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Use of the Supermolecule Approach To Model the Syn and Anti Conformations of Solvated Cyclic 3′,5′-Adenosine Monophosphate

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The supermolecule approach has been used to model the hydration of cyclic 3′,5′-adenosine monophosphate, cAMP. Model building combined with PM3 optimizations predict that the anti conformer of cAMP is capable of hydrogen bonding to an additional solvent water molecule compared to the syn conformer. The addition of one water to the syn superstructure with concurrent rotation of the base about the glycosyl bond to form the anti superstructure leads to an additional enthalpy of stabilization of approximately −6 kcal/mol at the PM3 level. This specific solute−solvent interaction is an example of a large solvent effect, as the method predicts that cAMP has a conformational preference for the anti isomer in solution. This conformational preference results from a change in the number of specific solute−solvent interactions in this system. This prediction could be tested by NMR techniques. The number of waters predicted to be in the first hydration sphere around cAMP is in agreement with the results of hydration studies of nucleotides in DNA. In addition, the detailed picture of solvation about this cyclic nucleotide is in agreement with infrared experimental results.

Introduction

Water is a unique solvent, providing a high degree of stabilization of the secondary and tertiary structure of macromolecules.[1−5] The electrostatic repulsion between phosphate groups in nucleic acids is reduced because of the high dielectric constant of water and the influence of hydrated counterions. The conformation of DNA depends on the degree of hydration, as high relative humidity favors the most common B-DNA helical structure. At roughly 80% relative humidity each nucleotide in DNA is surrounded with approximately 20 waters in the primary hydration shell.[1] Not all 20 waters are directly hydrogen bonded to a nucleotide as eight of them are hydrogen bonded to other waters that directly surround the nucleotide. The 11−12 water molecules that are directly hydrogen bonded to the nucleotide have been grouped into three classes according to their binding affinity for the phosphate, phosphodiester plus sugar, and base moieties. Approximately five water molecules are clustered about the phosphate group, which has the highest binding affinity for water.[1−5] Binding affinity decreases for the phosphodiester and sugar and decreases more for the base.

Infrared spectroscopy experiments have revealed that the primary hydration shell is different from bulk water.[6−8] The 11 or 12 water molecules in the inner layer of the first hydration sphere block cations from the nucleotide[9] and do not freeze into the characteristic ice structure when a DNA solution is cooled below 0 °C.[8] The second hydration shell is indistinguishable from bulk water as far as crystallization to ice and permeability to ions is concerned.[1]

Quantum mechanical studies on the explicit hydration of water to polar molecules are difficult because of the high level of ab initio theory necessary for accurate modeling of hydrogen bonding and because of basis set superposition error (BSSE).[10−12] Treatment of an entire solute−solvent system at the ab initio quantum mechanical level is currently impossible. The cost of this approach limits pure quantum mechanical studies to the inclusion of a few solvent molecules within the supermolecule approach, for the study of specific interactions within the solute.[13] Quantum mechanical techniques for solvation generally treat the solute quantum mechanically and the solvent classically, either as discrete microscopic particles in Monte Carlo or molecular dynamics simulations or as a continuous medium. A large number of models are available; for reviews see refs 13−15. Treatment of solvent as a continuous medium is advantageous in terms of cost, and it works well. Application of state-of-the-art ab initio methods to the study of the primary hydration sphere of a nucleotide using the supermolecule approach is currently not feasible. However, it has been shown that the PM3 semiempirical method[16] does a reasonable job of modeling intermolecular hydrogen bonding between small neutral molecules,[16−20] water clusters,[21,22] base pairs,[23] and nucleotide base pairs.[24] The success of the PM3 method stems from the reduction in the nuclear core−core repulsion term present in the Hamiltonian, and a better parametrization than other NDDO methods.[20] The reparametrization does have a cost, however, as the PM3 method overestimates the attraction between hydrogen centers and nonpolar atomic centers.[20,25]

The success of the PM3 method in modeling the intermolecular hydrogen bonding of water clusters[20−22] combined with the relatively low computational cost of the method led us to use PM3 and the supermolecule approach to model the syn and anti conformations of anionic adenosine 3′,5′-cyclic monophosphate (cAMP). Cyclic AMP is known as a hormonal second messenger and plays a key role in the regulation of many metabolic processes. Experimental studies in the 1970s on the conformation of cAMP in solution are contradictory.[26−31] Two lanthanide ion probe studies led to opposite conclusions about the preferred conformation of cAMP.[26,27] An NMR study of gadolinium complexes of cAMP suggested that the syn and anti conformers both exist in solution and are in rapid equilibrium.[28] This conclusion was also reached by an ultrasonic relaxation study.[29] A proton NMR study suggested that the anti conformer is dominant.[30] Perhaps the most thorough study in this time period was by Lee and Sarma, who recorded proton NMR spectra for cAMP at 100, 270, and 300 MHz.[31] They concluded that the syn conformational domain should be accessible for 3′,5′-cyclic nucleotides but that quantitative estimation of the syn and anti populations was not possible in their study because

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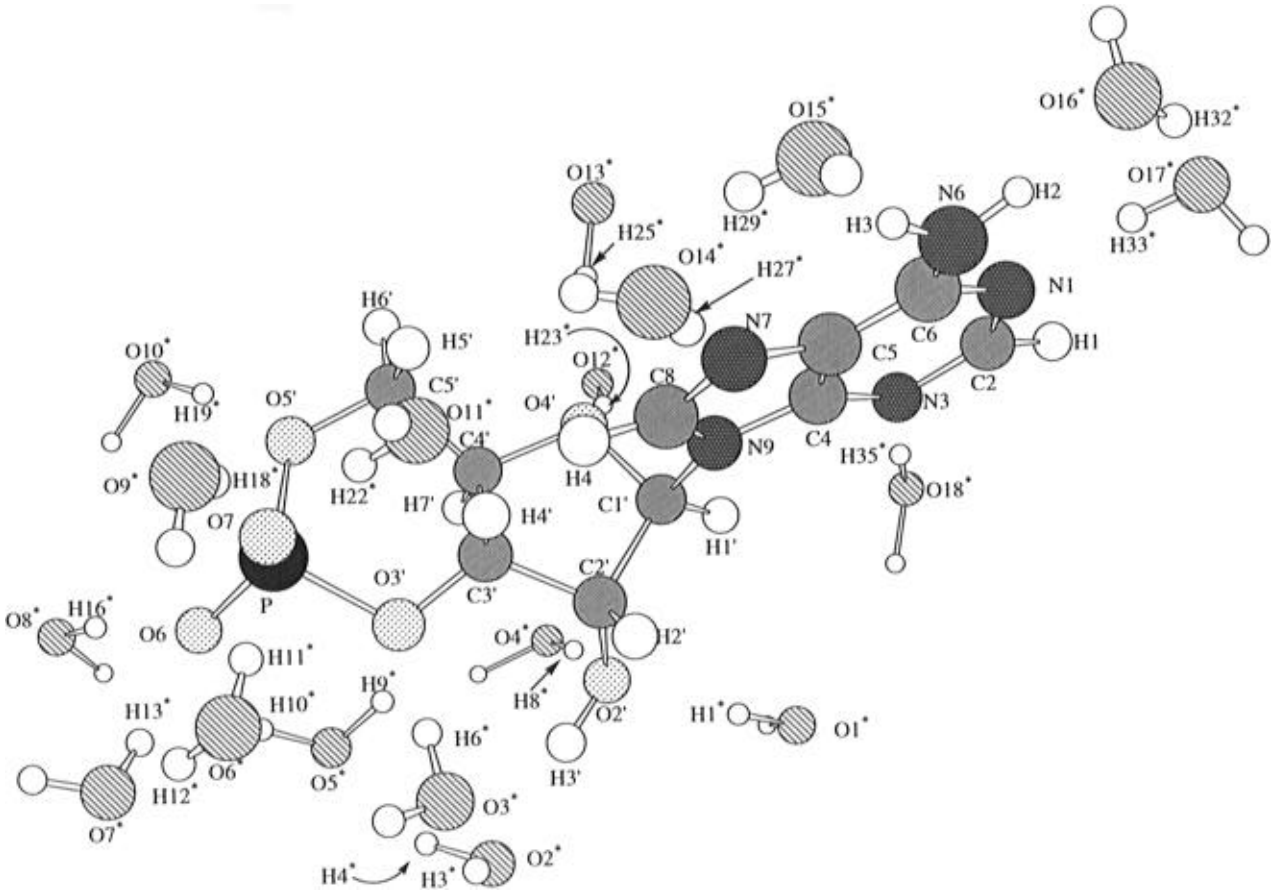

Figure 1. PM3 geometry-optimized superstructure of cAMP in the anti conformation with 18 hydrogen-bonded waters. The water oxygens are represented by a different shading pattern than are the oxygens that are covalently bonded within cAMP. The hydrogens involved in hydrogen bonding are labeled, to allow comparison of the figure with the tables.

limiting shift data in the pure syn and anti conformers were not available. They showed qualitatively that the population of syn conformers increases for cAMP compared to 3′-AMP and 5′-AMP. They expect that the cAMP base exists as an equilibrium mixture of syn and anti conformers but that the ratio of the two types of conformers remains uncertain.[31] At present there are no reliable NMR experiments on cAMP in solution that accurately specify the ratio of syn to anti.

An early molecular mechanics investigation of cyclic nucleotides predicted that cAMP shows a preference for the anti conformation by a ratio of 7:3.[32] A later molecular mechanics study was also consistent with the prediction that cAMP prefers to adopt an anti conformation.[33] Quantum mechanical computations at the AM1, PM3, and STO-3G//AM1 levels of theory were performed by Topiol et al.[34] They performed these calculations on cAMP in the chair−syn, chair−anti, twist boat−syn, and twist boat−anti conformations. The chair−anti conformation of cAMP was found to be the most energetically favorable by all three levels of theory, in agreement with experimental studies.[26−31]

In this paper the results of model building of chair−syn and chair−anti cAMP supermolecules, solvated with the primary hydration shell, combined with PM3 optimizations, are reported. The supermolecule approach is compared with the Cramer/Truhlar SM2 and SM3 methods.[35,36]

Method

The initial anionic cAMP structures used for explicit hydration were the PM3 optimized geometries for the syn and anti conformers.[37] The PM3 method was used instead of AM1 because of the ability of PM3 to model intermolecular hydrogen bonding.[16−24] Initial placement of waters was facilitated by making logical extrapolations from the results of experiments on the hydration of nucleotides.[1,6−8] An initial anti structure was built using the specific bonding motifs proposed in the above-mentioned research. This structure was optimized using SPARTAN 3.1[38] and frequency calculations were performed. If the resulting structure was a transition state, the first normal mode of the Hessian was examined to identify the transitory water molecule, and the structure was perturbed in an attempt to take it to a minimum. Waters that persisted as transition states were removed from the supermolecule. This trial and error procedure was repeated until the anti cAMP supermolecule was completely saturated with waters. During this process the number of intermolecular water−water hydrogen bonds was also maximized. This process was repeated for the syn conformer, using the results of the anti supermolecule as a guide.

Both supermolecules were built using Chem 3D Plus software (Cambridge Scientific Computing, Inc., Cambridge, MA) on a Macintosh II computer (Apple Computer, Inc., Cupertino, CA). The SPARTAN program,[38] implemented on Indy and Indigo2 workstations (Silicon Graphics, Mountain View, CA), was used to fully optimize all supermolecules. The semiempirical calculations were performed with the converge option selected and with one negative charge. Use of a unit Hessian matrix was often necessary in order to initially achieve a self-consistent field. The final anti and syn structures were established as stationary points and minima on the potential energy surface through frequency calculations.

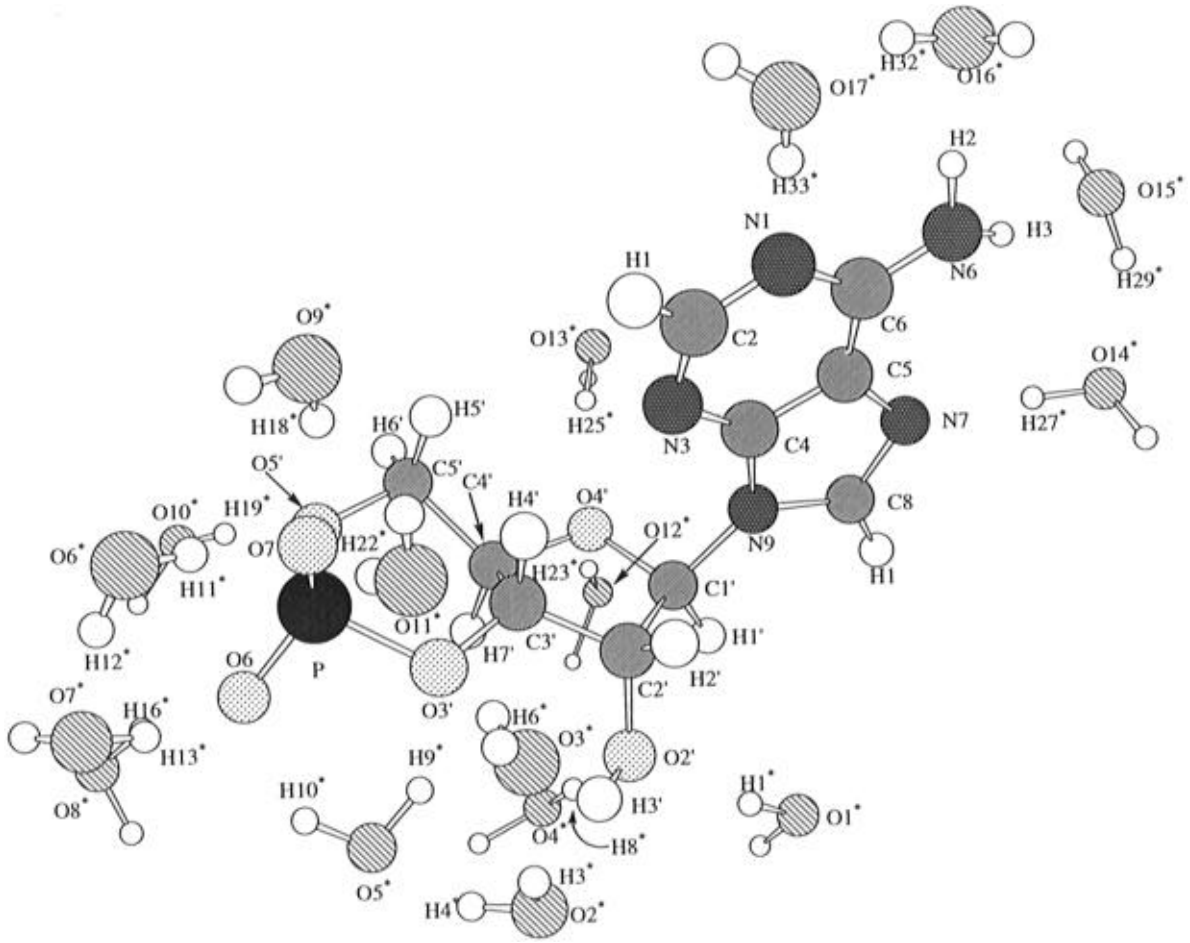

Figure 2. PM3 geometry-optimized superstructure of cAMP in the syn conformation with 17 hydrogen-bonded waters. The water oxygens are represented by a different shading pattern than are the oxygens that are covalently bonded within cAMP. The hydrogens involved in hydrogen bonding are labeled, to allow comparison of the figure with the tables.

The Cramer/Truhlar SM2 and SM3 methods[35,36] were applied to the previously obtained gas phase optimized geometries at the AM1, PM3, 3-21G*/HF, and 6-31G*/HF levels of theory.[37]

Results

Figures 1 and 2 show explicit hydration of 18 and 17 waters to cAMP in the anti and syn conformations, respectively. The water oxygens are represented by a different shading pattern than are the oxygens covalently bonded within cAMP. Only the hydrogens that are involved in hydrogen bonding are labeled. Figure 3 is a comparison of the anti and syn supermolecules with the unique anti water and hydrogen bond labeled. Table 1 displays all hydrogen bond distances, in angstroms, for both supermolecules. Table 2 displays all the hydrogen bond angles, in degrees, for both supermolecules. Hydrogen bond distances range from 1.76 to 1.87 Å, and hydrogen bond angles range from 140° to 179°. In Table 3 the results of the SM2 and SM3 continuum solvation calculations are displayed.

Discussion

Supermolecule Approach. The building of the two supermolecules was difficult, and the process will be briefly discussed here. It was suggested in the literature that one water bridges O2′ and O3′ of a nucleotide with the water acting as a hydrogen-bond donor to both oxygens.[39] We were not able to model this bridge in our hydration. However, an effective hydrogen-bond scheme was implemented by using two waters (H_2O2* and H_2-O3*) for the bridge.

Infrared spectroscopy investigations of DNA nucleotides are consistent with five to six waters hydrating the phosphate oxygens.[6−8] Results of Monte Carlo calculations agree with infrared spectroscopy.[40−43] The O7 atom in the crystal structure of cAMP accepts three hydrogen bonds from water molecules.[44] The anti supermolecule was constructed by first adding six waters to O7 and O6, three to each oxygen as suggested by previous results mentioned above. We then attempted to maximize the hydrogen bonds between these waters. The O6* water was addeded to form a hydrogen bond with the O7* water; these two waters form a five-membered ring with the phosphorous and its two axial oxygens (Figure 1). Upon optimization each of the waters stayed hydrogen bonded to cAMP. All attempts to add extra water around the axial oxygens failed as PM3 minimization removed the additional intended hydrogen bonds.

The final waters of the supermolecules were added to form hydrogen bonds with the remaining electronegative atoms of cAMP; O2′, O4′, O5′, N3, N1, N6, and N7. The O2′ and O4′ atoms of the furanose ring are sterically accessible to hydrogen bonding. Two waters were hydrogen bonded to each oxygen, by directing the water hydrogens toward the lone pairs of the furanose oxygens. This process was also applied to the O5′ and O3′ atoms of the phosphodiester, but upon minimization one water drifted away. We noticed that the angle between O6−P−O3′ and/or O6−P−O5′ might be appropriate for a water to bridge. This was attempted but upon minimization only the O5* water stayed in the hydration sphere, bridging the oxygens

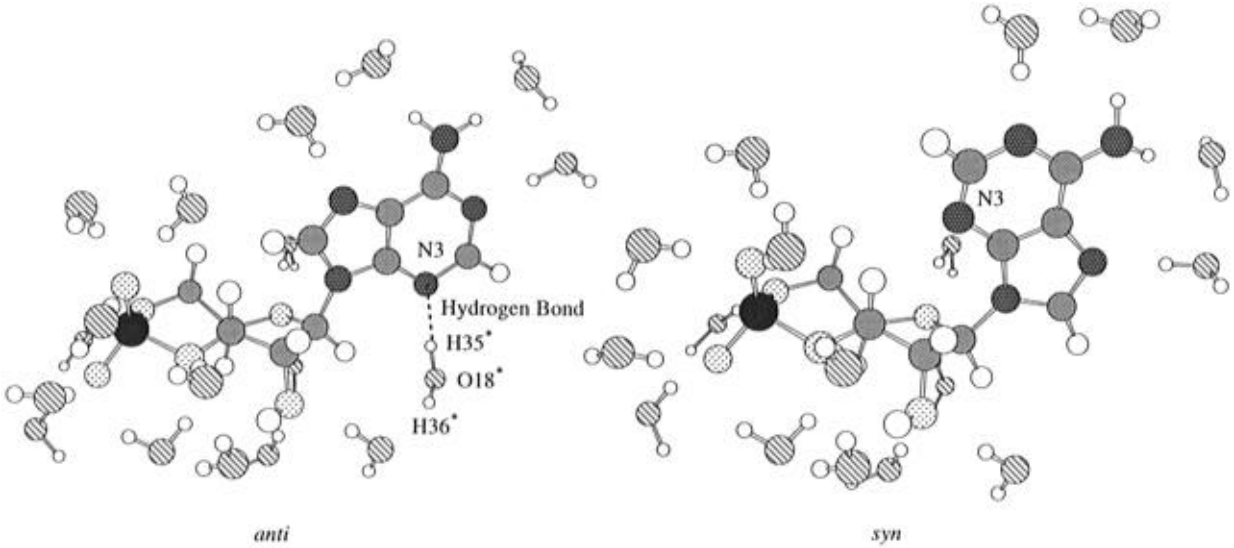

Figure 3. Comparison of the anti and syn supermolecules previously displayed separately in Figures 1 and 2. The unique anti water and its hydrogen bond are labeled on the left-hand side of the figure. The indicated hydrogen bond is not present in the syn supermolecule shown on the right-hand side of the figure.

TABLE 1: Hydrogen-Bond Distances (Å) for the PM3 Geometry-Optimized Supermolecules of Hydrated cAMP

	anti	syn
base to water		
H2−O16*	1.823	1.824
H3−O15*	1.820	1.828
water to base		
H33*−N1	1.803	1.805
H35*−N3	1.847	*a*
H27*−N7	1.805	1.793
sugar to water		
H3′−O2*	1.758	1.763
water to sugar		
H1*−O2′	1.819	1.826
H8*−O2′	1.816	1.814
H6*−O3′	1.824	1.830
H9*−O3′	1.854	1.852
H23*−O4′	1.867	1.859
H25*−O4′	1.856	1.853
H19*−O5′	1.846	1.844
water to axial phosphorus oxygens		
H10*−O6	1.852	1.834
H13*−O6	1.789	1.791
H16*−O6	1.785	1.786
H11*−O7	1.832	1.842
H18*−O7	1.810	1.807
H22*−O7	1.797	1.802
water to water		
H3*−O3*	1.797	1.799
H4*−O5*	1.808	1.808
H12*−O7*	1.823	1.830
H29*−O14*	1.772	1.776
H32*−O17*	1.779	1.776

[a] Bond distance applicable only for the hydrated anti structure.

TABLE 2: Hydrogen-Bond Angles (deg) for the PM3 Geometry-Optimized Supermolecules of Hydrated cAMP

	anti	syn
base to water		
N6−H2−O16*	173.1	174.0
N6−H3−O15*	161.6	159.7
water to base		
O17*−H33*−N1	171.4	170.4
O18*−H35*−N3	174.9	a
O14*−H27*−N7	171.1	174.2
sugar to water		
O2′−H3′−O2*	163.5	157.8
water to sugar		
O1*−H1*−O2′	161.2	155.0
O4*−H8*−O2′	171.7	178.8
O3*−H6*−O3′	164.8	161.0
O5*−H9*−O3′	140.2	142.6
O12*−H23*−O4′	166.3	172.2
O13*−H25*−O4′	167.8	160.5
O10*−H19*−O5′	171.9	174.7
water to axial phosphorus oxygens		
O5*−H10*−O6	140.8	139.9
O7*−H13*−O6	166.5	164.4
O8*−H16*−O6	162.0	162.4
O6*−H11*−O7	171.4	172.3
O9*−H18*−O7	160.1	157.9
O11*−H22*−O7	172.2	170.1
water to water		
O2*−H3*−O3*	146.3	143.1
O2*−H4*−O5*	153.9	154.2
O6*−H12*−O7*	157.0	156.7
O15*−H29*−O14*	154.5	154.7
O16*−H32*−O17*	154.2	155.2

[a] Bond angle is applicable only for the hydrated anti structure.

O6 and O3′. This water is unique because it is the only one that bridges two atoms of cAMP. It also has the smallest hydrogen bond angles, 140° and 141° (Table 2). The O5′ oxygen is incapable of accepting hydrogen bonds from two waters with the current hydration sphere.

The two superstructures that are displayed in Figures 1 and 2 have one major difference: the anti supermolecule has one extra water and subsequently one more hydrogen bond than the syn supermolecule. There are a total of 18 waters forming the anti supermolecule and 17 for the syn. The N3 atom on the base of the anti supermolecule is available as an additional hydrogen-bond acceptor for water. The N3 atom in the syn supermolecule is sterically excluded from this interaction. Aside from this one difference, the detailed interactions between the water molecules and the two cAMP conformers are quite similar. This is seen by comparing the figures and Tables 1 and 2.

The ΔH for the hydrogen bonding of one water to the syn supermolecule to form the anti supermolecule is easily estimated from the PM3 heats of formation of a water (−53.427 kcal/mol), and the two supermolecules (syn, −1293.901; anti, −1353.189). Thus ΔH for the isomerization of the syn supermolecule to the anti supermolecule upon incorporation of one additional water molecule is −5.86 kcal/mol. These results suggest that the conformation of anti cAMP is stabilized better

TABLE 3: Comparison of cAMP Energetics from Single-Point Calculations Using the Cramer/Truhlar SM2 and SM3 Solvation Methods[35,36] (Energies, in kcal/mol, Relative to Most Stable Species)

conformation	SM3//PM3	SM2//AM1	SM3//3-21G*	SM2//3-21G*	SM3//6-31G*	SM2//6-31G*
anti	0.0[a]	0.0[b]	0.0[c]	0.0[d]	0.0[e]	0.0[f]
syn	2.5	0.7	0.6	0.8	1.1	0.9

[a] Semiempirical heat of formation is −374.4 kcal/mol. [b] Heat of formation is −371.0 kcal/mol. [c] Heat of formation is −333.6 kcal/mol. [d] Heat of formation is −341.7 kcal/mol. [e] Heat of formation is −332.4 kcal/mol. [f] Heat of formation is −338.0 kcal/mol.

in aqueous solution than is the syn conformer, as a direct result of an additional specific hydrogen-bond interaction that is only available in the anti structure.

Cramer/Truhlar Results. Quantum continuum methods combine the quantum mechanical treatment of the solute with a continuum description of the solvent. In these methods the dielectric continuum reacts against the solute charge distribution generating a reaction field, which in turn interacts with the solute. Since the solute is treated quantum mechanically, a rigorous representation of the charge distribution is obtained, the mutual solute−solvent polarization is accurately included, and solvent-induced changes in the molecular properties of the solute can be evaluated.[13] The reaction-field methods developed by Cramer and Truhlar use semiempirical Hamiltonians to treat the solute, and the boundary with the dielectric continuum is defined by a molecular-shaped cavity built from the Born radii of the different atoms in the molecule. The reaction field is based on multipole expansions. Further details can be found in the literature.[13−15,35,36,45,46] The SM2 method[35,46] uses a modified AM1 Hamiltonian operator for single-point self-consistent-field calculations to allow solvent-induced electronic effects, and the SM3 method[35,36] uses a modified PM3 Hamiltonian operator in a similar manner. Both methods can also be used for complete geometry optimizations to probe solvent-induced geometric distortion of the solute.

Results of the application of the SM2 and SM3 single-point calculations to the anti and syn conformers of cAMP are given in Table 3. Complete SM3 geometry optimization on both the anti and syn structures took 1841 min of CPU time for each calculation, whereas the SM3 single-point calculation took 45 s. Full geometry optimizations decreased the energy difference between anti and syn conformers by just 0.2 kcal/mol with no significant changes in geometry. The SM3 single-point calculations adequately reveal the relative energies of the conformers with 0.04% of the CPU usage.

It has been shown previously that ab initio geometries, optimized at the 3-21G* and 6-31G* levels, compare better than semiempirical geometries, relative to the crystal structure of cAMP, particularly for the glycosyl torsion angle of the anti conformer.[37] This previous result, combined with the 1.8 kcal/mol difference between the SM3 and SM2 relative energies (Table 3), led us to apply the Cramer/Truhlar method to the previously determined gas-phase ab initio geometries. The single-point calculations presented in Table 3 on the ab initio geometries decrease the relative stabilities between the anti and syn conformers compared with the supermolecule approach, but still favor the anti conformation by roughly 1 kcal/mol. The anti conformer is predicted to be only slightly more favored in solution with the continuum approach.

Comparison between the Supermolecule and Continuum Approaches. Both approaches favor the anti conformer of cAMP. While the semiempirical results are necessarily qualitative, they do make an interesting prediction that can be tested experimentally. With the continuum model, the anti conformer is only slightly favored in solution. It is well-known that continuum methods are inexpensive yet accurate approaches for determining the effects of bulk solvent. However, they are not considered useful for the representation of specific solute−solvent interactions, which is what the supermolecule approach predicts in this system. With the supermolecule approach, the anti conformer is stabilized by an additional solvent molecule. Even if the details of the interactions of the water molecules in the first solvation shell are different, the models clearly predict that only the anti conformer has the N3 atom of the adenine base accessible to solvent. In the syn supermolecule, the N3 atom is screened from direct hydrogen-bond interactions with the solvent. Therefore the supermolecule approach predicts that the number of specific solute−solvent interactions changes by one during the conformational change from syn to anti. This is an example where one expects a large solvent effect, as the largest influence of solvent occurs either when there are great differences in polarities of the system of interest, or when the number of specific solute−solvent interactions changes in the system.[13] Besides suggesting an NMR experiment to test this prediction, it would be interesting to calculate the free energy of solvation difference and the equilibrium ratio for the isomerization of syn cAMP to anti cAMP, using the best theoretical methods available.[13]

Conclusions

Explicit hydration of cAMP with the PM3 method indicates that the anti conformer is capable of binding an additional water in the first hydration sphere, when compared with the syn conformer. The water bound to N3 of the adenosine base is sterically hindered from being a hydrogen-bond acceptor in the syn conformer. The number of waters predicted to be in the first hydration sphere around cAMP is consistent with the results of the hydration about nucleotides in DNA.[1] In addition, the detailed interactions are also consistent with experiment, where the binding affinity of water for nucleotides decrease in order from phosphate, to the phosphodiester plus sugar, to the base.[1−5] Application of the Cramer/Truhlar SM2 and SM3 continuum models to the gas-phase geometries suggests that the difference between the anti and syn conformers is about 1 kcal/mol. Use of the supermolecule approach with PM3 predicts that the addition of one water to the syn superstructure with concurrent rotation of the base about the glycosyl bond to form the anti superstructure has a ΔH of approximately −6 kcal/mol. We expect that the supermolecule approach makes the most accurate prediction, as this is an example of a specific solute−solvent interaction and is not a bulk solvent effect. Modern NMR techniques could be used to determine the ratio of syn to anti conformers in solution to check this prediction, and quantum discrete methods may give additional insight into the solvent effect for the cAMP system.

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