# Effects of Hydration on the Proton Transfer Mechanism in the Adenine−Thymine Base Pair

**J. P. Cerón-Carrasco* and A. Requena†**

*Departamento de Química Física Facultad de Química, Física Facultad de Química, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain*

**C. Michaux‡**

*Laboratorie de Chimie Biologique Structurale, Facultés Universitaires Notre-Dame de la Paix Rue de Bruxelles, 61. 5000 Namur, Belgium*

**E. A. Perpète§ and D. Jacquemin¶**

*Laboratoire de Chimie Théorique Appliquée, Facultés Universitaires Notre-Dame de la Paix Rue de Bruxelles, 61. 5000 Namur, Belgium*

*Received: January 27, 2009; Revised Manuscript Received: April 1, 2009*

We report the first density functional study of water catalytic effect in the double proton transfer (DPT) taking place in the adenine−thymine (AT) base pair. To gain more insight regarding the accuracy of several theoretical methods, the ability of various functionals and models for describing the geometry of this system has first been checked. According to our results, BP86/6-311++G(d,p) is the best option for describing the solvation effects in AT when applied to a two-water-molecule-featuring model. The two possible mechanisms for DPT in solution are explored: in the first one, water molecules only remain passive elements, whereas in the second one they are directly included in the reaction path. For the noncatalyzed mechanism, the stable structures constitute the canonical form of the base pair and the first proton transfer product. Nevertheless, by involving the two water molecules in the reaction, we found three stable species: canonical base pair, first proton transfer product, and double proton transfer product. Although the thermodynamic analysis confirms that AT does not contribute to spontaneous mutation through proton transfer catalyzed by surrounding water, our results suggest that microhydration may play a crucial role for DPT reaction in others DNA or RNA basis pair.

## I. Introduction

DNA-based system molecules are probably the most important biomolecules because, among other important functions, it stores and transfers the genetic information. Its chemical structure was already proposed in the seminal article by Watson and Crick in 1953[1] and consists of two double helixes of nucleotides linked by stabilizing hydrogen bonds formed between adenine−thymine and guanine−cytosine basis pairs (see Figure 1). However, it was more recently discovered that water is essential for DNA's stability.[2,3] In fact, DNA requires about 30% of water by weight to maintain its native conformation in the crystalline state. Of course, the water molecules surrounding DNA can also influence the equilibrium between the canonical form of the bases (Figure 1) and the possible tautomers, which probably play a key role in the formation of spontaneous mutations in DNA (see Gorb and co-worker's paper[4] and references therein). These (rare) tautomers have chemical structures, resulting from intra- or intermolecular proton transfer (Figure 2), that differ from the canonic forms described by Watson and Crick's DNA model. The mechanism proposed by Löwdin assumes that such tautomers are formed via the transfer of two protons in the interbase hydrogen bond[5,6] and suggests that the phenomenon of a spontaneous mutagenesis is induced by this variations of the tautomeric state of the nucleotide bases. Furthermore, rare tautomeric forms of DNA bases pairs can also play an important role in the formation of cyclobutane dimers and adducts, the most important products in mutagenesis of DNA under ultraviolet irradiation.[7] Consequently, proton transfer mechanism in DNA base pairs have been extensively studied using a wide range theoretical methods.[7−23]

However, most of this studies were carried out in gas phase, though DNA properties are highly sensitive to the environment. Physiological conditions are obviously closer to the condensed phase than to the gas phase, and consequently studying (partially) hydrated DNA bases has become a very important task for computational chemists. The influence of hydration on the tautomeric equilibrium has been tackled in several studies[4,24−35] With ab initio schemes,[33] Kabelác and Hobza have recently investigated the role of a solvent on the tautomerism in a nucleic acid base, and their results suggest that water strongly affects the tautomeric equilibrium of nucleic acid base. Lee and Cho have also quantified the importance of solvent effects for the vibrational properties of base pairs.[31] After DFT calculations they have found frequency shifts of the base modes, which are induced by the H-bonding interactions with surrounding water

* E-mail: jpceron@um.es, A. Requena.
† E-mail: rqna@um.es.
‡ Scientific Research Worker of the Belgian FNRS.
§ Senior Research Associate of the Belgian FNRS.
¶ Research Associate of the Belgian FNRS.

10.1021/jp900782h CCC: $40.75 
Published on Web 06/08/2009

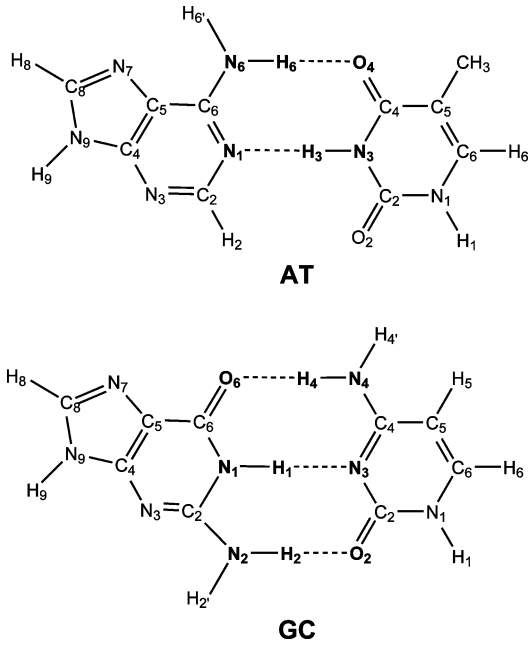


**Figure 1.** Watson−Crick adenine−thymine (AT) and ganine−cytosine (GC) base pairs structure.

molecules, are critical for accurate simulations of the IR absorption spectra of DNA. Other key works about chemical environment effects have been carried out by Herbert and co-workers, who evaluated the bond strengths for DNA base pairs using polarizable continuum models to evaluate solvation effects[32] and by Burda and co-workers, who focused their attention on the interactions between hydrated metal cations and the DNA bases.[36−38] In spite of these results, there is to the best of our knowledge no work considering the mechanism of the catalysis role of water for DPT in DNA base pairs, and only a few papers have considered water assisted proton transfer in a single base.[39−43] For instance, in a very recent study, Kim and co-workers report the first DFT calculations for the tautomerization of adenine facilitated by water molecules.[41]

Can surrounding water molecules affect the DPT reaction between DNA basis pairs and consequently influence the spontaneous mutation mechanism? To answer this question, we have investigated the water catalytic role during DPT in AT base pair using ab initio tools, more precisely density functional theory (DFT). We have focused our study on the AT basis pair because it contains only two hydrogen bonds, the DPT reaction in AT being consequently simpler than in the other guanine−cytosine basis pairs (see Figure 1). Although B3LYP can be considered as the most popular functional in the quantum chemistry studies, it is known that its fails for the prediction of chemical reaction barriers, or in the description of the intermolecular hydrogen bond.[44] Specifically, Wijst and co-workers demonstrated the inadequacy of B3LYP for DNA modeling because it significantly underestimates the hydrogen bond strength for basis pairs.[45] Therefore, to provide the most accurate results within DFT we initially checked the performance of several functionals for AT calculations. Furthermore, the theoretical model used for describing the hydration shell is of prime importance, and for this reason we compare the capabilities of several models for the interaction between AT and the surrounding water molecules.

## II. Theoretical Methods

Canonical Watson−Crick AT base pair structure has been fully optimized using the B3LYP,[46,47] BMK,[48] BP86,[46,49] PW91[50] and PBE0[51] functionals, in connection with Pople's 6-311++G(d,p) basis set, which can be considered large enough for DNA studies.[22,23,39,40,52] Moreover, results obtained with this basis compare with the previous data obtained by employing the cc-pVTZ and TZ2P basis set.[45] The optimizations have been carried out without any symmetry restriction (in $C_1$ symmetry group). The influence of the basis set superposition error (BSSE) in the final geometries and relative energies has been checked by computing the geometries in two different ways: (i) geometries obtained without BSSE correction are used for counterpoise (CP)[53] single point energy calculations; (ii) full BSSE corrections are performed during the optimization process,[54] as we recently demonstrated the importance of BSSE in water−amino acid interactions.[55,56] The correspondence of the stationary points to minimum or transition state has been checked by the analytic calculation of harmonic vibrational frequencies at the same level of the theory: absence of imaginary values for a minima, but one imaginary frequency for transition states. As an accurate ab initio benchmark for the gas phase AT base pair, we employ the MP2 complete basis set limit results obtained by Sponer et al.[57] We have to point out the additional high-level work by Jurecka et al., using coupled-cluster correction to the MP2 energies, and yielding similar results.[58] Here, we present only comparisons with the RI-MP2/aug-cc-pVQZ//RI-MP2/cc-pVTZ calculations from ref 57.

The experimental values for hydrogen bonds[59] are compared with the results obtained with BP86, PW91, and PBE0 functionals, using the 6-311++G(d,p) basis set, and considering different solvation models. We have selected as starting point the previous studies carried out by Guerra et al.,[45,60] who concluded that the AT-a and AT-c models provide the closest approach to the AT geometry in solution. Kumar and co-workers[61] designed a five-water-molecule AT-d model for successfully studying electron affinities of AT. While these previous studies have not been focused on DPT, the quality of the results obtained in both cases supports the validity of the solvation models for modeling the DPT process in solution. Furthermore, since the main environmental effect is the weakening of the N6−O4 hydrogen bond,[45,60] our AT-b model is built up from the original AT-d model with the aim of reproducing the experimental geometry of AT in solution by including only

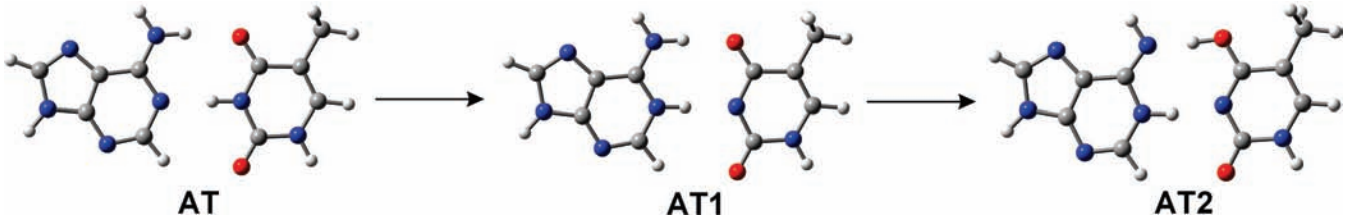


**Figure 2.** Double proton transfer via the two-step mechanism (see text for details).

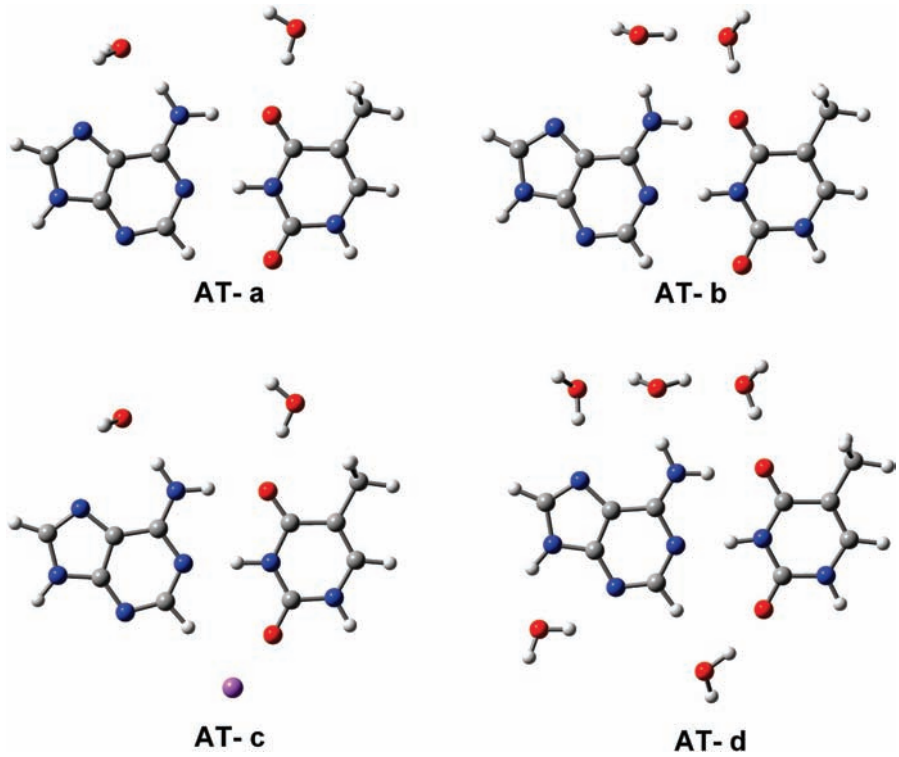


**Figure 3.** Theoretical models for AT in solution. AT-a and AT-c models are taken from ref 45 (reprinted with permission of Elsevier, copyright 2006), whereas AT-b and AT-d models are designed according to the Kumar method for first hydration shell (ref 61).

**TABLE 1: Hydrogen Bond Distances (Å) and Complexation Energies (in kcal/mol) for AT**

| | optimization without BSSE | | | | | | optimization with BSSE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| method | N6−O4 | N1−N3 | MAD[a] | $\Delta E$ | BSSE | $\Delta E_{BSSE}$[b] | N6−O4 | N1−N3 | MAD[a] | BSSE | $\Delta E_{BSSE}$[b] |
| RI-MP2/aug-cc-pVQZ//RI-MP2/cc-pVTZ[c] | 2.86 | 2.83 | | | | −15.1 | | | | | |
| cc-pVTZ basis set | | | | | | | | | | | |
| B3LYP[d] | 2.93 | 2.88 | 0.060 | −13.0 | 1.4 | −11.6 | | | | | |
| BLYP[d] | 2.94 | 2.90 | 0.075 | −12.5 | 1.7 | −10.8 | | | | | |
| BP86[d] | 2.87 | 2.83 | 0.005 | −13.7 | 1.5 | −12.2 | | | | | |
| 6-311++G(d,p) basis set | | | | | | | | | | | |
| B3LYP | 2.94 | 2.89 | 0.070 | −12.6 | 0.7 | −11.9 | 2.96 | 2.90 | 0.065 | 0.7 | −11.9 |
| BMK | 2.98 | 2.86 | 0.075 | −12.8 | 0.5 | −12.3 | 2.99 | 2.87 | 0.085 | 0.6 | −12.3 |
| BP86 | 2.89 | 2.83 | 0.015 | −13.2 | 0.8 | −12.4 | 2.90 | 2.85 | 0.030 | 0.8 | −12.4 |
| PW91 | 2.88 | 2.82 | 0.015 | −15.5 | 1.0 | −14.5 | 2.90 | 2.83 | 0.020 | 1.0 | −14.5 |
| PBE0 | 2.91 | 2.83 | 0.025 | −14.6 | 0.9 | −13.5 | 2.92 | 2.84 | 0.035 | 0.9 | −13.5 |

[a] Mean absolute deviation of the theoretical distances with respect to the RI-MP2/aug-cc-pVQZ values. [b] Bond energy with BSSE correction. [c] From ref 57. [d] From ref 45.

the water molecules which are complexed to that bond. All these models are shown in Figure 3. In this initial study, we have not taken into account larger hydration shells, as the interactions with the water molecules in the first hydration shell are much stronger than in the outer shells.

Then, we have computed the energies for the reactants, transition state, and products for DPT with BP86/6-311++G(d,p), the computational method providing the best hydrogen bond distances for AT base pair both in the gas phase and for the AT-b model. Throughout this paper, we assume the two-step mechanism for proton transfer in both the gas and condensed phases, which is the only possible mechanism according to the topology of the potential energy surface, in agreement with a recent the dynamical study.[4,18] Partial atomic charges were evaluated using the natural bond orbital (NBO)[62−64] and Merz−Singh−Kollman ESP[65,66] schemes. All calculations were carried out using the Gaussian 03 package,[67] while the normal vibration mode visualizations were performed using the Molekel program.[68]

## III. Results and Discussion

**A. AT in the Gas Phase.** In this section, we first discuss the H-bond lengths for all optimized structures in the gas phase. Table 1 shows the main geometric parameters involved in the two hydrogen bonds, together with previous benchmark MP2 results from the literature.[57] According to the mean absolute deviation (MAD) calculated for the hydrogen bond distances, DFT approaches are in very good agreement with MP2 results. Furthermore, DFT calculations using the 6-311++G(d,p) basis set do not provide significantly different results from larger basis sets such as cc-pVTZ, especially with the BP86, PW91, and PBE0 functionals. In an early stage we can neglect BSSE effects in geometry optimizations because the obtained bond distances differ less than 0.02 Å, and consequently the counterpoise correction would not yield results closer to the reference values. Regarding the complexation energies, using any of the functionals with the 6-311++G(d,p) basis set, the BSSE correction is less than 1 kcal/mol (<6.5% from the total energy), and thus

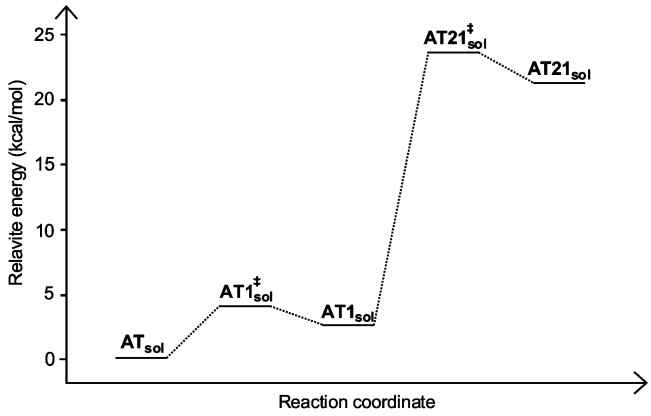


**Figure 4.** Reaction profile for DPT reaction for AT in solution. $AT_{sol}$, $AT1_{sol}$, and $AT21_{sol}$ denote adenine−thymine in the canonical form, first proton transfer product, and DPT with water catalysis, respectively. The total energies are given with respect to the canonical form.

it is suggested to omit the CP correction in the theoretical treatments of the DPT mechanism with these functionals combined to 6-311++G(d,p), a fortiori because we are interested in the relative energies between different tautomers. We are aware that the dispersion-corrected DFT (DFT-D) developed by Truhlar[69] and Grimme[70] is a highly promising method for the accurate description of noncovalent interactions,[71,72] mainly to describe van der Walls interactions. Unfortunately, the DFT-D approach also presents some shortcomings when used for the hydrogen bonded cases[73] like the one treated here. DFT-D does not guarantee, therefore, an improvement of the optimized geometry versus "conventional" functionals. For instance, when Lin et al.[74] considered dispersion corrections through BLYP-D and DCACP functionals for DNA base pairs, the MAD calculated for the hydrogen bond distances were 0.050 and 0.035 Å, respectively, while the BP86/6-311++G(d,p) method provides a much smaller deviation (0.015 Å). Accordingly, BP86 with the 6-311++G(d,p) basis set is a good option for the theoretical study of the tautomeric equilibrium in an AT base pair.

Consistently, we proceed to the investigation of the DPT reaction between the AT base pair in the gas phase at the BP86/6-311++G(d,p) level. Villani showed that the only possible mechanism for DPT is a two-step mechanism,[18] in which first the hydrogen H3 (see Figure 1) migrates from N3 to N1, achieving the AT1 structure with an almost unmodified H6−O4 hydrogen bond. Then, in a second stage, H6 moves from N6 to O4, reaching the final DPT product, that is, AT2. This mechanism is detailed in Figure 2. According to our calculations, the global minimum is found for the canonical structure of AT base pair, whereas neither the product for the first proton transfer (AT1) nor the DPT product (AT2) are stable structures. Let us briefly discuss the origin of this instability: AT1 is not a minimum because the first step implies the formation of a ion-pair-like intermediate (a zwitterionic tautomer), which is a very unstable form in the gas phase. Regarding AT2, our conclusion is consistent with the works of Florian et al.,[8] Gorb et al.,[4] and Villani,[18] who studied the gas phase DPT potential energy surface in AT base pair. In these studies, it is only possible to distinguish a clear minimum for the canonic species, as well as for a shallow local minimum for AT2 with practically the same energy as the transition state. In light of these respective energies, we can conclude that our computational scheme is totally in line with previous theoretical studies, as they all indicate that the DPT reaction between AT may not occur in the gas phase.

**B. Model for DPT in Solvated AT.** Despite the fact that DFT calculations are in very good agreement with the "best" benchmark values in the gas phase, discrepancies between theoretical geometries and the crystallographic data[59] pertains in most works dealing with DNA basis pair (see for instance refs 45 and 60). As noted by Guerra and co-workers,[60] these theory/experiment discrepancies are not due to a failure of the theoretical methods but to a deficiency in the model. Nevertheless, when a set of water molecules is added to the AT model to include environmental effects, the hydrogen bond distances come to be in much better agreement with experiment, whereas the effect of surrounding groups, such as methyl or ribose, is negligible. Therefore, to get meaningful insight about DNA base pairs, we selected the models published recently by the same authors (AT-a and AT-c),[45] together with the theoretical model for the first hydration shell considered by Kumar (AT-d).[61] In addition, a new model (AT-b) was built up, in which we have chosen to only include the two water molecules of the first shell that are closer to the N6 atom of adenine and to the O4 atom of thymine. Therefore, this model relies on the most hydration-sensitive hydrogen bond,[60] as shown in Figure 3.

The geometries of each hydrated AT base pair have been fully optimized with the functionals that provide the best results in gas phase (BP86, PW91, and PBE0). In Table 2, the calculated hydrogen bonds are compared both with the previous theoretical values available in the literature[45] and with the experimental data.[59] As one can deduce from this table, when two water molecules are in the vicinity of the N6−O4 hydrogen bond (models AT-a, AT-b and AT-c), this bond lengthens by 0.6−0.8 Å, whereas the N1−N3 distance remains almost unchanged. However, for the five-water-molecule model (AT-d), we observe the opposite effect: the N6−O4 bond distance is similar to that in the gas phase model, while the N1−N3 bond is elongated by 0.6−0.7 Å, so that the two hydrogen bond distances are practically equivalent in this model. This result is a logical consequence of the presence of water molecules forming hydrogen bonds with H2 and O2 atoms. From the data shown in Table 2, it can be concluded that compared to the two-water-molecule models, including a sodium cation (AT-c) or the whole first shell hydration with five water molecules (AT-d) does not improve the final description of the hydrogen bond distance involved in the DPT. Thus, both AT-a and AT-b are sufficient to model the environment impact on the AT pair geometry, with all these three functionals. For AT-a and AT-b models, the BP86 functional provides results close to reference data at a reasonable cost.

Now that the performances of several theoretical schemes have been studied, we are able to investigate DPT reactions between adenine and thymine in a microhydrated environment. The canonical structures for the two selected models are practically isoenergetic (AT-a is only 0.36 kcal/mol more stable than AT-b) and easily interconvertible one into the other at room temperature. Taking into account this result, together with the fact that only AT-b contains the appropriate positions to let act the water as catalyst, the AT-b model is selected for more refined study, and hereafter when we refer to AT in solution ($AT_{sol}$) we assume the AT-b model. Unlike in the gas phase, we have now two different reaction paths, depending on the role of the surrounding water molecules. On the one hand, water molecules could only be passive elements during the reaction, and therefore the mechanism is the same as in gas phase (see above). On the other hand, water molecules can act directly as a proton donor and as a proton acceptor[75] and subsequently catalyze the DPT process. To distinguish between these two mechanisms, we

**TABLE 2: Hydrogen Bond Lengths (Å) for Several AT Models in Solution**

| | | | BP86 | | | | |
|---|---|---|---|---|---|---|---|
| model | H-bond | exp[a] | cc-pVTZ | 6-311++G(d,p) | TZ2P | PW91 6-311++G(d,p) | PBE0 6-311++G(d,p) |
| | N6−O4 | 2.93/2.95 | 2.93[c] | 2.95 | 2.92[c] | 2.94 | 2.97 |
| AT-a | N1−N3 | 2.85/2.82 | 2.82[c] | 2.82 | 2.80[c] | 2.81 | 2.82 |
| | MAD[b] | | 0.015/0.010 | 0.025/0.000 | 0.030/0.025 | 0.025/0.010 | 0.035/0.010 |
| | N6−O4 | | | 2.95 | | 2.94 | 2.97 |
| AT-b | N1−N3 | | | 2.80 | | 2.79 | 2.80 |
| | MAD[b] | | | 0.035/0.010 | | 0.035/0.025 | 0.045/0.020 |
| | N6−O4 | | 2.96[c] | 2.97 | 2.93[c] | 2.95 | 2.97 |
| AT-c | N1−N3 | | 2.81[c] | 2.81 | 2.79[c] | 2.80 | 2.81 |
| | MAD[b] | | 0.035/0.010 | 0.040/0.015 | 0.030/0.025 | 0.035/0.010 | 0.040/0.015 |
| | N6−O4 | | | 2.90 | | 2.88 | 2.90 |
| AT-d | N1−N3 | | | 2.88 | | 2.88 | 2.89 |
| | MAD[b] | | | 0.030/0.055 | | 0.040/0.065 | 0.035/0.060 |

[a] X-ray crystallographic measurements from ref 59. Note that there are two experimental distances for N4−O6 and N1−N3, because Seeman et al. studied two pairs with different environments. [b] Mean absolute deviation in theoretical distances from MP2/aug-cc-pVQZ values. [c] From ref 45.

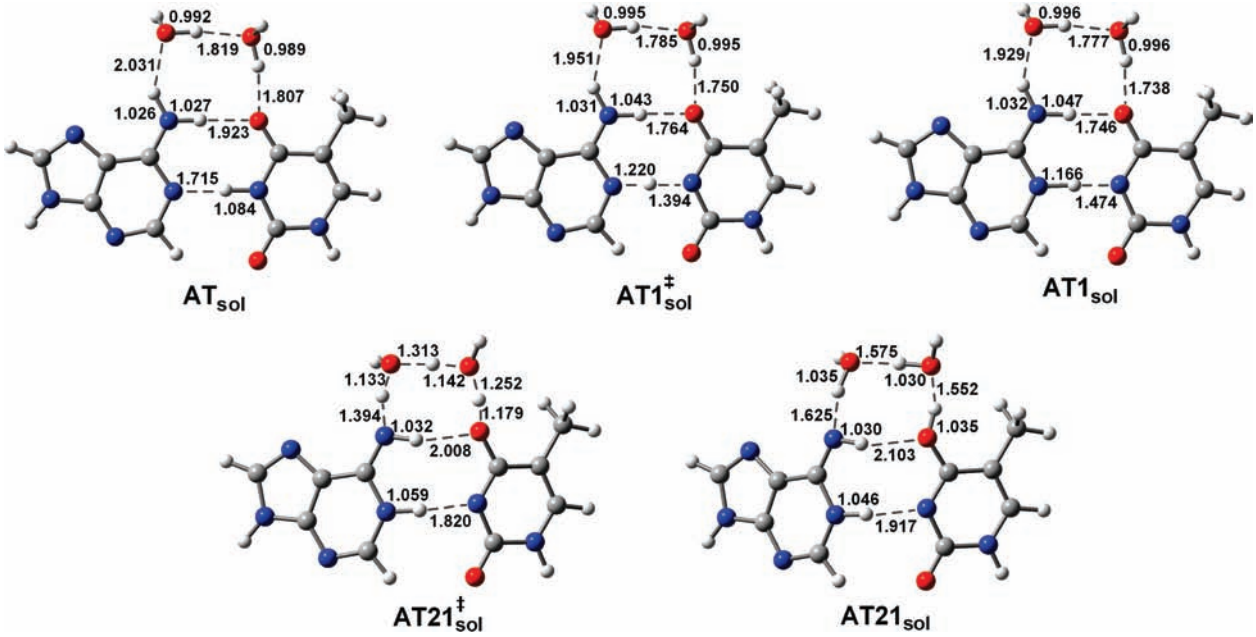


**Figure 5.** Optimized structures for DPT reaction in solution model. The values shown in the figure refer to theoretical distances for the bond implied in the reaction (in Å).

labeled the direct product as $AT2_{sol}$, whereas the water-assisted product is $AT21_{sol}$.

Figures 4 and 5 depict the reaction profile for the DPT process in solution, as well as the optimized structures for the stationary points. According to the reaction profile, and even though water molecules are not directly involved in the first proton transfer, the presence of explicit water affects the stability of the $AT1_{sol}$ product. As we previously pointed out, AT1 is an ion-pair-like intermediate, very instable in the gas phase, but stabilized by the presence of water molecules surrounding the base pair. $AT1_{sol}$ has a total energy similar to that for the transition state $AT1^{‡}_{sol}$, relating it to the canonical form (see Table 4). This first step is common for both direct and catalyzed DPT mechanisms, but we found a strong difference between the two mechanisms for the second step. In fact, for the direct reaction, no stable products were found (AT2 structure is not a minimum), whereas in the case of the water catalyzed process the DPT product (labeled as AT21) corresponds to a real minimum. In the transition states, all the atoms involved in the DPT mechanism are almost coplanar. The relevant associated frequency to $AT1^{‡}_{sol}$ is 373.8i $cm^{-1}$ and is mainly related to the H3−N3 stretching. For $AT21^{‡}_{sol}$, only one imaginary frequency shows up at 803.2i $cm^{-1}$, which can be interpreted as the simultaneous stretching N6−H6′, together with the stretching of water hydrogen atoms involved in the catalyzed proton transfer (vibrational modes analysis is detailed in Supporting Information). It is worth noting that the optimized structures along solvated DPT are nonplanar, especially for both the second proton transfer transition state and the products ($AT21^{‡}_{sol}$ and $AT21_{sol}$). This off-planarity of the hydrated AT is consistent with the previous results obtained by Kumar and co-workers,[61] who also found slightly bent structures for the AT base pair when surrounded by water molecules.

We have also studied the microsolvation effects on the charge distribution of the compounds. As we show in Table 3, both NBO and ESP charges show similar trends for the DPT in solution. When the first proton transfer is achieved, the expected ion-pair-like intermediate ($AT1_{sol}$) is reached, in which the adenine base has a partial positive charge, and the thymine has a negative charge. On the other hand, according to the results included in this table, the set of surrounding water molecules remains neutral. In this way, our results show that although

**TABLE 3: Total NBO and ESP Charges on the Hydrated Canonical, First Proton Product, and Catalyzed Second Proton Transfer Product in Solution ($AT_{sol}$, $AT1_{sol}$, and $AT21_{sol}$, Respectively) Calculated with the BP86/6-311++G(d,p) Method**

| | NBO charges | | | ESP charges | | |
|---|---|---|---|---|---|---|
| | $AT_{sol}$ | $AT1_{sol}$ | $AT21_{sol}$ | $AT_{sol}$ | $AT1_{sol}$ | $AT21_{sol}$ |
| adenine (A) | 0.054 | 0.711 | 0.029 | 0.117 | 0.663 | 0.028 |
| thymine (T) | −0.044 | −0.701 | −0.034 | −0.082 | −0.631 | 0.051 |
| water 1 (W1)[a] | −0.017 | −0.015 | −0.010 | −0.033 | −0.032 | −0.082 |
| water 2 (W2)[b] | 0.008 | 0.004 | 0.015 | 0.000 | −0.002 | 0.003 |

[a] Water molecule complexed to adenine base. [b] Water molecule complexed to thymine base.

**TABLE 4: Total Electronic Energy ($E$/au), Relative Energy ($\Delta E$/kcal mol$^{-1}$), and Relative Gibbs Free Energies at 298 K ($\Delta G$/kcal mol$^{-1}$) Calculated for the DPT Reaction in Solution**

| | $E$ | $\Delta E$ | $\Delta G$[a] |
|---|---|---|---|
| $AT_{sol}$ | −1074.71645 | 0.00 | 0.00 |
| $AT1^{\ddagger}_{sol}$ | −1074.71225 | 2.63 | 1.53 |
| $AT1_{sol}$ | −1074.71229 | 2.61 | 2.35 |
| $AT21^{\ddagger}_{sol}$ | −1074.68017 | 22.77 | 21.79 |
| $AT21_{sol}$ | −1074.68301 | 20.98 | 22.84 |

[a] Thermal corrections taken from unscaled vibrational calculations.

solvent effects on the charge distribution are negligible, water-surrounding molecules are likely to play an important role in the DPT for AT and other base pairs, because they stabilize the ion-pair-like intermediates and catalyze the second stage of the DPT. This result agrees with previous studies demonstrating that a very few water molecules are sufficient to stabilize the ionic intermediates in other related systems.[76−80]

Finally, the thermodynamics study is carried out by computing the relative Gibbs free energies at 298 K, which are presented in Table 4, and show that, even though we obtain three minima in the total energy profile (Figure 4), there is no minimum for $AT1_{sol}$ nor $AT21_{sol}$ based on $\Delta G$ obtained for catalyzed DPT process. This result coincides with the previous evidence for the DPT between AT in gas phase obtained by Gorb and co-workers,[4] who noted that $\Delta G$ computed for transition states are smaller than the relative total energies, and that the minimum corresponding to the DPT product disappears on the surface of the Gibbs free energy. Taking account the aforementioned results, the DPT mechanism in AT would not participate in hydrated DNA spontaneous mutation.

## IV. Conclusions

We have checked the ability of several theoretical methods, as well as molecular models, for describing the double proton transfer in adenine−thymine DNA basis pairs. The optimized geometries of our AT-b model are in very good agreement with the structures experimentally observed, in particular for calculations carried out at the BP86/6-311++G(d,p) level. Using this computational framework, all stable tautomers produced by DPT have been determined, both in the gas phase and in solution. Our results show that in the gas phase the only stable structure corresponds to the canonical AT. However, the DPT mechanism between the adenine and thymine basis pair is strongly affected by the environment in water solution. Thus, the zwitterionic tautomer $AT1_{sol}$ becomes a stable intermediate due to the interaction with the two side water molecules, and we obtain a stable DPT product through the catalysis of the surrounding waters, $AT21_{sol}$. In contrast, considering the relative Gibbs free energies, no minimum is obtained for $AT1_{sol}$, nor for $AT21_{sol}$. Accordingly, we conclude that AT is not involved in spontaneous mutation in hydrated DNA.

Although these calculations were carried out with relatively simple models, they deliver important insights into the DPT process between DNA base pairs, and spontaneous mutation in others base pairs, such as guanine−cytosine or adenine−uracil, are currently investigating.

**Acknowledgment.** The work was partially supported by the Ministerio de Educacion y Ciencia of Spain under Project CTQ2007-66528, by FEDER funds from the European Union, and by the Fundación Séneca del Centro de Coordinación de la Investigación de la Región de Murcia under Project 08735/PI/08. E.A.P., C.M., and D.J. thank the Belgian National Fund for their respective positions. Most calculations have been performed on the Interuniversity Scientific Computing Facility (ISCF), installed at the Facultés Universitaires Notre-Dame de la Paix (Namur, Belgium), for which we gratefully acknowledge the financial support of the FNRS-FRFC and the "Loterie Nationale" for the convention number 2.4578.02 and of the FUNDP. J.P.C.C. acknowledges a FPU fellowship provided by the Ministerio de Educación y Ciencia of Spain and also wishes to thank all members of the Laboratorie de Chimie Théorique Appliquée of the Facultés Universitaires Notre-Dame de la Paix for their warm hospitality.

**Supporting Information Available:** (1) Cartesian coordinates of the AT, $AT_{sol}$, $AT1^{\ddagger}_{sol}$, $AT1_{sol}$, $AT21^{\ddagger}_{sol}$, and $AT21_{sol}$ optimized with the BP86/6-311++G(d,p) method. (2) Visualization of the normal modes with an imaginary frequency for $AT1^{\ddagger}_{sol}$ and $AT21^{\ddagger}_{sol}$ transition states. This information is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Watson, J.; Crick, F. *Nature* **1953**, *171*, 964–967.
(2) Rueda, M.; Kalko, S.; Luque, F.; Orozco, M. *J. Am. Chem. Soc.* **2003**, *125*, 8007–8014.
(3) Rueda, M.; Luque, F.; Orozco, M. *J. Am. Chem. Soc.* **2006**, *128*, 3608–3619.
(4) Gorb, L.; Podolyan, Y.; Dziekonski, P.; Sokalski, W.; Leszczynski, J. *J. Am. Chem. Soc.* **2004**, *126*, 10119–10129.
(5) Löwdin, P. *Rev. Mod. Phys.* **1963**, *35*, 724–732.
(6) Löwdin, P. *Electronic Aspects of Biochemistry*; Academec Press: New York, 1964.
(7) Grebneva, H. *J. Mol. Struct.* **2003**, *645*, 133–143.
(8) Florián, J.; Hrouda, V.; Hobza, P. *J. Am. Chem. Soc.* **1994**, *116*, 1457–1460.
(9) Florián, J.; Leszczynski, J. *J. Am. Chem. Soc.* **1996**, *118*, 3010–3017.
(10) Marañón, J.; Fontani, A.; Grigera, J. *J. Theor. Biol.* **1999**, *201*, 93–102.
(11) Guallar, V.; Douhal, A.; Moreno, M.; Lluch, J. *J. Phys. Chem. A* **1999**, *103*, 6251–6256.
(12) Li, X.; Cai, Z.; Sevilla, M. *J. Phys. Chem. B* **2001**, *105*, 10115–10123.
(13) Kryachko, E.; Sabin, J. *Int. J. Quantum Chem.* **2003**, *91*, 695–710.
(14) Sobolewski, A.; Domcke, W. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2763–2771.
(15) Herrera, B.; Toro-Labbe, A. *J. Chem. Phys.* **2004**, *121*, 7096–7102.
(16) Danilov, V.; Anisimov, V.; Kurita, N.; Hovorun, D. *Chem. Phys. Lett.* **2005**, *412*, 285–293.
(17) Shimizu, N.; Kawano, S.; Tachikawa, M. *J. Mol. Struct.* **2005**, 243–248.
(18) Villani, G. *Chem. Phys.* **2005**, *316*, 1–8.
(19) Villani, G. *Chem. Phys.* **2006**, *324*, 438–446.
(20) Liu, F.; Qian, P.; Yan, S.; Bu, Y. *J. Mol. Struct.: THEOCHEM* **2006**, *760*, 209–217.
(21) Perun, S.; Sobolewski, A.; Domcke, W. *J. Phys. Chem. A* **2006**, *110*, 9031–9038.

(22) Noguera, M.; Sodupe, M.; Bertrán, J. *Theor. Chem. Acc.* **2007**, *118*, 113–121.
(23) Noguera, M.; Bertrán, J.; Sodupe, M. *J. Phys. Chem. B* **2008**, *112*, 4817–4825.
(24) Gorb, L.; Leszczynski, J. *J. Am. Chem. Soc.* **1998**, *120*, 5024–5032.
(25) Alemán, C. *Chem. Phys.* **1999**, *244*, 151–162.
(26) Alemán, C. *Chem. Phys.* **2000**, *253*, 13–19.
(27) Shishkin, O.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. B* **2000**, *104*, 5357–5361.
(28) Moroni, F.; Famulari, A.; Raimondi, M. *J. Phys. Chem. A* **2001**, *105*, 1169–1174.
(29) Sivanesan, D.; Sumathi, I.; Welsh, W. *Chem. Phys. Lett.* **2003**, *367*, 351–360.
(30) Podolyan, Y.; Gorb, L.; Leszczynski, J. *Int. J. Mol. Sci.* **2003**, *4*, 410–421.
(31) Lee, C.; Cho, M. *J. Chem. Phys.* **2006**, *125*, 114509.
(32) Herbert, H.; Halls, M.; Hratchian, H.; Raghavachari, K. *J. Phys. Chem. B* **2006**, *110*, 3336–3343.
(33) Kabelac, M.; Hobza, P. *Phys. Chem. Chem. Phys.* **2007**, *9*, 903–917.
(34) Park, H.; Nam, S.; Song, J.; Park, S.; Ryu, S. *J. Phys. Chem. A* **2008**, *112*, 9023–9030.
(35) Sukhanov, O.; Shishkin, O.; Gorb, L.; Leszczynski, J. *Struct. Chem.* **2008**, *19*, 171–180.
(36) Sponer, J.; Sabat, M.; Burda, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem. B* **1999**, *103*, 2528–2534.
(37) Sponer, J.; Burda, J.; Sabat, M.; Leszczynski, J.; Hobza, P. *J. Phys. Chem. A* **1998**, *102*, 5951–5957.
(38) Burda, J.; Shukla, M.; Leszczynski, J. *J. Mol. Model* **2005**, *11*, 362–369.
(39) Hu, X.; Li, H.; Liang, W.; Han, S. *J. Phys. Chem. B* **2005**, *109*, 5935–5944.
(40) Shukla, M.; Leszczynski, J. *J. Phys. Chem. A* **2005**, *109*, 7775–7780.
(41) Kim, H.; Ahn, D.; Chung, S.; Kim, S.; Lee, S. *J. Phys. Chem. A* **2007**, *111*, 8007–8012.
(42) Michalkova, A.; Kosenkov, D.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. B* **2008**, *112*, 8624–8633.
(43) Fogarasi, G. *Chem. Phys.* **2008**, *349*, 204–209.
(44) Jensen, A.; Gerhards, M. *J. Chem. Phys.* **2001**, *115*, 5445–5453.
(45) van der Wijst, T.; Guerra, C. F.; Swart, M.; Bickelhaupt, F. *Chem. Phys. Lett.* **2006**, *426*, 415–421.
(46) Becke, A. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
(47) Lee, C.; Yang, W.; Parr, R. *Phys. Rev. B* **1988**, *37*, 785–789.
(48) Boese, A. D.; Martin, J. M. L. *J. Chem. Phys.* **2004**, *121*, 3405–3416.
(49) Perdew, J. *Phys. Rev. B* **1986**, *33*, 8822–8824.
(50) Wang, Y.; Perdew, J. *Phys. Rev. B* **1991**, *44*, 13298–13307.
(51) Adamo, C.; Barone, V. *J. Chem. Phys.* **1999**, *110*, 6158–6170.
(52) Shukla, M.; Leszczynski, J. *J. Phys. Chem. A* **2002**, *106*, 4709–4717.
(53) Boys, S.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553–566.
(54) Simon, S.; Duran, M.; Dannenberg, J. J. *J. Chem. Phys.* **1996**, *105*, 11024–11031.
(55) Michaux, C.; Wouters, J.; Jacquemin, D.; Perpète, E. A. *Chem. Phys. Lett.* **2007**, *445*, 57–61.
(56) Michaux, C.; Wouters, J.; Perpète, E. A.; Jacquemin, D. *J. Phys. Chem. B* **2008**, *112*, 2430–2438.
(57) Sponer, J.; Jurecka, P.; Hobza, P. *J. Am. Chem. Soc.* **2004**, *126*, 10142–10151.
(58) Jurecka, P.; Sponer, J.; Cerny, J.; Hobza, P. *Phys. Chem. Chem. Phys.* **2006**, *8*, 1985–1993.
(59) Seeman, N.; Rosenberg, J.; Suddath, F.; Kim, J.; Rich, A. *J. Mol. Biol.* **1976**, *104*, 109–144.
(60) Guerra, C. F.; Bickelhaupt, F.; Snijders, J.; Baerends, E. *J. Am. Chem. Soc.* **2000**, *122*, 4117–4128.
(61) Kumar, A.; Mishra, P.; Suhai, S. *J. Phys. Chem. A* **2005**, *109*, 3971–3979.
(62) Foster, J.; Weinhold, F. *J. Am. Chem. Soc.* **1980**, *102*, 7211–7218.
(63) Reed, A.; Weinstock, R.; Weinhold, F. *J. Chem. Phys.* **1985**, *83*, 735–746.
(64) Carpenter, J.; Weinhold, F. *THEOCHEM* **1988**, *169*, 41–62.
(65) Singh, U.; Kollman, P. *J. Comput. Chem.* **1984**, *5*, 129–145.
(66) Besler, B.; Merz, K.; Kollman, P. *J. Comput. Chem.* **1990**, *11*, 431–439.
(67) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; 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.; Bakken, V.; 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 D.02; Gaussian, Inc.: Wallingford, CT, 2004.
(68) Flükiger, P.; Lüthi, H.; Portmann, S.; Weber, J. *Molekel*; Swiss Center for Scientific Computing: Manno, Switzerland; 2000.
(69) Zhao, Y.; Truhlar, D. G. *J. Phys. Chem. A* **2005**, *109*, 5656–5667.
(70) Grimme, S. *J. Comput. Chem.* **2004**, *25*, 1463–1473.
(71) Morgardo, C.; Vicent, M. A.; Hillier, I. H.; Shan, X. *Phys. Chem. Chem. Phys.* **2007**, *9*, 448–451.
(72) Lin, I. C.; Rothlisberger, U. *Phys. Chem. Chem. Phys.* **2008**, *10*, 2730–2734.
(73) Barone, V.; Biczysko, M.; Pavone, M. *Chem. Phys.* **2008**, *346*, 247–256.
(74) Lin, I. C.; von Lilienfeld, A.; Coutinho-Neto, M. D.; Tavernelli, I.; Rothlisberger, U. *J. Phys. Chem. B* **2007**, *111*, 14346–14354.
(75) Kryachko, E.; Nguyen, M.; Zeegers-Huyskens, T. *J. Phys. Chem. A* **2001**, *105*, 1934–1943.
(76) Podolyan, Y.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. A* **2002**, *106*, 12103–12109.
(77) Aikens, C. M.; Gordon, M. S. *J. Am. Chem. Soc.* **2006**, *128*, 12835–12850.
(78) Im, S.; Jang, S. W.; Lee, S.; Lee, Y.; Kim, B. *J. Phys. Chem. A* **2008**, *112*, 9767–9770.
(79) Bachrach, S. M. *J. Phys. Chem. A* **2008**, *112*, 3722–3730.
(80) Sobolewski, A. L.; Shemesh, D.; Domcke, W. *J. Phys. Chem. A* **2009**, *113*, 542–550.

JP900782H