformations, there is evidence for the formation of water bridges[58] between the various functional groups. In both the GA and GG conformations, there is a water bridge between the amino and aromatic groups, and in all cases water bridges are formed between the hydroxyl and aromatic groups. Similar water bridges were also observed in 1-(*R*)-phenylethanol.[35] The AG and GG conformations both provide examples of water molecules that bridge between the hydroxyl and amino groups.

3.2. APE-H$^+$. Bulk solvation serves to increase the propensity for conformational change in APE-H$^+$ (Figure 16). This contrasts with the cluster simulations, where addition of two water molecules increased conformational flexibility while four water molecules largely removed this conformational flexibility. The addition of four water molecules was sufficient to ensure

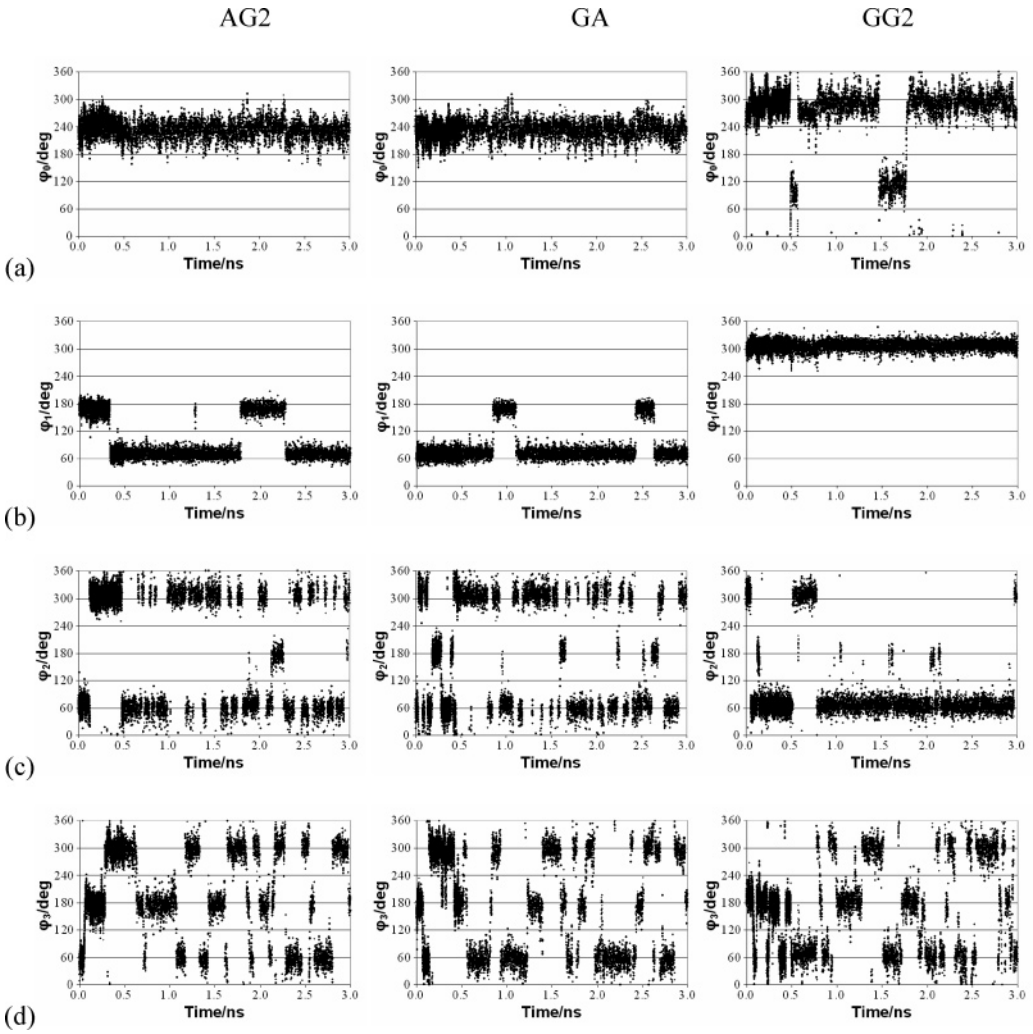

Figure 16. Dihedral angle monitors for 3 ns MD simulation of APE-H^+, commencing from AG2, GA, and GG2: (a) φ_0 (b) φ_1 (c) φ_2 (d) φ_3. $\varphi_1 = 60°$ corresponds to the GA conformation, $\varphi_1 = 180°$ corresponds to the AG conformation, and $\varphi_1 = 300°$ corresponds to the GG conformation.

that the four most stable hydration sites were occupied, imparting large barriers to conformational change. In aqueous solution, the presence of additional solvent molecules increases the possibility for conformational change by lowering energetic barriers. This result is in good agreement with results previously obtained for PEA,[37] where it was shown that the presence of a single catalytic water molecule can facilitate conformational change without disrupting the well defined solvation pattern around the solute.

The situation with respect to rotation about φ_1 in APE-H^+ is very similar to that in APE. Interconversions between AG and GA conformations are possible, whereas the GG conformation remains dynamically isolated. As with APE, however, it is important to note that the simulation length of 3 ns is not sufficient for complete sampling to have occurred, and the Monte Carlo results must be relied on for a realistic evaluation of the relative energies of the different conformations.

As in APE, φ_2 in APE-H^+ is destabilized by the presence of the solvent molecules, allowing for interconversion between the staggered forms. In the AG and GA conformations of APE-H^+, the most heavily populated of the staggered forms differ from those in APE, reflecting the fact that the hydroxyl group can no longer act as a hydrogen bond donor to the amino group. For the GG conformation, however, this is not the case; in both APE and APE-H^+ the simulations started from the GG conformations show the same orientation about φ_2, when one would expect to see a rotation of 60° because the hydroxyl group can no longer donate a hydrogen bond to the amino group. The solvent density around the GG conformation for APE-H^+ (Figure 15) reveals a broad region of water density located between the two polar groups, with the O density toward the solute. This site is occupied by a water molecule that accepts hydrogen bonds from both of the polar groups, forming a bridge between them and allowing the hydroxyl group to point toward the amino group.

While rotation about φ_3 does occur quite frequently in all three simulations, it is not nearly as common as in the APE simulations. This result is analogous to that found in the earlier work on PEA,[36] where the addition of an extra proton greatly increases the barrier to amino group rotation. This result arises because the NH_3^+ group has a much more rigidly defined solvation structure than does the NH_2 group (Figure 15). A water molecule is tightly bound to each of the amino hydrogen atoms, greatly increasing the barrier to rotation.

Further analysis of the simulation results reveals that the mechanism elucidated for rotation of the protonated amino group in PEA-H^+ can be extended to the equivalent rotation in APE-H^+, although the presence of the hydroxyl group adds extra complexity, and this mechanism has been described previously.[37]

From the results that have been obtained, it is possible to make some general comments about the effect of water on the conformational preferences of the APE molecule. The presence of the three functional groups in APE provides a framework around which water will arrange itself, with movements in any

TABLE 2: Experimental Conformer Populations in Aqueous Solution,[34] Illustrating the Effect of Addition/removal of a Functional Group (a More Recent Publication[67] has Disputed the Exact Figures for the Conformer Distribution in Amphetamine, but the Conclusions are Broadly Similar)

molecule	structure	P_{AG}	P_{GG}	P_{GA}
APE	$PhCH_2(OH)CH_2NH_2$	0.84	0.06	0.10
2-phenylethylamine	$PhCH_2CH_2NH_2$	0.56	0.44	
norephedrine	$PhCH_2(OH)CH_2(NH_2)CH_2$	0.21	0.79	
amphetamine	$PhCH_2CH_2(NH_2)CH_2$	0.50	0.05	0.45

one of these functional groups likely to disrupt the water structure and possibly the rest of the molecule with it. Similarly, movements in the water structure may disrupt the solute structure. Although there are significant similarities between the behavior of the simpler molecules 1-(*R*)-phenylethanol and 1-(*R*)-phenylethylamine studied previously[35,36] and those studied in this work, it is clear that there are also some major differences. Removal of any one of the functional groups within APE results in large disruptions to the conformational preferences and dynamic behavior that are observed (Table 2).

This observation raises the possibility that the results obtained here may be not be directly applicable to the catecholamine neurotransmitters; inclusion of the catechol hydroxyl groups to form noradrenaline will have an effect on the conformational behavior of the molecule. The role of the solvent in mediating interactions between the various functional groups within the molecule suggests that the catechol groups will be able to interact, albeit indirectly, with the other functional groups, thereby influencing the behavior of the molecule.

The conformer populations in Table 2 illustrate the significant effect that addition/removal of a functional group to/from APE can have. Removal of the hydroxyl group to form 2-PEA significantly reduces the stability of the anti conformation relative to the two gauche conformers, presumably because the absence of the hydroxyl group removes any possibility for the formation of water bridges, leading to a less well-defined water structure. In the gauche conformations, water bridges can still form between the amino and aromatic groups. Adding a methyl group to APE to form norephedrine has an even greater destabilizing effect on the AG conformation, a result which is harder to account for. Most puzzling of all though, is amphetamine, which differs from APE both in the removal of the hydroxyl group and the addition of a methyl group. That the AG conformer is destabilized relative to the gauche conformers is expected, but the significant preference for the GA conformation over the GG conformation is hard to rationalize, and illustrates the complexity of small molecules in aqueous solution, their conformational preferences being dictated by a subtle balance of forces arising from both the intrinsic properties of the solute as well as its interactions with the solvent.

Conclusions

Molecular mechanics simulations have been used to investigate the effect of bulk aqueous solvation on the conformational behavior of 2-amino-1-phenylethanol in both its neutral and protonated forms. The results obtained from gas-phase simulations are in line with previous experimental and theoretical work, suggesting that two well-defined conformations are present for each molecule, stabilized by the formation of an intramolecular hydrogen bond, and with barriers to rotation too high for any conformational change to be observed. The addition of just two water molecules is enough to destabilize the system, introducing some conformational change.

Bulk solvation serves to destabilize the molecule further still, allowing for a greater range of flexibility and conformational interconversion. These processes are mediated by the presence of water molecules. As well as affecting the energetics of the system, water also plays a significant role in the dynamic behavior of the solute. Analysis of the results of these simulations has allowed for the rationalization of previous experimental work at a molecular level, illustrating a range of effects that will help to explain the behavior of other, related, molecules.

Acknowledgment. CMB thanks the National Foundation for Cancer Research for funding, and GHG thanks Boehringer Ingelheim for a fellowship.

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