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Theoretical Estimations of the 298 K Gas-Phase Acidities of the Purine-Based Nucleobases Adenine and Guanine

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Theoretical estimations at both the B3LYP/6-31+G(d)//B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) levels for the 298 K gas-phase acidities (GPA) of all the possible deprotonation sites of the most stable tautomers of adenine and guanine are reported in terms of the enthalpy change of deprotonation. The gas-phase acidities of the nucleobases estimated by direct calculations and derived from isodesmic reactions are very similar. At the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory, for the most stable adenine tautomer, 9H-adenine, the GPAs of N9, $N4-H_a$, $N4-H_b$, C8, and C2 sites are 337.8 ± 2.1, 355.6 ± 2.1, 355.3 ± 2.1, 375.7 ± 0.5, and 401.2 ± 0.5 kcal/mol, respectively. For the most stable guanine tautomer, 7H-keto-guanine, the GPAs of N3, N7, $N2-H_a$, $N2-H_b$, and C8 sites are 336.4 ± 2.2, 338.0 ± 2.1, 347.7 ± 2.1, 341.9 ± 2.1, and 376.0 ± 0.5 kcal/mol, respectively. For the second most stable tautomer, 9H-keto-guanine, the GPAs of N3, N9, $N2-H_a$, $N2-H_b$, and C8 are 338.6 ± 2.2, 337.4 ± 2.1, 343.8 ± 2.1, 338.4 ± 2.1, and 379.9 ± 0.5 kcal/mol, respectively. The GPAs of N9, hydroxyl group, $N2-H_a$, $N2-H_b$, and C8 of 9H-*cis*-enol guanine are 338.7 ± 2.1, 340.2 ± 2.1, 358.0 ± 2.1, 357.7 ± 2.1, and 378.3 ± 0.5 kcal/mol, respectively. The corresponding GPAs of 9H-*trans*-enol guanine are 336.5 ± 2.1, 339.8 ± 2.1, 359.1± 2.1, 359.5 ± 2.1, and 374.6 ± 0.5 kcal/mol, respectively. The errors given above are based on the experimental uncertainty in the reference values used in calculations involving isodesmic reactions. In general, N9 is the most acidic site for 9H-adenine, followed by the amino group, while C2 is the least acidic site. For both 7H-keto- and 9H-keto-guanine, N3 and N7 (or N9) are the most acidic sites, followed by the amino group. For enol guanine tautomers, N9 is the most acidic site, followed by the hydroxyl group and the amino group. For all guanine tautomers mentioned above, C8 is the least acidic site. Differing from the situation of 9H-adenine and 9H-enol guanine, the acidities of the two amino group hydrogen atoms are significantly different in 7H-keto- and 9H-keto-guanine.

Introduction

The purine-based nucleobases adenine and guanine are components of nucleic acids. The protonation and deprotonation reactions of these nucleobases, which are of importance in many biological processes, are controlled by the intrinsic acid−base equilibrium properties of the nucleobases as well as their environment. The intrinsic acid−base properties of compounds can be characterized by their gas-phase acidity and basicity (or proton affinity).

Examination of the acid−base properties of adenine and guanine is complicated by the fact that they exist in various tautomeric forms. The structures of some of their important tautomers and the corresponding numbering schemes are shown in Figure 1. Many investigations have been carried out on the relative stabilities of these tautomers in both gas phase and solution. LeBreton et al. utilized ultraviolet photoelectron spectroscopy to study the electronic structures and tautomerism in adenine and other purine derivatives in the gas phase. They concluded that in an isolated environment, the 9H-adenine is more stable than 7H-adenine.[1] A microwave spectroscopy study carried out by Pierlot et al. resulted in the same conclusion.[2] Earlier semiempirical MNDO and HF calculations on adenine suggested a stability difference between 9H-adenine and 7H-adenine to be at least 6−7 kcal/mol, with 9H-adenine being more stable.[3,4] Fischer et al. investigated the tautomerism of adenine and its derivatives in solution by ^{15}N NMR spectroscopy and by theoretical calculations (in both gas phase and solution).[5] They calculated the relative free energies of 9H-, 7H-, and 1H-adenine at the B3LYP/6-311+G(2df,2p), MP2/6-311+G(2df,2p), and B3LYP/aug−cc−pVTZ levels of theory on B3LYP/6-31G(d) geometries. Their results showed that 9H-adenine is more stable than the other tautomers by at least 7 kcal/mol in the gas phase. In contrast, calculations using three different solvation models (Possion−Boltzman, PCM, and explicit solvent model) showed that 7H- and 1H-adenine are more stable than 9H-adenine in DMSO.[5]

Beauchamp et al.[6] used the AM1 theoretical method to estimate the gas-phase acidities (GPA) of N9 and the two amino hydrogen atoms of 9H-adenine to be 332.9, 349.0 ($N4-H_a$), and 350.0 kcal/mol ($N4-H_b$), respectively, in terms of ΔH of deprotonation. A comparison of the experimentally determined GPA and the semiempirically (AM1) calculated GPA of imidazole suggested that GPA(N9) of 9H-adenine obtained by using the AM1 model is likely to be 4−5 kcal/mol lower than the real value. Zeegers-Huyskens et al.[7] calculated the deprotonation enthalpy changes for the acidic sites N9, $N4-H_a$, and $N4-H_b$ of 9H-adenine to be 337.1, 356.1, and 355.5 kcal/mol, respectively, at the B3LYP/6-31+G(d,p) level of theory. Chen et al.[8] measured a GPA of 14.1 eV (325.2 kcal/mol) for the most acidic site of gaseous adenine by using negative chemical ionization mass spectrometry. Lee et al.[9] recently measured the gas-phase acidities of N9 and the NH_2 group by bracketing the gas-phase acidities (also in terms of deprotonation ΔH) of

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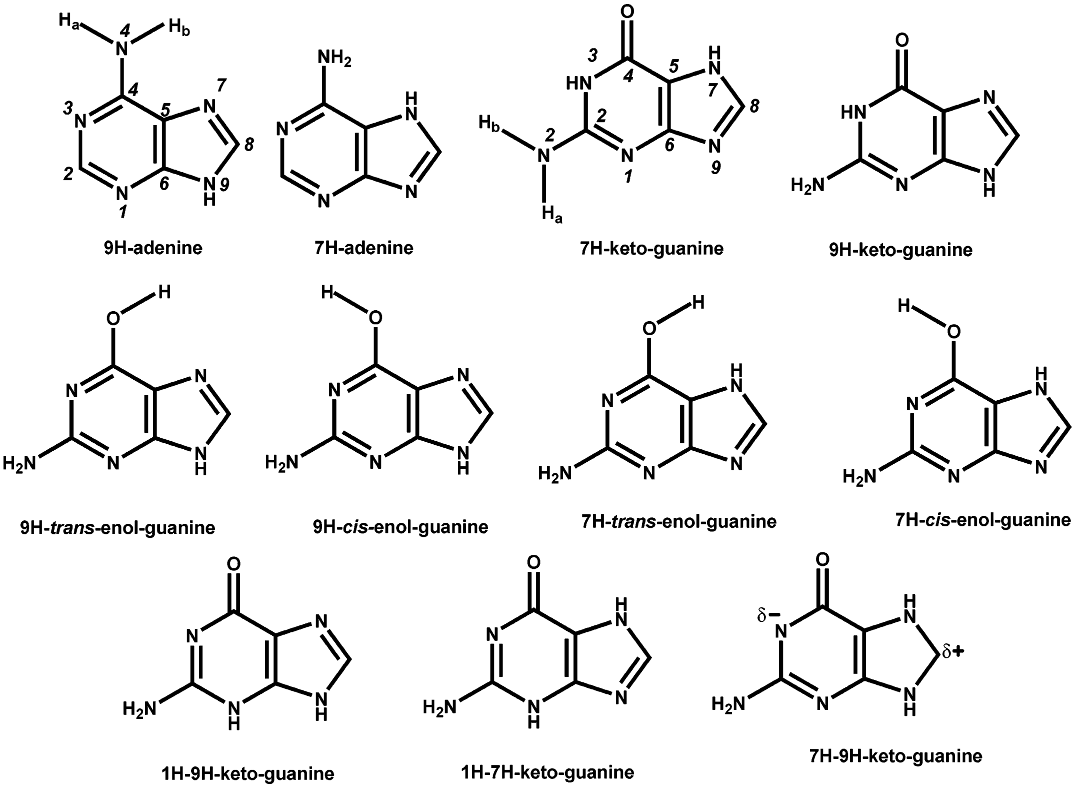

Figure 1. Structures and numbering schemes of important tautomers of adenine and guanine.

adenine, 9-ethyladenine, and 3-methyladenine by using a dual-cell Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR). They concluded that 9H-adenine has two acidic sites: N9 and NH_2. GPA(N9) was bracketed to be 333 ± 2 kcal/mol and GPA(NH_2) to be 352 ± 4 kcal/mol. They also calculated the gas-phase acidities of these sites at the B3LYP/6-31+G(d) level of theory. The corresponding GPAs are 333.3, 352.7, and 352.0 kcal/mol for N9, N4−H_a, and N4−H_b, respectively. All the values reported by Lee et al. are lower than the theoretical predictions given by Zeegers-Huyskens et al.[7] by about 3−4 kcal/mol. Since the calculated GPAs of N4−H_a and N4−H_b are very close, experiments probably cannot differentiate them, whether or not the C4−N4 bond has free internal rotation. No gas-phase acidities of the less acidic sites (C8 and C2) have been reported, mainly because of the experimental difficulties in bracketing very basic sites.

Guanine can exist in many tautomeric forms in the gas phase (Figure 1). Early theoretical calculations carried out at the HF/3-21G and HF/6-31G(d) levels of theory suggested that three tautomers, 7H-keto-, 9H-keto-, and 9H-enol-guanine, have a similar stability.[4] An ultraviolet photoelectron spectroscopy study showed that the 7H-keto tautomer is the most stable form of guanine in the gas phase.[10] Hanus et al.[11] performed various correlated ab initio calculations of guanine tautomers in the gas phase in a microhydrated environment and in aqueous solution. They concluded that, in the gas phase, the most stable five guanine tautomers are 7H-keto-, 9H-keto-, 9H-*cis*-enol-, 9H-*trans*-enol-, and 7H-*cis*-enol-guanine. The corresponding stabilites relative to 9H-keto-tautomer in terms of enthalpy at absolute Kelvin are −0.41, 0.00, 0.04, 0.29, and 2.76 kcal/mol, respectively. However, in bulk water, three tautomers rarely present in the gas phase (1H-7H-keto-, 1H-9H-keto-, and charge separated 7H-9H-keto-guanine, see Figure 1) are populated dominantly.

Chen et al. measured the gas-phase acidity of 14.1 eV (325.2 kcal/mol) for the most acidic site of guanine by using negative chemical ionization mass spectrometry.[8] Beauchamp et al. calculated the uncorrected GPAs of 330.5, 335.8, 337.3, and 333.4 kcal/mol for N9, N3, N2−H_a, and N2−H_b of 9H-keto-guanine, respectively, by using the AM1 model. Again, the real GPA(N9) was suggested to be 4−5 kcal/mol higher than the calculated value.[6] Zeegers-Huyskens et al.[7] calculated deprotonation enthalpy changes of N9, N3, N2−H_a, and N2−H_b of 9H-keto-guanine to be 336.7, 338.7, 343.3, and 337.9 kcal/mol, respectively, at the B3LYP/6-31+G(d,p) level of theory. If a 4−5 kcal/mol correction can be applied to other sites besides N9, then Beauchamp et al.'s results[6] are comparable to Zeegers-Huyskens et al.'s[7] results obtained at the higher level of theory.

To our best knowledge, no further experimental investigations have been reported on the gas-phase acidity of guanine or adenine. In addition, no comprehensive theoretical calculations *on all possible deprotonation sites* of *all the important tautomers* of adenine and guanine have been reported. We present here a comprehensive theoretical investigation on the gas-phase acidities of all possible deprotonation sites of the most stable tautomers of adenine and guanine, by performing both direct and isodesmic reaction-based calculations. The results of this investigation may facilitate the experimental determination of the gas-phase acidities of adenine and guanine.

Computational Methods

In this work, the relative gas-phase stabilities in terms of 298 K enthalpies of 7H- and 9H-adenine, 7H-keto- and 9H-keto-guanine, as well as 9H-*cis*(*trans*)-enol- and 7H-*cis*(*trans*)-enol-guanine, were estimated at the B3LYP/6-31+G(d)//B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) + ZPVE levels of theory. We chose Pople's diffuse and polarization function augmented double-ζ Gaussian 6-31+G(d) basis set to perform geometry optimizations and Dunning's diffuse function augmented triple-ζ aug−cc−pVTZ basis set to carry out single point energy calculations on the B3LYP/6-31+G(d) geometries. Aug−cc−pVTZ basis set is more flexible than double-ζ basis sets and is expected to yield a more accurate energy estimation. The 298 K gas-phase acidities of all possible deprotonation sites of 9H-adenine, 7H-keto-, 9H-keto-, 9H-*cis*-enol-, and 9H-*trans*-enol-guanine were evaluated theoretically by using both direct calculations and isodesmic reaction-based methodologies.

The gas-phase acidity (GPA) of a molecule HA is defined in this work as the 298 K enthalpy change of the reaction:

$$HA \rightarrow H^+ + A^- \tag{1}$$

The calculated 298 K enthalpies of H^+, A^-, and HA can be used to directly obtain the GPA via eq 1. GPA defined in this way is equivalent to the commonly used term ΔH_{acid}, and about 7 kcal/mol higher than ΔG_{acid} (free energy change of deprotonation).

Another way to estimate GPA is via an isodesmic reaction:

$$HA + R^- \rightarrow RH + A^- \tag{2}$$

wherein RH is a reference molecule whose 298 K GPA is known, and R^- is its conjugate base. The reference molecule and its conjugate base are chosen in such a way that the total number of each type of bond is identical before and after the reaction (2). The GPA of HA can be derived using the formula:

$$\mathrm{GPA(HA)} = \mathrm{GPA(RH)} + \Delta H_{298} \tag{3}$$

Here ΔH_{298} is the directly calculated 298 K enthalpy change of reaction (2).

Aniline, benzene, imidazole, pyrrole, and phenol were chosen as the reference molecules for isodesmic reaction calculations. All calculations reported in this work were performed using the Gaussian 98 program.[12] The geometries of all the isolated adenine and guanine tautomers mentioned above and all reference molecules, as well as the corresponding conjugate bases, were fully optimized by using density functional theory at the B3LYP/6-31+G(d) level. All optimized geometries were verified to be the real minima by the absence of imaginary frequencies. The single point energy calculations were carried out at the B3LYP/aug−cc−pVTZ level of theory on all B3LYP/6-31+G(d) geometries. B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) is currently our highest affordable level of theory for these systems. In both direct and isodesmic reaction-based calculations at the B3LYP/6-31+G(d) level of theory, 298 K enthalpies were estimated as the electronic energy modified by the 298 K enthalpy correction. In B3LYP/aug−cc−pVTZ single point calculations, 298 K enthalpies were estimated as the B3LYP/aug−cc−pVTZ level electronic energy corrected by the B3LYP/6-31+G(d) level 298 K enthalpy corrections. In direct GPA calculations, the enthalpy of the proton at 298 K was chosen to be 0.00236 hartrees (5*RT*/2).

Results and Discussion

1. Relative Gas-Phase Stabilities of the Different Tautomers of Adenine and Guanine. The calculated relative stabilities (298 K) of the 9H- and 7H-adenine, 7H-keto-, 9H-keto-, 9H-*cis*(*trans*)enol, and 7H-*cis*(*trans*)enol-guanine (Figure 1) in the gas phase are shown in Table 1. 9H-Adenine is about 8.14 and 7.75 kcal/mol more stable than 7H-adenine, as calculated at the B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) + ZPVE levels of theory, respectively. These values are in agreement with the previous results.[1−4] It is reasonable to assume that in the gas phase, 9H-adenine is the most important tautomer of adenine. Therefore, only the gas-phase acidities of 9H-adenine were calculated.

TABLE 1: The 298 K Relative Stabilities of Adenine and Guanine Tautomers

	relative stability (kcal/mol)	
	B3LYP/6-31+G(d)// B3LYP/6-31+G(d)	B3LYP/aug-cc-pVTZ// B3LYP/6-31+G(d)
	Adenine	
9H-tautomer	0.00	0.00
7H-tautomer	8.14	7.75
	Guanine	
7H-keto-taotomer	0.00	0.00
9H-keto-tautomer	0.54	0.64
9H-*cis*-enol-tautomer	2.51	1.48
9H-*trans*-enol-tautomer	3.26	1.87
7H-*cis*-enol-tautomer	5.33	4.15
7H-*trans*-enol-tautomer	13.40	11.09

The stability ordering of the different guanine tautomers mentioned above is 7H-keto- > 9H-keto- > 9H-*cis*-enol- > 9H-*trans*-enol- > 7H-*cis*-enol- > 7H-*trans*-enol-guanine, as predicted at the B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) + ZPVE levels of theory. On the basis of our calculations, the most stable tautomers in the gas phase are determined to be 7H-keto and 9H-keto-guanine, with an energy difference less than 0.7 kcal/mol. 9H-*cis*-Enol and 9H-*trans*-enol-tautomers are about 1−3 kcal/mol higher in energy than the 7H-keto-tautomer. 7H-Enol tautomers (especially the 7H-*trans*-enol tautomer) are much higher in energy than the other tautomers. Therefore, only the GPAs of 7H-keto-, 9H-keto-, 9H-*cis*-enol-, and 9H-*trans*-enol-gaunine were investigated in this work.

It is worthwhile to notice that *cis*-enol tautomers of guanine are lower in energy than the corresponding *trans*-enol counterparts. In particular, 7H-*trans*-enol-guanine lies 6−7 kcal/mol higher in energy than 7H-*cis*-enol-guanine. The reason for the lower stability of 7H-*trans*-enol-guanine is the repulsive interaction between the hydroxyl group and the N7-hydrogen atom. Such interaction is absent for 9H-*trans*-enol-guanine, and hence, 9H-*trans*-enol-guanine is only about 0.4 kcal/mol less stable than 9H-*cis*-enol-guanine.

2. Gas-Phase Acidities of Adenine. For both adenine and guanine, the difference in the GPA values estimated by direct and isodesmic reaction-based calculations at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory is less than 1 kcal/mol, except for the N3-site of 7H-keto- and 9H-keto-guanine, for which the difference is about 1.4 kcal/mol. At the lower B3LYP/6-31+G(d) level of theory, the differences of the results between the direct and the isodesmic reaction-based calculations sometimes exceed 3 kcal/mol, and the isodesmic reaction-based calculations give results that are in good agreement with those obtained at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory. Therefore, it is reasonable to assume that the direct GPA calculations at the B3LYP/6-31+G(d) level are not very reliable. The gas-phase acidities of adenine and guanine calculated by using isodesmic reactions (Figure 2) at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d)+ZPV level of theory are mainly discussed below.

The theoretically evaluated 298 K gas-phase acidities of different deprotonation sites of 9H-adenine are shown in Table 2. N9 is the most acidic site with a GPA of 337.8 ± 2.1 kcal/mol, as estimated by using imidazole/imidazole anion (with 298 K GPA of 350.1 ± 2.1 kcal/mol[13]) as the reference system. This value is greater than the experimental value obtained by Lee et al.[9] and Chen et al.,[8] but close to the GPA estimated by Beauchamp et al. on the basis of the corrected AM1 calcula-

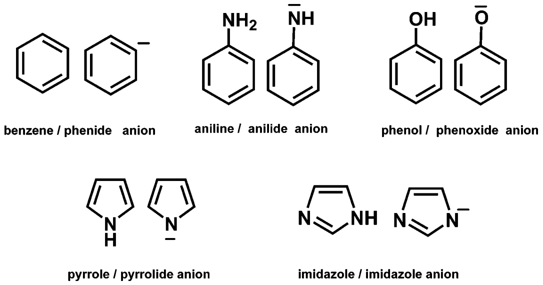

Figure 2. Structures of reference systems used for isodesmic reaction-based gas-phase acidity calculations.

TABLE 2: The 298 K Gas-Phase Acidities (Enthalpies of Deprotonation) of All Possible Deprotonation Sites of 9H-Adenine Calculated at the B3LYP/6-31+G(d)//B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) Levels of Theory

	298 K gas-phase acidity (kcal/mol)				
	B3LYP/6-31+G(d)//B3LYP/6-31+G(d)[d]		B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d)[d,e]		exptl results
deprotonation site	GPA[a]	GPA[b]	GPA[a]	GPA[b]	
C2	400.8 ± 0.5	399.0	401.2 ± 0.5	400.8	
N4−H_a	355.3 ± 2.1	354.2	355.6 ± 2.1	356.3	352 ± 4[c]
N4−H_b	354.6 ± 2.1	353.5	355.3 ± 2.1	356.0	352 ± 4[c]
C8	374.8 ± 0.5	373.0	375.7 ± 0.5	375.3	
N9	337.7 ± 2.1	334.8	337.8 ± 2.1	338.5	333 ± 2[c]

[a] GPA values were derived from isodesmic reactions. [b] GPA values were calculated directly from the enthalpies of neutral 9H-adenine, its conjugate base, and proton at 298 K. [c] Data from ref 9. [d] All errors given for the computational results are based on the uncertainty of the experimentally determined GPA of the reference systems. [e] GPA values calculated at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory are recommended.

TABLE 3: The 298 K Gas-Phase Acidities (Enthalpies of Deprotonation) of All Possible Deprotonation Sites of 7H-Keto-guanine Calculated at the B3LYP/6-31+G(d)//B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) Levels of Theory

	298 K gas-phase acidity (kcal/mol)			
	B3LYP/6-31+G(d)//B3LYP/6-31+G(d)		B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d)[d]	
deprotonation site	GPA[a]	GPA[b]	GPA[a]	GPA[b]
N2−H_a	347.7 ± 2.1	346.6	347.7 ± 2.1	348.4
N2−H_b	340.8 ± 2.1	339.7	341.9 ± 2.1	342.6
N3	336.1 ± 2.2	334.4	336.4 ± 2.2	337.8
N7	337.7 ± 2.1	334.8	338.0 ± 2.1	338.8
C8	375.2 ± 0.5	373.4	376.0 ± 0.5	375.6

[a] GPA values were derived from isodesmic reactions. [b] GPA values were calculated directly from the enthalpies of neutral 7H-keto-guanine, its conjugate base, and proton at 298 K. [c] All errors given for the computational results are based on the uncertainty of the experimentally determined GPA of the reference systems. [d] GPA values calculated at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory are recommended.

TABLE 4: The 298 K Gas-Phase Acidities (Enthalpies of Deprotonation) of All Possible Deprotonation Sites of 9H-Keto-guanine Calculated at the B3LYP/6-31+G(d)//B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) Levels of Theory

	298 K gas-phase acidity (kcal/mol)			
	B3LYP/6-31+G(d)//B3LYP/6-31+G(d)		B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d)[d]	
deprotonation site	GPA[a]	GPA[b]	GPA[a]	GPA[b]
N2−H_a	342.7 ± 2.1	341.5	343.8 ± 2.1	344.5
N2−H_b	337.2 ± 2.1	336.0	338.4 ± 2.1	339.1
N3	338.3 ± 2.2	336.7	338.6 ± 2.2	340.1
C8	379.2 ± 0.5	377.4	379.9 ± 0.5	379.6
N9	337.1 ± 2.1	334.8	337.4 ± 2.1	338.1

[a] GPA values were derived from isodesmic reactions. [b] GPA values were calculated directly from the enthalpies of neutral 9H-keto-guanine, its conjugate base and proton at 298 K. [c] All errors given for the computational results are based on the uncertainty of the experimentally determined GPA of the reference systems. [d] GPA values calculated at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory are recommended.

tions.[6] Our value is also in good agreement with the results reported by Zeegers-Huyskens et al.[7]

The N4 site contains two acidic hydrogen atoms, referred to as N4−H_a and N4−H_b (Figure 1). The isodesmic reaction-based calculations give 355.6 ± 2.1 and 355.3 ± 2.1 kcal/mol as the GPA(N4−H_a) and GPA(N4−H_b), respectively, by using the aniline/anilide pair as the reference system (GPA of 366.4 ± 2.1 kcal/mol[13]). These values are in good agreement with Zeegers-Huyskens et al.'s results[7] and Beauchamp et al.'s results[6] (if the 5 kcal/mol correction suggested by Beauchamp et al.[6] can be applied not only for the N9 site, but also for all other possible deprotonation sites). However, our results are about 3 kcal/mol higher than the experimental value of 352 ± 4 kcal/mol obtained by Lee et al.[9] H_b is calculated to be slightly more acidic than H_a. This is rationalized by the fact that the distance between N4 and N7 is about 3.11 Å in 9H-adenine-H_b anion while the distance between N3 and N4 in 9H-adenine-H_a anion is about 2.347 Å. Therefore, in the adenine-H_a anion, repulsion between the lone pair electrons of N3 and N4 is stronger than that of N4 and N7 in the adenine-H_b anion. Consequently, adenine-H_b anion is slightly more stable, which leads to the slightly lower gas-phase acidity of N4−H_b. However, as pointed out by Lee et al.,[9] even if the energy barrier for the rotation of the C4−N4 bond could not be overcome, and hence, it would be possible to differentiate the two "rotamers", the corresponding gas-phase acidities are predicted to be too close to be differentiated by experiment.

The C8 and C2 sites are much less acidic than N3 and N4. Therefore, their gas-phase acidities are difficult to measure experimentally. It is also difficult to find adequate reference systems to perform isodesmic reaction-based calculations. In this study, benzene was utilized as the reference molecule (298 K GPA of 401 ± 0.5 kcal/mol[13]). The results are compared with those obtained by direct calculations. At the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory, GPA(C8) is estimated to be 375.7 ± 0.5 and 375.3 kcal/mol, by using isodesmic reaction-based and direct calculations, respectively. The corresponding GPA(C2) is evaluated to be 401.2 ± 0.5 and 400.8 kcal/mol, based upon isodesmic and direct calculations, respectively. In general, N9 is the most acidic site in 9H-adenine, followed by the N4 site. C8 and C2 sites are much less acidic, with GPA(C2) larger than 400 kcal/mol.

3. Gas-Phase Acidities of Guanine. The theoretically evaluated 298 K GPA of different sites of 7H-keto-, 9H-keto-, 9H-*cis*-enol-, and 9H-*trans*-enol-guanine are listed in Tables 3, 4, 5, and 6, respectively. For 7H-keto-guanine, calculations using pyrrole/pyrrolide anion as the reference system predict a GPA(N3) of 336.4 ± 2.2 kcal/mol. This value is greater than the experimental value obtained by Chen et al. (325.2 kcal/mol).[7] The N7 site is slightly less acidic, with a calculated GPA of 338.0 ± 2.1 kcal/mol. The two hydrogen atoms of the amino group are more acidic than those in 9H-adenine. For 7H-keto-guanine, the GPAs of N2−H_a and N2−H_b are estimated to be 347.7 ± 2.1 and 341.9 ± 2.1 kcal/mol, respectively, by using aniline as the reference molecule. H_a is less acidic than H_b because of the repulsive interaction between H_b and the

TABLE 5: The 298 K Gas-Phase Acidities (Enthalpies of Deprotonation) of All Possible Deprotonation Sites of 9H-*cis*-Enol-guanine Calculated at the B3LYP/6-31+G(d)//B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) Levels of Theory

deprotonation site	298 K gas-phase acidity (kcal/mol)			
	B3LYP/6-31+G(d)//B3LYP/6-31+G(d)		B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d)[d]	
	GPA[a]	GPA[b]	GPA[a]	GPA[b]
N9	338.5 ± 2,1	335.6	338.7 ± 2.1	339.4
O	340.4 ± 2.1	334.7	340.2 ± 2.1	339.2
N2−H_a	357.4 ± 2.1	356.2	358.0 ± 2.1	358.7
N2−H_b	357.0 ± 2.1	355.9	357.7 ± 2.1	358.4
C8	377.5 ± 0.5	375.7	378.3 ± 0.5	377.9

[a] GPA values were derived from isodesmic reactions. [b] GPA values were calculated directly from the enthalpies of neutral 9H-*cis*-enol-guanine, its conjugate base, and proton at 298 K. [c] All errors given for the computational results are based on the uncertainty of the experimentally determined GPA of the reference systems. [d] GPA values calculated at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory are recommended.

TABLE 6: The 298 K Gas-Phase Acidities (Enthalpies of Deprotonation) of All Possible Deprotonation Sites of 9H-*trans*-Enol-guanine Calculated at the B3LYP/6-31+G(d)//B3LYP/6-31+G(d) and B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) Levels of Theory

deprotonation site	298 K gas-phase acidity (kcal/mol)			
	B3LYP/6-31+G(d)//B3LYP/6-31+G(d)		B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d)[d]	
	GPA[a]	GPA[b]	GPA[a]	GPA[b]
N9	336.1 ± 2.1	333.2	336.5 ± 2.1	337.3
O	339.7 ± 2.1	334.0	339.8 ± 2.1	338.8
N2−H_a	358.5 ± 2.1	357.4	359.1 ± 2.1	359.8
N2−H_b	359.0 ± 2.1	357.8	359.5 ± 2.1	360.2
C8	373.3 ± 0.5	371.5	374.6 ± 0.5	374.2

[a] GPA values were derived from isodesmic reactions. [b] GPA values were calculated directly from the enthalpies of neutral 9H-*trans*-enol-guanine, its conjugate base, and proton at 298 K. [c] All errors given for the computational results are based on the uncertainty of the experimentally determined GPA of the reference systems. [d] GPA values calculated at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory are recommended.

hydrogen at N3 in the guanine-H_a anion (in 7H-keto-guanine, the amino group is not in the plane of the purine ring, while in guanine-H_a and guanine-H_b anions, the remaining amino hydrogen atom is in the plane) as well as the repulsion between the electron lone pairs of N1 and the amino nitrogen atom.

The least acidic site of 7H-keto-guanine is C8. Isodesmic reaction-based calculations gave a gas-phase acidity of 376.0 ± 0.5 kcal/mol for this site. Benzene was utilized as the reference molecule since no better reference systems were found. The direct calculations gave the value of 375.6 kcal/mol at the same level of theory. These values are very close to the GPA-(C8) of 9H-adenine (375.7 ± 0.5 kcal/mol).

For 9H-keto-guanine, the gas-phase acidities of N9, N3, N2−H_b, and N2−H_a were estimated in a manner similar to that used for 7H-keto-guanine. The corresponding GPAs are 337.4 ± 2.1, 338.6 ± 2.2, 338.4 ± 2.1, and 343.8 ± 2.1 kcal/mol, respectively, as estimated at theB3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory. Imidazole, pyrrole, and aniline were utilized as the reference molecules. Our data are in good agreement with the results obtained by Zeegers-Huyskens et al.[7] The GPAs of N9 and the amino group suggested by Beauchamp et al.[6] are close to our data if the 5 kcal/mol correction is applied. Their GPA(N3) (after correction) is slightly greater than our result. C8 is the least acidic site, with GPA of 379.9 ± 0.5 kcal/mol, as calculated using benzene as the reference molecule.

For 9H-*cis*-enol-guanine, the hydroxyl group is slightly less acidic (GPA of 340.2 ± 2.1 kcal/mol, calculated by using the phenol/phenoxide anion reference system with a GPA of 349.2 ± 2.1 kcal/mol[13]) than the N9 site (GPA of 338.7 ± 2.1 kcal/mol). The amino group is less acidic than the hydroxyl group, with calculated GPA(N_2−H_a) and GPA(N_2−H_b) of 358.0 ± 2.1 and 357.7 ± 2.1 kcal/mol, respectively. The anions formed upon removal of either H_a or H_b of the NH_2 group each suffer from repulsion between two electron lone pairs (N2−N3 repulsion for 9H-enol-guanine-H_b anion and N2−N1 repulsion for 9H-enol-guanine-H_a anion). This repulsion results in a greater GPA for the amino group than for the hydroxyl group. C8 is the least acidic site with a GPA of 378.3 ± 0.5 kcal/mol.

The hydroxyl group and the C8 and C9 sites of 9H-*trans*-enol-guanine are slightly more acidic than those of the 9H-*cis*-enol-guanine, while the amino group of 9H-*trans*-enol-guanine is slightly less acidic than that of 9H-*cis*-enol-guanine. The conclusion can be drawn that the orientation of the hydroxyl group does not greatly affect the gas-phase acidities.

In general, unlike the situation of adenine, N3 (for keto tautomers), hydroxyl group (for 9H-enol tautomers), and imidazole nitrogen (N9 or N7) are the most acidic sites in guanine. The amino group is less acidic than N3 and the imidazole nitrogen. The two hydrogen atoms of the amino group in the keto tautomers have significantly different acidities and might be differentiated experimentally. This possibility does not exist for 9H-enol tautomers. The C8 site in guanine is almost as acidic as the same site in adenine.

Conclusions

1. The 298 K gas-phase acidities (in terms of ΔH of deprotonation) of all possible deprotonation sites of the most important tautomers of adenine and guanine were calculated by using direct and isodesmic reaction-based methods. The GPAs predicted by both methods are similar at the B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory. This level of theory is recommended for the gas-phase acidity calculations on purine-based nucleobases.

2. 9H-Adenine is the most stable tautomeric form of adenine in the gas phase. The N9 site is the most acidic site, with a calculated GPA of 337.8 ± 2.1 kcal/mol (B3LYP/aug−cc−pVTZ//B3LYP/6-31+G(d) level of theory). This value is 4−5 kcal/mol higher than the experimental value bracketed by Lee et al.[9] In agreement with the results of Lee et al.,[9] our calculations suggest that NH_2 is the second most acidic site. GPA(N−H_a) and GPA(N−H_b) are calculated to be too close (both about 355 kcal/mol) to be differentiated experimentally or theoretically. All our results for GPAs of N9 and of the amino groups are in good agreement with Zeegers-Huyskens et al.'s[7] and Beauchamp et al.'s results (if the 5 kcal/mol correction is applied[6]). The gas-phase acidities of C8 and C2 have not been reported before. They were calculated to be 375.7 ± 0.5 and 401.2 ± 0.5 kcal/mol, respectively. Hence, C2 is the least acidic site.

3. The 7H-keto- and 9H-keto-guanines are the most important guanine tautomers in the gas phase (with the former being slightly more stable than the latter), followed by 9H-*cis*-enol- and 9H-*trans*-enol-guanines. 7H-Enol-guanines are the least stable tautomers. Their GPAs were not calculated in this work.

4. For 7H- and 9H-keto-guanine, N3 and the imidazole nitrogen are the most acidic sites (with GPAs between 336 and 338 kcal/mol), followed by the amino group (with GPAs in the

range 341−348 kcal/mol for the 7H-keto-tautomer and 338−344 kcal/mol for the 9H-keto-tautomer). For 9H-enol-guanine, the imidazole nitrogen and the hydroxyl group are the most acidic sites (GPAs ranging 336−340 kcal/mol), followed by the amino group. The amino group is more acidic in the keto tautomers than in the enol tautomers. Unlike for 9H-enol-guanine, the acidities of $N2-H_a$ and $N2-H_b$ differ significantly for the keto guanine tautomers. In all guanine tautomers, C8 is the least acidic site, with a GPA of 375−378 kcal/mol.

5. In general, the most stable form of guanine, 7H-keto-guanine (with a GPA of 336.4 ± 2.1 kcal/mol for the most acidic site, N3), is slightly more acidic than 9H-adenine (with a GPA of 337.8 ± 2.1 kcal/mol for the most acidic site, N9). The second most stable form, 9H-keto-guanine (with a GPA of 337.4 ±2.1 kcal/mol for the most acidic site, N9), is almost as acidic as 9H-adenine.

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