Conjugated Dual Hydrogen Bonds Mediating 2-Pyridone/2-Hydroxypyridine Tautomerism

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Tautomeric equilibria of 2-pyridone (PD) and 2-hydroxypyridine (HP) dimeric forms as well as PD/HP complexes mediated by the conjugated dual hydrogen-bonding (CDHB) formation have been studied by ab initio molecular orbital calculations up to the 6-31+G** basis set at the Møller−Plesset level. The result in combination with the semiempirical solvation free-energy calculation reasonably predicts the relative free energy and consequently the tautomeric equilibria between PD, HP, their corresponding dimers and PD/HP complex in the gas phase as well as in solution. The results also indicate that the strength of dual hydrogen bonding resonantly affects the PD and HP electronic structures upon CDHB formation, resulting in an additional stabilization energy. Further calculation shows that the tautomeric equilibria can be fine-tuned by the formation of a hydrogen-bonded complex with guest molecules possessing bifunctional hydrogen bonds. The results lead to a possible mechanism suggesting that the tautomerization of a specific DNA base may be induced by forming a complex with an intruder, i.e., a specific molecule with multiple hydrogen-bonding capability, which triggers the mutation process.

Introduction

Through the courses of our studies as well as other efforts on the excited-state double-proton-transfer (ESDPT) reaction in 7-azaindole (7AI), the formation of a 7AI conjugated dual hydrogen-bonding (CDHB) complex has been concluded to be a key step to mediate the proton-transfer tautomerism.[1−14] In such a complex the partial transfer of a proton to the pyridinal nitrogen increases the electrophilicity of the nitrogen atom. Likewise, partial deprotonation of the amino group increases its nucleophilicity. These two processes take place cooperatively through the CDHB formation, consequently triggering the overall proton-transfer reaction. At the molecular level, this ESDPT reaction mediated by the CDHB formation has long been recognized as a model for the mutation of DNA resulting from the tautomerization of a specific base during replication.[2,14] However, while occurring in the excited state, this ESDPT mechanism only provides valuable information from the photodynamic viewpoint. If CDHB or even a multiple hydrogen-bonding formation, as proposed, plays a major role in mutation, it should not only dynamically catalyze the proton-transfer reaction but also thermodynamically manipulate the overall tautomeric equilibria.

It turns out that at the molecular level, 2-pyridone (PD), as well as its enolic isomer, 2-hydroxypyridine (HP), has been well-known as a prototype to study such a proton-transfer tautomerism mediated by the CDHB effect. The importance of the self-dimerization of HP and PD in determining the keto/enol tautomeric equilibria has long been recognized.[15−18] Unfortunately, available experimental information on the equilibria of dimeric forms with respect to their corresponding monomers is rare.[19−21] The overlap of electronic absorption spectra between dimers and their corresponding monomers makes the determination of equilibrium constants among various dimeric species in solution difficult. On the other hand, inaccuracy may be introduced in IR and NMR studies due to the necessity of high sample concentration, resulting in other competitive aggregation processes. Furthermore, the solubility prohibits these studies in hydrocarbon solvents where significant hydrogen-bonding association takes place. Among numerous theoretical approaches on the PD ⇌ HP tautomerism,[21−32] several have focused on calculating association energies of PD and HP dimers. MINDO/3 and MNDO methods have proven to be too inaccurate to even qualitatively reproduce the relative stability between PD and HP dimers.[21,23] Although CNDO/2 and CNDO/2 interfacing with the effective pair correlation energy can qualitatively explain the larger self-association energy for the PD dimer relative to the HP dimer, the calculated absolute self-association energy has also been reported to be unreliable due to the underestimated hydrogen-bonding distance.[23,24] The AM1 method can reproduce the relative trend of the self-dimerization effect reasonably well. Unlike its predecessor MNDO, which cannot even recognize the hydrogen bonds, the calculated tautomerization energy can correlate well with the CDHB formation. Unfortunately, the association energy has been underestimated. Consequently, the calculated hydrogen-bonding distance is not reliable.[25] On the other hand, due to their complicated molecular structures, only few reports have attempted to investigate the relative stability of dimeric forms based on the ab initio approach. Field and Hillier[27] have used the 3-21G basis incorporating the CI expansion to obtain the relative energy between PD and HP dimers. However, the lack of polarization functions in the basis set has biased the relative stability too much in favor of the PD dimer. To our knowledge, detailed ab initio calculations of various dimeric forms on high-level basis sets, especially those including polarized and diffuse functions have not been performed. Furthermore, regardless of many efforts to calculate the relative total molecular electronic energy and hydrogen-bonding effect, focus on tautomeric equilibria, i.e., the calculation of the free energy for these CDHB species, consequently the relative free energy with respect to PD and/or HP monomers, is still lacking.

In these studies, we have investigated the CDHB formation

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mediating tautomeric equilibria for PD and HP dimers, and the PD/HP complex by ab initio molecular orbital calculations up to 6-31+G** basis sets at the second-order Møller−Plesset perturbation level (MP2). We are especially interested in the PD/HP complex, of which the existence has never been recognized and/or considered. The calculated free energy will incorporate the solvation free energy obtained semiempirically (the PM3-SM*x* model)[33] to predict tautomeric equilibria among various species in solution. We have also investigated the possibility that certain biologically related guest molecules possessing dual hydrogen-bonding properties may alter the keto/enol tautomeric equilibria by forming CDHB complexes with PD and/or HP. The results may provide valuable information on the mutation process mediated by multiple hydrogen-bonding interaction.

Theoretical Methods

The methodology of the theoretical calculations involved the following sequences. The initially optimized structure used for the ab initio calculation was obtained by the semiempirical AM1 method using a Spartan package (Release 3.1.6, Wavefunction, Inc. Irvine, 1994) on a SiliconGraphics workstation. Ab initio molecular orbital calculations were performed using Gaussian 94 Rev D.3 programs.[34] Geometry optimizations for all structures were first carried out with the 6-31G** basis set at the Hartree−Fock (HF) level. This basis set that includes the p-polarization functions on the hydrogen atom has proven to be suitable for describing the hydrogen-bonding system.[32] As the next step, harmonic vibration frequencies were predicted at these equilibrium geometries. All molecules considered here were confirmed to be planar minima by the vibrational frequency calculation which should give all real frequencies. The directly calculated zero-point vibrational energies (ZPE) were scaled by 0.9181[35] to account for the overestimation of vibrational frequencies at the HF level. To investigate further the effect of basis set on the structures and tautomerization energy, additional optimizations and calculations of the total energy were performed at the HF-6-31G, HF/6-31G*, HF/6-31+G*, and HF/6-31+G** levels in the gas phase, in which the ZPE was scaled between 0.91 and 0.92 depending on the basis set used.[35] A calculation based on the MP2/6-31G** level has also been performed to examine the effect of electron correlation on the CDHB formation.

Since ab initio calculations using the SCRF method to calculate the solvent−solute interaction for various dimers and complexes are a time-consuming, possibly formidable process on the basis of our current computing facility, we will alternatively choose the semiempirical PM3-SM3 and PM3-SM4 solvation models proposed by Cramer and Truhlar[33] to calculate the solvation free energy. The solvation free energies were obtained with an AMSOL version 5.4 program[36] and then added to "gas-phase" energies obtained from the ab initio method. This combination method has proven to reproduce the experimental results, especially the relative stabilization energy of various proton-transfer tautomers in water, reasonably well.[37,38]

Results and Discussion

In the Gas Phase. The full geometry-optimized (6-31G** basis set) PD, HP, PD/PD dimer, HP/HP dimer, and PD/HP complex in the ground electronic state are shown in Figure 1a−e, where optimized geometries of PD and HP monomers have been reported by Wong et al.[32] It is certainly necessary to check whether the optimized geometrical structure for those dimeric and complex forms is at an energy minimum, transition state, or higher order saddle point. Therefore, the Hessians, and hence

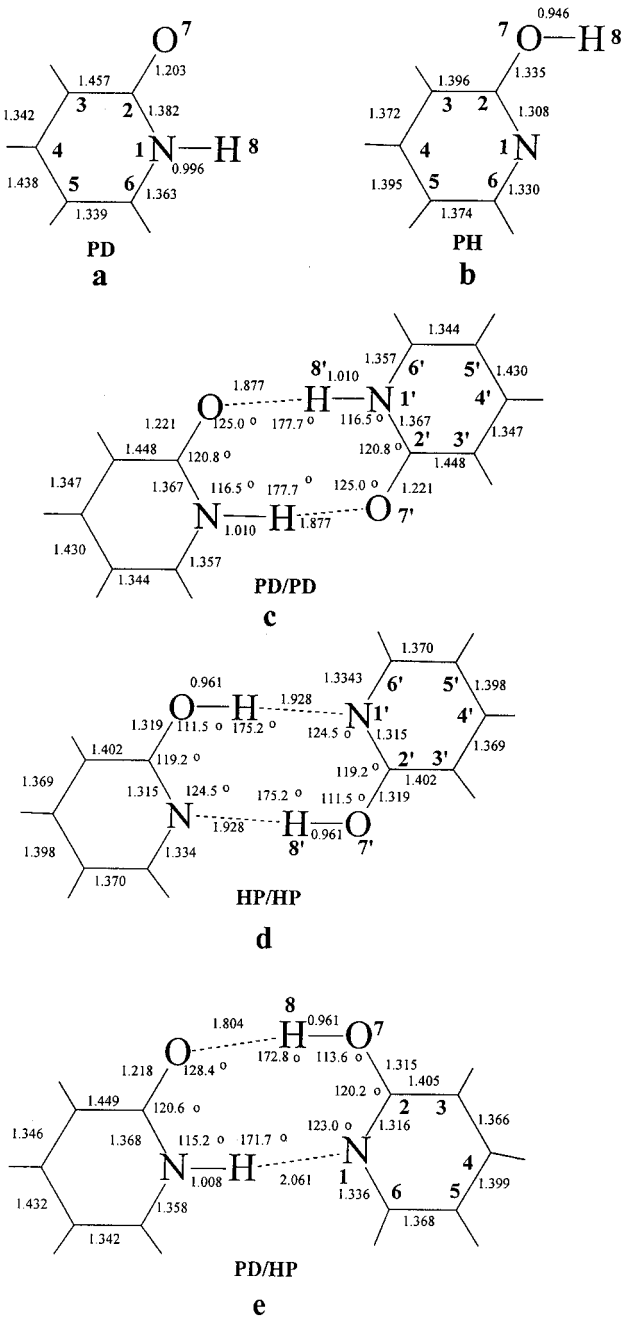

Figure 1. Optimized geometries based on the HF/6-31G** basis set (in Å and degrees, only critical angles are shown) of the ground states of (a) HP, (b) PD, (c) PD dimer, (d) HP dimer, and (e) PD/HP complex. For e the same numbering sequence used for the atoms in PD and HP is applied to simplify the comparison of bond lengths in Table 3.

vibrational frequencies, were calculated, and the result shows no imaginary vibrational frequencies, confirming the existence of local energy minima among all structures shown in Figure 1a−e.

The enthalpy of formation for various species was calculated by adding the total molecular energy with the zero-point energy followed by a correction contributing to the thermal energy at 298 K (see Table 1). The association energy, ΔH_{ac}, for various CDHB species was calculated as the change in the total molecular enthalpy of formation in the conversion of the optimized monomer individually into the optimized dimer, namely, $\Delta H_{ac} = -[H(\text{dimer or complex}) - 2H(\text{monomer})]$. However, this procedure may involve certain inconsistencies since the dimer basis set is larger than that of each monomer, resulting in an artificial lowering of the dimer energy. This

TABLE 1: Thermodynamic Properties of PD and HP Dimers and PD/HP Complex Calculated by the 6-31G Basis at 298 K (in the Gas Phase) in Combination with the PM3-SM*x* Method (in Solution)[a,b]**

	monomer		dimer		
	PD	HP	PD/PD	HP/HP	complex PD/HP
			RHF/6-31G**, Gas Phase		
total energy	−321.578 37	−321.580 83	−643.186 09	−643.182 30	−643.180 86
ΔE_{cp}(BSSE)			−2.33	−1.82	−2.40
ZPE	0.100 90	0.100 60	0.204 03	0.203 34	0.203 44
entropy	72.837	72.373	109.632	109.405	110.604
enthalpy	−321.479 85	−321.482 66	−642.986 53	−642.983 52	−642.981 83
free energy	−321.514 45	−321.517 05	−643.038 62	-643.035 50	−643.034 38
			Association Energy ΔH_{ac} (kcal/mol)		
			14.50	9.60	9.72
			Relative Free Energy in Gas Phase (kcal/mol)		
ΔG (1)	0.00	−1.63	−3.76	−2.32	−1.04
			Semiempirical Solvation Free Energy (kcal/mol)		
PM3 SM4 (2) (cyclohexane)	−8.14	−5.10	−10.07	−9.48	−10.09
PM3 SM3 (3) (water)	−18.61	−8.20	−12.39	−12.41	−14.08
			Relative Free Energy in Solution (Solvent = Cyclohexane) (kcal/mol)		
$\Delta G((1) + (2))$	0.00	1.41	2.45	4.48	5.15
			Relative Free Energy in Solution (Solvent = Water) (kcal/mol)		
$\Delta G((1) + (3))$	0.00	8.78	21.07	22.49	22.10

[a] Total energy, ZPE, enthalpy, and free energy in hartrees, entropy in cal mol^{-1} K^{-1}, ΔE_{cp}(BSSE) in kcal/mol. [b] BSSE has been applied in calculating ΔH_{ac} and ΔG.

error due to the basis set superposition (BSSE) can be corrected by a counterpoise correction procedure[39−41] in which the energy of the monomer is calculated by adding ghost atoms in space to the dimer (or complex), corresponding to the equilibrium positions of the counterpart monomer, i.e., $\Delta H_{ac} = -[H(\text{dimer or complex}) - 2H(\text{monomer}) - \Delta E_{cp}(\text{BSSE})]$, where ΔE_{cp}(BSSE) denotes the energy of monomers corrected by the counterpoise correction procedure. On the basis of this method, the association energy was calculated to be 14.50 kcal/mol for the PD dimer. Experimentally, the association energy of the PD dimer has been reported to be dependent on the solvent polarity, e.g. 5.9 kcal/mol in $CHCl_3$,[19] 14.8 kcal/mol in CCl_4,[20] and 15.5 kcal/mol in benzene.[21] The lower association energy in the high-polar medium can be rationalized by the calculated dipole moment of 4.586 D for the PD monomer with respect to that of zero for the PD dimer. Therefore, for the PD monomer the dipole−dipole interaction plays a major role in polar media, resulting in an additional stabilization energy. Accordingly, it is reasonable to predict that the association energy of the PD dimer in the gas phase should be close to that of 15.55 kcal/mol measured in benzene. The calculated association energy of 14.50 kcal/mol in the gas phase correlates reasonably well with the experimental results.

According to the bond distance of 1.877 Å between O(7) and H(8′) (and likewise O(7′) and H(8), see Figure 1c), it is reasonable to conclude that there exists two hydrogen bonds in the PD dimer, constructing a typical CDHB framework. Therefore, it seems that the association energy of 14.50 kcal/mol may be mainly due to the stabilization energy associated from the dual hydrogen-bonding formation. However, the calculation also shows a significant change in relative bond distances for the PD dimer in comparison to the PD monomer. For example, C(2)−O(7), C(3)−C(4), and C(5)−C(6) bond distances increase while the N(1)−C(6), C(4)−C(5), and C(2)−C(3) bond distances decrease upon forming the PD dimer (see Table 2). This result suggests a cooperative change in the electronic configuration of PD mediated by the CDHB formation. Figure 1d shows the optimized geometry of HP dimer with the association energy calculated to be 9.60 kcal/mol (see Table 1). The analysis of the relative distance for several crucial bonds with respect to the HP monomer is also shown in Table 2. Apparently, both an increase and decrease of the double-bond character are on the order of PD dimer > HP dimer, which corresponds to the decreasing trend of the calculated association energy. Thus, similar to the conclusion made in our previous studies on various 7-azaindole CDHB complexes,[12] *this result suggests that, in addition to the hydrogen-bonding stabilization, a cooperative change in the electronic configuration of PD and HP also takes place, resulting in additional stabilization energy.* In comparison to the HP dimer, the more than 5 kcal/mol association energy in the PD dimer may be rationalized by the weaker electronegativity of the N−H nitrogen than that of the O−H oxygen. Likewise, a similar mechanism can be applied to the C═O proton-accepting sites where the oxygen has stronger electronegativity than the pyridinal nitrogen. As a result, more electron density is shifted from the amino proton to the carbonyl oxygen in the PD dimer than from the hydroxyl proton to the pyridinal nitrogen, resulting in stronger hydrogen bond formation in the PD dimer.

TABLE 2: Relative Distances (in Å) for Some Crucial Bonds in PD, HP, and Their Corresponding Dimers and Complex

	N1−C6	C2−O7	C2−C3	C3−C4	C4−C5	C5−C6
PD (1)	1.363	1.203	1.457	1.342	1.438	1.339
PD dimer (2)	1.357	1.221	1.448	1.347	1.430	1.344
Δd (2) − (1)	−0.006	0.018	−0.009	0.005	−0.008	0.005
HP (3)	1.330	1.335	1.396	1.372	1.395	1.374
HP dimer (4)	1.334	1.319	1.402	1.369	1.398	1.370
Δd (4) − (3)	0.004	−0.016	0.006	−0.003	0.003	−0.004
PD in PD/HP(5)	1.358	1.218	1.449	1.346	1.432	1.342
Δd (5) − (1)	−0.005	0.015	−0.008	0.004	−0.006	0.003
HP in PD/HP (6)	1.336	1.315	1.405	1.366	1.399	1.368
Δd (6) − (3)	0.006	−0.020	0.009	−0.006	0.004	−0.006

Unlike the symmetric hydrogen-bonding formation in PD and HP dimers, unsymmetric dual hydrogen bonds are expected in the PD/HP complex. Evidence of the unsymmetric dual hydrogen-bonding formation is given by the calculated 2.061 Å hydrogen-bonding distance on the N−H----N site, which is ∼0.2 Å longer than that on the C═O----H−O site (1.804 Å). Such a large difference in the hydrogen-bonding distance may result from an unfavorable geometric effect in forming the PD/HP CDHB complex. Evidence can be shown from the hydrogen-bonding angle of 172.8° and 171.7° on O---H−O and N−H---N sites, respectively, which is far smaller than the nearly

TABLE 3: Calculated Thermodynamic Parameters for PD, PH, Their Corresponding Dimers, and PD/PH Complex by Different Basis Sets and Theoretical Levels at 298 K[a]

	PD	HP	PD/PD	HP/HP	PD/HP
		Total Energy (Hartrees)			
HF/6-31G//HF/6-31G	−321.434 16	−321.430 82	−642.906 17	−642.889 07	−642.892 90
HF/6-31G*//HF/6-31G*	−321.567 25	−321.567 10	−643.163 82	−643.154 93	−643.155 99
HF/6-31+G*//HF/6-31+G*	−321.578 06	−321.577 34	−643.183 55	−643.173 33	−643.174 91
HF/6-31+G**//HF/6-31+G**	−321.589 11	−321.591 05	−643.205 54	−643.200 34	−643.199 37
MP2/6-31G**//MP2/6-31G**	−322.569 27	−322.572 89	−645.177 39	−645.177 20	−645.174 12
		Enthalpy (Hartrees)			
HF/6-31G//HF/6-31G	−321.335 32	−321.332 90	−642.706 11	−642.690 98	−642.693 93
HF/6-31G*//HF/6-31G*	−321.468 97	−321.469 29	−642.964 61	−642.956 81	−642.957 49
HF/6-31+G*//HF/6-31+G*	−321.479 82	−321.479 53	−642.984 37	−642.974 61	−642.976 37
HF/6-31+G**//HF/6-31+G**	−321.490 63	−321.492 89	−643.006 05	−643.001 55	−642.000 37
MP2/6-31G**//MP2/6-31G**	−322.462 49	−322.466 48	−644.961 11	−644.961 76	−644.958 45
		ΔH_{ac} (kcal/mol)			
HF/6-31G//HF/6-31G			19.45	13.38	13.61
HF/6-31G*//HF/6-31G*			14.29	9.26	9.85
HF/6-31+G*//HF/6-31+G*			14.52	8.13	9.37
HF/6-31+G**//HF/6-31+G**			14.78	8.91	9.71
MP2/6-31G**//MP2/6-31G**			17.56	13.56	13.86
		Free Energy (hartrees)			
HF/6-31G//HF/6-31G	−321.369 67	−321.367 19	−642.756 93	−642.741 94	−642.745 44
HF/6-31G*//HF/6-31G*	−321.503 57	−321.503 67	−643.016 64	−643.008 77	−643.010 01
HF/6-31+G*//HF/6-31+G*	−321.514 44	−321.513 94	−643.036 39	-643.027 10	−643.028 85
HF/6-31+G**//HF/6-31+G**	−321.525 77	−321.527 31	−643.058 20	−643.053 65	−643.053 03
MP2/6-31G**//MP2/6-31G**	−322.497 10	−322.500 86	−645.013 20	−645.013 75	−645.010 98
		ΔG (kcal/mol)			
HF/6-31G//HF/6-31G	0.00	1.56	−8.23	0.79	−1.30
HF/6-31G*//HF/6-31G*	0.00	−0.06	−3.52	1.15	0.41
HF/6-31+G*//HF/6-31+G*	0.00	0.31	−3.72	2.73	1.33
HF/6-31+G**//HF/6-31+G**	0.00	−0.97	−3.41	−0.33	−0.07
MP2/6-31G**//MP2/6-31G**	0.00	−2.36	−6.81	−7.76	−5.90

[a] (BSSE) has been applied in calculating ΔH_{ac} and ΔG.

linear hydrogen-bonding formation in the PD dimer. As a result, the association energy for the PD/HP complex is calculated to be 9.72 kcal/mol, which is between that of PD and HP dimers. The good correlation between relative bond distances and the association energy can be further supported in the case of the PD/HP complex (see Table 2). The relative change of bond distances for the PD part is on the order of PD dimer > PD/HP. On the contrary, for the HP part, the relative change of bond distances in the PD/HP complex is greater than that in the HP dimer. Therefore, the CDHB formation mutually affects the electronic configuration for both chromophores in the PD/HP complex, and the degree of the relative change for the bond distance correlates well with the hydrogen-bonding strength.

We also calculated the entropy of formation for various CDHB species. This in combination with the calculated enthalpy of formation gives the free energy, and the results for these thermodynamic parameters are listed in Table 1. For the HP monomer, the free energy was calculated to be −1.63 kcal/mol relative to that of PD. This value is ∼1.0 kcal/mol lower than that of −0.64 kcal/mol reported by Wong et al.,[32] who applied a higher level basis set incorporating the electron correlation (QCISD/6-31+G**) for the calculation of the total molecular energy. However, such a basis set is unfortunately too expensive to calculate various dimeric and complex forms by our current computing system. Among various dimeric forms, the PD dimer turns out to be the most stable one, of which the free energy, after correction by the counterpoise correction procedure, was calculated to be −3.76 kcal/mol relative to two individual PD monomers. Since the entropy factor does not vary significantly among dimers and complexes (see Table 1), we may thus conclude that the alternation of the tautomeric equilibria can be fine-tuned mainly by the energies associated with the CDHB formation. It should be noted, however, that the entropy calculation is based on an infinitely diluted solute in which the correction term for the entropy due to the concentration effect has been neglected. Such an effect should lead to more favorable entropy upon the CDHB formation. The DG of −3.76 kcal/mol for the hydrogen-bonding association is much stronger than would be anticipated simply from the number and type of hydrogen bonds involved. For example, the pyrrole−pyridine complex was reported to have ΔG values of −1.9 kcal/mol.[42] Fritzche reported a ΔG value of 0.81 kcal/mol for the indole−pyridine complex in CCl_4.[43] To emphasize the role of the CDHB formation contribution to the keto−enol equilibrium, we have also investigated the PD/guest complex incorporating a single hydrogen-bonding formation. For this case, trimethylamine (TMA) was chosen as a guest molecule. Since TMA possesses only the proton-accepting site, the 1:1 PD/TMA complex, if it forms, should incorporate only a single hydrogen-bonding formation between TMA and the N−H hydrogen atom in PD. The calculated association energy of 4.91 kcal/mol for the PD/TMA complex is 2.34 kcal/mol smaller than half of that calculated for the PD dimer, in which the association involves two hydrogen bonds. Consequently, ΔG was calculated to be 4.84 kcal/mol endergonic for the formation of the PD/TMA hydrogen-bonded complex, giving further support that the CDHB formation in the case of the PD dimer resonantly induces the change in the electronic configuration of PD, resulting in additional stabilization energy.

Effect of Basis Sets. To investigate further the effect of basis sets on the calculated thermodynamic parameters, additional structure optimizations and free energy calculations were performed at the HF/6-31G, HF/6-31G*, HF/6-31+G*, and HF/6-31+G** levels in the gas phase. The calculated association energy as well as other thermodynamic parameters by various levels of theory are listed in Table 3. Similar to that calculated by the 3-21G basis,[27] the lack of polarization functions in the

6-31G basis overestimates the hydrogen-bonding strength, hence the association energy of the PD dimer, which is ∼4.0 kcal/mol larger than that measured in benzene.[21] In comparison to the results calculated at 6-31G** level, using the 6-31G* basis set results only in a change of the relative energy between two monomers, while the change of the relative energy between each monomer and its corresponding dimers and/or complex is small. Since the association energy is obtained by the change of enthalpy from the monomer to its corresponding dimer or complex, its value calculated by the 6-31G* basis set differs from those calculated by the 6-31G** basis set only by less than 0.5 kcal/mol. The small difference in the association energy, and in turn the hydrogen-bonding strength between these two basis sets, may be due to the strong, direct hydrogen-bonding formation. Thus, the contribution of the p-polarization function on the hydrogen atom to the total molecular energy has been significantly reduced. However, for the case of weak hydrogen-bonding formation, especially when the weakness of the hydrogen bond is due to steric effects, the incorporation of p-polarization functions on hydrogen atoms may become crucial in determining the hydrogen-bonding energy. Evidence can be given indirectly by calculating the relative energy between PD and HP. The weak four-member intramolecular hydrogen-bonding formation in HP is not well recognized by the 6-31G* basis set, resulting in only 0.06 kcal/mol lower free energy than PD. In contrast, the 6-31G** basis set predicts the more stable HP form by 1.63 kcal/mol. The inclusion of sp diffuse functions on heavy atoms has a small effect on the total energy of the dimeric and/or complex forms. Such a small effect has been recognized by calculating PD and HP monomers.[32] As a result, the relative association energies among three CDHB species are similar between 6-31G** and 6-31+G** basis sets.

Calculations based on the inclusion of electron correlation (MP2 perturbation) using the 6-31G** basis set have also been performed. Due to their complex molecular structures, in this study the inner shells have been excluded from the correlation energy calculation for all CDHB species studied. For consistency, similar methods have been applied for calculating both PD and HP monomers. Therefore, the total energies calculated for PD and HP (see Table 3) are different from those reported previously, in which all electrons are included in the correlation energy calculation.[32] Nevertheless, the relative total energy of 2.26 kcal/mol between PD and HP calculated in this study is only slightly different from that of ref 32 by 0.09 kcal/mol. In comparison to the RHF methods incorporating p-polarization functions on the hydrogen atom, the MP2/6-31G** method seems to overestimate the association energy for all CDHB species. For example, the calculated association energy of 17.56 kcal/mol is 3.06 and 2.78 kcal/mol larger than that extracted from RHF/6-31 G** and RHF/6-31+G** methods, respectively. In addition, the relative association energy between each CDHB species has been reduced. The association energy of the PD dimer is only 4 kcal/mol greater than that of the HP dimer. In comparison, all RHF basis sets give more than 5.0 kcal/mol association energy in favor of the PD dimer. In calculating the PD⇌HP tautomerism, the MP2/6-31G** method has been reported to bias the relative stability in favor of the HP monomer by ∼1.5 kcal/mol.[32] This, in combination with the smaller relative association energy, leads to the reverse stability between PD and HP dimers calculated by the MP2/6-31G** method (see Table 3). The results contradict the experimental observation[15−18] as well as those calculated by RHF basis sets, concluding that the PD dimer should be the most stable form among the three CDHB species. It seems that the electron correlation at the MP2 level may not describe the studied CDHB species well, possibly due to the overemphasized electronic correlation energy, which may suppress the contribution from the hydrogen-bonding effect. In calculating the relative energy between PD and HP monomers, Wong et al.[32] concluded that higher treatments of electron correlation at the MP4SDQ and QCISD levels reduced the relative energy, giving results closer to that obtained experimentally. Unfortunately, the inclusion of electron correlation on higher level basis sets is currently too expensive for the studied CDHB species.

In the Solution Phase. While most of the experiment was carried out in the solution phase where different degrees of solvent−induced dipole interaction need to be considered, an attempt has also been made to estimate how much inclusion of a dielectric continuum for the solvent would affect the results. In this study, semiempirical PM3-SM3 and PM3-SM4 solvation models[35] were used to calculate the solvation free energy in cyclohexane and water, respectively. In cyclohexane, the solvation free energies listed in Table 1 are on the order of 2(PD) < 2(HP) < PD/HP < PD dimer < HP dimer. The result indicates that the dipole−induced dipole interaction may play a major role for the first two cases. However, regardless of the large dipole moment of 4.959 μ for the PD/HP complex, the solvation free energy is calculated to be lower than PD and HP dimers by only 0.02 and 0.61 kcal/mol, respectively, indicating that for these CDHB species the differential solvation effect due to the local charge distribution is superior to that of the net dipole moment. Further details will be discussed in the following section upon calculating the solvation free energy in water environment. Adding the solvation free energy to the ab initio calculated free energy in the gas, the free energies of the formation for various species in cyclohexane are on the order of 2(PD) < 2(HP) < PD dimer < HP dimer < PD/HP complex. Although the calculated ΔG of 1.41 kcal/mol for PD → HP in cyclohexane is ∼1.0 kcal greater than the value of 0.36 kcal/mol based on the QCISD/6-31+G** basis set in combination with the SCRF method,[33] the prediction that PD is more stable than HP in cyclohexane follows the right trend. Experimentally, a ΔG of 0.33 kcal/mol in cyclohexane has been reported.[15] Obviously, the inclusion of the solute−solvent interaction greatly stabilizes the PD monomer relative to other species. Nevertheless, due to the calculated ΔG of 2.45 kcal/mol for the 2PD ⇌ PD dimer tautomerism, the existence of the PD dimer should be appreciable at high concentrations. While the actual equilibrium constants, hence the relative free energy, among PD, HP, and various CDHB species have not been reported in nonpolar solvents, the self-dimerization of PD in hydrocarbon solvents has been observed experimentally and therefore taken into account to avoid the deviation in calculating the tautomeric equilibrium between PD and HP monomers.[15−18]

The semiempirical calculation of solvation free energy was also carried out in a water environment, and similarly, the results were added to "gas-phase" energies to obtain the free energy for the tautomeric equilibria in water. A similar method has been used to calculate the relative free energy between PD and HP monomer in water.[37,38] The solvation free energies for the PD dimer, HP dimer, and PD/HP complex were calculated to be −12.39, −12.41, and −14.08 kcal/mol, respectively (see Table 1). Assuming that the stabilization energy due to the CDHB formation is independent of the solvation effect, it is intriguing that the solvation free energy for the DP/HP complex is only 1.69 and 1.67 kcal/mol smaller than that of the PD and HP dimers, respectively, while the difference in the dipole moment was calculated to be as large as 4.586 D. Similar to that proposed in the cyclohexane environment, the result indicates that the local charge distribution rather than the net

TABLE 4: Thermodynamic Properties of Various PD and HP Complexes in the Gas Phase Calculated by the RHF/6-31G Basis Set at 298 K[a,b,c]**

	ACID	LAM	DHP	PD/ACID	HP/ACID	PD/LAM	HP/LAM	PD/DHP	HP/DHP
total energy	−188.770 57	−323.93 47	−720.086 87	−510.376 62	−510.373 85	−645.535 84	−645.532 32	−1041.699 22	−1041.696 55
ΔE_{cp}(BSSE)				−2.26	−2.01	−2.30	−2.00	−2.46	−2.32
enthalpy	−188.732 48	−323.787 39	−719.972 38	−510.237 57	−510.235 13	−645.289 62	−645.286 65	−1041.483 84	−1041.480 69
entropy	59.045	78.032	91.806	96.051	95.809	115.571	116.389	128.194	128.528
free energy	−188.760 53	−323.824 47	−720.016 00	−510.283 20	−510.280 65	−645.344 53	−645.341 95	−1041.544 75	−1041.541 76
ΔH_{ac}				13.58	10.54	11.74	8.42	17.38	13.78
ΔG				−2.90	−1.55	−1.22	0.1	−6.51	−4.78

[a] The calculated thermodynamic properties for PD and HP can be referred to Table 1. [b] Total energy, enthalpy, and free energy in hartrees, entropy in cal mol^{-1} K^{-1}, ΔE_{cp}(BSSE), ΔH_{ac}, and ΔG in kcal/mol, c. BSSE has been applied in calculating ΔH_{ac} and ΔG, i.e., ΔH_{ac}(PD/ACID) = H(PD/ACID) − H(PD) − H(ACID) − ΔE_{cp}(BSSE), and ΔG(PD/ACID) = G(PD/ACID) − G(PD) − G(ACID) − ΔE_{cp}(BSSE). Similar procedures were applied to calculate ΔH_{ac} and ΔG for other complexes.

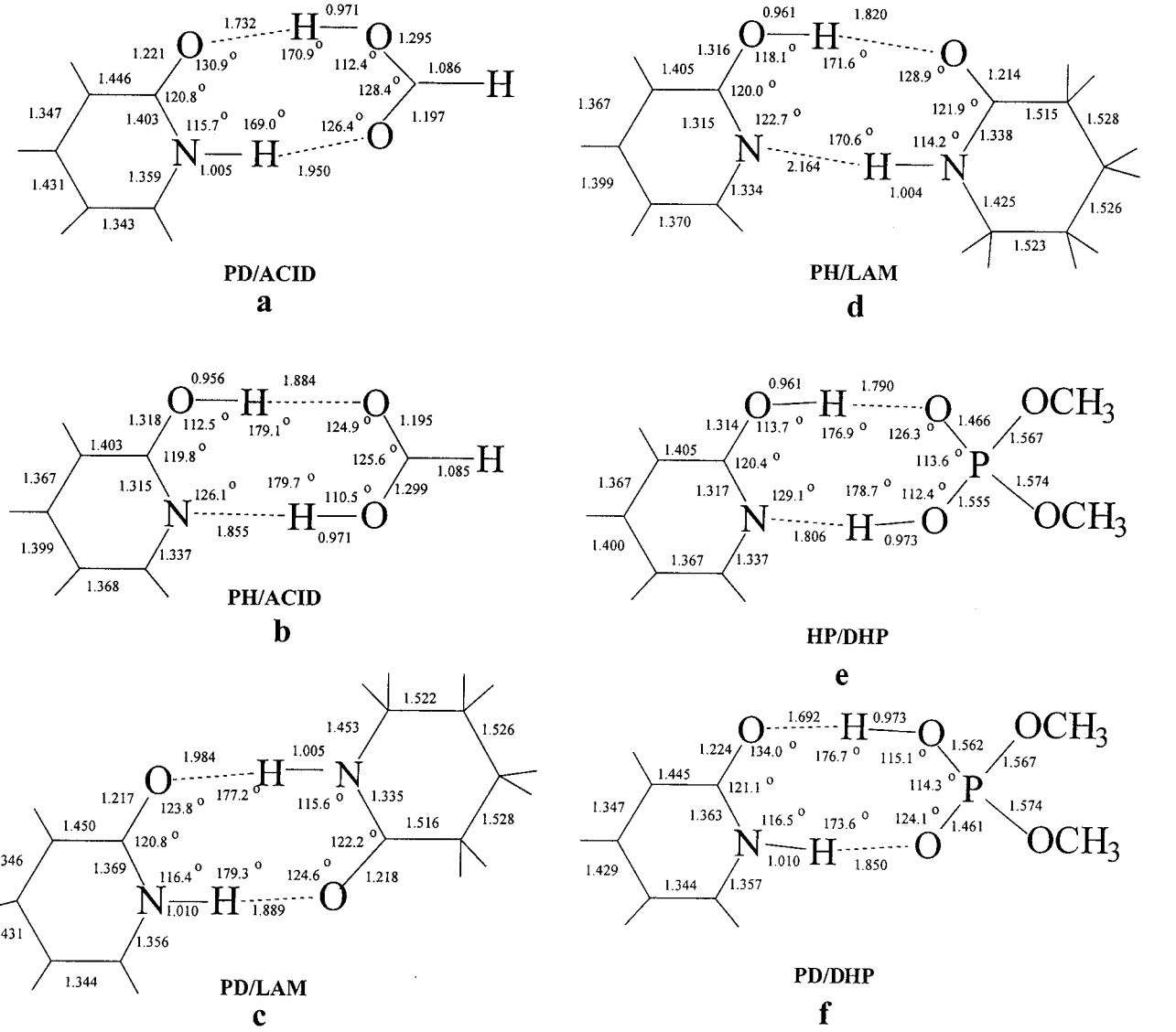

Figure 2. Optimized geometries based on the HF/6-31G** basis set (in Å and degrees, only critical angles are shown) of the ground states of (a) PD/ACID, (b) HP/ACID, (c) PD/LAM, (d) HP/LAM, (e) PD/DHP, and (f) HP/DHP complexes.

dipole moment plays a key role to determine the differential solvation effect. PD and HP dimers as well as the PD/HP complex can be treated as two separate parts in which each monomer loses two degrees of freedom due to the CDHB formation. Although the net dipole moment is zero in the case of PD (or HP) dimers, the local charge distribution in the rest of the non-hydrogen-bonded sites is similar to that of the PD/HP dimer. Thus, solvation free energy with the same magnitude can be predicted among the three CDHB species. On the other hand, the CDHB formation prevents the proton donor and acceptor functional groups, the largest local charge accumulated sites on either PD or HP monomer, from solvation by water. Such an effect is especially crucial in the case of the PD dimer (or the PD/HP complex) in which the PD monomer has been theoretically proven to exist as a dipolar species with positive and negative charges on nitrogen and oxygen sites, respectively, in protic media.[32] The CDHB formation significantly reduces the dipolar structure of PD in dimeric and complex forms, resulting in a poor solvation free energy. The largest solvation free energy calculated in the PD monomer is consistent with experimental data in that PD is the predominant species in water, and the existence of all other tautomeric species is negligible.[15−18]

Bifunctional Hydrogen-Bonding Complex. If HP and PD were one of the DNA bases and its malignant tautomer, respectively, and assuming that the situation resembles the equilibrium in the gas phase in which HP → PD is an endergonic process, it is of interest to investigate whether a guest molecule possessing bifunctional hydrogen-bonding capability, i.e., the existence of both proton-donating and -accepting sites in the guest molecule, can shift the tautomeric equilibria toward a keto form, consequently stimulating the mutation process. To examine such a possibility, thermodynamic parameters have been calculated for various types of PD and/or HP hydrogen-bonded complexes. In this study, three classes of guest molecules, formic acid (ACID), 2-azacyclohexanone (LAM), and a phospholipid-related molecule, dimethyl hydrogen phosphate (DHP), were selected, possessing the carboxylic acid, amide, and phosphoric acid bifunctional hydrogen-bonding sites, respectively. Table 4 shows the calculated thermodynamic parameters based on the 6-31G** basis set for PD and HP complexes associated with ACID, LAM, and DHP, of which the geometry-optimized structures are depicted in Figure 2a−f. Two remarks can be pointed out from Table 4. First, among those CDHB complexes, the association energy hosted by PD is generally ∼3.0 kcal/mol larger than that hosted by HP. The association energy is calculated to be 13.58 and 11.74 kcal/mol for PD/ACID and PD/LAM complexes, respectively, which are comparable with that calculated for the PD dimer. However, in the case of the PD/DHP complex the calculated association energy of 17.38 kcal/mol is even larger than that of the strongly associated PD dimer by 2.88 kcal/mol. Evidence of this unusually large association energy can be further supported by the short hydrogen-bonding distances of 1.850 and 1.692 Å for N−H---O═P and C═O---H−O−P sites, respectively, in the PD/BHP complex (see Figure 2e). Second, for the PD/ACID, PD/LAM, and PD/DHP CDHB complexes, the free energy was calculated to be −2.90, −1.22, and −6.51 kcal/mol lower than that of the PD monomer and its associated guest molecule. Therefore, it is concluded that the equilibria can be shifted toward the keto form not only by PD self-dimerization but also through the formation of PD/guest CDHB complexes, and the equilibrium constant can be fine-tuned by the CDHB strength mediated by the guest molecule. The results provide us with a more flexible approach to examine the hydrogen-bonding effect on the keto/enol tautomerization and may become more significant if the formation of a hydrogen-bonded complex is associated with triple or even multiple hydrogen bonds. Experimentally, we have recently investigated the keto−enol tautomerism in 3-hydroxyisoquinoline, an analogue of HP (or PD) with an additional fused benzene ring. Due to the significant spectral difference between keto and enol forms, the association constant, i.e., the free energy for the formation of various 3-hydroxyisoquinoline CDHB species, can be determined by either the absorption or the fluorescence titration experiment. The results show clear evidence that the keto−enol equilibrium can be fine-tuned by the CDHB strength,[44] giving further support for the mechanism of bifunctional hydrogen bonds mediating PD ⇌ HP tautomerism.

Conclusion

Our results, based on a theoretical approach, show that the tautomeric equilibria between PD and HP, in addition to being subjected to the solvation effect, can be strongly affected by the self-dimerization or complex formation associated with the CDHB effect. The result also shows that the CDHB formation, to a certain extent, will cooperatively induce charge redistribution of PD or HP, resulting in an additional stabilization energy. Although the significance of our results is only based on a simple molecular model, it clearly implicates that the tautomerization assisted by the CDHB or even multiple hydrogen-bonding formation may also be feasible in a biological system. If the mutation of DNA, at the molecular level, is due to the tautomerization of a specific DNA purine base during the replication, it may take place by intercalating an "intruder" possessing multiple hydrogen-bonding properties to stabilize the malignant tautomer. Such an effect may turn out to be crucial in the case of cytosine, which forms a base pair with guanine through a triple hydrogen bonds.

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