Protonation of Nucleic Acid Bases. A Comprehensive Post-Hartree−Fock Study of the Energetics and Proton Affinities

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The results of an ab initio post-Hartree−Fock study of the protonation of all nucleic acid bases are reported. Rare tautomers of guanine and cytosine, which coexist in the gas phase with the major forms, were also included in the study. The geometries of the local minima and transition states were optimized without symmetry restrictions by the gradient procedure at the HF and MP2 levels of theory and were verified by energy second-derivative calculations. The standard 6-31+G(d,p) basis set was used. The single-point calculations have been performed at the MP4(SDTQ)/6-31+G(d,p)//MP2/6-31+G(d,p) and MP2/6-311++G(d,p)//MP2/6-31+G-(d,p) approximations. The relative stabilities of the different protonated forms of all nucleic acid bases have been reported. The values of proton affinities (PA) for each base including contributions of rare tautomers and different protonated forms for guanine and cytosine have been calculated. We have shown that the calculated values of proton affinities are very close to the experimental data, and the differences are in the range of 0.0−2.1%. We have concluded that all levels of the Möller−Plesset theory considered in the study are able to describe the PA values of nucleic acid bases with experimental accuracy. The study has shown that the rare tautomers make up a significant portion of the gas-phase equilibrium mixture. Yet, the values of the proton affinities change only slightly with the inclusion of rare forms into the calculations.

Introduction

The interaction of DNA bases with a proton is, in some sense, one of the simplest acid−base chemical reactions that are very popular in both living systems and inorganic species. Following are several highlights of the importance of the protonation of DNA bases. The protonated cytosine contributes significantly to the stabilization of DNA triplexes.[1,2] The protonation can also cause mutations in the DNA via mispairing of complementary bases.[3−5] For example, the cytosine protonated at the O2 oxygen via the formation of the O2−H···N1 (adenine) hydrogen bond could be responsible for the stabilization of the adenine−cytosine mispair that was observed in single crystals of oligonucleotide duplexes.[6] Recently, it was also concluded that the structures of so-called rare tautomers stabilized by transition metals could be also presented as complexes between protonated bases and metal.[7]

Being so important, acid−base equilibria involving nucleic acid bases have been widely studied experimentally in gas and condensed phases.[3,8−11] In particular, the determination of the pK_a values and locations of the protonation sites of nucleic acid bases contributes to the understanding of chemical processes that occur in the DNA in condensed phase.[12] The latest experimental data were obtained with the development of desorption ionization methods in mass spectrometry,[13,14] which accelerate the gas-phase investigations in this field.[15] The most important outcome of these studies is the determination of the proton affinity (PA) values, which is the fundamental intrinsic property of the isolated (not surrounded by solvent or any interacting molecules) bases. These data are known for all DNA bases.[11,15,16] However, available experimental data do not always relate to a certain temperature. Another problem of any mass-spectrometry investigation is the lack of information about the geometrical structure of the investigated molecules and ions. In the case of the protonated nucleic acid bases, it means that the site of the protonation still remains unknown. In contrast to the experimental investigation, the theoretical quantum-chemical study is able to address these problems.

The studies of the protonation process of nucleic acid bases have been always of interest to the quantum-chemical community. The first ab initio study on this subject was performed in 1972.[17] Currently the most systematic theoretical study on the determination of proton affinities of nucleic acid bases has been performed by N. Russo et al.[18] The calculations have been carried out in the framework of density functional theory using Vosko−Wilk−Nusair[19] local density approximation and the nonlocal correction of Becke for the exchange[20] and Perdew for the correlation part of the DFT functional.[21] The most recent investigation of the protonation of nucleic acid bases was performed at the DFT/B3LYP level.[22] All nucleic acid bases were included, but unfortunately, the published values refer to 0 K temperature. The high-level calculations (MP4(SDQ)/6-311++G(d,p)//MP2/6-31G(d)) have also been reported only for protonated forms of guanine and cytosine.[23]

Even though recent ab initio data is in fairly good correspondence with the experimental data, there are several issues that have been addressed neither by experimental nor by theoretical investigations.

1. Evaporated in a vacuum, guanine and cytosine consist of the equilibrium mixture of different tautomers (so-called canonical and "rare" forms).[24−33] So, the protonated species of these nucleic acid bases might also consist of several protonated structures. Yet, it is not obvious that the equilibrium mixture of the protonated tautomers possesses the same composition as the mixture of the isolated guanine or cytosine tautomers.

2. A proper evaluation of the PA values (for both experimental and theoretical studies) should take into account not only

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one canonic form of the base and its most stable protonated form but also the equilibrium mixture formed by different tautomers of neutral and protonated nucleic acid bases.

3. For providing more reliable results, a comprehensive application to all considered forms of advanced theory such as MP4(SDTQ) with sufficient basis set is required.

All these issues are addressed by the current investigation. In this work we have calculated the relative stability (at room temperature) of the canonic and rare forms of neutral and protonated nucleic acid bases. On the basis of these data, we predicted the values of PA and compared them with those obtained earlier.

Computational Methods

The ab initio LCAO-MO method was used for the study of nucleic acid bases. The calculations were carried out with the Gaussian-94[34] and Gaussian-98[35] set of programs. The standard 6-31+G(*d*,*p*) basis set was used for the geometry optimizations. All the geometries of local minima structures were optimized without symmetry restrictions (C_1 symmetry was assumed) by the gradient procedure initially at the HF level and subsequently at the second order of closed shell restricted Möller−Plesset perturbation theory.[36] The characteristics of local minima were verified by establishing that the matrixes of the energy second derivatives (Hessians) (at the HF/6-31+G(*d*,*p*) level) have no negative eigenvalues. The single-point calculations were performed at the MP4(SDTQ)/6-31+G(*d*,*p*)//MP2/6-31+G(*d*,*p*) and MP2/6-311++G(*d*,*p*)//MP2/6-31+G(*d*,*p*) levels of theory for all studied systems (except for guanine where the MP4(SDTQ) level has been applied only to selected tautomers because of the computer expense of such calculations). To estimate the relative stability of different protonated forms (BH^+) and the composition of the equilibrium mixture, the values of the Gibbs free energies have been calculated by the standard formula $\Delta G_f = \Delta H_f - T\Delta S_f$ at room temperature (298.15 K). To evaluate the ΔH_f values, the thermal corrections to enthalpy calculated at the HF/6-31+G(*d*,*p*) level and scaled by a factor of 0.9 have been added to the total values of energies. The ΔS_f values have been calculated at the HF/6-31+G(*d*,*p*) level.

Gas-phase PAs were calculated as the negative of enthalpy of the process

$$B + H^+ \rightarrow BH^+$$

$$\Delta H = \Delta H_f(BH^+) - \Delta H_f(B) - \Delta H_f(H^+) + \Delta(PV)$$

and were calculated as follows

$$PA = -\Delta H = -[(E_{tot}(BH^+) + \tau(BH^+)) - (E_{tot}(B) + \tau(B))] + \frac{5}{2}RT$$

where E_{tot} is the total energy obtained from the MP2 or MP4 calculations, τ is the zero-point and thermal correction to enthalpy scaled by 0.9, and the term $^5/_2RT$ includes ΔnRT for the above reaction and translational energy of the proton. The average values of proton affinities for the mixtures of guanine and cytosine were calculated as follows

$$PA_{AV} = -[\sum_1^M (E_{tot}(BH^+) + \tau(BH^+))_M P_M - \sum_1^N (E_{tot}(B) + \tau(B))_N P_N] + \frac{5}{2}RT$$

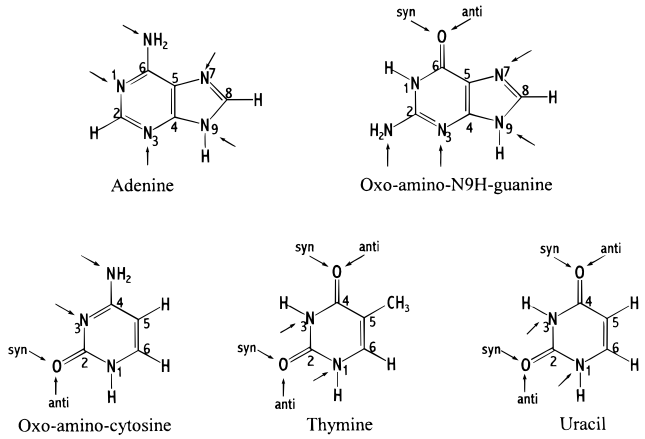

Figure 1. Protonation sites of canonic tautomers of nucleic acid bases.

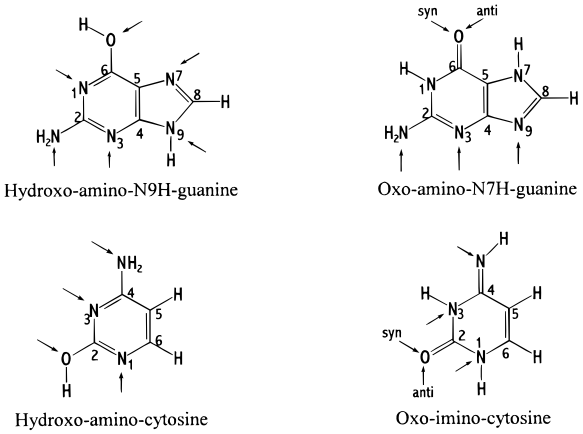

Figure 2. Protonation sites of tautomers of guanine and cytosine.

where *M* is the number of protonated bases in the mixture, *N* is the number of neutral bases in the mixture, and *P* is the fraction of particular base (protonated base) in the composition of the mixture. The values of *P* were obtained from the equilibrium constants for the processes of intramolecular proton transfer in the neutral and protonated bases and calculated using the room-temperature standard formula $\Delta G = -RT \ln K$.

Each nucleic acid base has several possible sites for proton attachment. In this work only the positions related to the O and N atoms have been considered.

Results and Discussion

Relative Stability of Protonated Nucleic Acid Bases. The protonation sites of the canonic and "rare" tautomeric forms of nucleic acid bases are presented in Figures 1 and 2. The total and relative Gibbs free energies of neutral and protonated nucleic acid bases are reported in Table 1. The predicted compositions of the equilibrium mixtures of the neutral and protonated nucleic acid bases in the gas phase are collected in Table 2.

Guanine. As mentioned above, guanine exists in the gas phase as a mixture of canonic and two rare forms[28−30,37,38] (see also Figures 1 and 2). Unfortunately, the exact concentrations of the tautomers are still not measured experimentally. According to our calculations, this equilibrium mixture consists of canonic (oxo−amino-N9H) and rare (oxo−amino-N7H− and hydroxo−amino-N9H−) tautomers. As follows from the obtained data (see Tables 1 and 2), the oxo−amino-N7H tautomer should slightly predominate.

As expected, the equilibrium mixture composition of protonated tautomers is significantly different from that of the neutral

TABLE 1: Relative (kcal/mol) and Total (au in parentheses) Gibbs Free Energies of Neutral and Protonated Nucleic Acid Bases at 298.15 K

level	MP2/6-31+G(d,p)	MP2/6-311++G(d,p)// MP2/6-31+G(d,p)	MP4(SDQ)/6-31+G(d,p)// MP2/6-31+G(d,p)	MP4(SDTQ)/6-31+G(d,p)// MP2/6-31+G(d,p)
		Oxo−amino-N9H-guanine		
neutral	0.5	0.2	0.1	0.6
N(C2)	33.9	32.7	37.4	
N3	15.9	15.0	15.4	
syn-(N1)O6	13.7	12.5	14.2	
anti-(N1)O6	6.0	5.1	6.2	
N7	(−541.356 01) 0.0	(−541.541 67) 0.0	(−541.387 56) 0.0	(−541.469 16) 0.0
N9	46.9	45.8	44.3	
		Hydroxo−amino-N9H-guanine		
neutral	1.0	0.3	1.6	1.8
N1	13.7	12.5	14.2	
N(C2)	30.1	29.1	33.4	
N3	10.4	9.4	10.7	
O6	46.9	45.0	50.7	
N7	2.9	2.6	4.1	3.6
N9	45.3	44.0	44.1	
N3−N7H	1.2	1.4	2.5	2.6
		Oxo−amino-N7H-guanine		
neutral	(−540.998 40) 0.0	(−541.185 33) 0.0	(−541.023 74) 0.0	(−541.109 16) 0.0
N(C2)	27.1	26.9	29.5	
N3	4.2	3.6	3.9	4.2
syn-(N1)O6	16.0	15.9	15.1	
anti-(N1)O6	15.2	15.0	14.0	
N9	*same as N7H$^+$-oxo−amino-N9H-guanine*			
		Oxo−aminocytosine		
neutral	1.3	2.5	−0.6	0.2
syn-(N3)O2	(−394.133 38) 0.0	(−394.271 55) 0.0	(−394.169 40) 0.0	(−394.222 94) 0.0
anti-(N3)O2	8.5	8.4	8.5	8.3
N3	0.9	1.2	0.1	0.1
N(C4)	28.4	28.3	30.4	27.6
		Hydroxo−aminocytosine		
neutral	(−393.775 45) 0.0	(−393.915 05) 0.0	(−393.804 30) 0.0	(−393.861 99) 0.0
N1	8.8	8.7	9.1	8.8
O2	42.6	41.4	46.6	43.6
N3	8.8	8.7	9.2	8.9
N(C4)	24.9	24.5	28.4	25.5
		Oxo−iminocytosine		
neutral	(−393.769 40) 3.8	(−393.907 88) 4.5	(−393.803 18) 0.7	(−393.858 64) 2.1
N1	48.1	47.3	46.1	45.1
syn-(N3)O2	28.0	27.5	26.1	26.9
anti-(N3)O2	29.3	28.8	27.2	28.1
N3	43.3	42.4	42.2	40.8
N(C2)	*same as N3H$^+$-oxo−aminocytosine*			
		Adenine		
neutral	(−465.946 93)	(−466.095 93)	(−465.970 35)	(−466.049 88)
N1	(−466.299 28) 0.0	(−466.447 17) 0.0	(−466.329 08) 0.0	(−466.404 78) 0.0
N3	2.3	2.4	1.8	2.0
N(C6)	17.2	17.0	20.0	17.5
N7	8.4	8.8	8.7	8.1
N9	45.5	44.9	43.4	42.8
		Thymine		
neutral	(−452.805 41)	(−452.972 27)	(−452.844 17)	(−452.906 29)
N1	26.6	26.2	25.0	25.3
syn-(N3)O2	5.8	5.9	5.1	5.6
anti-(N3)O2	7.0	7.0	6.1	6.7
N3	24.4	23.6	24.8	22.5
syn-(N3)O4	2.3	2.5	2.5	2.3
anti-(N3)O4	(−453.129 88) 0.0	(−453.298 16) 0.0	(−453.171 79) 0.0	(−453.232 50) 0.0
		Uracil		
neutral	(−413.639 68)	(−413.794 42)	(−413.667 88)	(−413.724 30)
N1	29.6	29.1	28.4	27.4
syn-(N3)O2	7.8	7.7	7.2	7.4
anti-(N3)O2	9.1	9.0	8.3	8.7
N3	25.0	24.4	25.6	23.0
syn-(N3)O4	2.8	2.9	2.9	2.7
anti-(N3)O4	(−413.961 91) 0.0	(−414.117 76) 0.0	(−413.993 78) 0.0	(−414.048 12) 0.0

ones. The most stable is the protonated form, which can be obtained by the protonation of the canonical structure of guanine in the position N7. This structure is the global minimum on the potential surface, which corresponds to the interaction between a proton and low-energy guanine tautomers. An analysis of the data presented in Table 1 enables us to conclude that there are

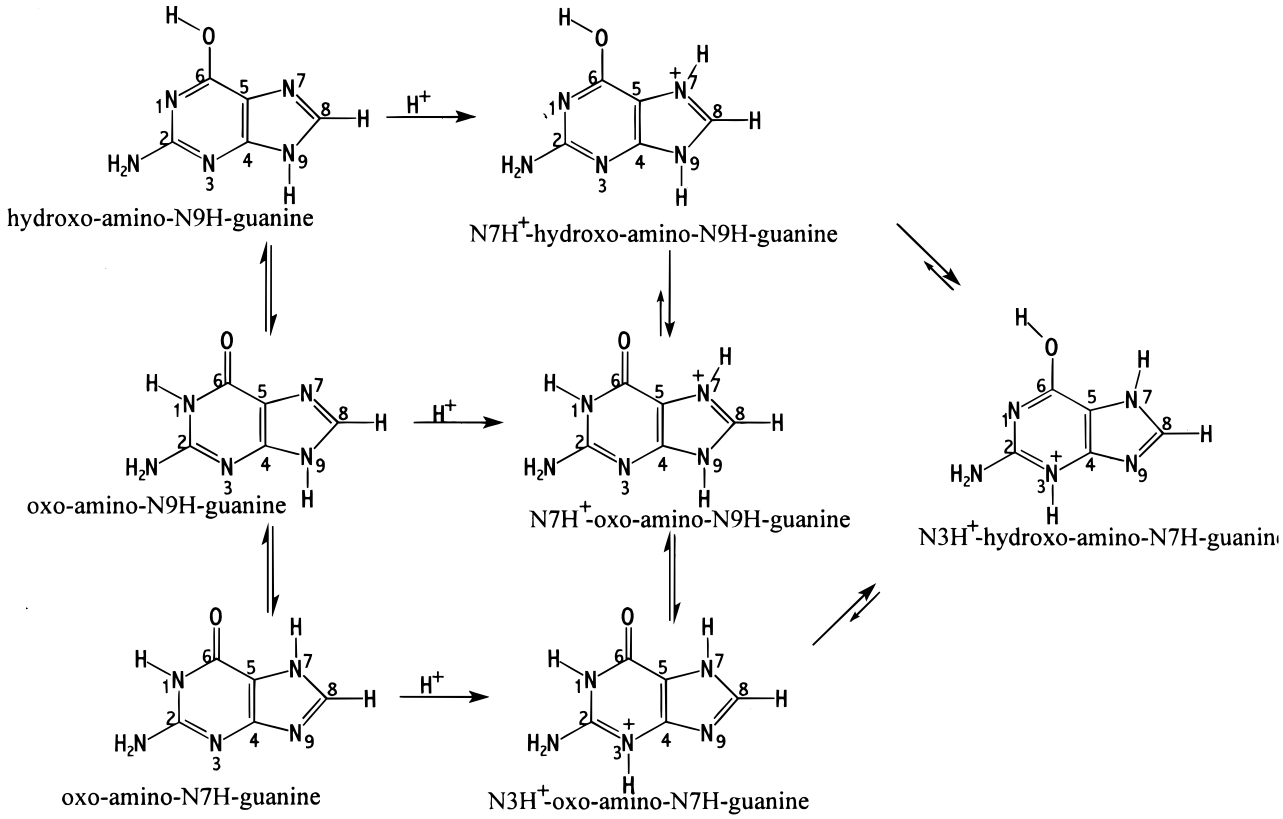

Figure 3. Protonation of canonic and rare forms of guanine.

TABLE 2: Compositions of Equilibrium Mixtures of Neutral and Protonated Cytosine and Guanine (%) at 298.15 K

	MP2/ 6-31+G(d,p)	MP2/ 6-311++G(d,p)// MP2/6-31+G(d,p)	MP4(SDQ)/ 6-31+G(d,p)//MP2/ 6-31+G(d,p)	MP4(SDTQ)/ 6-31+G(d,p)//MP2/ 6-31+G(d,p)
		Guanine		
oxo−amino-N9H-guanine	26.6	30.8	44.2	25.7
hydroxo−amino-N9H-guanine	11.4	26.0	3.5	3.4
oxo−amino-N7H-guanine	61.9	43.2	52.3	70.9
		Protonated Guanine		
$N7H^+$-oxo−amino-N9H-guanine	96.4	90.2	98.3	98.5
$N7H^+$-hydroxo−amino- N9H−guanine	0.7	1.1	0.1	0.2
$N3H^+$-oxo−amino-N7H-guanine	0.1	0.2	0.1	0.1
$N3H^+$-hydroxo−amino-N7H-guanine	2.8	8.5	1.4	1.2
		Cytosine		
oxo−aminocytosine	10.0	1.5	67.8	40.9
hydroxo−aminocytosine	89.9	98.5	24.6	57.4
oxo−iminocytosine	0.1	∼0.0	7.6	1.7
		Protonated Cytosine		
syn-(N3)O2H^+-oxo−aminocytosine	82.0	88.3	54.2	54.2
$N3H^+$-oxo−aminocytosine	18.0	11.7	45.8	45.8

no protonated low-energy structures that could be obtained as a result of the protonation of rare tautomers.

Since a previous study[23] has shown that another protonated form (hydroxo−amino-N3H−N9H-guanine) (this form can be formally obtained from the hydroxo−amino-N7H tautomer protonated at the N3 site or from the N7-protonated hydroxo−amino-N9H tautomer of guanine through the intramolecular proton transfer from the N9 to the N3 atoms) could be more stable than the protonated forms of the rare tautomers, we have also included this structure in our study. According to the data presented in Table 1, this structure definitely should be considered as the low-energy protonated form. Nevertheless, the canonic structure protonated at the N7 position strongly predominates in the predicted composition of the equilibrium mixture at all levels of our calculations (see Tables 1 and 2). Thus, we expect that in contrast to the composition of the equilibrium mixture of neutral guanine tautomers, the equilibrium mixture of protonated species contains mainly the protonated form that can be obtained by the protonation of the canonic structure in the N7 position.

The scheme of the most important equilibria related to the interaction of the guanine tautomer with the proton in the gas phase is presented in Figure 3.

Cytosine. Like in the case of guanine, cytosine exists in the gas phase as a mixture of canonic and rare tautomers.[24−27,39] To our knowledge the composition of the equilibrium mixture is unknown. The only experimentally estimated thermodynamic parameter that relates to the gas-phase equilibrium of cytosine is the free energy difference between the canonic and hydroxo−amino form.[40] It lies in the interval of 0.5−1.1 kcal·mol^{-1} in

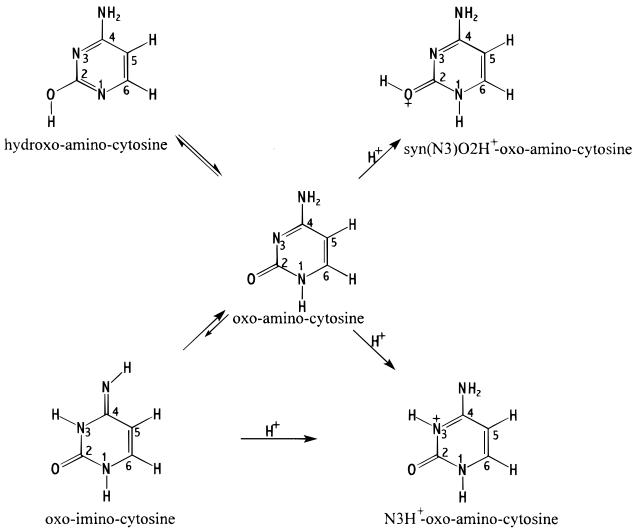

Figure 4. Protonation of canonic and rare tautomers of cytosine.

favor of the hydroxo form. The data presented in Table 1 are in line with the experimental estimation for all chosen levels of the Möller−Plesset theory except the MP4(SDQ)/6-31+G(d,p)//MP2/6-31+G(d,p) level, which slightly favors the canonic form of cytosine. The predicted compositions of the gas-phase equilibrium mixture of the cytosine tautomers are collected in Table 2. The highest level of the Möller−Plesset theory (MP4(SDTQ)/6-31+G(d,p)//MP2/6-31+G(d,p)) suggests a slight preference for the hydroxo form and the "trace" concentration of the imino- tautomer.

Let us now discuss the relative stability of the protonated forms. One may see that the situation here is very similar to the case of the protonated guanine. The most stable protonated forms are those that could be obtained from a protonation of the canonic form. However, there are also several peculiarities.

First, we predict that in contrast to guanine the equilibrium mixture of protonated cytosine tautomers will consist of a mixture of the protonated forms that could be obtained by attaching the proton in the N3 and the *syn*-(N3)O positions of the canonic form.

Second, the protonation sites in the cytosine are distributed between the heterocyclic ring and the carbonyl oxygen. Even though all considered approximations predict a preference for the *syn*-(N3)O protonated form, there is some discrepancy between the composition of the equilibrium mixtures predicted at the MP2 and the MP4 levels of theory (see Table 2). The MP2 level calculations predict a preference for the *syn*-(N3)O-protonated form. The data obtained at the MP4 level indicate the coexistence of the *syn*-(N3)O- and N3-protonated species with approximately equal concentrations.

Third, we would like to mention that the protonated species where the proton is attached to the N3 site of the canonic form can be also obtained by the protonation of the oxo−imino tautomer of cytosine at the N(C4) position. So, formally, this form can also be considered as the derivative of the rare oxo−imino tautomer protonated at the N(C4) site. (For other phenomena of this type related to the metal-stabilized forms of rare tautomers, see J. Sponer et al.[7])

Figure 4 depicts the most important equilibria related to the interaction of the cytosine tautomers with the proton in the gas phase.

Adenine. According to the experimental and recent quantum-chemical data, there are no gas-phase low-energy rare tautomers of adenine.[40] The data presented in Table 1 show that the same

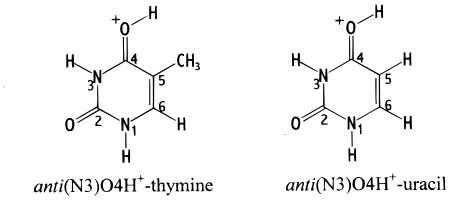

Figure 5. Major protonated forms of thymine and uracil.

TABLE 3: Theoretical and Experimental Values of the Proton Affinity of Nucleic Acid Bases (eV)

DNA base	Proton affinity					
	experimental data			ab initio data		
	ref 15	ref 11	ref 16	ref 18	ref 22	ref 23
guanine	9.86	∼9.67		9.99	9.95	9.79
cytosine	9.79	9.70	9.75	9.93	9.92	9.84
adenine	9.72	9.69	9.74	9.79	9.78	
thymine	9.09	9.05	9.15	9.05	8.97	

conclusions apply to the protonated species of adenine. The most stable protonated form of adenine is that with a proton attached to the N1 atom. This form is more stable by 1.8−2.4 kcal/mol than the species of adenine protonated at the N3 position. This means that the N1-protonated form completely predominates in the equilibrium mixture of protonated adenine. The same conclusion could be obtained from the data[18] predicted at the DFT level of theory.

Thymine and Uracil. Since thymine and uracil have similar chemical structures, it is not surprising that they have very close acid−base properties. Both of them do not possess gas-phase low-energy rare tautomers. It was confirmed by experimental and theoretical investigations.[40] These bases can be protonated at four different sites, viz., N1, O2, N3, and O4. Protonation at the N1 and N3 sites is an unfavorable process for both thymine and uracil. According to our calculations (Table 1), which are in agreement with previous ab initio studies,[18,22] the most stable protonated forms of thymine and uracil are those protonated at the *anti*-(N3)O4 position (Figure 5). This is the significant difference compared with the cytosine and guanine whose preferable protonated sites include the nitrogen atoms of the heterocyclic rings. With the exception of the species protonated at the *anti*-(N3)O4 position, we have not found any other forms that could be related to gas-phase low-energy protonated structures. Thus, we predict the *anti*-(N3)O4 position to be the major site for the protonation of thymine and uracil.

Proton Affinities. The values of proton affinities of all DNA bases obtained in previous experimental[11,15,16] and theoretical ab initio methods[18,22,23] are collected in Table 3. Even though, as mentioned above, experimental data do not always relate to a certain temperature, one may conclude that guanine, cytosine, and adenine are characterized by very similar PAs, which are quite different from thymine's. In our opinion the experimentally determined differences in the PAs of guanine, cytosine, and adenine are so small that there are not too many reasons to discuss the question of which DNA base is more protofilic. The aforementioned experimental results suggest the following pattern for the DNA bases' proton affinities:

$$\text{guanine} \geq \text{cytosine} \geq \text{adenine} > \text{thymine}$$

The calculated PA values of the canonic forms of nucleic acid bases and protonated low-energy species along with average PAs are presented in Table 4. Let us compare them with the most recent experimental data obtained by F. Greco et al.[15] One may see that the difference between the experimental results

TABLE 4: Values of Proton Affinities (eV) for the Most Important Tautomers of Nucleic Acid Bases

	MP2/ 6-31+G(d,p)	MP2/ 6-311++G(d,p)// MP2/6-31+G(d,p)	MP4(SDQ)/ 6-31+G(d,p)//MP2/ 6-31+G(d,p)	MP4(SDTQ)/ 6-31+G(d,p)// MP2/6-31+G(d,p)
$N7H^+$-oxo−amino-N9H-guanine	9.81	9.76	9.96	9.88
$N7H^+$-hydroxo−amino-N9H-guanine	9.69	9.65	9.84	9.77
$N3H^+$-oxo-amino-N7H-guanine	9.58	9.57	9.76	9.66
guanine average	**9.79**	**9.75**	**9.96**	**9.86**
oxo−amino-N3H−cytosine	9.86	9.85	10.01	9.93
oxo−amino-O2H−cytosine	9.90	9.91	10.0	9.93
cytosine average	**9.82**	**9.77**	**10.01**	**9.91**
adenine	**9.67**	**9.64**	**9.85**	**9.75**
thymine	**8.90**	**8.93**	**8.98**	**8.94**
uracil	**8.83**	**8.86**	**8.93**	**8.88**

and the results obtained at all considered levels of the Möller−Plesset theory are in the range of 0.0−2.1%, which is within a standard experimental error for this kind of measurement of 5%.[15] It indicates that all applied levels of the Möller−Plesset theory are able to describe the PA values of nucleic acid bases with experimental accuracy. Interestingly, the average PA values predicted for guanine at different levels of theory are very close to the values of the N7-protonated form of oxo−amino-N9H-guanine. This is because of the strong predominance of this form in the equilibrium mixture of guanine (see Table 2). The two most stable protonated forms of cytosine also have very close values of ΔH_f. However, because of the strong predominance of the rare form of the neutral cytosine predicted at the MP2 level (see Table 2), the PA_{AV} value of cytosine at this level is significantly lower than the proton affinity of the canonic cytosine. At the MP4(SDQ) and MP4(SDTQ) levels of theory, the rare tautomer of neutral cytosine does not have such prevalence in the composition of the equilibrium mixture. This is the reason the PA_{AV} of cytosine approaches the PA of the canonic cytosine.

Conclusions

In this study we have performed systematic calculations on the protonation sites and proton affinities of all nucleic acid bases at the MP2 and MP4(SDTQ) levels of theory. We have shown that the results on the energetics of the protonation of the bases are in excellent agreement with experimental data. The calculations of the proton affinities have been performed taking into account not only one tautomer but the equilibrium mixture of stable forms. The obtained values of proton affinities are very close to the experimental values with a deviation from 0.0% to maximum 2.1%. The results of this study explain why rare tautomers, which make a considerable part of equilibrium mixtures, do not change significantly the values of the proton affinities. We have concluded that all considered levels of the Möller−Plesset theory considered in this paper are able to describe the PA values of nucleic acid bases with experimental accuracy.

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