Nucleobases

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Specific Generation of 1-Methylcytosine Radicals in the Gas Phase**

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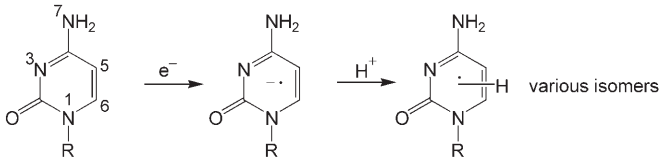
Nucleobase-derived radicals are crucial transient intermediates in the radiation damage of nucleic acids (RNA and DNA). Among the recognized mechanisms of radiation damage, capture of a low-energy electron followed by protonation of the transient anion radical results in the formation of hydrogen-atom adducts that can undergo further degradation reactions.[1] Radiation damage has been studied extensively by pulse radiolysis in solution and the solid state, in which characterization of the transient nucleobase radicals relied mainly on the detection of species with unpaired electrons by electron paramagnetic resonance (EPR) spectroscopy. In particular, cytosine and cytosine-containing nucleosides have been the subject of numerous studies that reported the formation of adducts with the hydrogen atom positioned at C5, C6, N3, and N7 in the solid state (Scheme 1).[2] However, the identity of these radicals has



R = H, CH_3, or deoxyribose
1: R = CH_3

Scheme 1. Formation of cytosine radicals by the direct mechanism.

been subject to discussion, and the factors that affect their formation and kinetic and thermodynamic stability have not been established unequivocally by EPR in the complex mixtures produced by pulse radiolysis. EPR analysis can be further complicated by the formation of cation radicals upon

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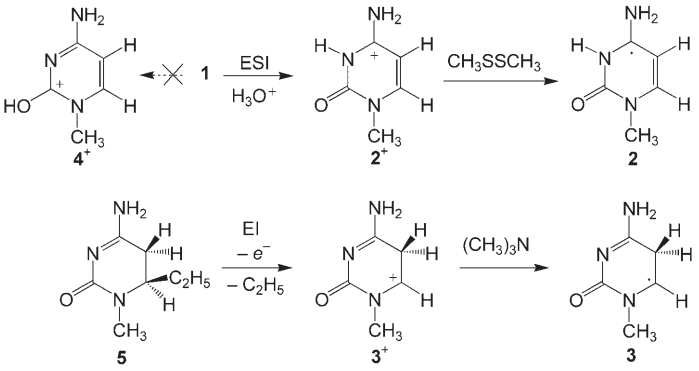
radiolysis.[2f,g] To our knowledge, the chemically specific generation of cytosine radicals has not been attempted.

In contrast to the condensed phase, the rarefied gas phase, which exists in the vacuum system of a mass spectrometer, offers an inert medium for the specific generation and investigation of highly reactive transient molecules, radicals, biradicals, and so forth.[3] We report herein on the targeted generation of two key hydrogen-atom adducts of 1-methylcytosine (**1**), namely, 1-methyl-3,4-dihydrocytosine-4-yl (**2**) and 1-methyl-5,6-dihydrocytosine-6-yl (**3**; Scheme 2). 1-Methylcytosine was selected as a simple model system that exists as a dominant tautomer and, thus, avoids the problems of gas-phase tautomerism that are present in the parent cytosine, which exists as a mixture of three major tautomers in the gas phase.[4] Our approach relies on the specific generation of 1-methylcytosine cations that have the same bond connectivity as the target radicals. The cations are accelerated to a high velocity (105 000 $m\,s^{-1}$) and discharged by a glancing collision with a molecular electron donor (dimethyl disulfide or trimethylamine). The nascent radicals are formed with the structure of the precursor cation because of the extremely short duration of the electron transfer (10–12 fs). The radicals are observed for several microseconds, during which time dissociation may occur. The neutral precursors and their dissociation products are then nonselectively ionized, the resulting cations are separated by mass and detected by neutralization–reionization (NR) mass spectrometry. The spectra, thus, provide a more-or-less complete analysis of all the reaction products that originate from the nucleobase radical.[5]



Scheme 2. Generation of 1-methylcytosine radicals **2** and **3** by collisional electron transfer in the gas phase.

The synthetic route to **2** utilizes electrospray ionization (ESI) of **1** to generate the pertinent ion $\mathbf{2}^+$ and transfer it in the mass spectrometer. Compound **1** is the most stable tautomer in solution[6] and is protonated exclusively at N3, thus specifically yielding $\mathbf{2}^+$.[7] Our concurrent ab initio calculations[8] have established that $\mathbf{2}^+$ is more stable (by 31–32 $kJ\,mol^{-1}$) than the tautomer protonated at O2 ($\mathbf{4}^+$), which is the second most stable in water and methanol. Tautomer $\mathbf{2}^+$ is also the most stable structure in gas-phase clusters with water, thus strongly indicating that $\mathbf{2}^+$ is transferred from solution to the gas-phase by electrospray without isomerization. Unimolecular isomerization $\mathbf{2}^+ \rightarrow \mathbf{4}^+$ requires a high-energy barrier (153 $kJ\,mol^{-1}$)[8] that is inaccessible to thermalized ions formed by electrospray ionization.[9]

Tautomer $\mathbf{3}^+$ is substantially less stable than $\mathbf{2}^+$ ($\Delta H_{g,0}(\mathbf{2}^+ \rightarrow \mathbf{3}^+) = 111\ kJ\,mol^{-1}$) and cannot be generated specifically by protonation of **1**.[8] However, electron-ionization-induced α-cleavage dissociation of a stable precursor, 1-methyl-6-ethyl-5,6-dihydrocytosine (**5**),[10] yields $\mathbf{3}^+$ as the most abundant ion in the mass spectrum and provides access to the generation of radical **3**.

Ions $\mathbf{2}^+$ and $\mathbf{3}^+$ were characterized by collisionally activated dissociation (CAD) mass spectra that showed characteristic differences in ion-dissociation patterns (Figure 1). Ion $\mathbf{2}^+$ dissociates by loss of NH_3 (m/z 109) and HO–C≡N (m/z 83), whereas $\mathbf{3}^+$ dissociates by loss of CH_2=C=NH (m/z 85) and HN=C=O (m/z 83). The identity of the fragments and the dissociation mechanisms have been established by deuterium labeling and ab initio calculations.[8] The important outcome from the CAD spectra and theoretical calculations is that $\mathbf{2}^+$ and $\mathbf{3}^+$ are distinct stable isomers that reside in potential-energy minima and do not interconvert under our experimental conditions.

Collisional neutralization of $\mathbf{2}^+$ and $\mathbf{3}^+$ followed by reionization yielded NR mass spectra that showed peaks of survivor ions at m/z 126 for both **2** and **3** (Figure 2). These signals indicate that a fraction of the cytosine radicals **2** and **3** did not dissociate on the 5.7-μs timescale of the measurement. Thus, **2** and **3** represent intrinsically stable neutral species in the gas phase. The NR mass spectra also show substantially different dissociations of **2** and **3**. In particular, **3** undergoes the facile loss of a hydrogen atom (m/z 125) and C_2H_4N (m/z 84) and the formation of C_2H_3N (m/z 41). The elimi-

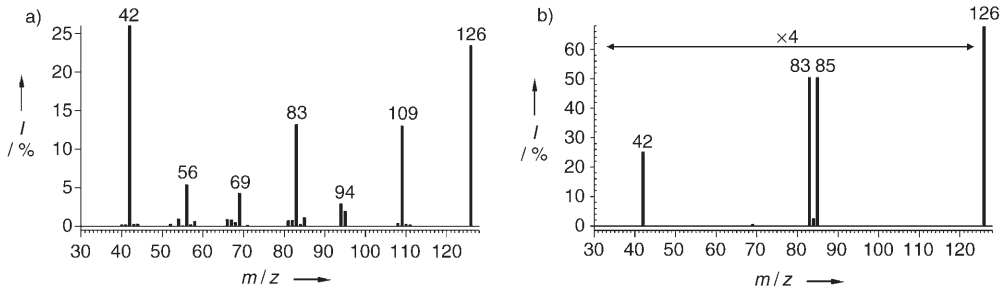


Figure 1. CAD mass spectra of a) $\mathbf{2}^+$ and b) $\mathbf{3}^+$ obtained at 5.5-eV center-of-mass collision energy in a radio-frequency-only quadrupole under multiple-collision conditions. The ion intensities are relative (%) to the total ion current.

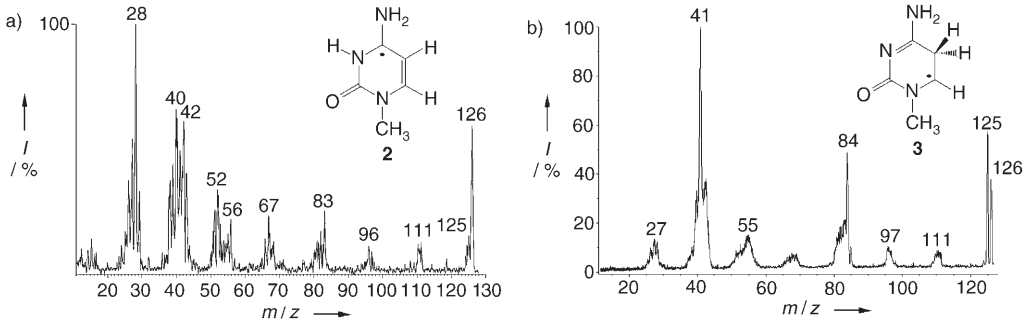
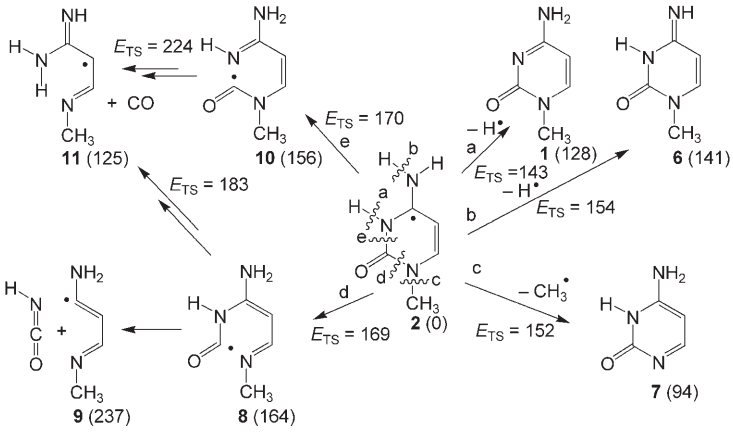


Figure 2. NR mass spectra. a) Neutralization of 2^+ with CH_3SSCH_3 and reionization with O_2 at 70% ion transmittance. b) Neutralization of 3^+ with $(CH_3)_3N$ and reionization with O_2 at 70% ion transmittance.

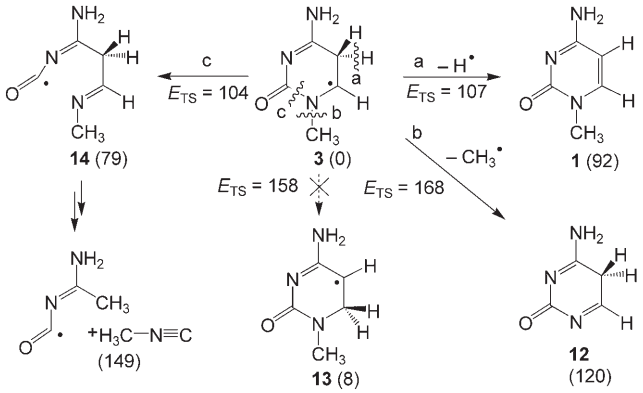
nation of C_2H_4N may be because of the combined losses of $(H + C_2H_3N)$ or $(CH_3 + HCN)$, which would require a detailed mechanistic study to be distinguished. In contrast to **3**, **2** shows only minor loss of hydrogen atoms, no fragment ion at *m*/*z* 84, and a dominant CO fragment ion at *m*/*z* 28. The differences in the spectra confirm that **2** and **3** do not interconvert by unimolecular isomerizations. This deduction was further corroborated by dissociations of deuterium-labeled radicals [3,7,7-D_3]-**2**, [6-D]-**3**, and [5,7-D_2]-**3**, of which [6-D]-**3** showed a specific loss of a hydrogen atom, thus indicating the absence of hydrogen/deuterium exchange at C5 and C6. A competitive loss of hydrogen and deuterium atoms from the methylene group at C5 was seen in [5,7-D_2]-**3**. These dissociations indicate that **1** forms as the stable product from the loss of a hydrogen atom from **3**. This conclusion is corroborated by the NR mass spectrum of $\mathbf{1}^{+\cdot}$, which showed a dominant survivor ion at *m*/*z* 125 and so confirmed that **1** was stable when formed by collisional electron transfer in the gas phase.



Scheme 3. The relative energies in parentheses (kJ mol^{-1}) are from B3-PMP2/6-311++G(3df,2p)+ZPVE calculations. E_{TS} = transition-state energy.

The relative stability and dissociation energetics of **2** and **3** were addressed by combined ab initio and density-functional theory calculations[6] to provide insight into the dissociation mechanisms. Both **2** and **3** are found to reside in potential-energy minima; whereby, **2** is more stable than **3** by 36 kJ mol^{-1}. The minimum-energy reaction pathway of **2** is the dissociation of the N3–H bond (path a) to yield **1** (Scheme 3). Competitive dissociations may involve the loss of a hydrogen atom from the amino group at N7 (path b) to yield the imine–oxo tautomer **6**. We note that it has been postulated that **6** is formed by tautomerization of **1** in the gas phase at 463 K.[11] Loss of the methyl group at N1 (path c) yields a cytosine tautomer **7**. The loss of the CH_3 group is seen to occur competitively with the loss of a hydrogen atom in the NR mass spectrum of **2** (Figure 2a). Ring opening by C2–N1 and C2–N3 bond dissociations (paths d and e) require somewhat higher transition-state (TS) energies (169–170 kJ mol^{-1}) to form **8** and **10**, respectively, as transient intermediates in the loss of CO and HNCO (Scheme 3). However, the ring cleavage of **2** and subsequent dissociations require several steps with high TS energies or product threshold energies (e.g., **9** and **11**) and can be expected to be kinetically disfavored on the potential-energy surface of the ground electronic state. The preponderance of small fragments of *m*/*z* 26–29 and 38–43 in the NR mass spectrum of **2** (Figure 2a) is probably because of a combination of dissociations that originate from the excited electronic states of $\mathbf{2}$[12] and post-reionization dissociations of the primary dissociation products. The calculations further indicate that vertical electron transfer in 2^+ results in only a modest vibrational excitation in the ground electronic state of **2** by Franck–Condon effects (E_{exc} = 39 kJ mol^{-1}), which is in keeping with the detection of a stable fraction of **2** in the spectrum.

The potential-energy surface for dissociations of **3** (Scheme 4) is substantially different from that of **2**. In particular, **3** shows a low-energy dissociation pathway for the loss of a hydrogen atom from the methylene group at C5 (path a), which is consistent with the prominent loss of a hydrogen atom in the NR mass spectrum (Figure 2b). The loss of the hydrogen atom competes with the loss of the CH_3 group (path b, **12**) and ring opening by cleavage of the weak C2–N1 bond (path c, **14**). The latter leads to expulsion of $CH_3N{\equiv}C$, which appears as the intense peak at *m*/*z* 41 in the NR mass spectrum of **3**. The formation of another major fragment at *m*/*z* 84 (Figure 2b) maybe because of combined losses of $(H + C_2H_3N)$ or $(CH_3 + HCN)$, which are currently unresolved and will require further study for satisfactory elucidation. In contrast, a 1,2-hydrogen migration between C5



Scheme 4. The relative energies in parentheses (kJ mol^{-1}) are from B3-PMP2/6-311++G(3df,2p)+ZPVE calculations.

and C6 in **3** that yields isomer **13** requires a high-energy TS to be crossed. This energy barrier explains why **3** does not exchange H5 and H6 atoms prior to the loss of the methylene hydrogen atom at C5.

In conclusion, transient radicals that correspond to adducts with a hydrogen atom positioned at N3 and C5 of the cytosine ring have been specifically generated in the gas phase for the first time. The isomeric radicals **2** and **3** are found to be intrinsically stable, distinct species that show specific unimolecular dissociations by loss of a hydrogen atom or the CH_3 group and ring cleavage followed by expulsion of small molecules. It can be expected that substituents at N1 larger than a methyl group (for example, 2′-deoxyribos-1-yl) would increase the propensity for N1–C bond dissociation in cytidine radicals, as the proposed mechanisms for nucleobase loss from radiation damage to DNA suggest.[1] Generation and computational studies of such nucleoside radical systems are underway.

Experimental Section

NR mass spectra were measured on a tandem-quadrupole mass spectrometer equipped with an electrospray ion source, as described previously.[13] Precursor ions $\mathbf{2}^+$ were produced by the electrospray of 50–100 μM solutions of 1-methylcytosine in methanol/water (70:30), desolvated, cooled to ambient temperature, transferred to high vacuum, accelerated to 7200 eV, and discharged by collisions with dimethyl disulfide under predominantly (83%) single-collision conditions. Radical **2** and its dissociation products were nonselectively ionized after 5.7 μs by collisions with O_2, decelerated to 70–75 eV, and analyzed by mass. Another set of NR mass spectra of $\mathbf{3}^+$ was measured at 8-keV ion kinetic energy on a ZAB2-SEQ double-focusing mass spectrometer that was furnished with an additional collision cell in the first field-free region. The spectra were obtained by a linked scan of the magnet and electrostatic sectors. Ion $\mathbf{3}^+$ was generated in the ion source by 70-eV electron ionization of 1-methyl-6-ethyl-5,6-dihydrocytosine. The details of the computational methods were described previously.[6,14]

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