Correlated ab Initio Study of Nucleic Acid Bases and Their Tautomers in the Gas Phase, in a Microhydrated Environment, and in Aqueous Solution. Part 3. Adenine

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Altogether, 14 amino and imino tautomers of adenine were studied theoretically in the gas phase, in a microhydrated environment (one and two water molecules), and in bulk water environment using the thermodynamic integration method (MD-TI), conductor-like polarizable continuum model (C-PCM, COSMO), and a hybrid model (C-PCM + one to three explicit water molecules). The structures and relative energies of various tautomers were determined at the RI-MP2 level using the TZVPP basis set. The relative enthalpies at 0 K and relative free energies at 298 K were based on relative energies and zero-point vibration energies, temperature-dependent enthalpy terms, and entropies evaluated at the MP2/6-31G** level. The effect of bulk solvent on the relative stability of adenine tautomers was studied by molecular dynamics free energy calculations using the thermodynamic integration method and self-consistent reaction field. The dipole moment of the canonical form is rather small (2.8 D) but three rare imino tautomers have very large dipole moments (more than 10 D). The canonical form is the global minimum at all theoretical levels in the gas phase, in a microhydrated environment, and in the bulk water. Two unusual rare amino tautomers having hydrogens at N3 and N7, respectively, are less stable in the gas phase by more than 7 kcal/mol and represent the first and the second local minimum. Microhydration, as well as bulk water, stabilizes these unusual tautomers, and the energy gap between them and the canonical form is reduced, but the canonical tautomer remains the global minimum in all three phases. Relative free energies ($T = 298$ K) of these two unusual tautomers in the bulk water evaluated by molecular dynamics free energy calculations are 2.5 and 2.8 kcal/mol, which supports their coexistence in this phase. The C-PCM results agree well with the MD-TI data, and the agreement became close when considering not only the bare tautomers but their complexes with several water molecules representing first solvation shell. Other tautomers are considerably less stable (by 12−45 kcal/mol), and neither a microhydrated environment nor bulk water can change this unfavorable tautomeric equilibrium. The theoretical data predicting the coexistence of the canonical form and the N3 and the N7 tautomers in bulk water nicely agreed with experimental data obtained from NMR measurements of the adenine tautomers in DMSO (Laxer, A.; Major, D. T.; Gottlieb, H. E.; Fischer, B. *J. Org. Chem.* **2001**, *66*, 5463.)

1. Introduction

Different tautomers of nucleic acid (NA) bases are obtained when considering different positions of hydrogen around the base. The rare tautomers may be involved in various biochemical processes including point mutations.[1,2] Their presence in biomolecules is nevertheless rather rare, and NA bases are dominantly present in the most stable canonical form (up to now there has not been found any direct evidence of the existence of tautomers in DNA). The situation is different with gas-phase experiments where various tautomers coexist. Passing from the gas-phase to bulk water requires a description of hydration effects. Providing the dipole moment of various tautomers differs considerably from that of the canonical form, water can dramatically change the relative stability of various tautomers. Many studies on the tautomeric equilibria of NA bases exist, and they directed attention particularly to cytosine and guanine[3−35] but also to adenine.[36−42] Our recent studies[43,44] have shown that bulk water can significantly change the relative stabilities, and with regard to cytosine in the gas phase, the canonical form (being the first local minimum destabilized over the global minimum by about 2 kcal/mol) becomes clearly favored in the water environment over other structures.[43] The situation with guanine is more complicated, and here the canonical form in the gas phase was energetically comparable to the other three tautomers. The bulk water, however, surprisingly stabilized the unusual rare tautomers (with very large dipole moments), which were energetically extremely disfavored (by about 20 kcal/mol) and made them the only existing tautomers in the water phase.[44]

In this paper, we present a study of the tautomerism of adenine (cf. also refs 28 and 32). Gas-phase calculations of the relative stability of 14 different tautomers is accompanied by microhydration studies, as well as studies taking the solvent effects of the bulk water fully into consideration. These calculations, for the first time systematically performed for all adenine tautomers, yield a final insight into the tautomeric preference of adenine in the gas phase, microhydrated environment, and bulk water.

Experimental evidence on the first solvation shell of NA bases is rare, but for adenine cation radical, it was recently shown[45] on the presence of four strongly bound water molecules.

2. Methods

Structures. Twelve different structures of the adenine tautomers were presented in ref 28, and the authors of ref 32

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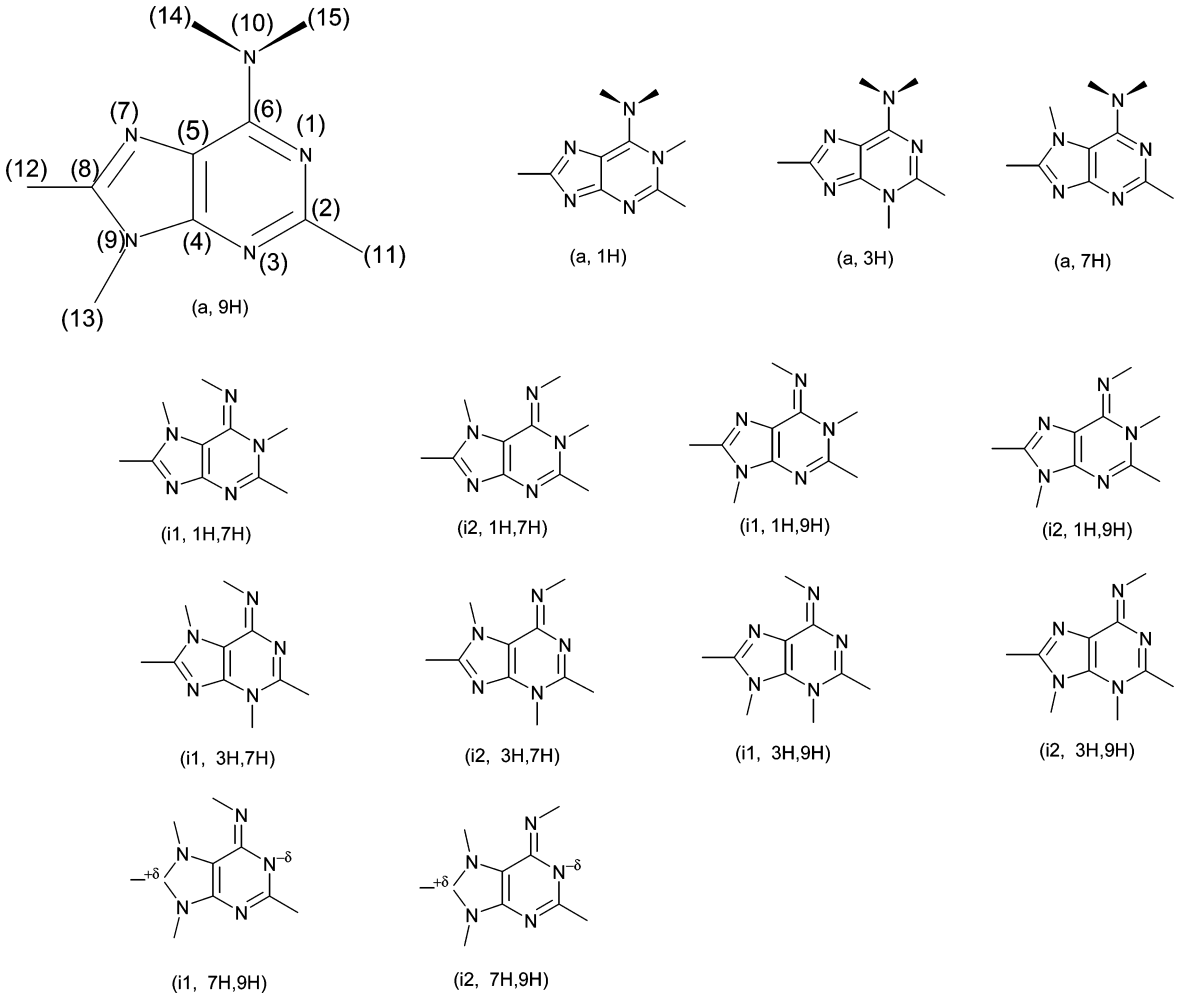

Figure 1. Fourteen adenine tautomers. Standard numbering and adopted nomenclature are presented.

claimed that these are all of the existing structures. This is true providing consideration of classical mesomeric structures is required; it was ascribed for 12 of these structures. With reference to the guanine tautomers,[44] we pointed out the existence of the unusual rare tautomers that could not be described by mesomeric structures and possess polar "zwitterion"-like structures (cf. also ref 46). Consistently and following expectations, relative gas-phase energies of these tautomers are much higher than the energy of the global minimum. These tautomers possess a rather large dipole moment and could be strongly stabilized by microhydration, as well as by bulk water or by covalent or noncovalent bonding with metal atom or ion.[46,47] The 12 "classical" and two unusual "zwitterion"-like structures of adenine investigated in this study are depicted in Figure 1, including the standard numbering of atoms.

Computation Strategy. The gas-phase geometry of the adenine tautomers was determined using the resolution of identity MP2 (RI-MP2) procedure.[48] Relative enthalpies and free energies were obtained by including the zero-point vibration energies (ZPVE), temperature-dependent enthalpy terms, and entropies. A molecular dynamics/quenching technique with the Cornell et al.[49] empirical potential was utilized to explore the potential energy surface (PES) of the adenine···water and adenine···$(water)_2$ clusters. The energy of the most stable structures was later recalculated at the RI-MP2/TZVPP level. The relative stability of various adenine tautomers in a water environment was deduced from the free energies determined as the sum of gas-phase free energies and free energy of hydration. The latter energies were determined using the self-consistent reaction field (SCRF), as well as molecular dynamics (MD) free energy calculations. In the case of the imino tautomers, where two different rotamers exist (and one is sometimes energetically considerably less stable), the molecular dynamics free energy calculations were performed only for the more stable rotamer.

In the case of a highly polar system (like NA bases), water might introduce some changes of geometry, and these changes are expected to be larger for more polar systems. Therefore, the effect of geometry optimization was studied for C-PCM (COSMO) and hybrid approaches.

Quantum Chemical Calculations. Energetical and geometrical characteristics of 14 adenine tautomers in the gas phase were investigated using the RI-MP2 procedure[48] with the double-polarized triple-ζ (TZVPP) basis set [5s3p2d1f/3s2p1d] and default auxiliary basis sets. Relative gas-phase free energies (ΔG_0^{298}) were determined as the sum of relative energy changes [ΔE(RI-MP2) or ΔE(MP2)], zero-point vibration energies (ΔZPVE) and temperature-dependent enthalpies and entropies [$\Delta(G_0^{298}-E)$]. Relative energy changes were calculated at the RI-MP2/TZVPP level. Harmonic vibration analysis providing the ZPVE characteristics and thermodynamic functions (via partition functions), as well as the character of the stationary point found, was computed from MP2/6-31G** characteristics (geometry, vibrations) according to a rigid rotor−harmonic oscillator−ideal gas approximation. Interaction energy of adenine···water and adenine···$(water)_2$ complexes was determined at the RI-MP2/TZVPP level with the inclusion of

corrections for basis set superposition error[50] and deformation energy. All calculations were performed with Gaussian[51,52] and Turbomole[53] suites of programs.

Molecular Dynamics/Quenching (MD/Q) Technique. The interaction of adenine with a microhydrated environment was investigated using a molecular dynamics/quenching technique with the modified Cornell et al. potential.[49] MD/Q simulations were performed in the *NVE* microcanonical ensemble within quaternion formalism. The respective code uses a fifth-order predictor-corrector algorithm with a 0.5 fs integration step. The MD simulations were performed at a constant total energy (corresponding to the average temperature of 298 K) that is high enough to allow crossing over relatively high-energy barriers and thus to sample the whole potential energy surface. Every 1 ps the MD run was interrupted, the kinetic energy was removed, and the structure of the cluster of adenine with one or two water molecules was optimized using the conjugate gradient method; the geometry and energy in the minimum was stored, while the MD run restarts from the point where it was interrupted.

Constants for geometrical parameters of the noncanonical tautomers (not parametrized in the standard Cornell et al. force field[49]) were derived from quantum chemical calculations. The atomic charges of the tautomers were generated with the electrostatic potential fitting procedure[54,55] (RESP) at the HF/6-31G* level.

Self-Consistent Reaction Field−Continuum and Hybrid Approaches. Bulk water was represented by a continuum model based on the C-PCM (COSMO)[56−58] methodology as implemented in Gaussian 03.[51] The C-PCM model was applied because it sufficiently describes solvation in the polar medium. The cavity was described by the UAHF (united atoms radii optimized for HF/6-31G* level of theory), as well as by the Pauling[59,60] vdW radii. The latter case was considered only for comparison to the literature data. Two phases were taken into account, water and dimethyl sulfoxide (DMSO) with their recommended permittivities. In the case of the hybrid method, solvation-first-shell effect was approximated considering one to three explicit water molecules. In all cases, we adopted the following strategy concerning the choice of geometry for C-PCM calculations:

A. Gas-Phase Geometry. Single-point (SP) calculations that considered gas-phase optimized geometries were carried out at HF/6-31G*/UAHF level using the Gaussian 03 standard parameters (UAHF, scaling 1.2, SES) as recommended.[58,61] SES means the solvent excluded surface.

B. C-PCM Optimized Geometry. The geometry of the adenine tautomers was optimized in the continuum solvent at the B3LYP/6-31G* level without considering any explicit water molecule. Small cluster geometries (one and two explicit waters) that were taken from gas-phase calculations were optimized at the B3LYP/6-31G* level; larger cluster geometries (three explicit waters) were superimposed from monohydrated gas-phase structures using the Insight 95.0 package,[62] and then the resulting structures were optimized at the B3LYP/6-31G* level of theory using the standard Gaussian 03 parameters and recommended optimization strategy[58] (iterative method, preconditioning, tessarea 0.4 Å^2). Additional nonelectrostatic first derivatives (related to cavitation, dispersion, and repulsion energies) were included in the SCF procedure. Reliable free energies of solvation are only obtained if the geometry optimization is followed by a single-point calculation at the HF/6-31G*/UAHF level.

Free Energy Calculations. The molecular dynamics-thermodynamic integration (MD-TI) method was applied to calculate relative free energy of hydration (ΔG^{HYD}) between the adenine tautomers. All calculations were performed using the GROMACS molecular modeling package[63,64] with our own code implementing the TI method; the soft core potential scaling[65] was systematically used. A detailed description of the procedure used can be found in our preceding study.[44]

The rectangular periodic box was filled with 412 TIP3P[66] water molecules and all perturbations were performed in the *NPT* canonical ensemble at 1.0 atm and 298.15 K. The time step was set to 1.0 fs, and a nonbonded cutoff of 9.0 Å was used for van der Waals and Coulombic interactions. Before perturbations, the typical 100 ps equilibration run was performed with 1.0 fs time step, 9.0 Å cutoff, and Berendsen temperature and pressure coupling schemes.[67] Perturbations were divided into 250 (shorter perturbations) or 500 (longer perturbations) sampling windows (cf. Supporting Information, Table 1). Vacuum calculations (representing the intermolecular terms in ΔG) were also performed according to the typical protocol: 250 windows, 1.0 fs time step, 100 ps of simulation time. Three values of soft core parameter α (0.60, 1.00, and 1.51) were used to prove theoretically derived independence on this parameter[65] (see Supporting Information, Table 1). The soft core parameter α was finally set to 1.00, and three various simulation times (2, 6, and 11 ns) for both forward and backward runs were used to ensure the convergence of the free energy and to estimate the error limits. All details of simulations performed are presented in the Supporting Information, Table 1.

The relative free energy of the adenine tautomers was finally determined as the sum of relative gas-phase free energies and free energy of hydration obtained from self-consistent reaction field (eq 1), hybrid method (eq 2), or MD-TI (eq 3) treatment:

$$\Delta\Delta G^{TAUT}(A\rightarrow B) = \Delta G^{GP}(B-A) + \Delta G^{SOL}_{SCRF}(B-A) \quad (1)$$

$$\Delta\Delta G^{TAUT}(A\rightarrow B) = \Delta G^{GP}(B-A) + \Delta E_{INT}[B\cdots(H_2O)_n - A\cdots(H_2O)_n] + \Delta G^{SOL}_{SCRF}[B\cdots(H_2O)_n - A\cdots(H_2O)_n] \quad (2)$$

$$\Delta\Delta G^{TAUT}(A\rightarrow B) = \Delta G^{GP}(B-A) + \Delta\Delta G^{SOL}_{MD-TI}(A\rightarrow B) \quad (3)$$

where ΔG and $\Delta\Delta G$ refer to free energy (in absolute scale) and relative free energy (representing difference between two states), A and B refer to the initial and the final states, GP denotes quantum-mechanical calculation in the gas phase, SOL denotes the solvation effect estimated by SCRF or MD-TI methods, INT denotes interaction energy, and *n* denotes the number of explicit water molecules considered.

It is worth noting that the incorrect MD-TI relative gas-phase free energies were replaced by accurate ab initio values (for detailed discussion of the discrepancy in empirical-potential and quantum-mechanical free energies, cf. ref 44, pages 7681 and 7683) and solvation free energies (ΔG^{SOL}) of explicit water molecules were not taken into account because of the mutual compensation within the relative $\Delta\Delta G$ calculation.

3. Results and Discussion

Gas-Phase Tautomers. The relative stability of four amino forms having hydrogen at nitrogen N9 (canonical form; a, 9H), nitrogen N1 (a, 1H), nitrogen N3 (a, 3H), and nitrogen N7 (a, 7H), eight standard imino forms having hydrogens at nitrogens N7 and N1 (i1, 1H,7H; i2, 1H,7H), nitrogens N9 and N1 (i1, 1H,9H; i2, 1H,9H), nitrogens N7 and N3 (i1, 3H,7H; i2, 3H,-7H), and nitrogens N9 and N3 (i1, 3H,9H; i2, 3H,9H), and two unusual imino tautomers having hydrogens at nitrogens N7 and

TABLE 1: Relative Energies (ΔE), Zero-Point Vibration Energies (ΔZPVE), and Free Energies (ΔG) (in kcal/mol) of Adenine Tautomers in the Gas Phase

method structure	ΔE(RI-MP2)[a]	ΔE(MP2)[b]	ΔZPVE[c]	$\Delta(G_0^{298}-E)$[c]	ΔG_0^{298} [c]
(a, 9H)	0.00	0.00	0.00	0.00	0.00
(a, 1H)	17.74	18.79	−0.26	−0.35	17.40
(a, 3H)	7.99	9.22	−0.56	−0.56	7.43
(a, 7H)	7.63	7.79	−0.12	−0.16	7.47
(i1, 1H,7H)	16.55	17.61	−0.30	−0.51	16.05
(i2, 1H,7H)	16.09	16.83	−0.25	−0.32	15.77
(i1, 1H,9H)	12.07	12.36	0.05	−0.03	12.05
(i2, 1H,9H)	18.53	19.08	−0.34	−0.44	18.09
(i1, 3H,7H)	24.29	25.84	−0.75	−1.22	23.07
(i2, 3H,7H)	17.47	18.24	−0.30	−0.54	16.93
(i1, 3H,9H)	31.56	32.55	−1.39	−1.68	29.89
(i2, 3H,9H)	31.96	32.56	−2.08	−1.77	30.19
(i1, 7H,9H)	44.96	47.96	−0.86	−1.17	43.79
(i2, 7H,9H)	35.54	37.56	−0.33	−0.44	35.10

[a] RI-MP2/TZVPP//RI-MP2/TZVPP. [b] MP2/aug-cc-pVDZ//RI-MP2/TZVPP. [c] MP2/6-31G**.

N9 (i1, 7H,9H; i2, 7H,9H) was examined (see Figure 1). The latter two tautomers possess "zwitterion"-like structures. Structures i1 and i2 differ just by the opposite orientation of the imino hydrogen. The geometries, rotational constants, and dipole moments of all of these tautomers are depicted in Supporting Information, Table 2, while Table 1 shows their (gas-phase) relative energies, enthalpies, and free energies. From Supporting Information, Table 2, it is evident that the canonical form has a small dipole moment, considerably smaller than that of cytosine and guanine. It has to be mentioned that a minimal change in the canonical structure (the prototopic tautomerism of amino group) leads to a dramatic increase of the dipole moment. The (i2, 7H,9H) and (i, 3H,9H) isomers possess dipole moments around 10 D and the (i1, 7H,9H) tautomer more than 12 D. A very large dipole moment of the latter structure corresponds to the fact that this structure does not have a standard mesomeric structure but a "zwitterion"-like one (cf. Figure 1). A significant increase of the dipole moment (by about 400%) indicates that these forms will be stabilized by interaction with polar systems. And we wish to add that a very similar dipole moment increase was found with various guanine tautomers.[44] Investigating the energy characteristics, we found that the canonical form clearly corresponds to the global minimum while the first and second local minima [(a, 7H) and (a, 3H) amino forms] are considerably less stable (by about 8 kcal/mol). The imino isomers (i1, 1H,9H), (i2, 1H,7H), (i1, 1H,-7H), (i2, 3H,7H), and (i2, 1H,9H) and amino isomer (a, 1H) are energetically less stable (by 12−19 kcal/mol). A very large energy difference of more than 30 kcal/mol was found for the rare unusual imino tautomers (i, 7H,9H) and (i, 3H,9H). The above-mentioned results were obtained from the RI-MP2/TZVPP calculations. In our previous papers on the cytosine and the guanine tautomers, we have shown[43,44] that these relative energies were accurate and very close to the MP2/aug-cc-pVTZ values. Furthermore, we have also shown that higher correlation energy contributions (CCSD(T) level) are practically negligible, and therefore, the RI-MP2/TZVPP data can be considered as the reference ones. To compare these values to experimental data, it is necessary to include the ZPVE, which means to pass from relative energies to relative enthalpies at 0 K. From Table 1, it is apparent that ZPVE corrections are systematically smaller than 1 kcal/mol and only for the (i, 3H,9H) imino tautomers is it larger than 1 kcal/mol. The relative stability of the adenine tautomers is thus not affected and changed when the ZPVE corrections were considered. Table 1 further shows that the temperature-dependent enthalpy terms and entropy reached comparable values, which means that the relative stability of the adenine tautomers remains at the free energy level (T = 298 K) the same as at the energy level.

Final relative energies (ΔE) and relative free energies at 298 K (ΔG_0^{298}) are summarized in Table 1. Relative free energies were determined from relative energies and $\Delta(G_0^{298}-E)$ terms (MP2/6-31G**). From the energy, enthalpy, and free energy results, we can conclude that only the canonical form can exist in the gas phase.

Microhydrated Tautomers. The MD/Q simulations on the monohydrated and the dihydrated tautomers yielded about four stable structures (cf. Table 2 and Figures2 and 3) with one water molecule and about 20 stable structures with two water molecules. The four energetically most stable structures were then studied using ab initio methods; their structures are presented in Figures 2 and 3. The stability of these structures decreases from left to right. For the imino tautomers, where two different orientations of imino hydrogen exist, only the more stable rotamer structure was studied further. The relative energies of these structures are presented in Table 2, while their interaction energies and relative energies of global minima are found in Table 3. Stabilization energies for adenine···water complexes are large, between 8.8 and 19.5 kcal/mol, and are fully comparable to those of guanine···water complexes.[44] Large stabilization energies are due to the favorable position of water that forms a bridge between adenine proton donor and acceptor positions. From Figure 2, it is evident that this is the case for a majority of monohydrated minima. Following expectation, the largest stabilization energy was found for the imino (i, 7H,9H) tautomers possessing the largest dipole moment. Interestingly, however, the second largest stabilization energy belongs to the (i2, 3H,7H) tautomer having only small dipole moment of 3.2 D. In addition, the stabilization energies for the dihydrated adenine tautomers are large (18.1−34.1 kcal/mol), and also here it is due to a very favorable orientation between tautomers and water molecules and among water molecules themselves. Figure 3 shows that the global minima of tautomers depicted contain mostly the water-dimer motif; for the tautomers (a, 1H), (i2, 3H,7H), and also (i2, 7H,9H), the most stable structure is the one in which the two water molecules do not form a dimer. As in the previous case, the largest stabilization energy was detected in the case of the imino (i2, 7H,9H) tautomer, which has very large dipole moment. Let us point out that the stabilization energy found is very large and because of the quality of the theoretical treatment it is not possible to expect that it will be considerably changed when passing to a higher theoretical level. The stabilization energies of the adenine tautomers with two

TABLE 2: Relative Energies ΔE (in kcal/mol) for Tautomers of Adenine with One and Two Water Molecules Evaluated at Different Levels of Theory[a]

structure	ΔE(MD/Q)[b]	ΔE(RI-MP2)[c]	structure	ΔE(MD/Q)[b]	ΔE(RI-MP2)[c]
(a, 9H)−(H_2O)			(a, 9H)−(H_2O)$_2$		
1	0	0	1	0	0
2	1.96	1.23	2	2.12	1.02
3	2.91	1.88	3	2.82	2.55
4	5.32	4.75	4	4.63	3.36
(a, 1H)−(H_2O)			(a, 1H)−(H_2O)$_2$		
1	0	0	1	0	0
2	2.23	4.29	2	1.45	0.64
3	11.03	9.33	3	2.37	2.39
4			4	4.23	5.50
(a, 3H)−(H_2O)			(a, 3H)−(H_2O)$_2$		
1	0	0	1	0	0
2	1.36	0.55	2	1.21	0.3
3	2.23	4.88	3	5.82	4.07
4	5.21	7.21	4	4.73	5.57
(a, 7H)−(H_2O)			(a, 7H)−(H_2O)$_2$		
1	0	0	1	0	0
2	−0.29	0.77	2	−0.12	0.53
3	1.13	2.52	3	1.86	2.33
4	4.49	3.23	4	3.98	3.25
(i1, 1H,7H)−(H_2O)			(i1, 1H,7H)−(H_2O)$_2$		
1	0	0	1	0	0
2	0.76	2.63	2	2.23	1.23
3	4.69	4.08	3	2.12	1.81
4	2.14	4.19	4	4.97	4.35
(i1, 1H,9H)−(H_2O)			(i1, 1H,9H)−(H_2O)$_2$		
1	0	0	1	0	0
2	0.54	1.98	2	1.58	0.86
3	2.89	4.6	3	1.35	1.83
4	5.00	5.43	4	3.41	4.08
(i2, 3H,7H)−(H_2O)			(i2, 3H,7H)−(H_2O)$_2$		
1	0.0	0	1	0	0
2	1.57	4.83	2	2.96	1.79
3	5.32	6.4	3	2.10	4.66
4	6.39	8.54	4	5.74	5.3
(i1, 3H,9H)−(H_2O)			(i1, 3H,9H)−(H_2O)$_2$		
1	0	0	1	0	0
2	1.95	0.71	2	−1.25	0.76
3	1.38	2.56	3	0.57	1.29
4	5.16	3.63	4	2.36	1.43
(i2, 7H,9H)−(H_2O)			(i2, 7H,9H)−(H_2O)$_2$		
1	0	0	1	0	0
2	3.33	5.62	2	1.94	2.95
3	8.78	11.82	3	6.23	6.04
4	10.36	12.16	4	5.08	7.03

[a] The order of the relative stability was referred to the most energetically preferable hydrated structure within complexes of one tautomer. [b] Modified Cornell et al. force field. [c] RI-MP2/TZVPP.

water molecules are in accord with those of the guanine tautomers, and similarly as in the case of monohydration, they are slightly larger.

The relative stabilities of mono- and dihydrated structures reflect the combination of the relative energies of bare (unsolvated) adenine tautomers (repeated in Table 3) and the interaction with water molecules. In general, hydration reduces the energy gap between global and local minima and could even change the order of stability. From Table 3, it follows that the stabilization energy of the (a, 3H) tautomer with one water is larger than that of the canonical form, which means that the energy gap between these two tautomers in the gas phase (7.43 kcal/mol) is reduced by 2.05 kcal/mol when monohydration is considered. The same applies for the (i2, 7H,9H) imino tautomer, where the largest stabilization energy found (9 kcal/mol) decreased enormous gas-phase energy penalization (34 kcal/mol in case of this tautomer). A similar trend is found for dihydration. For example, the gap between the canonical form and the (a, 3H) tautomer is reduced by 2.9 kcal/mol (from 7.4 kcal/mol in the gas phase to 4.5 kcal/mol). Considering microhydration results (and extrapolating dihydration to full bulk hydration), we must state that the energy gap between the canonical and the amino (a, 3H) tautomer is considerably reduced, which suggests that the latter form might coexist in a microhydrated environment with the canonical tautomer.

All hydration sites and water motifs identified by MD/Q were used in the hybrid model to approximate the first hydration shell.

Hydrated Tautomers. Relative hydration free energy for the adenine tautomers is shown in Table 4, which also gives the gas-phase free energy, hydration free energy, and free energy of tautomerization in an aqueous solution determined by MD-TI method, C-PCM (COSMO) procedure, and the hybrid model.

MD-TI Method. Relative hydration free energies determined by the MD-TI method (third column in Table 4) vary in the surprisingly broad range of −4 to −21 kcal/mol, and the largest values were found for the unusual rare imino (i, 7H,9H) tautomers that correspond with their largest dipole moments. Because, however, the energy destabilization of these two tautomers in the gas phase is in absolute value even larger, the resulting relative free energy of tautomerization (fourth column in Table 4) for these tautomers is still highly positive, which means they are (with respect to the canonical tautomer)

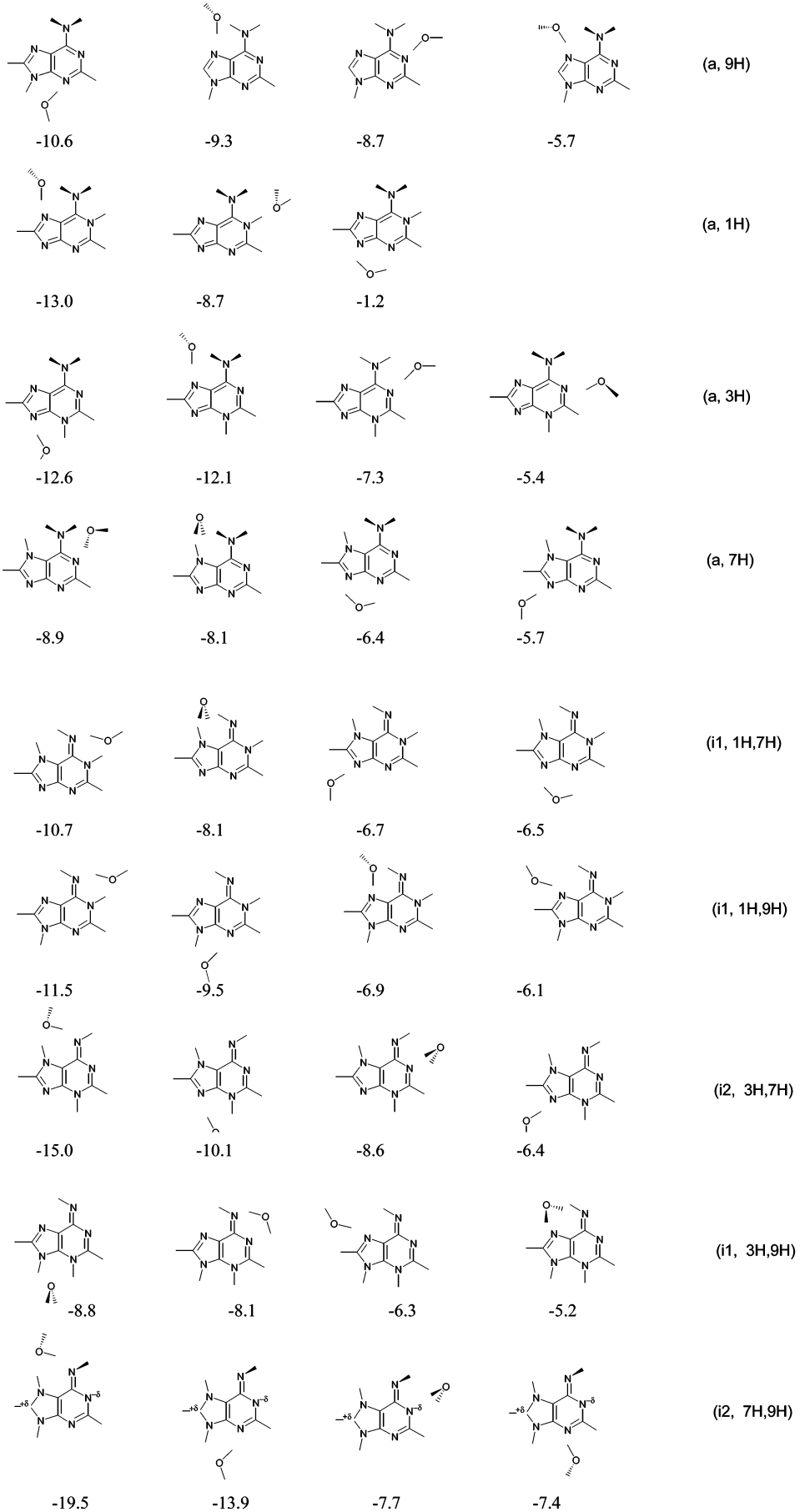

Figure 2. Nine of the most stable structures of adenine tautomers with one water molecule optimized at the RI-MP2/TZVPP level of theory. The stability is decreasing from left to right. The interaction energies in kcal/mol (cf. Table 3, last column, and Table 2, third column) are presented below the structures.

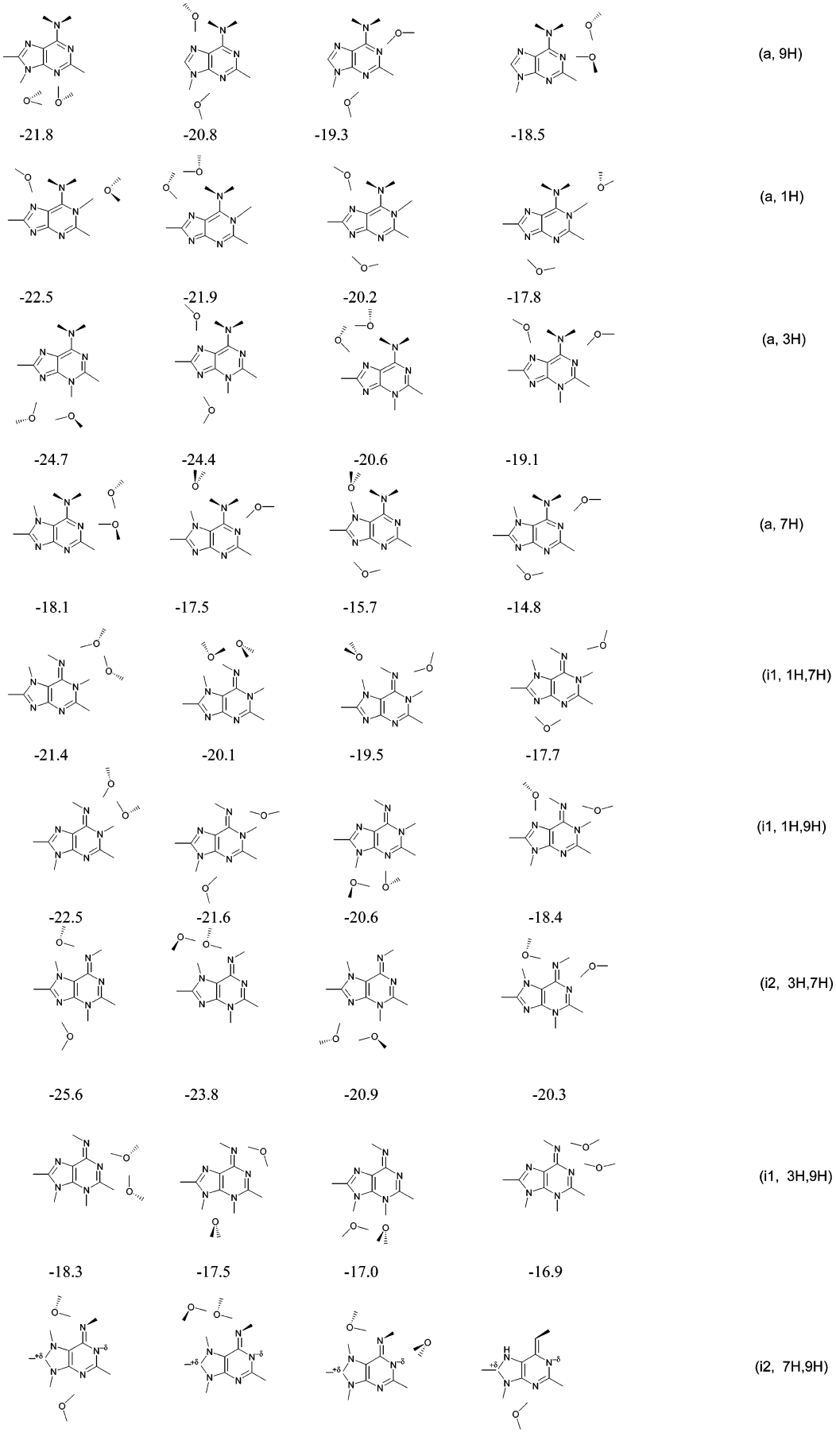

Figure 3. Nine of the most stable structures of adenine tautomers with two water molecules optimized at the RI-MP2/TZVPP level of theory. The stability is decreasing from left to right. The interaction energies in kcal/mol (cf. Table 3, last column and Table 2, last column) are presented below the structures.

TABLE 3: Relative and Interaction Energies (in kcal/mol) of Adenine Tautomers in the Gas Phase and Mono- and Dihydrated Environments (Global Minima for Each Tautomer−Water Complex)

	relative energies[a,b]		interaction energies[c,d]	
structure	RI-MP2	RI-MP2$_{ZPVE}$	RI-MP2	RI-MP2$_{TOT}$
(a, 9H)	0.00	0.00		
(a, 1H)	17.74	17.49		
(a, 3H)	7.99	7.43		
(a, 7H)	7.63	7.51		
(i1, 1H,7H)	16.55	16.23		
(i2, 1H,7H)	16.09	16.15		
(i1, 1H,9H)	12.07	11.22		
(i2, 1H,9H)	18.53	18.20		
(i1, 3H,7H)	24.29	24.04		
(i2, 3H,7H)	17.47	17.17		
(i1, 3H,9H)	31.56	30.82		
(i2, 3H,9H)	31.86	31.56		
(i1, 7H,9H)	44.96	42.88		
(i2, 7H,9H)	35.54	34.15		
(a, 9H)−(H_2O)	0.00		−11.21	−10.56
(a, 1H)−(H_2O)	14.95		−15.01	−12.96
(a, 3H)−(H_2O)	5.81		−13.29	−12.61
(a, 7H)−(H_2O)	9.04		−9.39	−8.90
(i1, 1H,7H)−(H_2O)	16.28		−11.43	−10.73
(i1, 1H,9H)−(H_2O)	10.97		−12.33	−11.52
(i2, 3H,7H)−(H_2O)	12.32		−16.28	−14.95
(i1, 3H,9H)−(H_2O)	33.42		−9.12	−8.81
(i2, 7H,9H)−(H_2O)	25.23		−23.21	−19.53
(a, 9H)−(H_2O)$_2$	0.00		−23.36	−21.81
(a, 1H)−(H_2O)$_2$	17.48		−24.87	−22.54
(a, 3H)−(H_2O)$_2$	4.89		−26.60	−24.71
(a, 7H)−(H_2O)$_2$	11.43		−19.81	−18.06
(i1, 1H,7H)−(H_2O)$_2$	16.98		−23.17	−21.35
(i1, 1H,9H)−(H_2O)$_2$	11.32		−24.46	−22.46
(i2, 3H,7H)−(H_2O)$_2$	13.43		−27.50	−25.59
(i1, 3H,9H)−(H_2O)$_2$	35.78		−18.99	−18.29
(i2, 7H,9H)−(H_2O)$_2$	23.56		−36.95	−34.13

[a] The order of the relative stability of each tautomer is given with respect to the canonical tautomer. Relative total energies of tautomers and mono- and dihydrated tautomers are considered. RI-MP2$_{ZPVE}$ is defined as a sum of relative RI-MP2 energy and $\Delta ZPVE$; the former energy is evaluated with the TZVPP basis set, while the latter are at the MP2/6-31G** level. [b] For description of abbreviations used for methods, see notes to Table 1. [c] Interaction energies were evaluated with the TZVPP basis set. [d] Total complexation energy, RI-MP2$_{TOT}$, is defined as a sum of the RI-MP2 interaction energy and deformation energies of the monomers.

destabilized. Very large relative hydration free energy found for these forms agrees with these energies calculated for the respective tautomeric form of guanine.[44] On the other hand, one of the lowest values of hydration free energies found for both the (a, 3H) and the (a, 7H) amino tautomers reduces the energy destabilization of these two forms in the gas phase. Consequently, their relative free energies of tautomerization in aqueous solution are less favorable than that of the canonical form by a relatively modest 2.5 and 2.8 kcal/mol, respectively. It should be mentioned that results concerning the (a, 3H) tautomer are supported by the microhydration results. The situation with the (a, 7H) tautomer is more complicated and will be further discussed in the hybrid model section.

The hydration free energies are determined in the present study using three different methods, MD-TI, C-PCM, and the hybrid model. None of these methods is parameter-free, and all of them depend critically on the choice of the starting configuration and parameters. It concerns especially the C-PCM and hybrid models where a little change of selected parameters (e.g., atomic radii, the respective scaling factor, or computational level and basis set) yields a rather large change of hydration free energy. On the other hand, the MD-TI method does not depend so critically on starting conditions and is more robust (but also more time-consuming). Therefore, the present MD-TI data (together with available experimental data) will be used as a reference for subsequent C-PCM and hybrid model calculations.

C-PCM (COSMO) Model. When investigating the hydration free energies obtained from the COSMO model (fifth column in Table 4), we found that with the exception of the (i2, 7H,9H) structure they agree reasonably with MD-TI data. The largest difference (∼1.8 kcal/mol) was found for the (i1, 3H,9H) tautomer, while for other tautomers, it is less than 1.5 kcal/mol. It should be noted that both methods give the same relative trends (similarly in the case of the guanine tautomers[44]) and they both predicted the same structures, which are the most solvent stabilized structures [(a, 1H), (i1, 3H,9H), and (i2, 7H,9H)]. The difference for the most stabilized structure (i2, 7H,9H) is, however, large (7.1 kcal/mol). Let us recall that the largest hydration free energies found for the three tautomers that were mentioned is not any artifact, but they are in full agreement with their largest dipole moments (cf. Supporting Information, Table 2), as well as with gas-phase microhydration results (cf. Table 3).

After performance of the optimization at the B3LYP/6-31G* level, followed by a single-point calculation at HF/6-31G*/UAHF level, slightly different solvation free energies resulted. From the sixth column of Table 4, it becomes clear that the "optimized" C-PCM free energies are systematically (in absolute value) larger than the "nonoptimized" values. The largest difference was found for (a, 1H), (a, 7H), and (i2, 7H,9H) structures, but the absolute average error (with respect to MD-TI data) remains practically unchanged. We can thus presently conclude that performing physically justified optimization resulted in no improvement but also no deterioration of "nonoptimized" gas-phase data.

Analyzing the effect of solvent on the tautomer dipole moments, we found that the dielectric medium in all cases notably increased the dipole moment (by 36%−49%) and geometry optimization further pronounced this effect (by an additional 1%−15% increase). Let us add that the change in nonelectrostatic energy upon optimization is small (in a narrow range of 0.7−1.1 kcal/mol) and its effect can be neglected. This conclusion allows direct comparison of our results (performed in water continuum) to published experimental and theoretical data made in DMSO[68] where only the electrostatic energy was considered (see section 4).

Hybrid Model. At first, we included the most strongly interacting water molecule (cf. Table 3) to the solvated system. Summing the relative interaction energies of each tautomer and the respective C-PCM (COSMO) free energy, we obtained the relative hydration free energies (seventh column in Table 4). Comparing these values to the C-PCM determined for the bare adenine tautomers, we found that the present values are closer to the "optimized" results. The difference is mostly small, and the only exceptions represent the (i2, 3H,7H) and the (i2, 7H,9H) tautomers where the monohydrated results are larger by more than 3 kcal/mol. The present data from the seventh column of Table 4 are also close to the MD-TI values and even the largest difference found for the (i2, 7H,9H) structure is notably reduced to about 2 kcal/mol.

A question arises whether it is correct to consider the most strongly bound water from the gas-phase microhydration. The other structures, being less stable in the gas-phase, can be favored in a continuum water environment. The eighth column of Table 4 shows that for the canonical, the (a, 3H), and the (a,

TABLE 4: Relative Gas-Phase Free Energies (ΔG_0^{298}), Relative Free Energies of Hydration Evaluated Using MD-TI Method (ΔG^{TI}), COSMO Method (ΔG^{C-PCM}), and Hybrid Model (ΔG^{HYB}), and Relative Free Energies in Aqueous Solution ΔG^{298}(TI) (in kcal/mol) of Adenine Tautomers

method structure[a]	ΔG_0^{298} [b]	ΔG^{TI}	ΔG^{298} (TI)	ΔG^{C-PCM}	ΔG^{C-PCM} opt[c]	ΔG^{HYB} (1w) vac min[d]	ΔG^{HYB} (1w) solv min[e]	ΔG^{HYB} (1w) opt[c,d]	ΔG^{HYB} (2w) vac min[d]	ΔG^{HYB} (2w) solv min[e]	ΔG^{HYB} (2w) opt[c,d]	ΔG^{HYB} (3w) sup[f]	ΔG^{HYB} (3w) opt[c,f]
(a, 9H)	0.00	0.00	0.00	0.00	0.00	0.00	−1.07	0.00	0.00	0.00	0.00	0.00	0.00
(a, 1H)	17.40	−11.49	5.91	−10.05	−14.36	−14.54	−14.54	−17.75	−11.34	−13.28	−13.90	−12.21	−12.30
(a, 3H)	7.43	−4.97	2.46	−3.49	−4.55	−4.46	−5.95	−4.87	−4.71	−4.71	−5.44	−4.82	−5.30
(a, 7H)	7.47	−4.68	2.79	−5.04	−6.88	−5.64	−7.31	−6.97	−4.18	−4.18	−5.53	−5.68	−7.33
(i1, 1H,7H)	16.05	−7.80	8.25	−7.07	−7.35	−7.41	−7.41	−7.69	−7.05	−7.05	−7.44	−4.99	−6.60
(i1, 1H,9H)	12.05	−4.04	8.01	−3.58	−3.70	−4.03	−4.03	−4.26	−3.67	−3.79	−4.11	−2.81	−3.74
(i2, 3H,7H)	16.93	−5.13	11.80	−3.64	−4.03	−7.75	−7.75	−8.82	−3.95	−5.33	−3.66	−5.38	−6.18
(i1, 3H,9H)	29.89	−12.18	17.71	−14.00	−14.83	−15.07	−15.07	−16.07	−10.43	−12.71	−10.07	−12.09	−14.53
(i2, 7H,9H)	35.10	−21.25	13.85	−14.16	−15.79	−18.94	−18.94	−22.44	−18.76	−18.76	−17.87	−17.72	−20.78

[a] See Figure 1. [b] See Table 1. [c] Geometries optimized in the continuum solvent. See Methods. [d] Global minimum in the gas phase. See Results. [e] Global minimum in the continuum solvent. See Results. [f] Superimposition of monohydrated structures. See Methods.

7H) tautomers the first local minimum from the gas-phase calculation becomes the global minimum upon the inclusion of the continuum solvent. The "additional" stabilization range (from 1.1 to 1.7 kcal/mol) is definitely not negligible.

Similarly as in the case of bare adenine, we performed the geometry optimization of monohydrated adenines in the presence of continuum solvent. From Table 4 (ninth column), it is clear that larger absolute values of hydration free energies resulted, but the difference is not dramatic. Worth mentioning is the fact that hydration free energy of the (i2, 7H,9H) tautomer is now comparable (in absolute value even slightly larger) to the respective MD-TI value, which strongly supports the reliability of MD-TI procedure.

Let us now briefly comment on the case of the (a, 7H) tautomer. As mentioned previously, the microhydration itself is not able to explain the magnitude of the hydration free energy of this tautomer (contrary to the (a, 3H) tautomer). From Table 4 it becomes, however, clear that the combination of the specific hydration (estimated including the geometry optimization in the continuum solvent) and C-PCM model yields satisfactory results comparable to MD-TI data.

Consideration of two and three specific water molecules in the C-PCM model yields basically similar results as in the case of monohydration. Here again, not only the global gas-phase minimum was considered, but also the other three local minima from Table 3 were taken into account. The "additional" stabilization obtained by passing from the global minimum to the local minimum was comparable to that found for monohydrated tautomers (the largest difference of 2.3 kcal/mol was found for the (i1, 3H,9H) tautomer).

Comparing the C-PCM results for adenine$\cdots(H_2O)_n$ complexes, where $n = 0$ (bare adenine), 1, 2, and 3, we can state that inclusion of water(s) does not deteriorate the hydration free energies obtained for bare adenines. We believe that the inclusion of a few specific waters is especially important if the solute dipole moment becomes large. In this case, the continuum model is not efficient enough to describe the solvation, and consequently too small (in absolute scale) hydration free energies resulted. This is the case for the (i2, 7H,9H) tautomer where C-PCM values were comparable to the MD-TI values only after consideration of explicit water(s). This conclusion is supported by recent results[69] and recent opinions[70] that the hybrid model considerably improves the accuracy of PCM methods in the case of charged systems. It must however be mentioned here that Saracino et al.[71] did not share this opinion and recommended only a geometry optimization within a "pure" continuum model.

We can conclude this section by stating that the continuum model and the hybrid model provide reliable values of hydration free energies comparable to the MD-TI ones. This result is very promising for evaluation of hydration free energy of larger systems and is being currently systematically tested in our laboratory.

4. Comparison with Experiment

Experimental data on population of NA base tautomers in solution are very rare, and to our best knowledge, they only exist for uracil/thymine[72] and adenine.[68] Fischer et al.[68] measured using NMR technique the population of various adenine tautomers in DMSO ($T = 298$ K) and found the coexistence of the canonical form and N3 and N7 tautomers. This finding nicely agrees with our MD-TI results obtained from water phase. To demonstrate the similarity of water and DMSO phases, we calculated the hydration free energies (continuum C-PCM model) in both solvents. As expected, we found that there is only a slight difference between water ($\epsilon = 80$) and DMSO ($\epsilon = 50$) hydration free energies. We performed a single-point calculation at B3LYP/6-31G* level with Pauling radii in DMSO and also in water for both vacuum and water-optimized geometries with good agreement with the H-NMR results. The water and DMSO hydration free energies differ by less than 1%.

5. Conclusion

Theoretical relative energies, enthalpies, and free energies for isolated adenine tautomers support the existence of the canonical form only. The microhydrated environment and bulk solvent stabilize the canonical form, and it remains the global minimum in both phases. The relative hydration free energies of (a, 3H) and (a, 7H) tautomers are higher than that of the canonical form, and their energy destabilization in the gas phase is only modest. Consequently, the relative free energies of tautomerization of (a, 3H) and (a, 7H) tautomers in an aqueous solution are rather small (below 3 kcal/mol), which supports the coexistence of these tautomers with the dominant canonical form. This finding is fully supported by the NMR experiment and theoretical calculations in DMSO, which clearly show the coexistence of these three tautomers. Let us note that continuum solvent model yields for DMSO and water practically identical results (the difference is smaller than 1%).

This work is another theoretical prediction (cf. ref 44) of coexistence of several tautomers in the water phase, which could be very important in the specificity of nucleotide incorporation during DNA replication as mentioned in a very recent work,[1] which supports the rare tautomer hypothesis of substitution mutagenesis.[73,74]

Hydration free energies of adenine tautomers evaluated by MD-TI and C-PCM models agree well, and a significant difference was found only in the case of a polar (i2, 7H,9H) tautomer having "zwitterion-like" structure. This difference becomes smaller (or even vanishes) when optimization of the geometry in the water phase was performed or when a few (one to three) water molecules were considered explicitly. Inclusion of explicit water molecules in other cases neither improves nor deteriorates results obtained for rare tautomers. The use of the hybrid model is thus recommended if the solute dipole moment becomes very large (approximately >10 D).

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Supporting Information Available: Details of simulations performed (Table 1) and geometries, rotational constants, and dipole moments of all of the tautomers. This material is available free of charge via the Internet at http://pubs.acs.org.

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