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Single- and Double-Proton-Transfer in the Aggregate between Cytosine and Guaninediazonium Ion†,§

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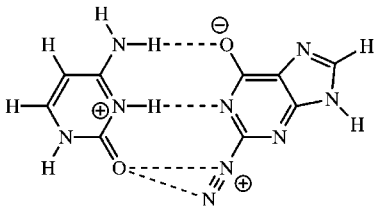
ABSTRACT



The structure of "guaninediazonium ion" in its aggregate with cytosine has been explored with ab initio and density functional methods. The hydrogen-bonded aggregate between cytosine and guaninediazonium ion, 1, is a stable minimum, 3. While the isolated enol tautomer of guaninediazonium ion, 2, is significantly more stable than 1, the tautomeric aggregate 4 that results from double-proton-transfer in 3 is almost isoenergetic with 3. Most interesting and entirely unexpected is the finding that neither 3 nor 4 is predicted to be the thermodynamically predominant structure. Instead, single-proton-transfer to cytosine results in the most stable cytosinium–guaninediazo complex, 5.

A variety of disorders in people are likely to result from DNA base deamination and interstrand cross-linking due to reaction with HNO_2 or NO.[1,2] It had been generally assumed that the nitrosation of guanine leads to the formation of the guaninediazonium ion which would then react with water to give xanthine. Alternatively, the guaninediazonium ion might form cross-links such as dG-to-dG and dG-to-dA by attack of an amino group of a neighboring nucleoside on the guaninediazonium ion.[3] The assumption of guaninediazonium ions as the reactive intermediate has been generally accepted, and this hypothesis has been employed in the QM-MM studies[4] of DNA sequence effects on cross-linking.

The mechanistic hypotheses invoking guaninediazonium ions as the reactive species in guanosine deamination and cross-linking are based on product analyses and deduction and analogy to the chemistry of aromatic primary amines. Yet, guaninediazonium ion has never been observed directly. The proposed mechanisms for the reaction of water with guaninediazonium ion *all* result in the replacement of the $-N_2^+$ function by the −OH group, followed by tautomerization to xanthine, and they differ in the timing of the elemental steps. We have carried out ab initio studies of diazonium ions of the DNA base pairs and examined their thermodynamic and kinetic stabilities with regard to loss of

† Part 3 in the series Theoretical Studies of DNA Base Deamination. For parts 1 and 2, see ref 5.

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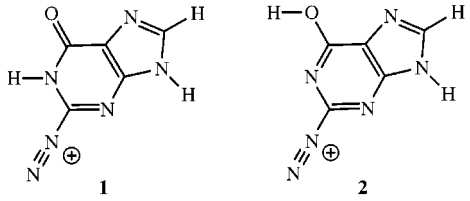
N_2.[5] The free guaninediazonium ion was found to be kinetically and thermodynamically unstable toward dediazoniation with concomitant pyrimidine ring opening.[5] These theoretical results suggested a mechanism for nitrosative guanine deamination that also accounts for the recently discovered formation of more than 20% of 2′-deoxyoxanosine in the nitrosations of 2′-deoxyguanosine, oligodeoxynucleotide, and calf thymus.[6]

In the course of our studies of guaninediazonium ion, **1**, we also considered its enol tautomer **2** (Scheme 1 and Figure

Scheme 1



1). Surprisingly, at the RHF/6-31G* level we found **2** to be more than 20 kcal/mol *more* stable than **1** (Table 1). Even though **2** is more stable than **1**, the kinetic barrier to dediazoniation of **1** is much less than in the case of **2**.[5b] For the isolated ion, it is therefore the dediazoniation of **1** that is pertinent. Yet, in the anisotropic environment of DNA it might not be warranted to assume that the relative stabilities of the free ions persist, nor is it warranted to assume that the dediazoniation will emanate necessarily from the same species. With the present paper we begin to address these questions related to the chemical effects of the anisotropic environment and we consider the effects exerted by the presence of a single cytosine.

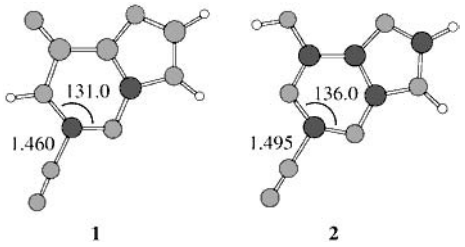


Figure 1. The RHF/6-31G* optimized structures of guaninediazonium ion (**1**) and its enol tautomer (**2**).

The RHF/6-31G* optimized structures of **1** and **2** are displayed in Figure 1. Guaninediazonium ion is perfectly set

Table 1. Relative Isomer Stabilities As Computed at the RHF/6-31G* and the B3LYP/6-31G*//RHF/6-31G* Levels[a]

compd	RHF ΔE_0	RHF ΔE_0 + ZPE[b]	RHF ΔG_{298}[c]	B3LYP ΔE_0	B3LYP ΔE_0 + ZPE	B3LYP ΔG_{298}
1	0.00	0.00	0.00	0.00	0.00	0.00
2	−22.51	−22.15	−22.14	−15.27	−14.91	−14.90
3	0.00	0.00	0.00	0.00	0.00	0.00
4	−3.22	−3.04	−2.92	−0.19	0.00	0.11
5	−13.04	−13.33	−13.20	−8.95	−9.23	−9.10

[a] Relative energies in kilocalories per mole relative to **1** for the free guanine derivative and relative to **3** in the case of the aggregate. [b] Vibrational zero-point energies were computed at the RHF/6-31G* level, and they were not scaled. [c] The free enthalpy corrections include corrections for all thermal contributions to the partitioning function.

up to engage in two hydrogen bonds with cytosine to form the aggregate **3** (Scheme 2). The third strong hydrogen bond present in the CG base pair, between the C's carbonyl and the G's amino group, has been replaced by a bridging interaction between the carbonyl and the diazonium function. Aggregate **4** can be seen as the result of double-proton-transfer in the two hydrogen bonds of **3**. The equilibration between isomers **3** and **4** does not need to involve a single step. Instead, the two hydrogen shifts might occur successively and of the two stepwise options only the path via **5** is reasonable. The alternative stepwise option would involve protonation of a cation.

We have studied the structures and stabilities of the aggregates **3**−**5** with ab initio methods at the restricted Hartree−Fock level (RHF).[7] All molecules involved are highly polar, and their adequate functional description requires employment of a split-valence basis set in conjunction with sets of polarization functions on all heavy atoms. The basis set 6-31G* satisfies these needs, and structure optimizations and vibrational analyses were performed at the RHF/6-31G* level. The vibrational analyses, while computationally demanding and expensive, were deemed necessary for several reasons. First, the structure optimizations were performed assuming planarity. Amino groups in DNA[8] always have the potential to pyramidalize, and the assumption of planarity therefore requires validation. All vibrational analyses returned only real frequencies, thereby establishing the C_s symmetry of **3**−**5**. Second, the aggregates exhibit several "soft modes", and these low-frequency vibrations greatly matter for binding energies. The large effects of thermal motion nearly cancel for the relative energies pertinent to the present discussion as is shown in Table 1. At the Hartree−Fock level, one neglects large amounts of

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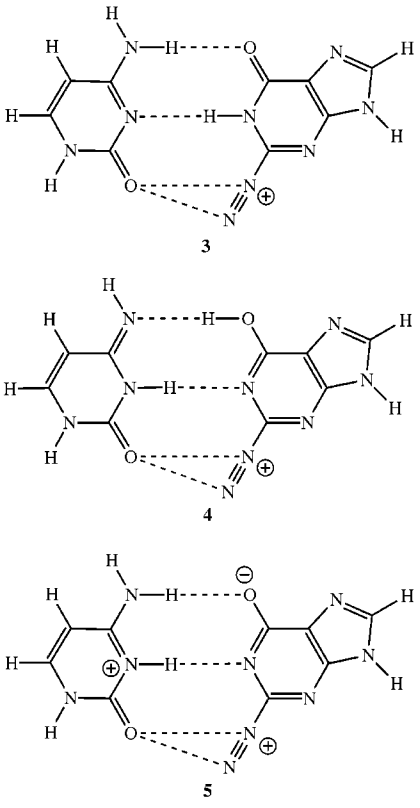
(7) (a) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; John Wiley & Sons: New York, 1986. (b) Computations were carried out with Gaussian94 on a Silicon Graphics PowerChallenge L system.

(8) Sponer, J.; Hobza, P. *Int. J. Quantum Chem.* **1996**, *57*, 959, and references cited in this review.

electron correlation. This approximation often is justified so long as the numbers and types of bonds are the same among the entities being compared. We are interested in tautomers, and there are significant changes in bonding associated with the single- and double-proton-transfers in **3**−**5**. Density functional theory has proven to be a cost-efficient and accurate way to account for parts of the electron correlation effects in a semiempirical fashion.[9] The B3LYP functional was used, which combines Becke's three-parameter exchange functional[10] with the correlation functional of Lee, Yang, and Parr.[11] These are both nonlocal functionals whose combination is widely used and accepted. The density functional calculations employed the 6-31G* basis set and were carried out with the RHF/6-31G* optimized structures. This level of theory is referred to as B3LYP/6-31G*//RHF/6-31G*.

Scheme 2



The structure of **3** exhibits an interesting distortion from ideal linear hydrogen bonds.[12] In **3**, **1** appears shifted relative to the cytosine in a perpendicular fashion relative to the direction of the hydrogen bonds in a way that results in short contacts between the cytosine carbonyl-O and the N_2 fragment. We attribute this distortion to strong 1,3-attractive interactions associated with the incipient nucleophilic attack of the carbonyl-O on the diazonium function.[13]

The structure of the aggregate **4** also indicates a strong interaction between the carbonyl group of the cytosine tautomer and the diazonium function of the enol tautomer **2** of guaninediazonium ion. The lengths of the contacts suggest, however, that this interaction is weaker in **4** compared to that in **3**. The geometry of **4** is such that the two hydrogen bonds are essentially linear. The effects of the cytosine aggregation on the isomer stabilities of **1** and **2** are stunning: The large preference for the free aromatic ion **2** over the amide **1** all but disappears in the aggregate! The free enthalpy preference of 14.9 kcal/mol for **2** is reduced to essentially zero in the aggregate (Table 1).

Most interesting and entirely unexpected is the finding that neither **3** nor **4** is predicted to be the thermodynamically predominant structure. Instead, single-proton-transfer from **1** or **2** to cytosine results in the most stable cytosinium−diazoguanine complex **5** (Figure 2). At the level B3LYP/6-31G*//RHF/6-31G*, the aggregate **5** shows a free enthalpy preference of 9.1 kcal/mol with respect to **3**. The proton transfers are fast processes with activation barriers of $\Delta E_A(\mathbf{3}\rightarrow\mathbf{5}) = 5.7$ and $\Delta E_A(\mathbf{4}\rightarrow\mathbf{5}) = 2.2$ kcal/mol and show that the chemical formation of the aggregates **3** or **4** will be

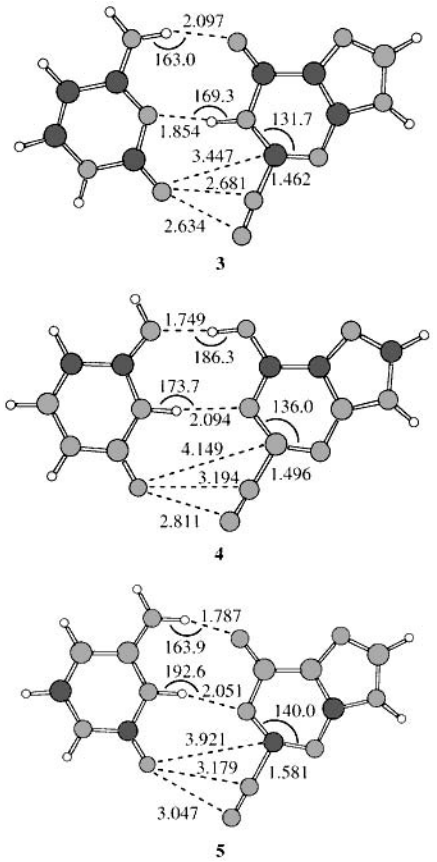


Figure 2. The RHF/6-31G* optimized structures for cytosine−guaninediazonium ion (**3**), the tautomer derived from double-proton-transfer (**4**), and the complex derived from single-proton-transfer (**5**).

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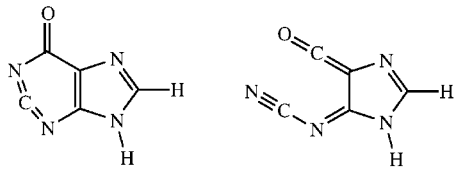
followed by a fast and quantitative conversion into aggregate **5**.

The description of the diazoguanine **5** requires zwitterionic Lewis structures, such as the one shown in Scheme 2, and one can write resonance forms that place negative charge on both of the heteroatoms that are engaged in hydrogen bonding to the cytosinium ion. The structure of **5** is distorted in a fashion that is reminiscent of that in **3**, but the distortion occurs for a different reason. The distortion in **5** likely occurs to allow for some bifurcation of the hydrogen bonding.

Current studies of the diazoguanine moiety in **5** show that the dediazoniation is facile[14] and the guanine derivative **6** easily undergoes ring opening to **7** (Scheme 3). With the recognition that the pyrimidine ring-opened product of the dediazoniation of **1** is identical to protonated **7** comes the realization that our previous mechanistic discussion fully applies to the aggregate.

Scheme 3



6 **7**

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(14) The loss of dinitrogen occurs spontaneously for the free diazoguanine and with only a small kinetic barrier in the presence of the cytosinium ion. To be published.