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Theoretical study of tautomeric equilibria of 2,6-diamino-8-azapurine and 8-aza-iso-Guanine

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ABSTRACT

Tautomeric equilibria of amino forms of 2,6-diamino-8-azapurine and 8-aza-iso-Guanine is revealed by DFT computations. The most populated tautomer of 2,6-diamino-8-azapurine, in agreement with available experimental data, is protonated at position N(9). The lowest free energy tautomer of 8-aza-iso-Guanine is protonated at positions N(3) and N(8). For biologically more important tautomer N(9)-H, probability of N(3) protonation is higher than N(1). This result, observed also for iso-Guanine, shows reversed probability of protonation at positions N(3) and N(1), compared to Guanine and decreased specificity of pairing of 8-aza-iso-Guanine with iso-Cytosine should be expected. Computed electronic excitation energies well match available experimental data.

1. Introduction

The tautomeric equilibrium of nucleobases is one of key factors responsible for correct base–base recognition in nucleic acids [1] and its disturbance has a large impact on biological processes as it can lead to genome mutations [2]. The tautomerization process of purines and nucleobases has been extensively studied by both theoretical and experimental methods [3–11]. Development of the expanded genetic code raised natural question about tautomeric equilibrium of its new letters – iso-Guanine and iso-Cytosine [12].

Recently synthesized derivative of iso-Guanine (isoGua) called aza-iso-Guanine (z8isoGua) was recognized as a promising fluorescent probe [13], which can be used for studies of important biological processes. The replacement of C8 carbon of isoGua does not directly influence both Watson–Crick and Hoogsteen interfaces, although it can possibly change tautomeric equilibrium of the new compound compared to unmodified isoGua.

In this letter results of quantum chemistry methods applied to various tautomeric forms of z8isoGua are presented. The picture of tautomeric equilibrium of this fluorescent probe is revealed. The results are compared to those obtained for natural Guanine and isoGua. The method applied to z8isoGua was also tested on 2,6-diamino-8-azapurine (DaaPur) molecule. The reliability of our methodology was checked by computation of electronic excitation energies and their comparison with available experimental data [13–15].

2. Methods

Quantum chemistry methods have been successfully applied to the description of tautomerization phenomena in small molecules [16–18]. It was shown previously [16,19–23], that density functional theory (DFT) with B3LYP functional and 6-311+G (d,p) basis set [24–27] is sufficient for correct description of tautomerization phenomena and results generated by DFT method are close to those obtained with the higher level methods like MP2, MP4 and CCSD. Although chemical compounds studied in publications above do not include keto-enol tautomerism, characteristic for z8isoGua molecule. Therefore, only preliminary calculations in both the gas phase and water (PCM method [28,29]) were performed at the DFT/B3LYP method and 6-311+G (d,p) basis set level of theory with GAMESS package [30,31]. These calculations served as the preliminary sieve and structures of the lowest energy tautomers were also optimized with higher level DFT-BHandHLYP method [32] combined with cc-pVTZ basis set [33], the method/basis-set combination, which was successfully applied to benzoderivatives of nucleic acid bases [34]. Geometries of the selected tautomers were optimized in neutral form and ground state and the vibrational analysis of each tautomer was carried out. The latter step

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served two purposes. First, vibrational analysis proved that all structures reached energy minima in the optimization process. Second, the output of vibrational analysis contains information about thermochemistry of the investigated system. The optimization of water-solvated systems was speed up by using final structures from the optimization in vacuum as initial structures for optimization in solvent.

The following thermodynamic parameters were obtained from thermochemistry calculations: energy (E – including electronic energy and zero point energy), enthalpy ($H = E + k_BT$), entropy (S) and Gibbs free energy ($G = H - TS$). The tautomerization preference was determined by calculation of the relative thermodynamic parameters (ΔE, ΔH, ΔG, $T\Delta S$) with respect to the lowest-energy tautomer. The relative free energy ΔG depends on electronic energy, zero point energy, thermal corrections to energy and entropy. The solvation free energy $\Delta\Delta G$ was calculated according to the equation:

$$\Delta\Delta G = \Delta G_{water} - \Delta G_{gas} \quad (1)$$

where ΔG_{water} and ΔG_{gas} are relative free energies of selected tautomer in water and gas phase, respectively. Furthermore, tautomeric equilibrium constants ($pK = \Delta G/2.303RT$) and percentage contents of individual tautomer ($x = K/(1 + K)$) were computed.

The validity of our theoretical prediction was checked by comparison of the energies of electronic excitation with available positions of absorption peaks. For this purpose first three singlet electronic excited states (vertical states) were computed using time-dependent DFT [35] and BHandHLYP/cc-pVTZ functional/basis-set methodology. All excited-state calculations were performed for ground-state-optimized geometries. The computed excitation energies were scaled by factors specific for each tautomer in the same manner as described by Shukla et al. [36–38]. Scaling factors were set to match excitation energies of the experimentally determined and computed first singlet exited states (S1). Then agreement between scaled excitation energies of third singlet excited state (S3) and experimentally determined one was checked.

Various tautomers of DaaPur and z8isoGua molecules considered in this publication are shown in Figures 1 and 2, respectively. In both cases only tautomers with amino groups were considered. It was shown that tautomers with amino groups are dominant for both adenine [16] and isoGua [17], so imino tautomers are presumably less important for DaaPur and z8isoGua molecules, also. Nevertheless, calculations of imino and charged tautomers are in progress in our laboratory.

Table 1
Relative thermodynamic parameters (kcal/mol) for DaaPur tautomers in the gas phase. For the assignment of symbols see Figure 1. The relative energies shown in parenthesis were computed with BHandHLYP/pVTZ level of theory.

Tautomer	ΔE	ΔH	ΔG	$T\Delta S$
D1	28.26	28.26	28.49	−0.24
D3	15.13	15.13	15.40	−0.55
D7	10.02	10.02	10.47	−0.45
D8	3.86 (5.13)	3.86 (5.13)	4.04 (5.16)	−0.18 (−0.18)

Positive values are related to less preferred tautomers than reference D9 tautomer. Thermochemistry was calculated at 298.15 K.

3. Results and discussion

3.1. DaaPur tautomeric system

The neutral amino form of DaaPur molecule is the simpler of two investigated tautomeric systems, as it has only one tautomeric proton, which can occupy one of five available positions as shown in Figure 1. The relative thermodynamic parameters such as: energy (ΔE), enthalpy (ΔH), free energy (ΔG), parameter related to entropy ($T\Delta S$) and free energy of solvation ($\Delta\Delta G$) of DaaPur molecule in gas phase and in water are collected in Tables 1 and 2, respectively.

Gas phase. The D9 tautomer is the most stable one and its free energy is around 4 kcal/mol lower than D8 – the second lowest energy tautomer. The population of various tautomers, shown in Table S1, seems to be closely related to the distance between tautomeric proton and two amino groups of DaaPur molecule. This observations are supported especially by a very large ΔG value obtained for D1 tautomer, in which proton is located between two amino groups of DaaPur molecule. Higher-level calculations performed for two lowest energy tautomers (D8 and D9) do not change their positions as D9 tautomer is still clearly dominant (see Table 1) and the energy gap between two lowest-energy tautomers increased by 1 kcal/mol.

Solvent effect. Immersion of DaaPur molecule in water significantly decreases the free energy difference between lowest (D9) and highest (D1) free energy tautomers, which dropped from 28.49 to 11.84 kcal/mol. D3 and D7 tautomers remain in the middle of the energy scale, although, because of a very strong solvation effect of D3 ($\Delta\Delta G = -10.33$ kcal/mol), they exchanged positions. It should be stressed here that, in agreement with the experimental data [14], D9 remains the lowest energy tautomer in water. As in the case of vacuum calculations, application of the higher-level method left

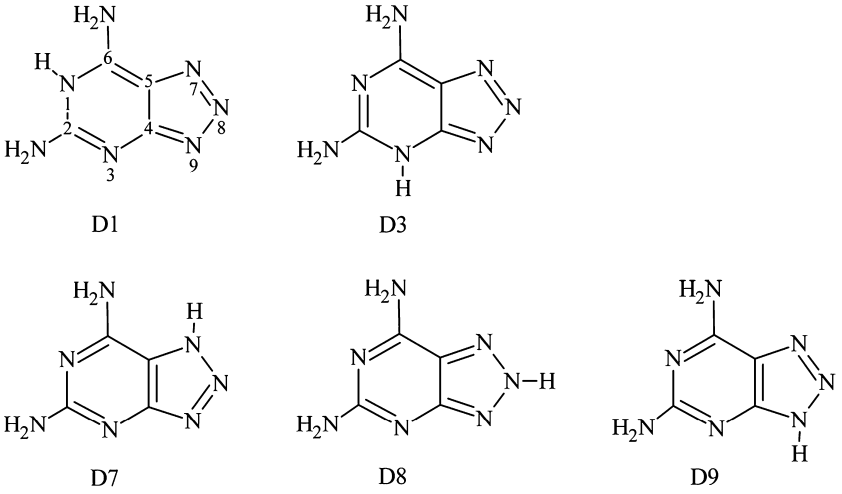

Fig. 1. DaaPur tautomeric structure. In contrast to z8isoGua, only one tautomeric proton is located on the entire structure.