DOI 10.5012/bkcs.2010.31.01.168

Notes

Study of 7-Methylguanine on pK_a Values by Using Density Functional Theoretical Method

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Received August 25, 2009, Accepted October 19, 2009

Key Words: 7-Methylguanine, Acid dissociation constant (pK_a), Density functional theory, Poisson-Boltzmann continuum-solvation model

Methylation of DNA by endogenous methylating agents generates a variety of genotoxic adducts.[1] The N7 position of guanine is known as the most nucleophilic site within the heterocyclic bases of DNA.[2] Accordingly, N7-methylguanine (7MeG) is the one of the most prevailing forms of the methylated guanine. 7MeG is also generated by methylation in order to be used as a probe of protein-DNA interactions and DNA sequencing. Recently, a DNA double helical structure of a 25-mer containing a 7MeG was reported, which revealed the base pairing characteristics of 7MeG.[1] Information on the acid dissociation constants and the relative populations of various tautomers would provide valuable clues toward optimizing analysis of 7MeG and to understand the biological consequences of the modified base.

We have developed a scheme based on density-functional theoretical calculations in combination with the Poisson-Boltzmann continuum solvation model for water to predict pK_a values and the major tautomeric forms of a number of nucleobases, their oxidative damage products,[3-5] and heteronuclear aromatic compounds.[6,7] As well as macroscopic pK_a values, we reproduced the micro pK_a values of individual protonation sites of purine nucleobases.[8] Hydrogen transfer between paired nucleobases was also investigated using the same computational model.[9]

Among methylated guanine species, we have already reported computations on the tautomerism and pK_a values of 9-methylguanine (9MeG) and 7,9-dimethylguanine.[8] Gas-phase vibrational experiments and computation results on 1,7-dimethylguanine, 7MeG, and 9MeG were also reported.[10]

In the current work, we report calculated relative stabilities of the tautomers of 7MeG at each ionization stage and the pK_a values of 7MeG in aqueous solution.

pK_a calculations on the nucleobases and their derivatives are complicated due to the presence of multiple tautomers with different site-specific microscopic pK_a values. A way to estimate the overall macroscopic pK_a from the site-specific pK_a's of the tautomers devised in our previous studies and the details of the computation scheme have been presented elsewhere.[3,4]

For a deprotonation process leading the i-th tautomer of an acid HA into the j-th tautomer of the conjugate base A^-, the Gibbs energy of deprotonation reaction is calculated as

$$\Delta G^{0,ij}_{\mathrm{deprot},aq} = \Delta G^0_{aq}(\mathrm{A}^-_j) + \Delta G^0_{aq}(\mathrm{H}^+) - \Delta G^0_{aq}(\mathrm{HA}_i) \qquad (1)$$

and the corresponding micro pK_a^{ij} values is given by

$$\mathrm{p}K_a^{ij} = \Delta G^{0,ij}_{\mathrm{deprot},aq}/2.303RT, \qquad (2)$$

where R is the gas constant and T is 298.15 K. From this micro pK_a^{ij} value, the partial population of the i-th tautomer out of all of the acid species (f_i), and the partial population of the j-th tautomer out of all of the conjugate base species (f_j'), the macro pK_a value is estimated as[4]

$$\mathrm{p}K_a = \mathrm{p}K_a^{ij} - \log f_i + \log f_j'. \qquad (3)$$

The standard free energy of each species (HA, A^-, and H^+) in water, ΔG^0_{aq}, can be written as the sum of the gas-phase standard Gibbs energy ΔG^0_g and the standard Gibbs energy of solvation in water ΔG^0_{solv}:

$$\Delta G^0_{aq} = \Delta G^0_g + \Delta G^0_{solv}. \qquad (4)$$

The standard Gibbs energy of each species in the gas phase, ΔG^0_g, is obtained by

$$\Delta G^0_g = E_{0\,\mathrm{K}} + \mathrm{ZPE} + \Delta\Delta G_{0\rightarrow 298\,\mathrm{K}}. \qquad (5)$$

The total energy of the molecule at 0 K ($E_{0\,\mathrm{K}}$) is calculated at the optimum geometry from quantum mechanics (QM). The zero-point energy (ZPE) and the Gibbs free energy change from 0 K to 298 K ($\Delta\Delta G_{0\rightarrow 298\,\mathrm{K}}$) are calculated from the vibrational frequencies calculated using QM. The translational and rotational free energy contribution is also calculated according to the ideal gas approximation. We used $\Delta G^0_g(\mathrm{H}^+) = 2.5\,RT - T\Delta S^0 = 1.48 -$

7.76 = –6.28 kcal/mol from the literature.[11,12]

All QM calculations used the Jaguar v 5.5 quantum chemistry software.[13] To calculate the geometries and energies of the various molecules, we used the B3LYP flavor of density functional theory (DFT), which includes the generalized gradient approximation and a component of the exact Hartree-Fock (HF) exchange.[14-17] Since calculations of vibration frequencies are generally quite time-consuming, the calculations were carried out in two steps. The 6-31G** basis set was first used to optimize the geometry and calculate the vibration frequencies. Then the 6-31++G** basis set was used for the final geometry optimization started from the 6-31G** geometry:

$$\Delta G_g^0 = \mathrm{ZPE}^{6\text{-}31\mathrm{G}^{**}} + \Delta\Delta G_{0\to 298\,\mathrm{K}}{}^{6\text{-}31\mathrm{G}^{**}} + E_{0\,\mathrm{K,g}}{}^{6\text{-}31{+}{+}\mathrm{G}^{**}}. \quad (6)$$

This strategy for the gas-phase calculation was chosen after considering several basis sets for the calculation of gas-phase proton affinity (PA) and gas-phase basicity (GB) of guanine 298 K, which are defined as the enthalpy change and the free energy change, respectively, during the protonation process in gas-phase.[4]

Details for the solvation energy calculation are given elsewhere,[18-20] so we here briefly describe the overall procedure. The continuum solvent model was applied to the calculations. The solvation Gibbs free energy was given by the sum of the non-electrostatic contribution due to the creation of the solute cavity in the solvent and the electrostatic interaction between solute and solvent. The electrostatic part of the solvation free energy was evaluated by a self-consistent formalism, which involves quantum mechanical calculations in the solvent reaction field generated from the numerical solution of the Poisson-Boltzmann (PB) equation.[18] The non-electrostatic contribution is taken into consideration by a term proportional to the solvent-accessible surface (SAS) area of the solute defined by the surface traced out by the center of a sphere of probe radius (1.4 Å for water) as it is rolled around the solute, which is usually built up as a van der Waals (vdW) envelope of the solute with a chosen set of atomic radii. The atomic radii used to build the vdW envelope of the solute were taken from our previous work on guanine: 1.88 Å for sp^2-hybridized carbon, 1.79 Å for sp^3-hybridized carbon, 1.46 Å for oxygen, 1.41 Å for nitrogen, 1.175 Å for hydrogen attached to sp^2-hybridized carbon, and 1.08 Å for other types of hydrogen.[4] All solvation energy calculations were carried out at the B3LYP/6-31++G** level, and the geometry was re-optimized in solution. We took the solvation free energy of a proton in water ($\Delta G_{solv}^0(\mathrm{H}^+)$) to be –263.47 kcal/mol, which gave the best fit of pK_as of guanine to experimental values.[4]

In summary, we used the following scheme to calculate the solution phase Gibbs energy of a chemical species:

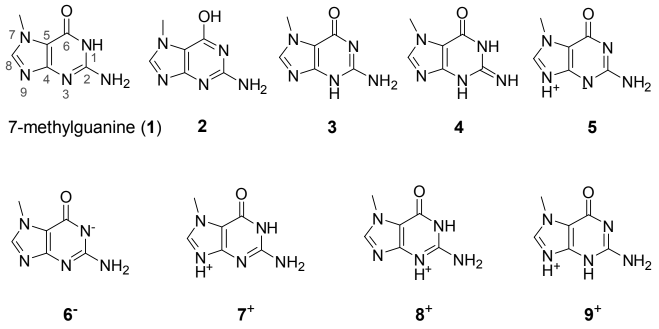
$$\begin{aligned}\Delta G_{aq}^0 &= \mathrm{ZPE}^{6\text{-}31\mathrm{G}^{**}} + \Delta\Delta G_{0\to 298\,\mathrm{K}}{}^{6\text{-}31\mathrm{G}^{**}} \\ &\quad + E_{0\,\mathrm{K,g}}{}^{6\text{-}31{+}{+}\mathrm{G}^{**}} + \Delta G_{solv}^0{}^{6\text{-}31{+}{+}\mathrm{G}^{**}}.\end{aligned} \quad (7)$$

Tautomers of neutral 7MeG considered in this study (**1**-**5**) are shown in Scheme 1 and their relative free energies and relative populations in equilibrium in gas and aqueous phases are given in Table 1. The number of tautomers is smaller than those of guanine[4] and 8-oxoguanine[3] due to the methyl group's blockage of prototropies.[10]

The free energy of tautomers of neutral 7MeG in the gas phase increases in the following order: **1** < **2** < **4** < **3** < **5**. Among the five tautomers of neutral 7MeG, the amino-oxo form (**1**) was calculated to be the most stable in the gas phase. The other tautomers were found to be at least ~5 kcal/mol less stable than **1**. Thus, 7MeG would exist as amino-oxo form in the gas phase. This result is consistent with the previously reported vibrational spectroscopy and computational study[10] on 7MeG. This relative stability of keto-enol, amino-imino, and prototropic tautomers are also in accordance with guanine[4] and oxoguanine.[3]

In the aqueous phase, the free energy of the tautomers increases in the following order: **1** < **3** < **5** < **4** < **2**. Still, the amino-oxo form **1** predominates, and the other amino-oxo form **3** produced by the proton migration from N1 to N3 became more stable than in the gas phase. This stabilization of **3** in the aqueous solution indicates a lessening of the intramolecular repulsion between the N3 proton and amine protons of the 2-amino group, as in the case of guanine.[4] The zwitterionic form **5** was also stabilized in the aqueous solution due to the high dielectric constant of water. The enol (**2**) and imino tautomer (**4**) were less stable than amino-oxo or zwitterionic forms, as in the previous studies on guanine[4] and oxoguanine.[3]

Due to the N7-methylation, there is no tautomeric equilibrium



Scheme 1. Tautomers of neutral (1-5), anionic, and cationic (7^+-9^+) 7-methylguanine

Table 1. Relative free energies (kcal/mol) of tautomers of neutral 7-methylguanine and their relative Boltzmann populations in equilibrium. (a) Gas phase and (b) aqueous phase.

name	1	2	3	4	5
type	Amino-oxo	Amino-hydroxy	Amino-oxo	Imino-oxo	Zwitter-ionic
(a) gas					
$\Delta G_{g,rel}^{0\ a}$	0.0	4.9	6.6	5.7	18.0
Population	1.0	3×10^{-4}	1×10^{-5}	7×10^{-5}	6×10^{-14}
(b) aqueous					
$\Delta G_{aq,rel}^{0\ b}$	0.0	9.5	2.8	8.4	5.4
Population	1.0	1×10^{-7}	9×10^{-3}	6×10^{-7}	1×10^{-4}

[a] Relative free energies with respect to $\Delta G_g^0(\mathbf{1})$ [b] Relative free energies with respect to $\Delta G_{aq}^0(\mathbf{1})$.

Table 2. Relative free energies (kcal/mol) of tautomers of cationic 7-methylguanine and their relative Boltzmann populations in equilibrium. (a) Gas phase and (b) aqueous phase.

	$\mathbf{7^+}$	$\mathbf{8^+}$	$\mathbf{9^+}$
(a) gas			
$\Delta G^{0}_{g,rel}{}^{a}$	0.0	6.2	20.2
Population	1.0	3×10^{-5}	2×10^{-15}
(b) aqueous			
$\Delta G^{0}_{aq,rel}{}^{b}$	0.0	1.6	5.9
Population	0.94	0.06	5×10^{-5}

[a]Relative free energies with respect to $\Delta G^{0}_{g}(\mathbf{7^+})$ [b]Relative free energies with respect to $\Delta G^{0}_{aq}(\mathbf{7^+})$.

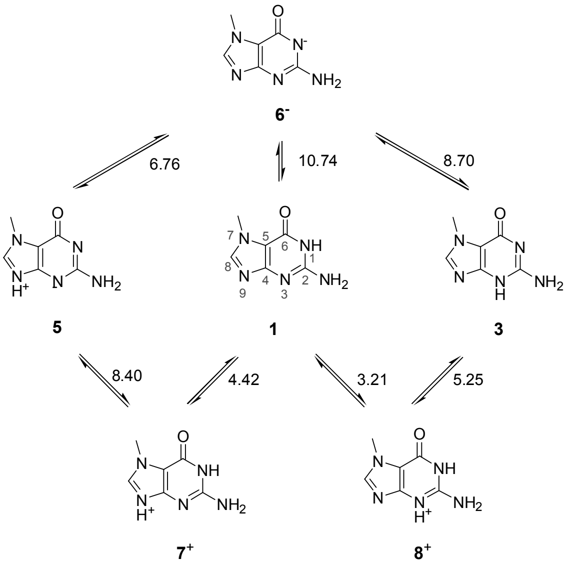


Figure 1. Calculated micro pK_a values of 7-methylguanine.

for anionic 7MeG ($\mathbf{6^-}$).

The tautomers of cationic 7MeG considered in this study ($\mathbf{7^+}$-$\mathbf{9^+}$) are also shown in Scheme 1, and their relative Gibbs energies and relative populations in gas and aqueous phases are given in Table 2. The N7 site, which was the major protonation site in guanine, is not available in 7MeG, since it is already methylated in its neutral state. Instead, N9 is protonated in the gas phase ($\mathbf{7^+}$) and N3, with a population of 6%, can also be protonated in the aqueous phase ($\mathbf{8^+}$).

Macroscopic pK_a values were calculated by Eq (3). The pK_{a1} value of 7MeG was estimated to be 4.44 and the pK_{a2} value 10.75. The pK_{a2} value of 7MeG was almost the same as that of 9MeG (pK_{a2} = 10.72) in our previous study,[8] since this value is related to the deprotonation from N1. The pK_{a1} value of 7MeG is larger than that of 9MeG (pK_{a1} = 3.51), reflecting the fact that the N7 position of 9MeG is more susceptible to deprotonation than is the N9 position of 7MeG, which is also consistent with our previous study on guanine.[4]

Microscopic pK_a values are shown in Figure 1. These microscopic pK_a values give clues toward better understanding the chemistry of nucleobases, such as metal ion-binding properties and proton transfer. The microscopic pK_a value corresponding to the deprotonation from $\mathbf{7^+}$ to **5** (deprotonation of N1 proton) is calculated to be 8.40, which suggests that 7MeG is more facile to proton-transfer to cytosine than from guanine to cytosine (The corresponding micro pK_a value is 9.65 for guanine. See reference 4).

In summary, various tautomers of 7-methylguanine were investigated using the B3LYP flavor of DFT in combination with the Poisson-Boltzmann continuum-solvation model. We show that the major tautomer of neutral 7-methylguanine in aqueous solution is the amino-oxo form **1** with a minor contribution from the other amino-oxo form **3**. The N9 site (94%) is more suitable for protonation than the N3 site (6%). Macroscopic pK_{a2} value of 7MeG is similar to that of 9MeG, and the pK_{a1} value of 7MeG is larger than that of 9MeG.

This work was supported by the Program for Integrated Molecular systems (PIMS) of GIST.

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