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Cytosine neutral molecules and cation–radicals in the gas-phase Structures, energetics, ion chemistry, and neutralization–reionization mass spectrometry

Jill K. Wolken[a], Chunxiang Yao[a], František Tureček[a,*],
Michael J. Polce[b], Chrys Wesdemiotis[b,*]

[a] Department of Chemistry, Bagley Hall, Box 351700, University of Washington, Seattle, WA 98195-1700, United States
[b] Department of Chemistry, University of Akron, Akron, OH, United States

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Abstract

Gas-phase cytosine molecules and cation–radicals represent a complex system of several nearly isoenergetic tautomers within each group. Computational methods differ in ordering the relative enthalpies of neutral cytosine tautomers. At our highest level of theory, CCSD(T)/aug-cc-pVTZ calculations find an enol form, *anti*-2-hydroxy-4-aminopyrimidine (**2**), to be the most stable neutral tautomer in the gas-phase, followed by its rotamer, *syn*-2-hydroxy-4-aminopyrimidine (**3**), the canonical oxo-form, 4-amino-1,2-dihydropyrimidin-2(*1H*)-one (**1**), imino-forms, 2-oxo-4-iminodihydro(*1H*,*3H*)pyrimidine (**4** and **5**), and another oxo-form, 4-amino-dihydropyrimidin-2(*3H*)-one (**6**). Other tautomers, such as *anti-anti*, *syn-syn* and *syn-anti*-2-hydroxy-4-iminodihydro(*3H*,*4H*)pyrimidines (**7**–**9**), are less stable. The adiabatic ionization energies of the major cytosine tautomers have been calculated to be 8.71, 8.64, 8.62, 8.58, 8.64, and 8.31 eV for **1**, **2**, **3**, **4**, **5**, and **6**, respectively. Cytosine cation–radicals show very close relative energies that increase in the order of $\mathbf{6}^{\bullet+}$ (most stable) $< \mathbf{2}^{\bullet+} \approx \mathbf{3}^{\bullet+} < \mathbf{4}^{\bullet+} \approx \mathbf{7}^{\bullet+} \approx \mathbf{1}^{\bullet+} < \mathbf{5}^{\bullet+}$. In addition, distonic ions having radical centers at C-5 ($\mathbf{10}^{\bullet+}$) and C-6 ($\mathbf{11}^{\bullet+}$ are found as low-energy isomers of $\mathbf{1}^{\bullet+}$–$\mathbf{7}^{\bullet+}$. Metastable cytosine cation–radicals undergo ring-cleavage dissociations by eliminations of CO (major) and HN=C=O (minor). The energetics of these and other higher-energy dissociations, including the pertinent transition states, have been established by high-level ab initio and density functional theory calculations and plausible mechanisms have been proposed. Collisional neutralization of cytosine cation–radicals with trimethylamine and dimethyldisulfide as electron donors forms stable molecules that are detected as cation–radicals following collisional reionization. The dissociations observed upon neutralization–reionization mainly include ring-cleavages followed by loss of N=C=O, HN=C=O, and formation of C_2H_3N, C_2H_2N, and CO neutral fragments that are assigned to ion dissociations following reionization.

Keywords: Cytosine tautomers; Cytosine cation–radicals; Ionization energies; Neutralization–reionization; Ab initio calculations

1. Introduction

Ionization of DNA is the primary event that triggers processes contributing to DNA damage via the so-called direct mechanism [1]. The π-electronic systems in the nucleobases are the most likely sites of ionization, and the electron deficiency is presumed to progress along the DNA strand according to the nucleobase ionization energies (IE) that follow the trend IE(thymine) > IE(cytosine) > IE(adenine) > IE(guanine), as established by photoionization measurements [2–6] and theory [7–9]. While bimolecular reactions of nucleobase cation–radicals have been studied extensively by radiolytic methods in solution or solid state [10–14], relatively little is known about unimolecular dissociations of nucleobase cation–radicals in the gas-phase [15–17]. Density functional theory calculations have been reported for the canonical tautomers of nucleobase cation–radicals, and structure changes upon ionization have been discussed [8]. Ionization energies have been reported by both experiment [2–6] and theoretical calculations [7–9], as reviewed recently [18]. There is also a wealth of computational data on the structures and relative stabilities of nucleobase molecules in the gas-phase and clusters

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[*] Corresponding authors. Tel.: +1 206 685 2041; fax: +1 206 685 3478.
E-mail address: turecek@chem.washington.edu (F. Tureček).

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with water molecules [19]. Cytosine, in particular, has been the subject of detailed computational studies that were motivated by the existence of four tautomers of very similar relative enthalpies that posed a challenge to computational methods. In spite of this wealth of data, there are no studies aimed at the unimolecular reactivity of cytosine cation–radicals and their interactions with electrons. We now report a combined experimental and theoretical study of cytosine molecules and cation–radicals in the gas-phase using high-level ab initio theory and neutralization–reionization mass spectrometry [20–23].

2. Experimental

2.1. Materials

Cytosine (Sigma–Aldrich) was used as received and sampled to the mass spectrometers from heated solid probes. Cytosine-d_3 was prepared by dissolving cytosine (100 mg, 0.9 mmol) in 5 mL D_2O and allowing to stand at 20 °C for 3 days. D_2O was evaporated in vacuo and the solid cytosine-d_3 was used without further purification. A 70 eV electron ionization mass spectrum showed 6% d_1, 29% d_2, 61% d_3 and 4% d_4 species. Collisionally-activated dissociation (CAD) spectra were measured on a JEOL HX-110 double focusing mass spectrometer of EB geometry (electrostatic analyzer E precedes magnetic sector B). Air was admitted to the first field free region at pressures to achieve 70% or 50% transmittance of the precursor ion beam at 10 keV. The CAD spectra were recorded by scanning E and B while maintaining a constant B/E ratio (B/E linked scan). The mass resolution in these linked scans was >500. Metastable ion and CAD spectra (collisions with He) were also obtained as kinetic energy scans on the VG-Autospec tandem mass spectrometer at the University of Akron operating at 8 keV [24]. Neutralization–reionization ($^+NR^+$) mass spectra were measured on the University of Washington (UW) tandem quadrupole acceleration–deceleration mass spectrometer [25]. Cytosine cation–radicals were produced in an electron-impact ion source (200 °C, 1 mA emission current) that was floated at 75–80 V. The ions were transmitted by a wide-open quadrupole mass filter (55–60 V bias) onto an acceleration lens that was floated at −8150 V. A 8225 eV ions were discharged by collisions with dimethyl disulfide that was admitted to the vacuum system at pressures to achieve 70% transmittance of the precursor ion beam. Residual ions were reflected by a special electrostatic lens and the neutral products were allowed to drift to a reionization cell where they were reionized by collisions with molecular oxygen at 70% beam transmittance. The ions were decelerated to 70–75 eV and mass analyzed by a second quadrupole mass analyzer that was floated at 70–75 V and scanned at unit mass resolution. Neutralization–collisional–activation–reionization ($^+NCR^+$) mass spectra were measured by admitting He at 50% beam transmittance into the conduit of the UW instrument, as described previously [26]. Typically, 50–60 scans were collected and averaged to provide the NR and NCR mass spectra presented here. Additional neutralization–reionization mass spectra were measured at 8 keV ion kinetic energy on the VG-Autospec tandem mass spectrometer. Ions were prepared by electron ionization at 200 °C, selected by mass, and neutralized by collisions with trimethylamine at 70% ion beam transmittance. Oxygen was used for reionization at 70% beam transmittance. The spectra were obtained as kinetic energy scans.

2.2. Calculations

Standard ab initio calculations were performed using the Gaussian 03 suite of programs [27]. Optimized geometries were obtained by density functional theory calculations using Becke's hybrid functional (B3LYP) [28–30] and the 6-31+G(d,p) basis set. Another set of structures for several neutral cytosine tautomers were obtained by optimizations that used the Møller–Plesset theory [31] with all-electron excitations (MP2(FULL)) and the 6-31+G(d,p) basis set. Spin unrestricted calculations were performed for all open-shell systems. Stationary points were characterized by harmonic frequency calculations with B3LYP/6-31+G(d,p) as local minima (all real frequencies) and first-order saddle points (one imaginary frequency). The optimized geometries and uncorrected harmonic frequencies are available from the corresponding author (F.T.) upon request. The calculated frequencies were scaled with 0.963 and used to obtain zero-point energy corrections, enthalpies, entropies. The rigid-rotor harmonic oscillator (RRHO) model was used in thermochemical calculations except for low frequency modes where the vibrational enthalpy terms that exceeded $0.5RT$ were replaced by free internal rotation terms equal to $0.5RT$. It has been shown previously [32] that enthalpies and entropies based on the RRHO and free rotation approximations bracket the more accurate values calculated with the hindered internal rotor model, and the small differences cancel out in calculations of relative enthalpies and entropies. Improved energies were obtained by single-point calculations that were carried out at several levels of theory, including split-valence triple-ζ basis sets of increasing size furnished with polarization and diffuse functions, e.g., 6-311G(d,p), 6-311+G(3df,2p), 6-311++G(3df,2p), and 6-311G(3df,2pd), and correlation consistent basis sets cc-pVTZ and aug-cc-pVTZ. The B3LYP and MP2 energies calculated with the large basis set were combined according to the B3-MP2 scheme, as described previously [33,34]. Second, single-point energies were calculated with coupled-cluster theory [35] including single, double, and disconnected triple excitations (CCSD(T)) [36] and the 6-31G(d,p) basis set. These were then extrapolated to effective CCSD(T)/6-311++GG(3df,2p) using the standard formula (Eq. (1))

$$\begin{aligned}\text{CCSD(T)/6-311}++\text{G(3df, 2p)} &\cong \text{CCSD(T)/6-31G(d, p)}\\ &+\text{MP2/6-311}++\text{G(3df, 2p)}-\text{MP2/6-31G(d, p)}\end{aligned}\qquad(1)$$

For selected systems, single-point CCSD(T) calculations were also carried out with Dunning's correlation consistent triple-ζ basis set, cc-pVTZ and aug-cc-pVTZ [37].

RRKM calculations were performed using Hase's program [38] that was recompiled for Windows NT [39]. Unimolecular rate constants were obtained by direct count of quantum states at internal energies that were increased in 2 kJ mol^{-1} steps from the transition state up to 350 kJ mol^{-1} above the reactant.