http://dx.doi.org/10.5012/bkcs.2012.33.12.4255

Density Functional Theoretical Study on the Acid Dissociation Constant of an Emissive Analogue of Guanine

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Received August 7, 2012, Accepted September 21, 2012

Key Words : DFT, Acid dissociation constant, Guanine

Fluorescence spectroscopy has played an important role in modern research especially in biological areas.[1] However, the purine and pyrimidine bases in nucleic acids are practically nonemissive in physiological condition. Accordingly, the emissive analogues of nucleic acid bases have been the target of extensive studies because they can facilitate the fabrication of biophysical and discovery assays.[1-3] Recently, a fluorescent guanine analogue, 2-aminothieno[3,4-*d*]pyrimidine (thG), was developed.[4] One of the essential criteria of a successful analogue is to minimize the structural and functional disturbances when replacing any native residue. Along with many structural, photochemical, and biophysical characteristics, the acid dissociation constant, pK_a, would be an important parameter to be monitored, particularly when an aqueous solution is used in the assay because p*K*a value is related to the stability of nucleic base, Watson-Crick base pairing, proton migration, and the mispair formation during the DNA replication.[5,6]

We have developed a protocol based on a density functional theory (DFT) quantum mechanics method in order to understand the keto-enol and amino-imino tautomeric equilibrium of normal and damaged DNA bases in various environments and to calculate their pK_a values in water.[7-9] Computational procedure adapted in this paper was developed to reproduce the pK_a values of guanine.[8] It was applied to damaged DNA bases,[7,9] and methylated purine nucleobases,[10,11] and the macroscopic and microscopic pK_a values were reproduced successfully. In this study, the same method was used to calculate the pK_a values of a sulfur containing guanine analogue.

The site-specific microscopic pK_a value is related to the Gibbs energy of the deprotonation process. For deprotonation of the *i*-th tautomer of an acid HA into the *j*-th tautomer of the conjugate base A^-, the Gibbs energy of the deprotonation reaction was calculated using the following equation:

$$\Delta G^{0,ij}_{\text{deprot},aq} = \Delta G^0_{aq}(A^-_j) + \Delta G^0_{aq}(H^+) - \Delta G^0_{aq}(HA_i) \quad (1)$$

The corresponding micro pK_a^{ij} values are given by the following equation:

$$pK_a^{ij} = \Delta G^{0,ij}_{\text{deprot},aq}/2.303RT, \quad (2)$$

where R is the gas constant and T is 298.15 K. This micro pK_a^{ij} value, partial population of the *i*-th tautomer of the acid species (f_i), and partial population of the *j*-th tautomer of the conjugate base species (f_j') were used to measure the macro pK_a value.[4]

$$pK_a = pK_a^{ij} - \log f_i + \log f_j' \quad (3)$$

The standard Gibbs 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}:

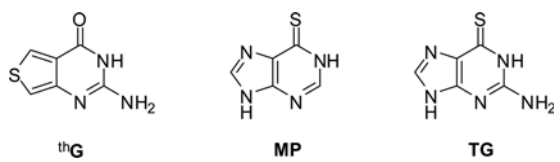
$$\Delta G^0_{aq} = \Delta G^0_g + \Delta G^0_{solv} \quad (4)$$

The standard Gibbs energy of each species in the gas phase, ΔG^0_g, was obtained using the following equation:

$$\Delta G^0_g = E_{0\,\text{K}} + \text{ZPE} + \Delta\Delta G_{0\to 298\,\text{K}} \quad (5)$$

The total energy of the molecule at 0 K ($E_{0\,\text{K}}$) was calculated at the optimal geometry from quantum mechanics (QM). The zero-point energy (ZPE) and Gibbs energy change from 0 to 298 K ($\Delta\Delta G_{0\to 298\,\text{K}}$) were calculated from the vibrational frequencies obtained using QM. The translational and rotational contributions were also calculated according to the ideal gas approximation. The expression $\Delta G^0_g(H^+) = 2.5RT - T\Delta S^0 = 1.48 - 7.76 = -6.28$ kcal/mol was obtained from the literature.[7-9] All QM calculations used Jaguar v5.5 quantum chemistry software. The B3LYP/6-31G** level was used to optimize the geometry and calculate the vibrational frequencies. The 6-31++G** basis set was used for the final geometry optimization based on the 6-31G** geometry.

The solvation energy was calculated according to the con-



thG MP TG

Scheme 1

tinuum solvent model. The solvation energy was given as the sum of the two terms. The electrostatic part of the solvation energy was evaluated using a self-consistent formalism with a numerical solution from the Poisson-Boltzmann (PB) equation. The non-electrostatic contribution was considered using a term that was proportional to the solvent-accessible surface (SAS) area of the solute. The atomic radii used to build this vdW envelope for the solute were taken from a previous study of guanine:[8] 1.880 Å for sp^2-hybridized carbon, 1.410 Å for nitrogen, 1.175 Å for hydrogen attached to the sp^2-hybridized carbon, and 1.080 Å for the other types of hydrogen.

The acid dissociation constants of mercaptopurine (MP) and thioguanine (TG) were calculated to determine the atomic radius for sulfur. As reported previously for selenoguanine,[6] various tautomers of neutral, cationic, and anionic MP and TG were considered, and their relative free energies and relative populations in equilibrium in the gas and aqueous phases were calculated. The optimized atomic radius of sulfur was 2.05 Å. Table 1 lists the acid dissociation constants of the molecules calculated with this parameter.

The pK_a values of thG were calculated using the optimized parameter of sulfur. In Scheme 2, the neutral tautomers of thG were depicted. Table 2 lists their relative free energies and relative populations in equilibrium in the gas and aqueous phases. Tautomer **1** has the lowest energy in the gas phase. On the other hand, both keto-amine forms **1** and **2** showed similar stability in water. The enol and imine forms make little contribution to the population in both phases.

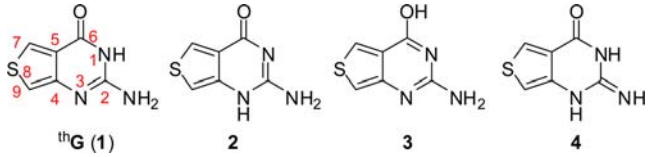
Scheme 3 show the tautomers of anionic ($\mathbf{5}^-$) thG considered in this study. Unlike the other cases considered previously,[6-8,10,11] only one tautomer exists.

Scheme 4 presents the tautomers of cationic ($\mathbf{6}^+$-$\mathbf{8}^+$) thG considered in this study, and Table 3 lists their relative free energies and relative populations in equilibrium in the gas and aqueous phases. Enolic tautomer $\mathbf{8}^+$, which was protonated at O6, was most stable in the gas phase, and the keto-amino form $\mathbf{6}^+$ was most stable in the aqueous phase. This shows that the main protonation site of thG in the aqueous phase is N1 rather than O6.

Table 1. Calculated and experimental acid dissociation constants

pK_a	Experimental[a]	Calculated[b]
pK_{a1} (MP)	7.77	7.27
pK_{a1} (TG)	8.2	8.40
pK_{a2} (MP)	11.17	10.78
pK_{a2} (TG)	11.6	11.71

[a]Reference.[12] [b]Present study

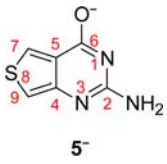


Scheme 2. Tautomers of neutral thG.

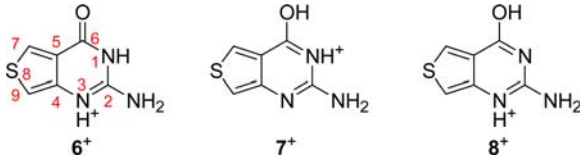
Table 2. Relative free energies (kcal/mol) of the tautomers of neutral thG and their relative Boltzmann populations in equilibrium: (a) Gas phase and (b) aqueous phase

Name	**1**	**2**	**3**	**4**
type	Keto-amine	Keto-amine	Enol-amine	Keto-imine
(a) Gas				
$\Delta G^0_{g,rel}$[a]	0.0	8.3	5.9	6.5
Population	1.0	8×10^{-7}	4×10^{-5}	2×10^{-5}
(b) Aqueous				
$\Delta G^0_{aq,rel}$[b]	0.1	0.0	5.8	5.6
Population	0.45	0.55	3×10^{-5}	4×10^{-5}

[a]Relative free energies with respect to ΔG^0_g(**1**). [b]Relative free energies with respect to ΔG^0_{aq}(**2**)



Scheme 3. Tautomers of anionic thG.



Scheme 4. Tautomers of cationic thG.

Table 3. Relative free energies (kcal/mol) of the tautomers of cationic thG and their relative Boltzmann populations in equilibrium: (a) Gas phase and (b) aqueous phase

Name	$\mathbf{6}^+$	$\mathbf{7}^+$	$\mathbf{8}^+$
type	Keto-amine	Enol-amine	Enol-amine
(a) Gas			
$\Delta G^0_{g,rel}$[a]	2.6	14.8	0.0
Population	0.01	0.0	0.99
(b) Aqueous			
$\Delta G^0_{aq,rel}$[b]	0.0	18.0	8.6
Population	1.0	0.0	5×10^{-7}

[a]Relative free energies with respect to $\Delta G^0_g(\mathbf{7}^+)$. [b]Relative free energies with respect to $\Delta G^0_{aq}(\mathbf{6}^+)$

The macroscopic pK_a values were calculated using Eq. (3). The pK_{a1} and pK_{a2} of thG were found to be 4.84 and 10.73, respectively, which are approximately 1.5 larger than those of guanine. (pK_{a1}: 3.2-3.3 and pK_{a2}: 9.2-9.6)[8] The ratio of deprotonated anionic form of thG relative to the neutral form is smaller compared with guanine. On the other hand, the major contribution under physiological conditions would still be the neutral form for both thG and guanine.

In summary, the relative stabilities of the tautomers of thG were calculated. In the aqueous phase, keto-amino tautomers **1** and **2** showed similar stability in neutral form. The presence of the enolic and imine forms was negligible from the

calculations. The computational result showed that the main protonation site of thG in the aqueous phase is O6 rather than N3. The pK_a in the aqueous phase was calculated from this scheme, which shows that the pK_a is larger than guanine.

Acknowledgments. This study was supported by a 2-Year Grant of Pusan National University.

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