Pyrimidine Ring Opening in the Unimolecular Dediazoniation of Guanine Diazonium Ion. An Ab Initio Theoretical Study of the Mechanism of Nitrosative Guanosine Deamination

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DNA base deamination and interstrand cross-linking due to reaction with HNO₂ or NO has been linked to a variety of disorders in people.¹ Nitric oxide deaminates nucleosides, nucleotides, and DNA in vitro, and similar DNA damage also occurs in vivo.² The amino group of adenine can be eliminated via diazotization reactions,³ and deamination of cytosine to uracil is a well-known mutagenic event.⁴,⁵ The deamination of guanine with HNO₂ leads to xanthine formation (Scheme 1) or cross-linking⁶ to a proximate guanine or adenine.⁷

The deaminations of the DNA bases are thought to involve diazonium ions as the crucial reactive intermediate, and the mechanistic hypotheses are based on product analyses and their rationalization in analogy to the chemistry of aromatic primary amines.⁸ In contrast to aniline, however, the diazonium ions of the DNA bases have never been observed directly, and their properties, stabilities, and reactivities are not known. The mechanistic hypotheses for the reaction of water with the guanine diazonium ion GN₂⁺ (1) are outlined in Scheme 1. The three principle mechanisms previously discussed all result in the replacement of the N₂⁺ function by the OH group, followed by tautomerization to xanthine, and they differ in the timing of N₂ elimination and hydroxyl group addition (+H₂O/H⁻⁺). If H₂O attacks the C-atom to which the diazonio function is attached, nucleophilic aromatic substitution occurs with N₂ loss and formation of xanthine in a uni-(S₉Ar1) or bimolecular (S₉Ar2) fashion. Nitroguanine is a known side product, and its formation is indicative of an S₉1 type process. Alternatively, a nucleophile may add to N₂ and the diazene may undergo N₂ expulsion. In cross-link formations, it is thought that the amino group of another DNA base serves as the nucleophile, and the Shapiro mechanism is consistent with the Verly kinetic data.¹⁰ The cross-linking was studied with oligodeoxynucleotide duplexes,¹¹a and the observed sequence preferences were rationalized by proximity effects involving the diazonium ion¹¹a and corroborated by theoretical study.¹¹b On this background, Makino et al.¹² have discovered that in excess of 20% of 2°-deoxoxyosanosine was formed in the nitrosations of 2°-deoxyguanosine, oligodeoxynucleotide, and calf thymus. No currently accepted mechanism for guanine deamination accounts for the oxanose product, and no postulates have been advanced. Here, we report the results of an ab initio study of the unimolecular dediazoniation of 1 (Figure 1) that explains the experimental findings by Makino et al.¹² and provides a mechanistic hypothesis for future experimental investigations.

Structure optimizations and vibrational analyses were carried out at the RHF/6-31G* level, and electron correlation effects were approximated with third-order Möller–Plesset perturbation theory in the frozen core approximation and with the RHF/6-31G* structures (Table 1). Systematic studies of theoretical model dependencies of RN₂⁺ (R = H,¹³b Me,¹³c Et¹³a, Ph¹³d) show excellent agreement between experiment and theory at this theoretical level [MP3(fc)/6-31G*/RHF/6-31G*+1.0DZPPE-] and...
The binding energy of the benzenediazonium ion PhN₂⁺ is the pertinent reference, and it is the reaction energy for dissociation to form singlet phenyl cation and N₂ which is the lowest energy path for dissociation. We determined E₂(PhN₂⁺) = 26.6 kcal/mol[13] at the well-correlated level QCISD(T,fc)/6-31G**(full)/6-31G*, and this value is in excellent agreement with experimental dissociation energies (25.8–28.3 kcal/mol).[16] The reaction enthalpy of 31.6 kcal/mol for the process 1 → 2a + N₂ would suggest that 1 is more stable toward N₂ loss than PhN₂⁺. However, since the unimolecular dissociation leads to 2b instead of 2a, ion 1 is thermodynamically unstable by ~30.4 kcal/mol with regard to the reaction channel 1 → 2b + N₂ and this diazoniation is kinetically hindered by only about 10 kcal/mol. The stability of 1 toward diazoniation is not measured by the binding energy but rather by this kinetic barrier. The stabilities of the DNA base diazonium ions toward unimolecular diazoniation follow the order CyN₂⁺ (3.7 kcal/mol) < AdN₂⁺ (9.0) ≈ GuN₂⁺ (<10) ≈ PhN₂⁺ (26.6). According to comparative kinetic analysis of nitrous acid deaminations,[16] the reactivities followed the order guanosine > adenosine > cytidine and parallel (with few exceptions) the reactivities observed in intact nucleic acids or whole viruses. In light of the computed stabilities of the diazonium ions, it appears that these reactivities are not determined by diazonium ion stabilities but more likely reflect the rates of their formations. Our calculations suggest that only about 10 kcal/mol are required to elongate the CN₂ linkage to such a degree that C6—N1 amide bond cleavage occurs. Isotropic and anisotropic effects of the environment will have to be included in future refinements of the model, but it is very likely that the qualitative essentials will persist. This finding provides a consistent explanation for the formations of all observed products. Hydrolysis of 2b to form the carboxylic acid 3 should be facile and strongly suggests intramolecular addition of the acid onto the carbodiimide as the most likely route to oxanosine. Two-fold hydrolysis of 2b to 4 followed by amide formation constitutes a reaction channel for xanthine formation via 2b and a second path for oxanosine.[17] The formations of xanthine via S₈1 or S₈2 type reactions at C₈₅₀ should by no means be considered exclusively, and xanthine might well arise from 2b. Such condensation reactions are preceded and may occur under the reaction conditions used in the diazotizations. The direct S₈2 type replacement of the N₂ group by weak nucleophiles (H₂O, NO₂⁻) as well as the intermediacy of 2a both seem unlikely in light of the results presented. Instead, our results suggest that experimental and theoretical studies of guanine deamination should focus on investigations of the reactivity of 2b. Preliminary results of electronic structure analyses indicate that 2b is well described as a ketene—carbodiimide-substituted tertiary (C₄-centered) carbocation and that this description is superior to the acylium ion resonance form. We are currently addressing regiochemical issues of the reactivity of 2b.

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Supporting Information Available: Details of computations and results of electron density analysis of 2b (6 pages). See any current masthead page for ordering and Internet access instructions.

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Table 1. Binding, Relative, and Vibrational Zero-Point Energies

<table>
<thead>
<tr>
<th></th>
<th>VZPE</th>
<th>Eₐ(ROHF)</th>
<th>Eₐ(MP2)</th>
<th>Eₐ(MP3)</th>
<th>AVZPE</th>
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<tr>
<td>1</td>
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<td>18.11</td>
<td>52.80</td>
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<td>2a</td>
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<td>2b</td>
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<td>−75.76</td>
<td>−63.06</td>
<td>1.18</td>
</tr>
<tr>
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<td>−1.18</td>
<td>−0.08</td>
<td>−0.43</td>
<td>−0.02</td>
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<tr>
<td>N₂</td>
<td>3.94</td>
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</table>

*All data based on RHF/6-31G* structures. Vibrational zero-point energies (VZPE, not scaled), VZPE corrections (AVZPE, scaled, factor 0.9), and relative energies are in kilocalories per mole. Eₐ(calc) = Eₑ(calc) + AVZPE. Relative to 2a + N₂. **Relative to 2a. ***Relative to 2b.

Figure 2. Potential energy diagrams for the unimolecular diazoniation of 1 as a function of the CN bond length.

(RHF/6-31G*]). The linear unimolecular diazoniation path was examined, and several structures of 1 with fixed CN bond lengths were optimized. Molecular models of the stationary structures are shown in Figure 1, and cross sections of the potential energy surface (PES) as a function of rCN are depicted in Figure 2. The PES cross section of 1 provided a surprise: For structures with CN bond lengths greater than 2.2 Å, cleavage of the C₆—N₁ bond occurred (Figure 2). The cation 2a—the expected product of heterolytic CN bond cleavage—does exist as a stable structure on the potential energy surface. However, 2a is not formed in the unimolecular diazoniation! Instead, the N₂ elimination is correlated with amide bond cleavage and leads to the pyrimidine-ring-opened cation 2b. All attempts to find a reaction channel connecting 1 directly to 2a failed. Structure 2b (Figure 1) was optimized and is preferred over 2a by 63.0 kcal/mol. The rotational isomer 2b' is nearly isoenergetic. One might expect an allene type structure for the carbodiimide moiety of 2b as for HN=C=NH itself,[14] but the planar structures 2b and 2b' are indeed minima. The N₁—C₆ inversion barrier of carbodiimide is known to be rather low.[15]