A Study of the Tautomeric Equilibria of 2-Hydroxypyridine/2-Oxopyridine and of Cytosine in Water Using the Coupled Reference Interaction Site Model(RISM)/Molecular Dynamics (MD) Approach

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We present an application of the recently proposed coupled reference interation site model (RISM)/molecular dynamics (MD) solvation free-energy method (Freedman, H.; Truong, T. N. *Chem. Phys. Lett.* **2003**, *381*, 362; *J. Chem. Phys.* **2004**, *121*, 2187) to study the hydration effect upon the tautomeric equilibria of 2-hydroxypyridine and 2-oxopyridine and of cytosine in water. In this methodology, simulation trajectories of solvated systems are computed and averaged to determine radial distribution functions, which are then inserted into a RISM expression for the solvation free energy. The computed differences in free energy of hydration for the two tautomerizations agree well with the experimental data as well as results from previous free-energy simulations. This is particularly encouraging since the coupled RISM/MD method requires a single MD simulation as compared to the more complicated and computationally demanding free-energy simulation methods.

1. Introduction

Both of the molecules 2-oxopyridine, which has often been studied as a minimal nucleic acid prototype, and the nucleic acid base cytosine can undergo tautomerizations in solution. There is a marked sensitivity of these tautomeric equilibria to solvation. The relative stabilities of the two tautomers 2-oxopyridine and 2-hydroxypyridine in a polar solvent are reversed from the order occurring in vacuo. Various computational models have been applied to determine the free-energy differences between these tautomeric pairs in solution in an attempt to understand the effect of a solvent on liquid-phase tautomeric reactions. These have included classical molecular dynamics (MD) free-energy simulation,[1−3] quantum mechanical/molecular mechanical (QM/MM) free-energy simulations,[4,5] continuum models,[2,3,6−13] reference interation site model (RISM),[14,15] and quantum chemical studies of H-bonded water complexes.[16]

The most stable, amino form of cytosine can undergo tautomerization to form an imino tautomer. The particular tautomers of cytosine being considered here are depicted in Figure 1. Figure 1 also shows the tautomerization of the related molecule 2-hydroxypyridine to 2-oxopyridine. Alternative hydrogen-bonding patterns caused by tautomerization of nucleic acid bases in DNA may lead to base-pair mismatch. Such mispairing can then be followed by DNA mutation. Water has a far larger stabilizing effect upon the amino form of cytosine than upon the imino form. This is significant in that it leads to reduced rates of mutation; any situation, however, in which the molecule is less hydrated may result in more frequent occurrences of mutations.[2,3]

Solvent treatment within a computational model can range from a less-detailed description such as by a continuum model to a more explicit, atomistic description, such as is employed in a molecular dynamics simulation. In a continuum model, solvent atoms are treated implicitly as a continuum environment.

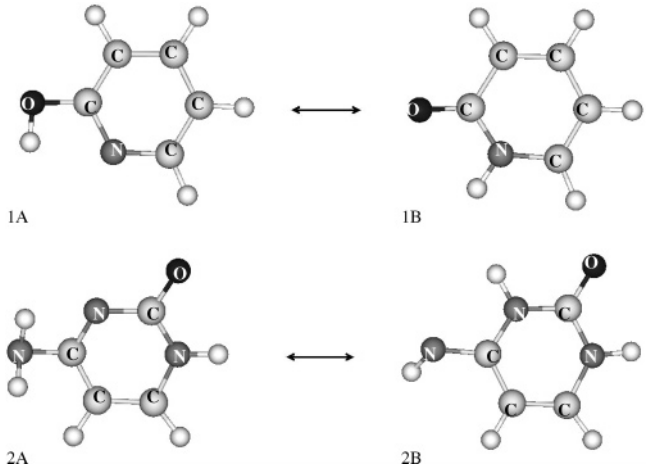

Figure 1. Structures of tautomers averaged over a 2 ns MD trajectory using restrained geometries. (1A) 2-hydroxypyridine; (1B) 2-oxopyridine; (2A) amino-cytosine; (2B) imino-cytosine.

The electrostatic potential surrounding a solute is solved for and used to determine the electrostatic component of the Gibbs free energy of solvation. However, this approach discounts specific solute−solvent interactions, which may be important, such as hydrogen bonding; for example, in both tautomerizations being considered here, studies have demonstrated the marked effect on the equilibrium of the presence of a single water molecule interacting with the solute molecule.[2,16] This issue has been addressed by the application of improved continuum models using nonspherical solute cavities, with solute charge distributions represented as point charges on the surface. Several such continuum methods have been applied to the 2-hydroxypyridine/2-oxopyridine and cytosine equilibria in the past and have had some success.[2,6,9,11,12,16] However, continuum methods are dependent upon the availability of a range of parameters suitable to the system being considered.[17,18]

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In a molecular simulation, solvent molecules are explicitly included. Although more realistic, the use of an explicit solvent drastically increases the computation time. The methods known as free-energy perturbation (FEP) and thermodynamic integration (TI) can be used to determine solvation free-energy differences from simulations. Free-energy simulation calculations are difficult to carry out, however, requiring that a range of simulations be carried out as one of two solutes being compared is mutated into the other.[18,19]

An alternative approach for modeling solvation is the use of probabilistic methods from statistical mechanics. The reference interaction site (RISM) model is a statistical mechanical model in which the radial distribution functions of solvent sites surrounding solute sites are calculated.[20−25] Perturbation theory has been used to derive an expression for solvation free energy as a function of these correlations.[26−28] Thus in the RISM theory, specific solute−solvent interactions are accounted for in solvation free-energy calculations without the intense computational effort associated with the free-energy perturbation simulation approach.[29,30]

Simulation results are generally looked upon as highly accurate and dependable. On the other hand, RISM-calculated radial distribution functions may differ appreciably from those determined by simulation. This factor can affect the accuracy of RISM-calculated solvation free energies.[31,32] In the tautomerization of 2-oxopyridine, two sets of RISM calculations have been carried out, one of these, by Shao et al.,[14] giving close agreement with experiment. In this RISM study, however, some parametrization of adjustable van der Waals radii and well depths had to be performed first to yield an optimal fit of RISM-calculated solvation free energies for a set of small organic molecules.[14] In a second study performed by Sato et al.,[15] in which the RISM/SCF method was applied to obtain ab initio partial atomic charges for the solute, the solvation free-energy difference determined was noticeably overestimated.

We have recently proposed the coupled RISM/simulation approach for the determination of solvation free energies. In this method, radial distribution functions are calculated by averaging over a Monte Carlo (MC) or molecular dynamics (MD) simulation, using an explicit solvent representation. These are then used to calculate the solvation free energy from a RISM expression. In contrast to standard free-energy simulation methods, the coupled RISM/simulation methodology requires only a single MD simulation. Our past results have demonstrated that this approach is feasible, and moreover it can calculate reasonably accurate results for small molecules.[33,34]

In this study, we apply the recently proposed coupled RISM/MD method to computing solvation free energies for the tautomeric equilibria of 2-hydroxypyridine → 2-oxopyridine and of amino-cytosine → imino-cytosine in water. We are particularly interested in developing the coupled RISM/MD method for applications to biological systems. The standalone RISM method runs into difficulty when solving for the radial distribution functions of solutes with over a few hundred sites,[35] and it may be that, for this reason as well as its improved accuracy, the coupled RISM/simulation methodology will serve as a useful alternative to the standalone RISM/HNC approach for large systems. This study would establish a framework for applications of this coupled RISM/MD method to biophysical problems involving DNA.

2. Methodology

In a coupled RISM/simulation calculation, radial distribution functions are first calculated by averaging over a simulation trajectory. In addition to radial distribution functions, g, direct correlation functions are also needed to find a solvation free energy by the RISM expression. Two approaches are used for computing these direct correlation functions. One form of the direct correlation function, c^{HNC}, is solved for which satisfies the HNC equation, and a second form, c^{RISM}, is solved in reciprocal space from the RISM equation. Solving the RISM and HNC equations yields the following equations used to determine the separate components c^{HNC} and c^{RISM}:[33,34]

$$c_{uv}^{\mathrm{HNC}}(r) = -\beta u_{uv}(r) + h_{uv}(r) - \ln(h_{uv}(r) + 1) \quad (1)$$

$$\hat{c}_{uv}^{\mathrm{RISM}} = [\hat{\omega}_u]^{-1}[\hat{h}_{uv}][\hat{\omega}_v + \rho_v \hat{h}_{vv}]^{-1} \quad (2)$$

where $h = g - 1$ is the pair correlation function, and ω is the intramolecular correlation function. The expression in 2 is evaluated with the aid of the renormalization technique.[22−24,36]

A RISM expression for solvation free energy was derived by Singer and Chandler[26] and later modified by Zichi and Rossky,[28] making use of the hypernetted chain (HNC) closure relation and is referred to as the HNC solvation free-energy expression. Later, this expression was improved by Tenno et al. to the more accurate partial wave (PW) solvation free-energy expression.[37,38] We rewrote these solvation free-energy expressions as depending upon both the solution c^{HNC} to the HNC equation and the solution c^{RISM} to the RISM equation in such a way as to minimize contributions from errors in c.[33,34] This was accomplished by consideration of the invariance principle for the RISM solvation free-energy expression, shown by Singer and Chandler.[26] These two expressions are given by

$$\Delta\mu^{\mathrm{HNC}} = \frac{\rho}{2\beta}\sum_{\alpha\gamma}\int 4\pi r^2[-2c_{\alpha\gamma}^{\mathrm{HNC}}(r)(1 + h_{\alpha\gamma}(r)) + [h_{\alpha\gamma}(r)]^2 + h_{\alpha\gamma}(r)c_{\alpha\gamma}^{\mathrm{RISM}}(r)]dr$$

$$\Delta\mu^{(\mathrm{PW})} = \frac{\rho}{2\beta}\sum_{\alpha\gamma}\int 4\pi r^2[-2c_{\alpha\gamma}^{\mathrm{HNC}}(r)(1 + h_{\alpha\gamma}(r)) + h_{\alpha\gamma}(r)h_{\alpha\gamma}^{\mathrm{PW}}(r) + h_{\alpha\gamma}(r)c_{\alpha\gamma}^{\mathrm{RISM}}(r)]dr \quad (3)$$

where

$$\hat{h}_{uv}^{\mathrm{PW}} = [\hat{\omega}_u]^{-1}[\hat{h}_{uv}][\hat{\omega}_v]^{-1} \quad (4)$$

A third expression, the Gaussian fluctuation (GF) expression, has also been derived for solvation free energy within the RISM formalism:[39]

$$\Delta\mu^{\mathrm{GF}} = \frac{\rho}{2\beta}\sum_{\alpha\gamma}\int 4\pi r^2[-2c_{\alpha\gamma}^{\mathrm{HNC}}(r)(1 + h_{\alpha\gamma}(r)) + h_{\alpha\gamma}(r)c_{\alpha\gamma}^{\mathrm{RISM}}(r)]dr \quad (5)$$

In computing eq 2, because of the ill-conditioning of the matrixes ω and $\omega + \rho h$, approximations had to be used in which the dimensionalities of these matrixes were decreased to 1 in the limit as k approaches 0. A second effect of this ill-conditioning is that for k close to 0 we cannot expect the reciprocal space solutions c^{HNC} and c^{RISM} to match closely. The HNC closure makes the approximation that the so-called bridge function, consisting of a subset of the diagrams in the diagrammatic expansion of the radial distribution function, is zero. This bridge function corresponds to the difference $c^{\mathrm{RISM}} - c^{\mathrm{HNC}}$. In the low-$k$ range, this HNC approximation can be corrected by

TABLE 1: Solvation Free-Energy Differences (kcal/mol)

	flexible solute		restrained solute				
	2.0 ns	average[a]	2.0 ns	average[a]	expt	FEP or TI	RISM
2-HO-pyridine → 2-O-pyridine	−3.96	−4.08 ± 0.24	−3.80	−3.85 ± 0.18	−4.3[b]	−5.3[c] −5.74[d] −2.23[e]	−3.9[f] −4.4[f] −9.7[g]
imino cytosine → amino cytosine	−6.69	−6.82 ± 0.25	−5.62	−4.77 ± 0.78	−(5.5−6.9)[h]	−4.2[c] −6.1[i] −5.2[j] −6.0[k]	

[a] Taken over values at 1.6, 1.7, 1.8, 1.9, and 2.0 ns; the error corresponds to the nonbiased standard deviation from the average. [b] From ref 47. [c] MD/FEP from ref 1. [d] MC/FEP from ref 5. [e] MC/FEP from ref 4. [f] From ref 14. [g] From ref 15. [h] From experimental equilibrium constants in solution reported in ref 48 and gas-phase estimates presented in ref 1, on the basis of ref 49. [i] MC/FEP from ref 3. [j] MD/TI from ref 2. [k] MC/FEP from ref 2.

accounting for the bridge function in the solvation free-energy expression.[34,40−42]

Finally, an adjustment was added to the solvation free energy to account for nonstoichiometric numbers of water hydrogen and oxygen atoms lying within the distance bounded by the integration limit from each solute site. This amounts to the implementation of a "soft sphere" boundary condition. More details on the formalism of the coupled RISM/MD method can be found elsewhere.[33,34]

3. Computational Details

In this study, solute/solvent radial distribution functions were calculated by MD simulations using the AMBER package.[43] Solute structures were optimized at the B3LYP/6-31G(d,p) level. However, since it has been well known that atomic charges from the HF/6-31G(d,p) level are quite accurate for simulations of solvated systems, in this study we also used the same HF level for solutes' atomic charges. This was done using the RESP method, accomplished with the Antechamber suite of the AMBER package.[43] All quantum calculations were done using the GAUSSIAN98 package.[44] Solvent water molecules were then added at distances of up to 12 Å in the *x*, *y*, or *z* direction from the center of any solute atom. The general AMBER force field (GAFF) was used for solutes and the TIP3P model was used for water in all MD simulations. Solvated systems were first allowed to relax for 500 steps. Thermalization from an initial temperature of 0 K to the final temperature of 298 K was carried out over 25 ps, and equilibration over 5−10 ps, using 1 fs time steps. Averaging was then performed over a trajectory of 2 ns, in 2 fs time steps, sampling every 100 configurations, or every 0.2 ps. The solvent density used was 0.0333/Å^3. Two separate simulations of each solute molecule were performed using two different strategies. In one set of simulations, the solute structure was kept restrained, after the initial relaxation, using a harmonic potential with a force constant of 400−500 kcal/mol during the equilibration and thermalization, as well as throughout averaging. In a second scheme, solute molecules were consistently given full flexibility. Solvent/solvent radial distribution functions were taken from our previous simulations using the BOSS package,[45] with the TIP3P water model. Calculated radial distribution functions were sampled at intervals of 0.04 Å and were gradually smoothed to zero at distances exceeding 11 Å.

The GAFF force field was also used to obtain the pair potential when solving for *c* from the HNC equation. This force field represents the pair potential as a Coulomb potential with the usual 12−6 van der Waals interactions and uses the Lorentz−Berthelot mixing rules.

The Fourier transformations for the RISM calculations were carried out on a linear grid of 537 points. In carrying out the numerical integration for the solvation free energy, radial distribution functions were cut off at 11 Å. Further computational details have been provided in our earlier work.[33,34]

4. Results and Discussion

A. 2-Hydroxypyridine/2-Oxopyridine. In 2-hydroxypyridine, tautomerization occurs by a hydrogen transfer between the ring nitrogen and the neighboring oxygen atom (Figure 1). 2-Oxopyridine is more favored by solvation because of the free carbonyl oxygen available for strong hydrogen bonding in a polar solvent such as water.

Calculated solvation free-energy differences are given in Table 1 along with those from earlier simulations and RISM calculations. Solvation free-energy differences calculated for the 2-hydroxypyridine/2-oxopyridine tautomerization using radial distribution functions averaged over a 2 ns trajectory are −3.96 and −3.80 kcal/mol for the flexible and restrained solute geometries, respectively, and both are in good agreement with the experimental value of −4.3 kcal/mol. There is also fairly good agreement with the results of earlier simulation studies. In fact, although FEP results are generally fairly accurate, some noticeable disparity exists between the FEP results shown here for the 2-oxopyridine/2-hydroxypyridine equilibrium and the experimental values. This could possibly result from simulation runs having been carried out for an insufficiently large number of configurations. We also report in Table 1 average values calculated by letting the time length of simulation vary from 1.6 to 2.0 ns, with errors shown as standard deviations from the average. The small observed standard deviations are indicative of converged radial distribution functions at or soon after the first 1.6 ns of simulation.

We plotted some radial distribution functions obtained from our MD simulations. Two different patterns are observed for the distributions of water molecules about the substituted oxygen and ring nitrogen atoms between 2-hydroxypyridine and 2-oxopyridine. The oxygen in 2-oxopyridine can act as a hydrogen-bond acceptor and, accordingly, the O−H(water) radial distribution function displays a sharp first peak at around 2 Å (see Figure 2b). This peak is much lower in magnitude in 2-hydroxypyridine (Figure 2a) as a result of the presence of a hydrogen atom bonded to the oxygen. On the other hand, the ring nitrogen in 2-hydroxypyridine hydrogen-bonds with water hydrogen atoms, as is seen from the peak in the O−H(water) radial distribution function in Figure 2a, while in 2-oxopyridine this nitrogen is precluded from forming such hydrogen bonds by the substituted hydrogen atom.

Table 2 shows a comparison of the main features of some RISM calculated radial distribution functions for 2-hydroxypyridine and 2-oxopyridine taken from the two studies mentioned above with MD-calculated radial distribution functions

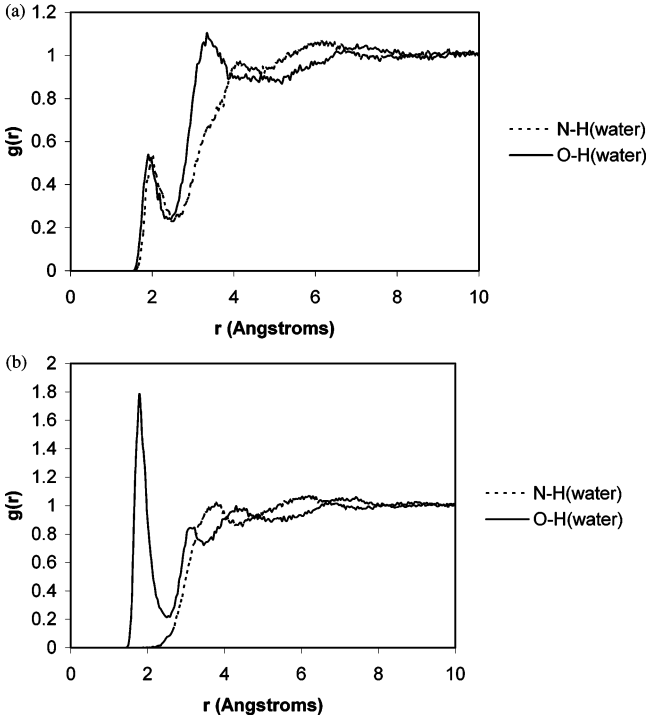

Figure 2. Calculated solute/solvent radial distribution functions for N and O atoms of (a) 2-hydroxypyridine and (b) 2-oxopyridine with H of water solvent.

TABLE 2: Heights and Positions of Local Maxima and Minima of Solute/Solvent Radial Distribution Functions for 2-Hydroxypyridine and 2-Oxopyridine in Water

		1st max		1st min		2nd max
		position	height	position	height	position
2-hydroxypyridine						
hydroxyl O/water H	MD	1.9	0.5	2.4	0.2	3.4
	RISM[a]	1.9	0.1	2.2	0.1	3.9
	RISM[b]	1.8	1.0	2.3	0.3	3.6
amide N/water H	MD	2.0	0.5	2.5	0.2	4.1
	RISM[a]	2.0	0.7	2.4	0.2	4.2
	RISM[b]	1.8	2.7	2.4	0.2	3.9
hydroxyl O/water O	MD	2.8	1.3	3.4	0.8	4.2
	RISM[a]	3.2	1.7	4.2	0.8	5.0
	RISM[b]	3.0	2.0	3.9	0.7	5.8
2-oxopyridine						
hydroxyl O/water H	MD	1.8	1.8	2.5	0.2	3.1
	RISM[a]	1.9	1.5	2.3	0.2	4.0
	RISM[b]	1.7	>4.0	2.3	0.2	3.7
amide N/water H	MD	3.7	1.0	4.4	0.9	6.2
	RISM[a]	4.0	1.0	4.6	0.9	6.1
	RISM[b]	3.7	1.2	4.4	0.9	5.8
hydroxyl O/water O	MD	2.7	1.8	3.2	0.6	5.0
	RISM[a]	3.3	2.0	4.1	0.7	4.9
	RISM[b]	2.9	2.6	3.8	0.5	5.5

[a] From ref 14. [b] From ref 15.

used in this work. (We use the restrained solute model here, as in Figure 2 also.) It can be seen from the table that there is a lot of agreement between our MD-calculated distribution functions and those of Shao et al.,[14] although some minor differences may be noticed. As mentioned above, the free-energy result found in this RISM study for the tautomeric equilibrium was very reasonable. This can be explained by the invariance of the RISM solvation free-energy expression about the solution to the RISM and HNC equations with respect to not only the direct correlation functions but also the radial distribution functions which may be a little erroneous and still yield reasonable free-energy results.[26] There is a lot more discrepancy

TABLE 3: Solvation Free Energies (kcal/mol)

	$\Delta\mu^{HNC}$	$\Delta\mu^{GF}$	$\Delta\mu^{PW}$	$\Delta\mu^{PW}$ (no bridge)
2-hydroxypyridine	20.10	−11.26	−6.98	−4.96
2-oxopyridine	17.75	−15.25	−10.78	−8.75
2-hydroxypyridine → 2-oxopyridine	−2.35	−3.99	−3.80	−3.79
cytosine b	8.12	−26.38	−19.69	−17.46
cytosine a	1.32	−32.08	−25.31	−22.88
cytosine b → cytosine a	−6.80	−5.70	−5.62	−5.42

between our radial distribution functions and those determined by Sato et al.[15] For this work, the calculated solvation free-energy difference differs considerably from the experimental value. This demonstrates that the accuracy of solvation free-energy differences calculated using the RISM formulation is dependent upon the degree of deviation of calculated radial distribution functions from their true values.

B. Cytosine. The amino group of amino-cytosine, which extends in a pyramidal projection from the plane of the conjugated ring, can transfer a hydrogen to the neighboring ring nitrogen to form the imino tautomer, as is seen in Figure 1. A possible explanation for the lower solvation free energy of the amino cytosine tautomer compared to the imino tautomer was suggested by Cieplak et al.[1] The arrangement of charges in the amino tautomer includes two proximal negative charges on oxygen and ring nitrogen atoms. This contributes to the polarizability of the surrounding water. On the other hand, the same proximal oxygen and nitrogen atom have negative and positive partial charges, respectively, in the imino tautomer, interfering with the alignment of the surrounding water molecules.[1]

The calculated solvation free-energy differences shown in Table 1 between the amino tautomer and the imino tautomer are −6.69 kcal/mol for the flexible solute model and −5.62 kcal/mol for the restrained solute model. Both of these values agree well with the experimentally determined range of −5.5 to −6.9 kcal/mol, as well as with the data displayed in the table from earlier simulation studies. Again, averages over data collected during the first 1.6−2.0 ns are also shown with errors expressed as standard deviations. Although the error for the flexible model is again small, the value calculated for the restrained molecule is 0.78 kcal/mol, showing that radial distribution functions converge slower.

C. Variations in the Solvation Free-Energy Expressions. In addition to finding tautomeric solvation free-energy differences using the PW form of the RISM free-energy expression, we also examined these free-energy differences using each of the other two formulations, HNC and GF. In addition, we calculated solvation free-energy differences for both tautomerizations leaving out the bridge function correction. The restrained solute model was used during these computations. The results are compared in Table 3.

It may be noticed that the absolute solvation free-energy results determined by the three different expressions, HNC, GF, and PW, are rather different from each other. Inspection of the free-energy expressions reveals that this difference is accounted for by the second term occurring under the integral sign in the expression for $\Delta\mu^{HNC}$, which is somewhat modified in the expression for $\Delta\mu^{PW}$, and is absent in that for $\Delta\mu^{GF}$. The term added to the HNC expression has the effect of making these results too positive, and the nonappearance of such a term in the GF expression makes the results of this expression rather more negative. Because, in the derivation of the PW expression, the solvation free-energy expression is decomposed into site−

site terms which are dependent upon the positions of other solute and solvent sites, that is, upon the molecular structures, this expression is exact within the HNC approximation.[37,38] Thus, the PW expression represents an improvement over the HNC expression, while the GF expression was derived using a somewhat different approach from the other two, which treats the solvent distribution as perturbed in a linear response to the solute.[39]

Very little difference is seen in relative solvation free energies calculated by GF, PW, or PW disregarding the bridge function, in either tautomerization. However, HNC free-energy results are inconsistent with those from the other formulations. The same pattern of similar free-energy differences determined by the PW, GF, and PW expression without a bridge correction was observed in our previous study of the alanine dipeptide, and there too, HNC results showed variation from these values.[46] As in that work, the unimportance connected with the bridge function correction can be attributed here to the cancellation of errors in the low-k range.

The HNC free-energy formulation was employed by Sato et al., and this may partly account for the lack of accuracy in the values obtained in that work.[15] The GF formalism was utilized in the work of Shao et al.[14]

5. Conclusions

We have presented our results for calculating the solvation effect on the tautomeric equilibria of 2-hydroxypyridine → 2-oxopyridine and amino-cytosine → imino-cytosine in water using the recently proposed coupled RISM/MD method. The calculated difference in free energy of hydration for the former tautomeric equilibrium of 2-hydroxypyridine → 2-oxopyridine in water is −3.8 or −4.0 kcal/mol with the restrained or flexible solute model, respectively, which is found to be in good agreement with the experimental value of −4.3 kcal/mol. For the tautomeric equilibrium of cytosine, the calculated solvation free-energy difference was −5.6 or −6.7 kcal/mol with the restrained or flexible solute models in favor of the amino tautomer. This value also compares well with the experimental data ranging from −5.5 to −6.9 kcal/mol. Our study reaffirms the accuracy of the coupled RISM/MD method in obtaining solvation free energies and studying reactions in solution.

We have shown a comparison between both radial distribution functions and solvation free energies from this study and two previous RISM studies of the 2-hydroxypyridine/2-oxopyridine tautomeric equilibrium. When RISM-calculated radial distribution functions differ by only a little from the more accurate MD-calculated functions, accurate solvation free energies can be determined. However, larger deviation from the true values of the radial distribution functions correlates with imprecision in the RISM-calculated solvation free-energy difference. This reliance of the accuracy of solvation free energies calculated by the RISM expression upon having adequate input radial distribution functions illustrates the advantage in better dependability offered by combining the two formerly developed methodologies of molecular simulations and RISM integral equation theory into the coupled RISM/MD approach.

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