DFT Calculation of Site-specific Acid Dissociation Constants of Purine Nucleobases

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The intrinsic proton affinities of individual basic sites, so-called micro acid dissociation constants, of purine nucleobases in aqueous solution are calculated using an ab initio quantum-mechanical method in combination with the Poisson–Boltzmann continuum solvation model. Calculated micro acid dissociation constants as well as macro acid dissociation constants (pK_a) agree with recent experimental measurements mostly within a pH unit.

Acid dissociation constants (pK_a) and predominant tautomeric forms of nucleobases in aqueous solution were established nearly five decades ago.[1–5] A great deal of effort has also been made to predict their pK_a's and major tautomeric forms from theory. We have used a first-principles quantum mechanics method (density functional theory in combination with the Poisson–Boltzmann continuum solvation model for water) to predict pK_a values and proton configurations for a number of nucleobases.[6–8] The calculation also presents more detailed information such as intrinsic proton affinities of individual basic sites (site-specific acid dissociation constants) and relative populations of various tautomers, which provides valuable clues to understand the biological consequences of base modifications and mutations. However, quantitative confirmation of predicted values has been hampered by lack of experimental data. Recently, Kampf and co-workers[9,10] estimated the micro pK_a values and the relative tautomer populations of 9-methylguanine (9MeGua), 9-methylhypoxanthine (9MeHx), and 9-methyladenine (9MeAde). Now given the experimental data, we calculate their micro pK_a's as well as the overall macro pK_a's.

First the relative free energies and Boltzmann populations of tautomers are calculated in the same way as in our previous studies on purine bases.[6,7] The proton configurations of major tautomers of 9MeGua, 9MeHx, and 9MeAde are shown in Figures 1 and 2. Each name designates where the ring protons are bound. For example, a ring proton is bound to N1 in **1**, and two ring protons are bound to N1 and N7 in **1·7**. No proton is bound to any ring nitrogen atom in **0**. The previous studies have shown that their amino–keto tautomers are significantly more stable than enol and imino tautomers, indicating that the ring nitrogens are more prone to de/protonation than oxo and amino groups in aqueous phase. Based on this result, only the amino–keto tautomers are considered and we only consider de/protonation processes occurring on the ring nitrogens (N1, N3, and N7). This restriction leads us to consider only three tautomers (**1**, **3**, and **7**) for 9MeGua, 9MeHx, and 9MeAdeH$^+$, and three (**1·7**, **1·3**, and **3·7**) for the conjugate acids and one (**0**) for the conjugate bases.

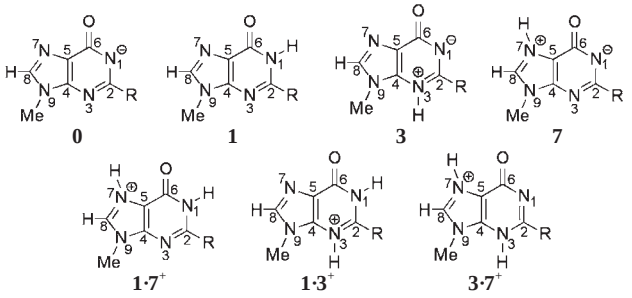

Figure 1. Major tautomers of 9MeGua (R = NH_2) and 9MeHx (R = H).

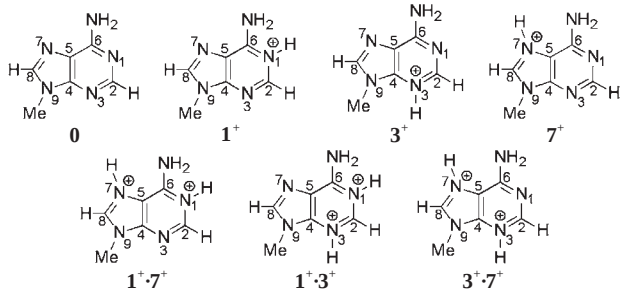

Figure 2. Major tautomers of 9MeAde.

The standard free energy of each tautomer in water is calculated as the sum of the gas-phase free energy and the free energy of solvation in water:

$$\Delta G^0_{aq} = \Delta E_0 + \Delta(\mathrm{ZPE}) + \Delta G_{0\to 298} + \Delta G^0_{solv}. \quad (1)$$

The total energy of the solute at 0 K [ΔE_0] is calculated at the B3LYP/6-31++G** level with the final geometry optimized at the B3LYP/6-31G** level. The zero-point energy [ΔZPE] and the free energy change from 0 to 298 K [$\Delta G_{0\to 298}$] are calculated from the vibration frequencies obtained at the B3LYP/6-31G** level with the ideal gas approximation for translational and rotational free energy contributions. The standard free energy of solvation (ΔG_{solv}) is calculated using the Poisson–Boltzmann continuum solvation approach[11–13] at the B3LYP/6-31++G** level. The atomic radii (in Å) used to build the solute envelope are taken from the previous studies: 1.88 (sp^2 C), 1.79 (sp^3 C), 1.41 (sp^2 N), 1.457 (sp^2 O), 1.175 (H attached to sp^2 C), and 1.08 (other Hs). All calculations use the *Jaguar v4.1* quantum chemistry software (Schrödinger, Portland, OR, USA).

Table 1 lists the relative free energy ($\Delta\Delta G^0$) of each tautomer as well as its partial population (f). Tautomer **1** is predom-

Table 1. Relative free energies (kJ/mol) and populations of tautomers

		Protonation stage 1			Protonation stage 2		
		1	**7**	**3**	**1·7**	**1·3**	**3·7**
9Me-Gua	$\Delta\Delta G^0$	0.0[a]	21.5	28.4	0.0[b]	18.6	30.4
	f	1.0[c]	2×10^{-4}	1×10^{-5}	1.0	5×10^{-4}	5×10^{-6}
9Me-Hx	$\Delta\Delta G^0$	0.0	20.9	26.8	0.0	27.4	34.0
	f	1.0	2×10^{-4}	2×10^{-5}	1.0	2×10^{-5}	1×10^{-6}
9Me-AdeH$^+$	$\Delta\Delta G^0$	0.0	8.4	14.3	0.0	29.1	18.5
	f	0.965	0.003	0.032	1.0	8×10^{-6}	6×10^{-4}

[a]Free energy relative to **1**. [b]Free energy relative to **1·7**. [c]Partial population over all the tautomers at the same protonation stage.

Table 2. Micro pK_a's with the experimental values[9] in parentheses

pK_a^{ij}	9MeGua	9MeHx	9MeAdeH$^+$
1·7 → 1	3.51 (3.11)	2.15 (1.87)	−1.02 (−0.61)
1 → 0	10.72 (9.56)	9.10 (9.21)	3.90 (4.07)
1·7 → 7	7.28 (7.22)	5.81 (6.46)	0.45 (0.50)
7 → 0	6.96 (5.45)	5.44 (4.62)	2.43 (2.96)

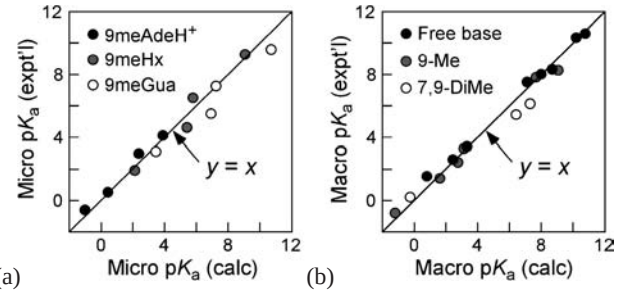

Figure 3. Calculation vs. experiment:[9] (a) micro pK_a and (b) macro pK_a.

inant for 9MeGua, 9MeHx, 9MeAdeH$^+$, and tautomer **1·7** for their conjugate acids. This confirms again that the N1 (**0** → **1**) and then the N7 (**1** → **1·7**) sites are the most basic sites for the nucleobases. The relative population of **1** with respect to the next probable tautomer **7** is calculated to be 60000, 50000, and 30 for 9MeGua, 9MeHx, and 9MeAdeH$^+$, respectively. This agrees quite well with the estimate of Kampf and coworkers (10000, 40000, and 10).[9]

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

$$\Delta G^{0,ij}_{\mathrm{deprot,aq}} = \Delta G^0_{\mathrm{aq}}(\mathrm{A}^-_j) + \Delta G^0_{\mathrm{aq}}(\mathrm{H}^+) - \Delta G^0_{\mathrm{aq}}(\mathrm{HA}_i) \quad (2)$$

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

$$\mathrm{p}K_a^{ij} = \Delta G^{0,ij}_{\mathrm{deprot,aq}}/2.303RT, \quad (3)$$

where R is the gas constant, T is 298.15 K, and the standard free energy of the proton in water is chosen as −1129 kJ/mol from the previous studies. From the micro pK_a^{ij} value, the partial population of the i-th tautomer over all the acid species (f_i) and the partial population of the j-th tautomer over all the conjugate base species (f'_j), the macro pK_a value is finally estimated as

$$\mathrm{p}K_a = \mathrm{p}K_a^{ij} - \log f_i + \log f'_j. \quad (4)$$

The micro pK_a values corresponding to the deprotonation processes of (**1·7** → **1**), (**1·7** → **7**), (**1** → **0**), and (**7** → **0**) are shown in Table 2 and Figure 3a. The calculation agrees well with the measurement of Kampf and co-workers[9] within root-mean-square (RMS) error of 0.67-pH units. The macro pK_a values of 9MeGua, 9MeHx, and 9MeAde are shown in Table 3 and Figure 3b. Those of nonsubstituted free bases (Gua, Hx, and Ade) and 7,9-dimethyl bases (79DiMeGua, 79DiMeHx, and 79DiMeAde) are also shown (Detailed calculations not shown; See Ref. 9 for guanine). The calculated values show good correlation with experimental data[1–5,9] within RMS error of 0.61-pH unit. Only 79DiMeGua and 79DiMeHx show a mismatch larger than a pH unit. 7-Methylguanosine, the nucleoside analog of 79DiMeGua, is prone to the scission of the imidazole ring in neutral or alkaline solution.[4,14,15] The same reaction is expected for 7,9-dimethyl purine nucleobases in high-pH solution. Since the problematic pK_{a1} of 79DiMeGua and 79DiMeHx fall in the range of pH 7–9, a ring-opening reaction might happen during the titration and this effect is not considered in our calculation.

Table 3. Macro pK_a's with the experimental values[1–5,9] in parentheses

	Gua (ref 6)	Hx	AdeH$^+$
pK_{a1}	3.15 (3.2–3.3)	1.22 (2.0)	−1.54 (< 1)
pK_{a2}	9.44 (9.2–9.6)	8.47 (8.8–8.9)	4.16 (4.1–4.2)[c]
pK_{a3}	12.61 (12.3–12.4)	12.00 (12.0–12.1)	10.24 (9.8)
	9MeGua	9MeHx	9MeAdeH$^+$
pK_{a1}	3.51 (2.9, 3.11)	2.15 (1.87)	−1.04 (−0.64)
pK_{a2}	10.72 (9.9, 9.56)	9.10 (9.21)	3.92 (3.9, 4.10)
	79DiMeGua	79DiMeHx	79DiMeAdeH$^+$
pK_{a1}	8.70 (7.22)	7.65 (6.46)	0.01 (0.50)

Summarizing, we developed a first principles scheme to calculate the micro pK_a values of individual protonation sites of purine nucleobases as well as the overall macro pK_a values and confirmed the applicability of this scheme by successfully reproducing recent experimental estimates.

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