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Theoretical Study of Direct and Solvent–Assisted Proton Transfer and Tautomerism in the Ground State of 8–Hydroxyadenine

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Theoretical Study of Direct and Solvent–Assisted Proton Transfer and Tautomerism in the Ground State of 8–Hydroxyadenine

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Abstract

Motivation. Due to the potential misreading of unusual nucleoside the presence of the modified adenine may be the reason for errors occurring during the replication and transcription processes. So that 8–hydroxyadenine is an interesting subject for theoretical studies.

Method. DFT method with the hybrid functional denoted B3LYP have been used throughout this work. In order to take into account the bulk of the solvent we have used the self–consistent isodensity polarized continuum model (SCI–PCM).

Results. The energy barrier for proton transfer process is quite high in both electronic states (183.3 kJ/mol), but decreases significantly to 50.2 kJ/mol when one water molecule is used as solvent or 39.6 kJ/mol for two water molecules. The same trend is observed in going from gas phase to aqueous phase. Furthermore, in going from gas phase to aqueous phase, the dipole moments of the enolic, keto and TS complexes increase. Their values go respectively from 2.83 D, 2.35 D and 2.60 D to 3.87 D, 3.11 D and 3.47 D, showing that both water–complexed tautomers become more polar. Consequently, it is clear that the effect of solvent bulk is to favor more polarized structures so that charge separation and the dipole moment are clearly higher.

Conclusions. The energy barrier for proton transfer process decreases significantly in addition of one and two water molecules in gas phase, and this behavior is more pronounced in aqueous phase.

Keywords. 8–Hydroxyadenine; proton transfer; tautomerization; DFT; B3LYP; 6–311+G**.

Abbreviations and notations

DNA, deoxyribonucleic acid	DFT, Density Functional Theory
B3LYP, Becke Three Lee–Yang–Parr	NBO, Natural Bond Orbital
SCRF, Self–Consistent Reaction Field	SCI–PCM, Self–Consistent Isodensity Polarized Continuum Model

Dedicated to Professor Lemont B. Kier on the occasion of the 75th birthday.

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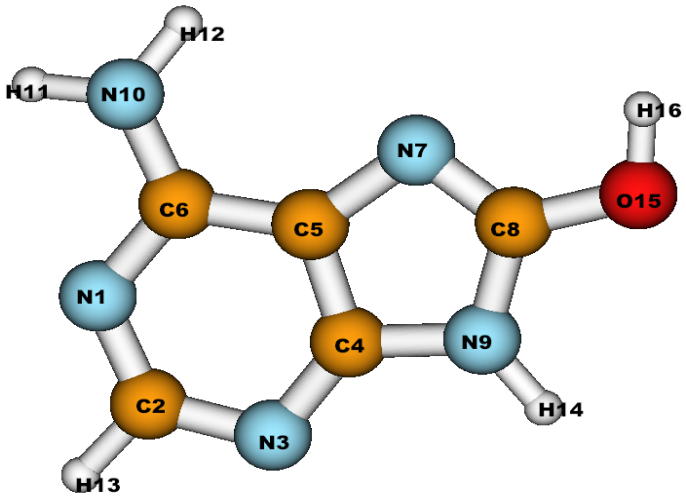
1 INTRODUCTION

A significant fraction of biological lesions undergone by living tissues after exposure to ionizing radiation result from oxidative modifications of DNA molecules. The reaction of the hydroxyl radical HO, produced by water radiolysis with DNA, the oxidative stress developed in response to environmental pollutants, chronic inflammation, and various degenerative diseases may be cited as examples of oxidative DNA damages. These oxidative DNA damages are believed to increase the risk of malignant cell transformation and the development of many human cancers [1–3].

Evidence exists showing that free–radical–induced DNA damage occurs in vivo, and there is a steady–state level of the free–radical–modified bases in cellular DNA. The adenine molecule may undergo different modifications as the result of the hydroxyl attack. The hydroxylation of adenine at the C_8 position leads to 8–hydroxyadenine (8–HA) [4,5]. This molecule and many of its derivatives possess interferon (IFN) inducing activities, and in combination with ribavirin they play an interesting role in the treatment of cancer [6–8].

Recently, a lot of interest has been focused not only on the possibility of tautomerism in various *N*–heterocycles of biological interest, but also on the mechanism of intramolecular and intermolecular proton transfer. It has been demonstrated that nucleic acid bases exist predominantly in keto–amino forms [9–11]. This shows that the hydroxyl radical modified adenine probably undergoes additional tautomeric transitions. The tautomeric form is crucial for the determination of miscoding properties since there is a direct correlation between intermolecular hydrogen bond formation and the position of donor and acceptor site. Tautomeric systems such as 8–HA, 2–hydroxypyridine [12,13], 7–hydroxyquinoline [14], 7–hydroxyimidazo[1,2–a]pyridine [15] etc, are bifunctional hydrogen–bonding hydroxyaromatics molecules. They can act as proton donor at the –O–H group, and as proton acceptor at the N_7 atom in the enol form (see Figure 1)

Due to the potential misreading of unusual nucleoside the presence of the modified adenine may be the reason for errors occurring during the replication and transcription processes. As illustration, it has been shown by Switzer *et al*. [16] that the formation of 2–hydroxyadenine in DNA induce promutagenic properties in cells such as adenine → guanine transition, adenine → thymine and adenine → cytosine transversions. Due to its miscoding potential 8–HA are interesting subjects for theoretical studies. The aim of this work is to investigate intramolecular (*i.e*., investigate the direct and solvent–assisted) proton transfer process in 8–HA. We therefore examine the alternative pathways for proton transfer and study the relative tautomer energies and calculate the barriers.



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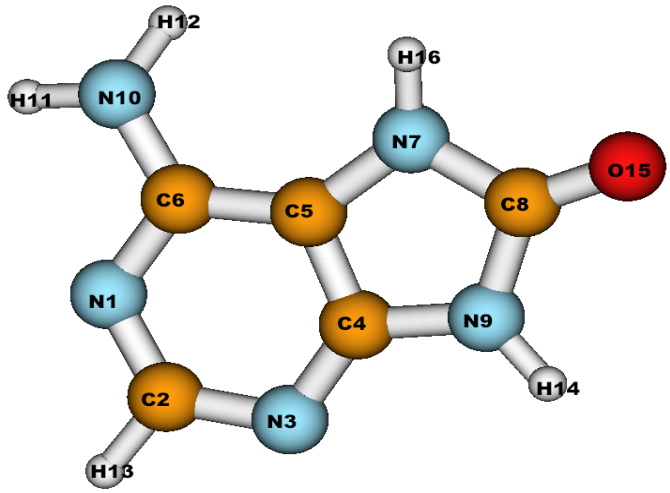

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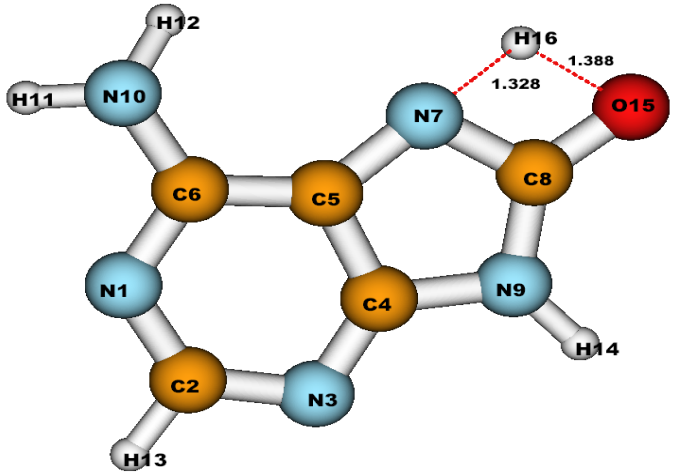


(*c*)

Figure 1. Equilibrium geometries of the enol tautomer (*a*), the keto tautomer (*b*) and the transition state (*c*) of 8–hydroxyadenine (8HA) as optimized with B3LYP/6–311+G**.

2 COMPUTATIONAL METHODS

Calculations of the equilibrium structures and harmonic vibrational frequencies of 8–HA·$(H_2O)_n$ (n = 0–3) have been undertaken. Geometry optimisations for all structures were carried out using the hybrid functional Becke's three parameters (B3) [17] combined with the gradient corrected correlation functional of Lee–Yang–Parr [18]. In order to keep the computational parameters (CPU time and disk storage) to reasonable limits and to maintain the average error under a threshold, the standard split valence triple zeta gaussian basis set 6–311+G** of McLean and Chandler [19] with polarization functions on all atoms and diffuse functions on heavy atoms was chosen and used throughout this work. In the optimisations, both inter– and intra–molecular degrees of freedom were optimised without any symmetry restriction and converged until the largest component of nuclear gradient was 10^{-6} a.u./bohr and the change in total energy was less than 10^{-7} a.u. In order to calculate the natural charges and describe some hydrogen bondings, we have used the natural bond orbital (NBO) procedure [20], implemented in the Gaussian03W computational package [21].

In order to take into account the bulk of the solvent, we have performed an SCRF calculation using a cavity determined self–consistently from an isodensity surface, *i.e.*, the self–consistent isodensity polarized continuum model (SCI–PCM) [22]. All the calculations have been performed with Gaussian03W computational package [21].

3 RESULTS AND DISCUSSION

In the gas phase, the B3LYP/6–311+G** optimized geometries (keto tautomer, enol tautomer and transition state (TS)) are displayed in Figure 1, and their structural parameters listed in Table 1. The energy difference between the two locally stable conformers (enol and keto) is calculated to be 40.4 kJ/mol, the keto tautomer being the most stable. The search for the TS serving for interconversion between the enol and the keto minimized structures gave the fully optimized geometry displayed in Figure 1(c). This optimization has been achieved by using the Opt=QST2 command (in the Gaussian 03W computational package), with the keto and enol stationary geometries used as the starting points on the potential energy surface. The obtained TS structure has been characterized by one imaginary vibrational frequency (ω = –1937.9 cm^{-1}, I = 864.9 km/mol) in the normal coordinate analysis based on the analytic second derivatives. The major contribution to the normal mode associated with this frequency comes from the detachment of the hydrogen atom (H_{16}, see Figure 1.c) from the –O–H group to the nitrogen atom N_7, *i.e.*, N–H–O stretching. The barrier for the enol–keto interconversion is calculated to be 183.3 kJ/mol. As could be observed, this barrier is very high, showing and confirming that this enol–keto tautomerization takes place with difficulties (or does not take place) in vacuum, and that this molecule (8–HA) exists predominantly in the keto–amino form as many nucleic acid bases [23–25].

Table 1. Some selected bond lengths, bond angles and dihedrals as derived from DFT/B3LYP (6–311+G**) geometry optimization of 8–hydroxyadenine·$(H_2O)_n$ (n = 0, 1, 2).

Parameters	8–HA			8–HA·H_2O			8–HA·$(H_2O)_2$		
	Enol	TS	Keto	Enol	TS	Keto	Enol	TS	Keto
Bond length (Å)									
N_1C_2	1.337	1.334	1.331	1.337	1.335	1.332	1.337	1.335	1.333
C_2N_3	1.338	1.340	1.341	1.338	1.339	1.340	1.338	1.339	1.340
N_3C_4	1.330	1.323	1.325	1.329	1.327	1.326	1.329	1.328	1.327
C_4C_5	1.396	1.403	1.397	1.397	1.397	1.396	1.395	1.395	1.393
C_5C_6	1.403	1.399	1.391	1.402	1.399	1.394	1.401	1.398	1.394
C_6N_1	1.344	1.345	1.347	1.344	1.344	1.346	1.344	1.345	1.347
C_6N_{10}	1.360	1.365	1.377	1.365	1.369	1.373	1.367	1.371	1.369
C_5N_7	1.394	1.395	1.396	1.394	1.391	1.393	1.394	1.392	1.392
N_7C_8	1.302	1.333	1.398	1.315	1.348	1.384	1.317	1.347	1.377
C_8N_9	1.372	1.359	1.399	1.371	1.373	1.393	1.374	1.382	1.393
N_9C_4	1.387	1.404	1.379	1.385	1.388	1.382	1.385	1.383	1.383
C_8O_{15}	1.338	1.277	1.210	1.323	1.276	1.223	1.315	1.271	1.228
$H_{16}\cdots O_{15}$	0.966	1.388	–	–	–	–	–	–	–
$N_7\cdots H_{16}$	–	1.328	1.007	–	–	–	–	–	–
$N_7\cdots H_{18}O_{17}$	–	–	–	1.966	1.328	1.017	–	–	–
$O_{17}\cdots H_{16}O_{15}$	–	–	–	1.754	1.189	1.940	–	–	–
$N_7H_{18}\cdots O_{17}$	–	–	–	–	1.182	1.999	–	–	–
$O_{17}H_{16}\cdots O_{15}$	–	–	–	–	1.256	1.939	–	–	–
$O_{15}H_a$	–	–	–	–	–	–	1.001	1.261	1.789
$N_7\cdots H_c$	–	–	–	–	–	–	1.772	1.327	1.027
$O_A\cdots H_a$	–	–	–	–	–	–	1.642	1.157	–
$O_B\cdots H_b$	–	–	–	–	–	–	1.775	1.265	–
$O_A\cdots H_b$	–	–	–	–	–	–	–	1.155	1.768
$O_B\cdots H_c$	–	–	–	–	–	–	–	1.168	1.814
Bond angle (º)									
$N_1C_2N_3$	128.1	127.7	127.7	128.0	127.8	127.7	128.0	127.9	127.9
$C_2N_3C_4$	111.6	112.3	112.6	111.7	112.1	112.5	111.7	112.0	112.3
$N_3C_4C_5$	128.9	126.2	125.4	126.9	126.4	125.6	126.7	126.3	125.5
$C_4C_5C_6$	115.9	116.1	117.0	115.8	116.1	116.9	116.1	116.3	117.3
$C_5C_6N_1$	118.7	118.6	118.5	118.8	118.7	118.4	118.6	118.7	118.2
$C_5C_6N_{10}$	122.6	123.1	123.8	122.9	123.2	123.8	122.9	123.1	123.4
$C_5N_7C_8$	103.5	106.9	110.3	104.1	106.4	110.0	104.5	106.5	109.7
$N_7C_8N_9$	114.7	112.1	104.4	113.6	110.4	105.3	112.9	109.7	105.6
$C_8N_9C_4$	106.0	106.4	111.1	106.7	108.1	110.6	107.0	108.7	110.5
$H_{16}O_{15}C_8$	107.5	–	–	108.1	101.6	104.9	–	–	–
$H_{18}N_7C_8$	–	–	–	102.6	103.5	119.5	–	–	–
$H_aO_{15}C_8$	–	–	–	–	–	–	111.7	116.4	121.3
$H_cN_7C_8$	–	–	–	–	–	–	128.4	122.7	123.4
$O_BO_AO_{15}$	–	–	–	–	–	–	94.9	93.9	91.3
$O_AO_BN_7$	–	–	–	–	–	–	76.9	84.9	80.8
Dihedral (º)									
$H_{12}N_{10}C_6C_5$	12.3	17.5	30.8	18.5	22.3	26.0	20.7	23.8	21.9
$H_{11}N_{10}C_6C_5$	170.2	169.0	169.9	167.9	167.8	169.1	168.1	167.8	168.5
$O_{17}H_{18}N_7C_8$	–	–	–	5.5	5.4	7.8	36.1	22.0	65.5
$H_{19}O_{17}H_{16}O_{15}$	–	–	–	113.4	118.9	144.0	68.2	–14.0	9.3
$N_7O_BO_AO_{15}$	–	–	–	–	–	–	–10.8	–5.3	–6.6
$O_BO_AO_{15}C_8$	–	–	–	–	–	–	14.8	6.9	12.0
$O_AO_BN_7C_8$	–	–	–	–	–	–	15.9	6.8	7.6

It is also found (see Table 2) that the oxygen atom from which the hydrogen migrates becomes more negatively charged in the TS, while the migrating hydrogen (H_{16}) becomes more positively charged, due to its state of simultaneous bonding to two electronegative atoms, nitrogen N_7 and oxygen O_{15}. The positive charge on the migrating hydrogen (H_{16}) confirms that this is a proton transfer reaction. The carbon C_8 in the four–centered structure is also positively charged, showing that the energy of the TS is high because of the strain in the four–membered ring (N_7, C_8, O_{15}, H_{16}) and also because of the interatomic repulsion between the two oppositely charged atoms.

Table 2. NBO charges on some selected atoms of 8–hydroxyadenine·$(H_2O)_n$ (n = 0, 1, 2) at their respective keto, enol and TS equilibrium geometries

Atoms	8–HA			8–HA·H_2O			8–HA·$(H_2O)_2$		
	Enol	TS	Keto	Enol	TS	Keto	Enol	TS	Keto
N_7	–0.296	–0.444	–0.428	–0.634	–0.668	–0.614	–0.643	–0.654	–0.617
C_8	0.272	0.433	0.369	0.736	0.780	0.799	0.751	0.789	0.803
O_{15}	–0.306	–0.425	–0.392	–0.679	–0.699	–0.672	–0.682	–0.713	–0.694
H_{16}	0.296	0.341	0.307	0.502	0.484	0.454	–	–	–
O_{17}	–	–	–	–0.962	–0.936	–0.971	–	–	–
H_{18}	–	–	–	0.522	0.494	0.495	–	–	–
H_a	–	–	–	–	–	–	0.525	0.498	0.506
O_A	–	–	–	–	–	–	–0.968	–0.922	–0.981
H_b	–	–	–	–	–	–	0.511	0.499	0.510
O_B	–	–	–	–	–	–	–0.983	–0.975	–0.975
H_c	–	–	–	–	–	–	0.499	0.474	0.455

Table 3. Dipole moments (in Debye) of the corresponding tautomers of 8–hydroxyadenine·$(H_2O)_n$ (n = 0–3) in gas and aqueous phases.

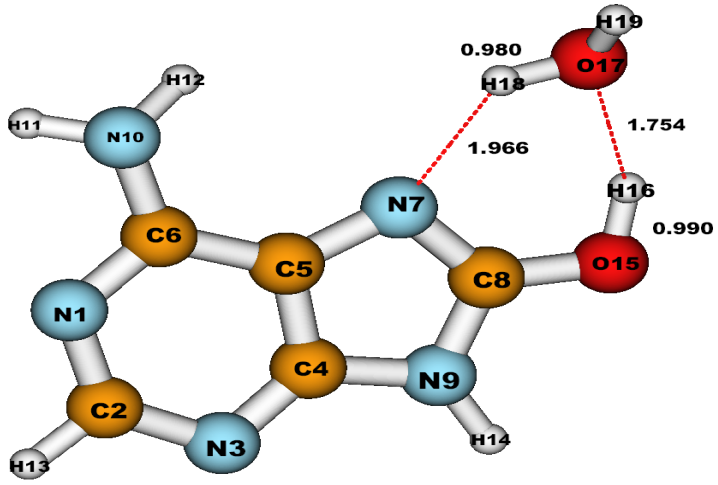
tautomers	8–HA		8–HA·H_2O		8–HA·$(H_2O)_2$		8–HA·$(H_2O)_3$	
	Gas Phase	Aqueous phase	Gas Phase	Aqueous phase	Gas Phase	Aqueous phase	Gas Phase	Aqueous phase
Enol	3.0938	4.0947	2.8271	3.8702	3.3985	4.2525	3.8179	4.6723
Keto	3.1934	4.7732	2.3466	3.1067	2.4860	3.1786	3.2580	3.9557
TS	1.5083	1.8989	2.5982	3.4706	3.2781	un.	3.1197	3.9461

un.: unsuccessful

Table 4. Relative energies (in kJ/mol) of the corresponding tautomers of 8–hydroxyadenine·$(H_2O)_n$ (n=0–3) in gas and aqueous phases.

tautomers	8–HA		8–HA·H_2O		8–HA·$(H_2O)_2$		8–HA·$(H_2O)_3$	
	Gas Phase	Aqueous phase	Gas Phase	Aqueous phase	Gas Phase	Aqueous phase	Gas Phase	Aqueous phase
Keto	–40.4	–50.1	–34.3	–38.0	–39.1	–38.3	–41.9	–41.7
Enol [a]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TS	183.3	174.6	50.2	48.2	39.6	un.	53.0	55.1

[a] The enol electronic energy is taken as reference
un.: unsuccessful



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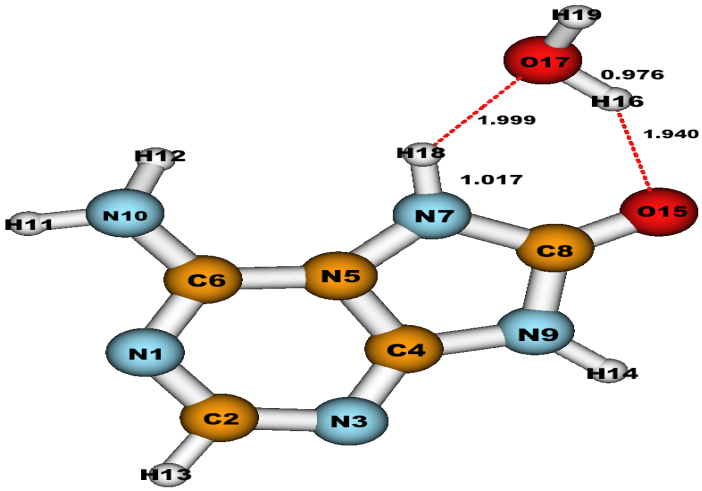

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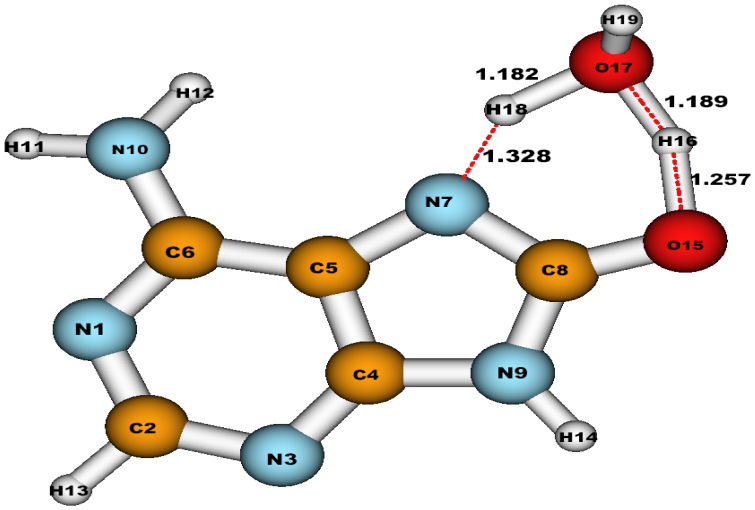

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Figure 2. Equilibrium geometries of the enol tautomer (a), the keto tautomer (b) and the transition state (c) of 8–hydroxyadenine·H_2O (8HA·H_2O) as optimized with B3LYP/6–311+G**.

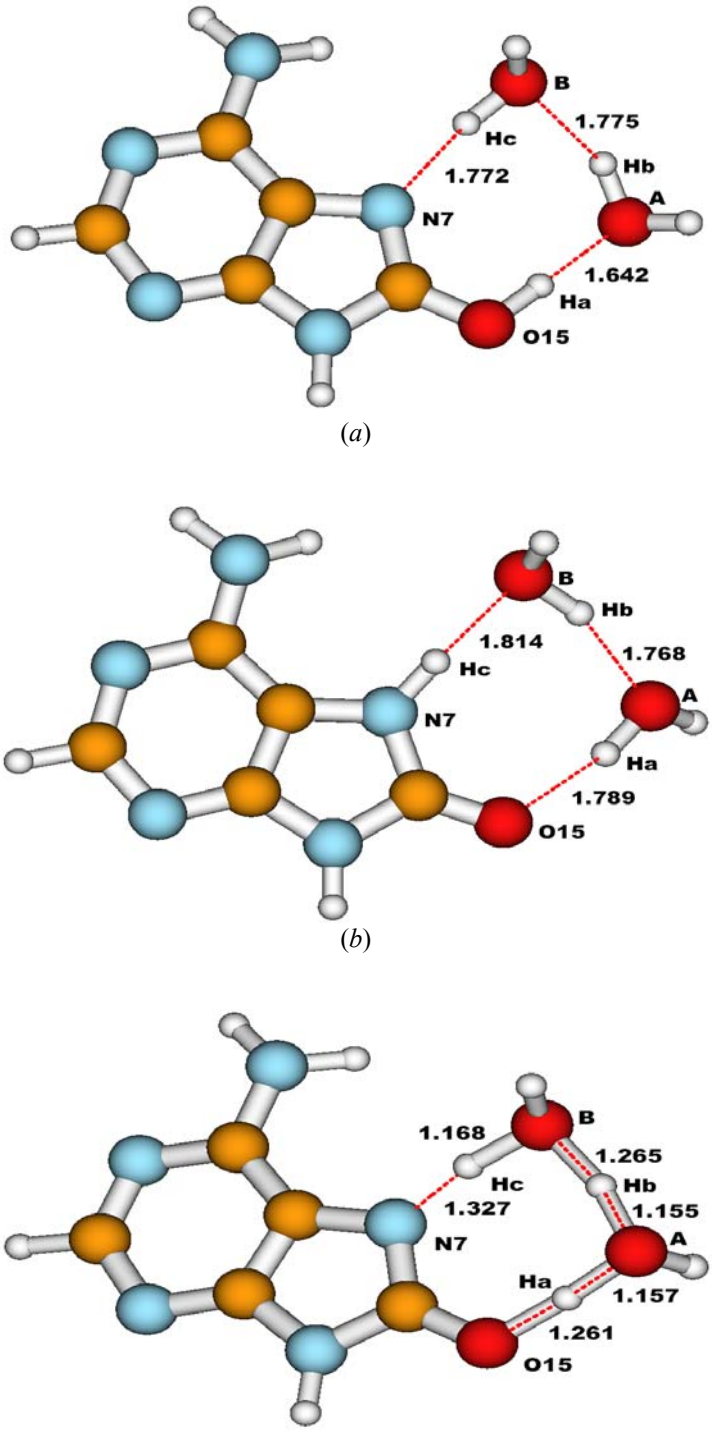


Figure 3. Equilibrium geometries of the enol tautomer (a), the keto tautomer (b) and the transition state (c) of 8–hydroxyadenine·$(H_2O)_2$ (8HA·$(H_2O)_2$) as optimized with B3LYP/6–311+G**.

Complexation with a single water molecule has a significant effect on the geometries of the enol and the keto tautomers and the TS. The charge densities on the atoms involved in the proton transfer (PT) process (N_7, C_8, O_{15}, H_{16}, O_{17}, H_{18}) change. It is found that the total electronic energies of the locally stable geometries of the enol tautomer, the keto tautomer and the TS are respectively calculated to be –619.1856 a.u., –619.1987 a.u. and –619.1664 a.u. Their structural parameters

(bond lengths, bond angles and dihedrals) are listed in Table 1 and their corresponding equilibrium geometries displayed in Figure 2. It also turns out from these electronic energy values that the intervention of one water molecule brings down the activation barrier considerably. This barrier is calculated to be 50.2 kJ/mol (see Table 4). In this solvent–assisted case, the imaginary frequency and its corresponding intensity, for the normal mode associated with this TS, are –1521.6 cm^{-1} and 317.4 km/mol. The values of these parameters are significantly lower than for the direct process. These observations may be explained by the fact that the water molecule plays the role of a bifunctional catalyst. It transfers one proton and accepts another one from the adduct. The water molecule connects the hydrogen–donor group to the hydrogen–acceptor atom of 8–hydroxyadenine through the intermolecular hydrogen–bonds. The analysis of the normal mode associated with the imaginary frequency of the TS, interconverting the enol·H_2O cluster to the keto·H_2O cluster, shows that the two migrating protons of the complex jump at the same time.

In the preceding paragraph, we have seen that one water molecule has a significant effect on the 8–HA tautomerization process. However, it is also evident that one water molecule does not play the role of bulk solvent. To progressively include the effect of the rest of the solvent, we have first considered the introduction of a second water molecule in the system. In gas phase, the optimization process turns out to be successful. An overview of important parameters of the three structures (enol·$(H_2O)_2$, keto·$(H_2O)_2$ and TS) is given in Table 1 and Figure 3 displays the resulting equilibrium geometries. As for the preceding case of one water molecule, the keto tautomer turns out to be more stable than the enol tautomer. Their electronic energies are calculated to be –695.6791 a.u. and –695.6642 a.u. respectively. The transition state interconverting the enol·$(H_2O)_2$ cluster to keto·$(H_2O)_2$ cluster is located at –695.6491 a.u. This leads to a barrier of 39.6 kJ/mol, which is lower than that of the system with a single water molecule. The frequency and the intensity associated with the normal mode interconverting the enol·$(H_2O)_2$ tautomer to the keto·$(H_2O)_2$ are calculated to be ω = –1354.8 cm^{-1} and I = 224.6 km/mol. Here again, each water molecule acts as a bifunctional catalyst as clearly mentioned in the above case of a single water molecule. Figure 3(c) is a good illustration of this behavior. One could obviously observe that hydrogen atoms H_a, H_b and H_c are respectively at intermediate distances. These intermediate distances are 1.261 Å and 1.157 Å with respect to O_{15} and O_A for H_a, 1.155 Å and 1.265 Å with respect to O_A and O_B for H_b, and 1.168 Å and 1.327 Å with respect to O_B and N_7 for H_c. This also confirms that hydrogen atoms involved in the proton transfer chain jump at the same time. Each water molecule transfers one proton and accepts another one from the adduct or the second water molecule. The picture of the proton transfer process is also clearly pointed out in Figure 3(c) by the simultaneous lengthening of the $O_{15}H_a$, O_AH_b and O_BH_c bonds and the shortening of the H_aO_A, H_bO_B and H_cN_7 distances. The intrinsic reaction path (IRC) displayed in Figure 4 indicates the proton transfer path between enol and keto tautomers. Attempts to introduce a third water molecule in a cyclic way (forming hydrogen bond cycle linking the donor and acceptor sites) were unsuccessful (see Figure 5), probably because

the space between the proton donor and the proton acceptor sites is not adequate (too short). In this case (see Figure 5), the oxygen atom O_c of the third water molecule interacts with the hydrogen atom H_{12} of the amino group leading to a hydrogen bond. The consequence of this cooperativity is a slight increase of the energy barrier (53 kJ/mol), compared with the case of two water molecules.

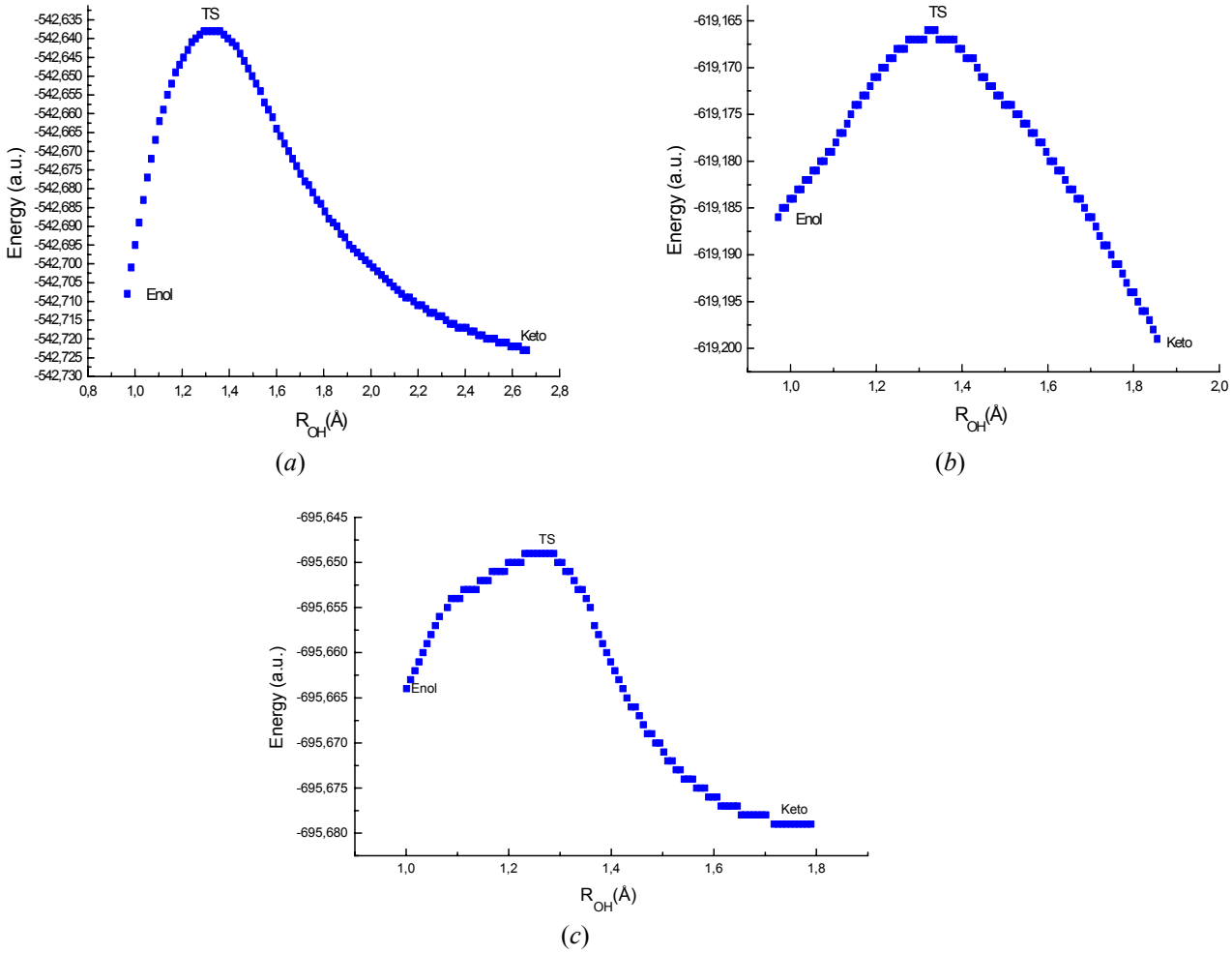
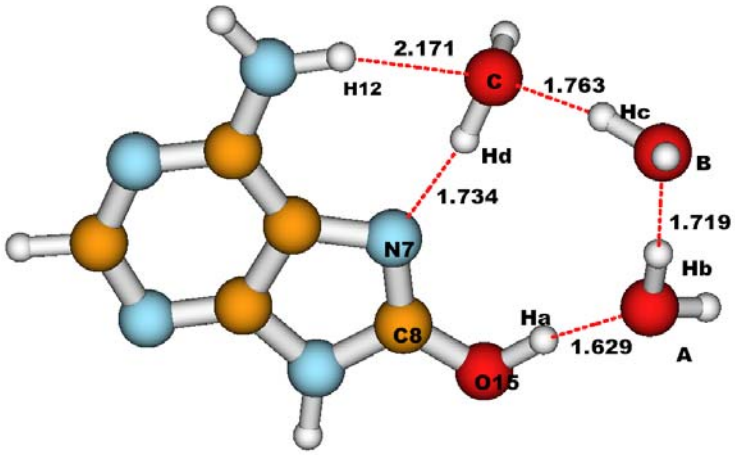
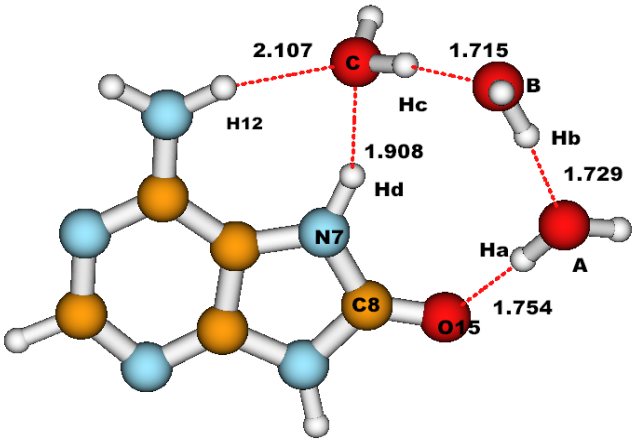


Figure 4. Intrinsic reaction path (IRC) for: the direct enol–keto proton transfer (*a*), the solvent–assisted enol–keto proton transfer for one water molecule (*b*) and the solvent–assisted enol–keto proton transfer for two water molecules (*c*).

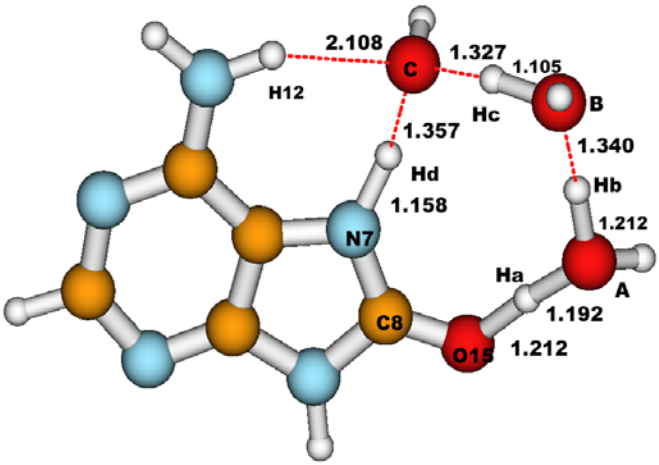
To introduce the effect of the rest of solvent without hydrogen bonding interaction, we have opted for the self–consistent isodensity polarized continuum model (SCI–PCM) [22]. Upon addition of solvent, the stability of each tautomer of the 8–HA·H_2O changes. The electronic energies of the enol, keto and TS tautomers are calculated to be –619.2011 a.u., –619.2156 a.u. and –619.1827 a.u. respectively. Thus, the barrier slightly decreases and becomes 48.2 kJ/mol as compared with that obtained in gas phase. As illustrated in Table 3, in going from gas phase to aqueous phase, the dipole moments of the enolic, keto and TS complexes increase. Except for the non–complexed case (n = 0) where the keto tautomer is more polar than the enol tautomer, in the water–complexed case (n = 1, 2, 3) the enol tautomer is always more polar than the keto tautomer.



(*a*)



(*b*)



(*c*)

Figure 5. Equilibrium geometries of the enol tautomer (*a*), the keto tautomer (*b*) and the transition state (*c*) of 8–hydroxyadenine·$(H_2O)_3$ (8HA·$(H_2O)_3$) as optimized with B3LYP/6–311+G**.

It is also obvious from our calculations (see in Table 3) that the dipole moment decreases when adding one water molecule and increases in addition of more water molecules. Consequently, it is

clear that the effect of bulk solvent is to favor more polarized structures so that charge separation and the dipole moment are clearly higher. Attempts to carry out a single point calculation in aqueous phase on the system with two water molecules (previously optimized in gas phase) were unsuccessful for the TS geometry. So, the analysis of the barrier for two water molecules in aqueous phase will not be discussed. It is also important to emphasize that the correction of electronic energies of the enol and keto equilibrium structures by taking the Zero Point Energy (ZPE) into account does not change the behavior of the barrier. The corrected values of the barrier in gas phase are 169.1, 34.6, 18.3, and 27.7 kJ/mol respectively for n = 0, 1, 2, and n = 3. The corrected energy difference between enol and keto locally stable structures are calculated to be –40.1, –35.9, –39.5, and –41.5 kJ/mol.

Table 5. A part of calculated second order perturbation stabilization energies $E^{(2)}$ (kJ/mol) for donor–acceptor natural orbital interactions for 8–hydroxyadenine·$(H_2O)_n$ (n = 0–3) enol and keto tautomers.

n [a]		Donor NBO(i)	Acceptor NBO(j)	$E^{(2)}$ (kJ/mol)	
				Gas Phase	Aqueous phase
1	enol	$LP(2)O_{17}$	$BD^*(1)O_{15}–H_{16}$	75.4	77.4
		$LP(1)N_7$	$BD^*(1)O_{17}–H_{18}$	35.9	34.8
	keto	$LP(2)O_{15}$	$BD^*(1)O_{17}H_{16}$	26.3	26.2
		$LP(1)O_{17}$	$BD^*(1)N_7–H_{18}$	27.0	27.1
2	enol	$LP(2)O_A$	$BD^*(1)O_{15}–H_a$	110.0	121.9
		$LP(2)O_B$	$BD^*(1)O_A–H_b$	64.0	64.8
		$LP(1)N_7$	$BD^*(1)O_B–H_c$	94.2	93.2
	keto	$LP(2)O_{15}$	$BD^*(1)O_A–H_a$	43.0	43.1
		$LP(2)O_A$	$BD^*(1)O_B–H_b$	61.7	61.9
		$LP(1)O_B$	$BD^*(1)N_7–H_c$	74.0	75.3
3	enol	$LP(2)O_A$	$BD^*(1)O_{15}–H_a$	120.1	121.0
		$LP(2)O_B$	$BD^*(1)O_A–H_b$	80.3	81.5
		$LP(1)O_C$	$BD^*(1)O_B–H_c$	69.6	70.5
		$LP(1)O_C$	$BD^*(1)N_{10}–H_{12}$	11.5	11.7
		$LP(2)O_C$	$BD^*(1)N_{10}–H_{12}$	4.7	5.1
		$LP(2)N_7$	$BD^*(1)O_C–H_d$	108.9	106.5
	keto	$LP(1)O_{15}$	$BD^*(1)O_A–H_a$	24.1	24.0
		$LP(2)O_{15}$	$BD^*(1)O_A–H_a$	39.7	39.6
		$LP(2)O_A$	$BD^*(1)O_B–H_b$	72.5	72.9
		$LP(2)O_B$	$BD^*(1)O_C–H_c$	79.0	70.4
		$LP(2)O_C$	$BD^*(1)N_7–H_d$	49.2	50.6
		$LP(1)O_C$	$BD^*(1)N_{10}–H_{12}$	17.8	18.4
		$LP(2)O_C$	$BD^*(1)N_{10}–H_{12}$	4.6	4.7

[a] number of water molecules in the complex.

However, it is clear from our results that the increase of the number water molecules stabilized by hydrogen bonds between the proton donor and proton acceptor sites contributes to the decrease of the activation barrier. What leads to the conclusion that the PT reaction may easily occur at room temperature, and an enol–keto equilibrium may be established, in solvent bulk.

Let us now analyze the –O–H bond in the locally stable enol tautomers assisted by one or two water molecules (see Figures 1–3) using the natural bond orbital (NBO) technique. In this NBO analysis, we focus on the stabilization energy $E^{(2)}$. This energy is calculated by the second–order

perturbation theory analysis of the Fock matrix [20]. We are interested in the $E^{(2)}$ for the lone pair orbital of the oxygen atom of the nearest water molecule transferred to the acceptor antibonding orbital of the –O–H bond. The calculated values of $E^{(2)}$ are listed in Table 5. These values of $E^{(2)}$ increase with increasing the number of water molecules, showing for these complexes that the electron density is directly transferred to the –O–H bond. Accordingly, the –O–H bond is elongated (0.990 Å for the monomer and 1.001 Å for the dimer) and the hydrogen bond $O_{17}\cdots H_{16}$ is shortened (they are calculated to be 1.754 Å for n = 1 and 1.642 Å for n = 2). This shortening of the $O_{17}\cdots H_{16}$ hydrogen bond confirms its progressive strengthening. Interestingly, one could observe that the frequency of the $O_{15}H_a\cdots O_A$ stretching decreases (3332.7 cm^{-1} for n = 1 and 3109.3 cm^{-1} for n = 2); showing its red shift when one or two water molecules are attached. The increase of the strength of the hydrogen bond (as determined by ν(–O–H) stretching frequency) is accompanied by an extremely large increase of the intensity of the stretching vibration of the proton donor (–O–H). This intensity grows from 647.9 km/mol for n = 1, to 863.3 km/mol for n = 2. It is clear that these observations are typical for classical hydrogen bonding and become more pronounced as the interaction strength increases. It is thus clear that the noticeably larger shortening of the $O_A \cdots H_a$ hydrogen bond in the dimer complex as compared with the analogues in the monomer, indicates the strong hydrogen bond cooperativity in the above–cited complex. Added to the lengthening of the –O–H bond, both processes may be interpreted as the preparation of the 8–HA for the proton transfer to the solvent, as was clearly pointed out by Nsangou *et al* [15] for 7–hydroxyimidazo[1,2–a]pyridine ammonia clusters. Moreover, One notes for n = 2 that $E^{(2)}$ decreases in going from the –O–H group end to the nitrogen atom, except for the following NBO donor → acceptor, $LP(1)N_7 \rightarrow BD^*(1)O_C–H_d$, $LP(1)N_7 \rightarrow BD^*(1)O_B–H_c$. The consequence is that hydrogen bonds or distances between water molecules increase in going from the hydroxyl group to the nitrogen atom N_7 (for n = 1, $O_{15}–O_{17}$ and $N_7–O_{17}$ are respectively 2.668 Å and 2.774 Å; and for n = 2, $O_{15}–O_A$, $O_A–O_B$ and $O_B–N_7$ are respectively 2.643 Å, 2.709 Å, 2.759 Å) meaning that the hydrogen bond strength diminishes.

4 CONCLUSIONS

In this paper, the direct and solvent–assisted proton transfer between the enol and keto tautomeric forms of 8–HA have been investigated at the B3LYP/6–311+G** level of theory. Our results show that the keto tautomer is more stable than the enol tautomer in their respective ground states. In gas phase, the transition state, which is intermediate between the two locally stable enol and keto tautomers and through which the proton is interchanged, is located at 183.3 kJ/mol above the enol locally stable geometry. This barrier becomes 50.2 kJ/mol when a single water molecule is added and 39.6 kJ/mol for two water molecules. The same trend is observed in solvent bulk, confirming that the solvent–assistance plays an important role in the proton transfer process by

reducing the barrier height, and that the water molecule facilitates the transfer of a proton by using its own hydrogen atoms.

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