The Structures and Proton Transfer Barriers in Proton-bound Homodimers of Aromatic Molecular Bases: Implication of Zero-point Energies for the Proton-transfer Reaction

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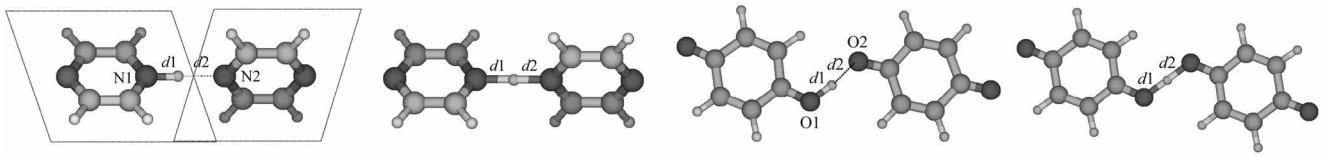
This study examines the energetics and structures of proton-bound complexes with an ionic hydrogen bond, which, in recent years, has attracted increasing attention due to their biological implications. Hydrogen bonding, as an important intermolecular chemical interaction, plays a significant role in many of chemical and biological phenomena. Structures and dynamics, specifically concerning solvation and chemical reactions, are strongly influenced by hydrogen bonding. Extensive studies, both experimental and theoretical, have been performed in order to understand hydrogen bonding in molecular systems of biological significance, such as DNA base pairs.[1-9] In those investigations, stacking interactions between the DNA base molecules, within a doublestrand manifold, were also found to be important. In addition, proton transfer energetics (energy barrier) and dynamics have been studied in depth. In particular, double proton transfer within a DNA base pair has been a subject of great research focus.[4-6] For example, the barrier heights for the double proton transfer are calculated to be 16.1 and 6.1 kcal/mol [B3LYP/6-311++ G(d,p)] for forward and backward proton transfers in the canonical GC base pair, respectively.[4] As for the transfer mechanism, whether the double proton transfer takes place synchronously or asynchronously is the main research issue in this field. On the other hand, isolated protonated ion-molecule complexes of small molecules, *e.g.* $NH_4^+(NH_3)_n$, have also been extensively studied, as they represent ideal microscopic model systems for solvation involving hydrogen bonding and proton transfer reactions.[10-12]

In recent years, hydrogen bonding in a protonated species has become an important issue in molecular biology. Protonated DNA bases have been known to participate in the formation of DNA triple helices that may have specific roles *in vivo*.[13,14] A potential use of the triplex as therapeutics has also prompted active research on this subject.[15,16] Besides, it was suggested that protonation of a DNA base may lead to incorrect pairing, or mismatch, as in the case of the methylated guanine base, which shows the mutagenic potential of guanine alkylation.[17] Among the four DNA bases, protonation of cytosine has been a focus of many studies, as the protonated cytosine-cytosine (denoted as C^+C or CH^+•••C) base pairs with three hydrogen bonds. This molecular interaction was first experimentally observed in fiber X-ray analysis and provides a prototypical system for hydrogen-bonded proton-bound base systems. A previous theoretical study on this protonated dimer noted that the triple hydrogen bonding within the CH^+•••C pair was predicted to be very strong; the interaction energy at the MP2/6-31G* theory level was estimated to be −44.8 kcal/mol, while the doubly-bonded neutral G•••C Watson-Crick, A•••T Watson-Crick, and C•••C base pairs were found to be stabilized only by −25.8, −12.4, and −18.2 kcal/mol, respectively.[7] The stronger interactions (>20 kcal/mol), arising largely from the additional hydrogen-bonding by protonation, were viewed simply due to charge-dipole interactions in the optimized geometry.[7]

In the field of biology, much research has focused on the formation of DNA triple helices within a manifold of longer multiple strands. In the case of C^+GC and TAT triplets, the protonated pyrimidine was shown to form Hoogsteen hydrogen bonds with a purine base, whereby it participates in binding of a single strand of DNA to the major groove of a double helix.[18-20] In this binding interaction, the presence of a proton on N3 is critical to the triplet stability. X-ray crystallography also indicated that the added proton in the protonated cytosine can contribute to conformational variability of DNA by offering extra hydrogen bonding to the base pairing and, thus, strengthening the DNA base pairing stabilization.[21-24]

In spite of its own significance in biological systems, the intrinsic property of hydrogen bonding in proton-bound dimer systems was rarely elucidated. The reason for this is largely due to the complexity of biological molecules. Such factors as the presence of multiple hydrogen bonds generally involved in base pairing and rather strong dipole moments of DNA base molecules (~2.5-5 Debye) often cause complexity. Thus, in this paper, the first theoretical attempt, examining hydrogen bonding at the detailed molecular level



(a) PHP Min. [PH⁺···P] (b) PHP TS [P···H⁺···P] (c) QHQ Min. [QH⁺···Q] (d) QHQ TS [Q···H⁺···Q]

Figure 1. Optimized structures for PH⁺P [(a) PHP Min. and (b) PHP TS], and QH⁺Q [(c) QHQ Min. and (d) QHQ TS] complexes were obtained using the B3LYP density functional method: The structures for PHP were found to be perpendicular, while those for QHQ were co-planar. Further details are given in Table 1.

and involving protonation of a representative model system of DNA base molecules, is described. As a simple model system that represents the essence of DNA base pairing interactions, the proton-bridged dimers of pyrazine ($C_4H_4N_2$: P) and quinone ($C_6H_4O_2$, p-Benzoquinone: Q) were chosen (see Figure 1). These molecules are symmetric and, thus, have a negligible dipole moment. In addition, they also include functional groups that can accommodate the hydrogen bonding of proton-bound base molecules. By choosing homogeneous dimers, we also avoid asymmetry in the proton transfer barrier, *e.g.* forward and backward transfer, which arises from chemically different protonation sites in a heterogeneous dimer. In the present work, among many interesting aspects of this system, the structures and energetics of the hydrogen-bonded dimers and the associated proton transfer barrier are the central focus.

Density functional theory using B3LYP functional methods was employed in the present computational study. This theory level has been known to be reliable in predicting the structures and vibrational frequencies of molecular systems with hydrogen bonds in a cost-effective way. For example, this method has been in broad use for a wide range of investigations on hydrogen bonding and proton transfer dynamics in DNA base pairs.[4,5,8,9,11] The standard basis set of 6-31G(d) was used for geometry optimization, which was further examined using a larger basis set of 6-311+G(2d,p). These levels of calculations were generally known to produce comparable results to *ab initio* calculations at the MP2 theory level. The Gaussian 03 program suite was used for the caluclations.[25] Geometries were optimized using analytical gradients without any constraint on geometrical parameters. When a stationary point was reached, the geometry was further examined by calculating vibrational frequencies. All non-zero vibrational frequencies at the stationary point ensure that a given geometry stands for a minimum energy structure, while a single imaginary frequency indicates that the predicted geometry represents a transition state connecting two minimum energy structures. The frequency calculation is also useful for zero-point energy correction. The obtained zero-point energy corrections were scaled by a factor of 0.98 to adjust for the deviation due to the harmonic oscillator approximation.

Figure 1 represents the stationary structures calculated for the proton-bridged dimers of pyrazine and quinone, PH⁺P and QH⁺Q, respectively. As for the pyrazine dimer, two stationary structures, as given in Figure 1, are found to pose a perpendicular geometry between the two constituent molecules. The global minimum energy structure is the one with the excess proton being bound to one molecule [PH⁺•••P] [Figure 1(a)]. This is a typical structure for ion-molecule complexes, which is generally expected, for example, from the known $NH_4^+(NH_3)$ structures.[10-12] The other is a transition state (TS) structure [Figure 1(b)], characterized by a single imaginary frequency, in which the proton is shared equally by the two molecules in the complex [P•••H⁺•••P]. Normal mode analysis of the calculated imaginary frequency indicates that the TS structure is the transition state connecting the two chemically identical minimum energy structures in the course of proton transfer from the one pyrazine molecule to the other. When the proton is transferred between the two nitrogen atoms, N1 and N2, the distance between N1 and N2 shortens from 2.72 to 2.58 Å as the molecule passes through the TS in which the proton and the extra charge are shared equally by the two molecular moieties.

Energy changes were also considered upon rotation of the plane about the axis connecting N1 and N2 in Figure 1(a).

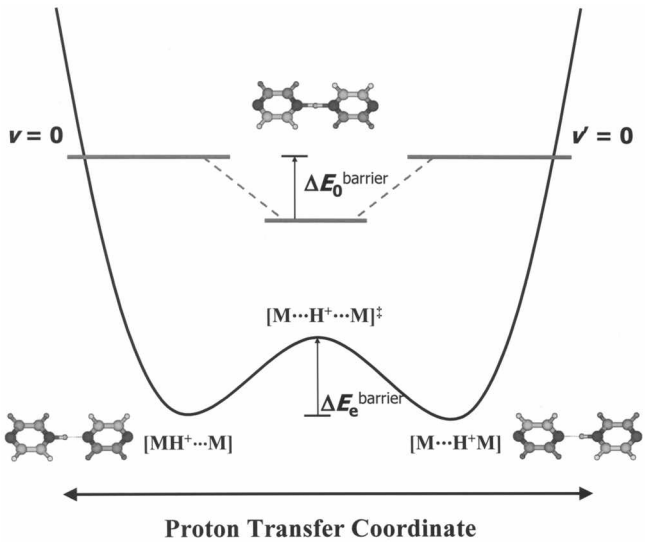


Figure 2. Schematic energy diagram for proton transfer reactions in the proton-bound complexes of PH⁺P and QH⁺Q investigated by the present study. The lower trace stands for the electronic potential energy surfaces, which has a positive energy barrier for proton transfer. As found in the present PH⁺P and QH⁺Q cases, when one considers zero-point energy, the proton-shared structure (TS) represents the minimum structure for the proton-bound complexes.

For the two species [PH^+•••P] and [P•••H^+•••P], the structures in which the two monomers are co-planar were of particular interest. It was revealed that the co-planar [PH^+•••P] constitutes the TS state between the perpendicular lowest energy structures [Figure 1(a)] with respect to the rotation of the plane. The same also holds true for the co-planar [P•••H^+•••P]. The rotation barrier around the symmetry axis of [P•••H^+•••P] presumably arises from steric effects, with its value estimated to be about 76 meV.

On the other hand, in the case of the quinone dimer, the stationary structures corresponding to the minimum-energy and TS states were all found to be planar, as shown in Figure 1(c) and (d). When the proton is attached to the carbonyl group of one moiety, [QH^+•••Q], it constitutes the minimum energy structure [Figure 1(c)]. The proton-shared structure represents the transition state [Q•••H^+•••Q] for the proton transfer reaction. Non-planar initial structures were also explored and were found to be repulsive, all of which lead to the planar minimum structure in the geometry optimization.

Table 1 shows the calculated dissociation energies, ΔD_e, for the lowest energy structures of [PH^+•••P] and [QH^+•••Q]. For [PH^+•••P], the calculations at B3LYP/6-31G(d) and B3LYP/6-311+G(2d,p) levels gave rise to dissociation energies of 23.0 and 21.1 kcal/mol, respectively, which are comparable to the estimated contributions of ionic hydrogen bonding arising from protonation of the cytosine dimer.[7] It is also notable that these estimated dissociation energies are also comparable to the threshold dissociation energies (E_o) obtained in blackbody infrared radiation dissociation experiments for amino acid homodimers; for example, for ArgME (ME: methyl ether), Gly, GlyME, and Ala, E_o values were observed between 23.2 and 27.8 kcal/mol.[26,27] This may indicate that the hydrogen bond involving a proton generally imparts a ~20 kcal/mol binding energy contribution. Furthermore, it is noteworthy that, even without the appreciable charge-dipole interactions, as in the complexes examined here, a single ionic hydrogen bond can offer proton-bound complexes a stability as large as ~20 kcal/mol.

The energy difference between the TS and the minimum energy structure gives rise to the energy barrier for the proton transfer reaction between two monomers. Those energy barriers ($\Delta E_e^{barrier}$) were calculated to be 1.2 and 0.3 kcal/mol at the theory level of B3LYP/6-31G(d) for PH^+P and QH^+Q, respectively. These barriers are significantly low when compared with those of neutral DNA base pairs. For example, in the case of the isolated GC base pair, the barrier heights for the forward and backward double proton transfer were predicted to be as large as 16.1 and 6.1 kcal/mol, respectively, at the B3LYP/6-311++G(d,p) theory level.[4] However, it should be noted that those barrier heights in PH^+P and QH^+Q systems are still positive and tangible in the thermal energy regime of ~RT (0.59 kcal/mol). Thus, it is expected that the energy barrier can still affect the reaction dynamics that follow the adiabatic electronic potential energy surface obtained using the computational chemistry method.

However, in real chemical reactions, the zero-point energy should always be considered. In a real chemical system, the actual reaction proceeds through a zero-point energy corrected potential energy surface rather than a genuine adiabatic electronic potential energy surface. Therefore, the unavoidable zero-point energy must be taken into account in order to correctly describe reaction dynamics, particularly when the barrier height is significantly low. When zero-point energies are considered for the four stationary structures of PH^+P and QH^+Q examined above, the two nominal TS structures turn out to be more stable than the nominal minima by 1.1 and 1.5 kcal/mol for PH^+P and QH^+Q, respectively. As shown in Table 1, consideration of zero-point energy drastically decreases the proton transfer barrier heights ($\Delta E_e^{barrier}$) of 1.2 and 0.3 kcal/mol, resulting in −1.1 and −1.5 kcal/mol ($\Delta E_0^{barrier}$) for PH^+P and QH^+Q, respectively. In effect, the proton transfer in PH^+P and QH^+Q is a barrierless transition. After all, the structure of the proton-shared TS may now represent the true lowest energy geometry at the theory level of the current calculations.

In summary, the density functional study of the PH^+P and QH^+Q hydrogen-bonded complexes reveals strong hydrogen

Table 1. Optimized geometries and energies: Results were obtained with full optimizations at the B3LYP/6-31G(d) theory level. The results obtained with a larger basis set of 6-311+G(2d,p) are also given in parenthesis. *d*1 and *d*2 denote the distance of the proton from N1 and N2 in PH^+P, or from O1 and O2 in QH^-Q, respectively. *L* is the distance between N1 and N2 in PH^+P and that for O1 and O2 in QH^-Q. The dissociation energy defined by $\Delta D_e = [E_e(MH^+) + E_e(M)] - [E_e(MH^+M)]$ is obtained by taking the energy difference of the sum of monomer (M) and protonated monomer (MH^+) energies, and the energy of the corresponding dimer geometry, without BSSE corrections. Using zero-point energy corrections, the corrected dissociation energy is calculated by $\Delta D_0 = [E_0(MH^+) + E_0(M)] - [E_0(MH^+M)]$. The zero-point energy was scaled by 0.9804

	Geometry (Å)			Energy (kcal/mol)			
	*d*1	*d*2	L	ΔD_e	ΔD_0	$\Delta E_e^{barrier}$	$\Delta E_0^{barrier}$
[PH^-···P] Min	1.10	1.62	2.72	23.0	23.0	1.2	−1.1
	(1.11)	(1.58)	(2.69)	(21.1)	(21.3)	(0.8)	(−1.2)
[P···H^-···P] TS	1.29	1.29	2.58	21.9	24.2		
	(1.29)	(1.29)	(2.58)	(20.3)	(22.5)		
[QH^-···Q] Min	1.08	1.40	2.48	28.0	28.3	0.3	−1.5
	(1.10)	(1.34)	(2.44)	(25.6)	(26.1)	(0.1)	(1.5)
[Q···H^-···Q] TS	1.21	1.21	2.42	27.8	29.8		
	(1.21)	(1.21)	(2.41)	(25.5)	(27.3)		

bonding interactions, arising from protonation, with hydrogen bonding contribution being more than 20 kcal/mol and the low energy barriers for proton transfer reaction being ~1 kcal/mol. Interestingly, it was also shown that the zero-point energy effects can alter the overall proton-transfer reaction energetics so that the lowest energy structure for the proton-bound dimer of aromatic molecular bases takes a form of the proton-shared structure. In this specific example of aromatic base molecular species, zero-point energy consideration predicts that no energy barrier exists for proton transfer reactions, suggesting a dynamic reactivity of the proton in the molecular systems.

Since the barrier height is only 1 kcal/mol, much caution should be paid to invoking the reversal of the reaction energetics between the minimum and TS states even though state-of-the-art theoretical methods are employed. In general, true corrections for zero-point energy are only possible with a numerically reconstructed potential energy surface. Other corrections, such as those for BSSEs (basis set superposition errors) and quantum mechanical tunneling effects, can also affect the reaction energetics by up to a few kcal/mol. As for the BSSEs, the errors due to different basis set sizes, involved when predicting a dissociation energy, were calculated to be only a small amount of the +0.1 and 0.5 kcal/mol for PH^+P and QH^+Q, respectively, at the B3LYP/6-311+G(2d,p) theory level. But we deliberately avoid the corrections, as its validity for the strongly bonded complexes of $[P\cdots H^+\cdots P]$ and $[Q\cdots H^+\cdots Q]$ is not clear. However, we would like to point out that, for low energy barriers, as found in this work for the proton-bound aromatic base molecular complexes, the contribution of zero-point energy should be considered because it influences the energetics (barrier height) and, thus, the overall dynamics of the proton transfer. This may have some implications for the spectroscopically observed proton-shared structure, for example, $[H_3N\cdots H^+\cdots NH_3]$ in the case of the gas phase $NH_4^+(NH_3)$ complex.[10] Numerous theoretical results indicate that the minimum energy structure for an ion-molecule complex type of $[NH_4^+\cdots NH_3]$ has a barrier of 2.3 kcal/mol [B3LYP/6-31G(d,p)] after BSSE corrections.[11] This may also have some implications for the reactivity of the proton in a proton-bound biological system, such as C^+C, where the proton transfer reaction is believed to participate actively in many biologically important processes.

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References

1. Hobza, P.; Sponer, J. *Chem. Rev.* **1999**, *99*, 3247.
2. Lee, K.-M.; Park, S.-W.; Jeon, I.-S.; Lee, B.-R.; Ahn, D.-S.; Lee, S. *Bull. Korean Chem. Soc.* **2005**, *26*, 909.
3. Hobza, P.; Sponer, J. *J. Am. Chem. Soc.* **2002**, *124*, 11802.
4. Zoete, V.; Meuwly, M. *J. Chem. Phys.* **2004**, *121*, 4377.
5. Li, X.; Cai, Z.; Sevilla, M. D. *J. Phys. Chem. B* **2001**, *105*, 10115.
6. Florián, J.; Leszczyński, J. *J. Am. Chem. Soc.* **1996**, *118*, 3010.
7. Sponer, J.; Leszczynski, J.; Vetterl, V.; Hobza, P. *J. Biomol. Struc. & Dyn.* **1996**, *13*, 695.
8. Dannenberg, J. J.; Tomasz, M. *J. Am. Chem. Soc.* **2000**, *122*, 2062.
9. Müller, A.; Talbot, F.; Leutwyler, S. *J. Am. Chem. Soc.* **2002**, *124*, 14486.
10. Price, M.; Crofton, M. W.; Lee, Y. T. *J. Phys. Chem.* **1991**, *95*, 2182.
11. Meuwly, M.; Karplus, M. *J. Chem. Phys.* **2002**, *116*, 2572.
12. Park, J. K. *Bull. Korean Chem. Soc.* **1999**, *20*, 1067.
13. Frank-Kamenetskii, M. D.; Mirkin, S. M. *Annu. Rev. Biochem.* **1995**, *64*, 65.
14. Soyfer, V. N.; Potaman, V. N. *Triple-Helical Nucleic Acid*; Springer-Verlag: New York, NY, 1996.
15. Chubb, J. M.; Hogan, M. E. *Trends Biotechnol.* **1992**, *10*, 132.
16. Gee, J. E.; Miller, D. M. *J. Med. Sci.* **1992**, *304*, 366.
17. Williams, L. D.; Shaw, B. R. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 1779.
18. Völker, J.; Klump, H. H. *Biochemistry* **1994**, *33*, 13502.
19. Roberts, R. W.; Crothers, D. M. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 4320.
20. Scaria, P. V.; Shafer, R. H. *Biochemistry* **1996**, *35*, 10985.
21. Chen, L.; Cai, L.; Zhang, X.; Rich, A. *Biochemistry* **1994**, *33*, 13540.
22. Berger, I.; Kang, C. H.; Fredian, A.; Ratliff, R.; Moyzis, R.; Rich, A. *Nature Struct. Biol.* **1995**, *2*, 416.
23. Kang, C. H.; Berger, I.; Lockshin, C.; Ratliff, R.; Moyzis, R.; Rich, A. *Proc. Natl. Sci. Acad. USA* **1995**, *92*, 3874.
24. Jaskolski, M.; Gdaniec, M.; Gilski, M.; Alejska, M.; Bratek-Wiewioroska, M. D. *J. Biomol. Struct. Dyn.* **1994**, *11*, 1287.
25. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.; *Gaussian 03, Revision C.02*; Gaussian, Inc.: Wallingford, CT, 2004.
26. Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1997**, *119*, 11988.
27. Strittmatter, E. F.; Williams, E. R. *J. Phys. Chem. A* **2000**, *104*, 6069.