Gas-Phase Reactions of Deprotonated Nucleobases with H, N, and O Atoms

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Supporting Information

ABSTRACT: Complex organic molecules, the hallmark of terrestrial life, are increasingly detected in exotic environments throughout the universe. Our studies probe the ion chemistry of these biomolecules. We report gas-phase reaction rate constants for five deprotonated nucleobases (adenine, cytosine, guanine, thymine, and uracil) reacting with the atomic species H, N, and O. Hydrogen atoms react at moderate rates via associative electron detachment. Oxygen atom reactions occur more rapidly, generating complex product distributions; reaction pathways include associative electron detachment, substitution of the hydrogen atom by an oxygen atom, and generation of OCN−. Nitrogen atoms do not react with the nucleobase anions. The reaction thermodynamics were investigated computationally, and reported product channels are exothermal. Many of the proposed products have been observed in various astrochemical environments. These reactions provide insight into chemical processes that may occur at the boundaries between diffuse and dense interstellar clouds and in complex extraterrestrial ionospheres.

The purine nucleobases (adenine and guanine) and the pyrimidine nucleobases (cytosine, thymine, and uracil) are essential subunits of deoxyribonucleic acid and ribonucleic acid (DNA and RNA); as such, they are critical to the chemistry of life on Earth. The origin of the nucleobases and other biomolecules remains an area of intense interest and research. However, the detection of nucleobases in comets and meteorites, as well as their extraterrestrial isotopic abundances, supports the hypothesis of their exogenous production and delivery to early Earth. Moreover, nucleobases and their precursors may exist in other environments within our solar system, including the atmosphere of Titan, Saturn’s largest moon. The recently completed Cassini–Huygens mission to Titan revealed the presence of complex carbon and nitrogen-containing molecules, as well as positive and negative ions. Moreover, experimental studies of the irradiation of a simulated Titan atmosphere demonstrate the formation of nucleobases.

The interstellar medium is home to a wide array of organic molecules; polycyclic aromatic hydrocarbons (PAHs) and nitrogen-containing polycyclic aromatic hydrocarbons (PANHs) are the likely sources of ubiquitous infrared emissions, and C60 has recently been identified as the first carrier of the Diffuse Interstellar Bands, whose origins have eluded astronomers for almost a century. Dark molecular clouds are especially intriguing, as the birthplace of both stars and planets but also as reservoirs of exotic species and chemical processes. In these interstellar clouds, about 200 molecules, cations, and anions have been detected. These species range from simple diatomics to complex polyatomic molecules that represent many functional groups, including alcohols, acids, amines, ketones, aldehydes, esters, ethers, and nitriles.

Although nucleobases have not yet been observed in the interstellar medium, many complex molecules including possible biomolecule precursor compounds containing carbon, hydrogen, nitrogen, and oxygen are present in abundance. Our experimental studies have focused on characterizing the structures, energies, fragmentations, and reactions of interstellar species with H, N, and O atoms, which are among the most abundant atomic species in the universe, in order to understand the synthesis and destruction mechanisms of astrochemical processes. Our recent work has involved nitrogen-containing negative ions, for which laboratory results are lacking, including cyanoacetylene, cyanate, dicyanamide anions, azoles, azines, and amino acids. Our recent development of an electrospray ionization source (ESI) for the selected ion flow tube (SIFT) has enabled our first experimental studies of the reactions of nucleobase anions with atomic reactants. This Letter reports our findings that these relatively robust ions undergo efficient and intriguing reactions with hydrogen and oxygen atoms. The SIFT technique, which has been described in detail previously, allows quantitative kinetic measurements, including rate constants and product distributions, for a wide variety of ions reacting with neutral reagents. Figure 1 shows...
nucleotide bases were obtained commercially as powders from Sigma-Aldrich and diluted in HPLC-grade acetonitrile to a final concentration of \( \sim 5 \times 10^{-6} \) M. To promote solubility and deprotonation of the nucleobases, reagent-grade sodium hydroxide was added to the solution in a 1:1 molar ratio (nucleobase:NaOH). The acetonitrile solution was then infused through a heated capillary at a flow rate of \( \sim 1-10 \) \( \mu \)L min\(^{-1} \) using a syringe pump; ions were extracted at high potential and concentrated by an ion funnel.\(^{27} \) The desired nucleobase anions were mass-selected with the SIFT quadrupole mass filter and injected into the reaction flow tube, where they were entrained in helium carrier gas (UHP, 99.9999%, Airgas). The ions underwent thousands of collisions with helium before undergoing reaction with added neutrals; thus, this technique provides a stable, continuous flow of deprotonated nucleobases for kinetic measurements at thermal energy.

The H, N, and O atoms are generated downstream using a microwave discharge coupled to the reaction flow tube (Figure 1). This technique is well described in the general literature\(^{28} \) and thoroughly characterized in our previous papers.\(^{20} \) To form H atoms, purified molecular hydrogen is passed through an Evenson cavity supplied with 50 W of microwave radiation; the H\(_2\) flow is regulated with a metering valve and monitored with a flow meter. The reaction rate constants between the deprotonated nucleobases and H atoms are measured relative to the reaction Cl\(^+\) + H, \( k_{\text{expt}} = 9.6 \times 10^{-10} \) cm\(^3\) s\(^{-1}\).\(^{29} \) The values in parentheses indicate one standard deviation of the mean \( \sigma \) of 15 or more measurements. Theoretical energies are computed with the B3LYP/6-311++G(d,p) level of theory and reported at \( T = 298 \) K by including thermal corrections under the rigid rotor–harmonic oscillator approximation. O-atom rate constants are measured using a gas-phase titration as described previously.\(^{20} \) The values in parentheses indicate one standard deviation of the mean \( \sigma \) of nine or more measurements. Associative electron detachment distribution for O-atom reactions.\(^{2} \) The spin state is a singlet unless indicated with a superscript 2 for a doublet. Tonic product distributions are determined by monitoring the relative intensities of each product ion.

![Figure 1. Electrospray ionization-selected ion flow tube with a microwave discharge atom source.](image1)

![Figure 2. Five nucleobases deprotonated at the most acidic site.](image2)

<table>
<thead>
<tr>
<th>deprotonated nucleobase</th>
<th>( \Delta H ) theory (kcal/mol)</th>
<th>( k_{\text{expt}} ) (10(^{-11}) cm(^3)/s)</th>
<th>( k_{\text{fl}} ) (10(^{-11}) cm(^3)/s)</th>
<th>AED (%)</th>
<th>proposed products</th>
<th>distribution</th>
<th>( \Delta H ) theory (kcal/mol)</th>
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<tr>
<td>adenine ( \text{C}<em>{11}\text{H}</em>{10}\text{N}_{3}\text{O}^- ) m/134</td>
<td>4.9 (1.9)</td>
<td>-20.5</td>
<td>26.1 (5.2)</td>
<td>0.2</td>
<td>42 OCN(^-) + 4HCN</td>
<td>0.10</td>
<td>-15.1</td>
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<td></td>
<td>80</td>
<td>C(_2)H(_3)N(_3)O(^-) + HNCO + HCN</td>
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<td></td>
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<td>C(_2)H(_4)N(_3)O(^-) + 2HCN</td>
<td>0.10</td>
<td>-44.7</td>
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<td></td>
<td>123</td>
<td>C(_2)H(_5)N(_3)O(^-) + HCN</td>
<td>0.35</td>
<td>-65.8</td>
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<tr>
<td></td>
<td>149</td>
<td>C(_2)H(_4)N(_2)O(^2-) + H(^+)</td>
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<td>-30.0</td>
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<td>cytosine ( \text{C}<em>{10}\text{H}</em>{10}\text{N}_{3}\text{O}^- ) m/110</td>
<td>23.6 (1.9)</td>
<td>-23.2</td>
<td>39.0 (4.1)</td>
<td>0.7</td>
<td>42 OCN(^-) + 2HCN + H(_2)CO</td>
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<td>C(_2)H(_3)N(_2)O(^-) + H(^+)</td>
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<td>guanine ( \text{C}<em>{10}\text{H}</em>{10}\text{N}_{3}\text{O}^- ) m/150</td>
<td>4.9 (1.4)</td>
<td>-18.9</td>
<td>48.1 (6.2)</td>
<td>0.6</td>
<td>26 C(_2)N(_2) + 2HCNO + 2HCN</td>
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<td>-19.7</td>
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<td>OCN(^-) + HNCO + 3HCN</td>
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<td>C(_2)H(_4)N(_3)O(^-) + HNCO + 2HCN</td>
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<td>thymine ( \text{C}<em>{12}\text{H}</em>{12}\text{N}_{3}\text{O}_2^- ) m/125</td>
<td>5.1 (1.6)</td>
<td>-18.3</td>
<td>29.7 (5.5)</td>
<td>0.4</td>
<td>42 OCN(^-) + HNCO + C(_2)H(_4) + CO</td>
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<td>-48.2</td>
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<td>-28.1</td>
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<td>uracil ( \text{C}<em>{12}\text{H}</em>{12}\text{N}_{3}\text{O}_2^- ) m/111</td>
<td>5.2 (1.6)</td>
<td>-16.0</td>
<td>19.1 (9.1)</td>
<td>0.7</td>
<td>42 OCN(^-) + C(_2)H(_2)O + HNCO</td>
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<td>-54.9</td>
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<td>C(_2)H(_2)N(_2)O(^2-) + H(^+)</td>
<td>0.55</td>
<td>-30.3</td>
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</table>

\(^{2}\) Deprotonation assumed to occur at the most acidic site.\(^{31} \) H-atom rate constants are measured relative to the reaction Cl\(^+\) + H, \( k_{\text{expt}} = 9.6 \times 10^{-10} \) cm\(^3\) s\(^{-1}\).\(^{29} \) The values in parentheses indicate one standard deviation of the mean \( \sigma \) of 15 or more measurements. Theoretical energies are computed with the B3LYP/6-311++G(d,p) level of theory and reported at \( T = 298 \) K by including thermal corrections under the rigid rotor–harmonic oscillator approximation. O-atom rate constants are measured using a gas-phase titration as described previously.\(^{20} \) The values in parentheses indicate one standard deviation of the mean \( \sigma \) of nine or more measurements. Associative electron detachment distribution for O-atom reactions.\(^{2} \) The spin state is a singlet unless indicated with a superscript 2 for a doublet. Tonic product distributions are determined by monitoring the relative intensities of each product ion.
to the well-characterized reaction between H and Cl−, which occurs exclusively through associative electron detachment, H + Cl− → HCl + e−, with a rate constant of kexp = 9.6 × 10−10 cm3 s−1. For these studies, reagent-grade NaCl is added to the ESI acetoneitrile solution in a 1:1 molar ratio (nucleobase: NaOH:NaCl), and the SIFT quadrupole is set to transmit all masses in order to simultaneously introduce Cl− and the deprotonated nucleobase, X−, into the reaction flow tube. The depletions of both Cl− and X− are monitored with the downstream triple-quadrupole mass analyzer, allowing a relative rate constant for X− + H to be determined. N atoms are generated by passing molecular nitrogen through the microwave discharge. O atoms are formed by introducing NO (4% in He, Matheson) immediately downstream of the discharge to initiate the reaction N + NO → N2 + O. This titration allows the determination of nitrogen and oxygen atom concentrations as a function of NO flow.

The deprotonated nucleobases were found to be unreactive with H2, N2, and N atoms. However, all five nucleobase anions reacted readily with H atoms and O atoms; the reaction rate constants and product distributions were measured and are summarized in Table 1.

The thermochemical values, ΔH, theory, listed in Table 1, were determined with the computational program suite Gaussian 09b6 and the B3LYP/6-311++G(d,p) level of theory with thermal corrections using the rigid rotor–harmonic oscillator approximation to provide ΔH theory values at 298 K. For these calculations, the parent nucleobases were assumed to be deprotonated at the most thermodynamically favorable position,31 that is, N9 for adenine and guanine and N1 for cytosine, thymine, and uracil (Figure 2). Previous studies demonstrated that gas-phase biological ions formed by ESI generally retain their most stable solution structure.32

The reactions of the nucleobase anions with H-atoms proceed exclusively by associative electron detachment, in which a bond is formed to hydrogen and the electron is detached. We have observed this mechanism for a wide variety of negative ions, and indeed, we have found only two extremely stable anions (C2N3− and C3N4−) that are unreactive with H-atoms.33,34 Associative electron detachment processes are critical in the interstellar medium where they play a major role in determining the balance between negative ions and electrons. For the thermochemical values listed in Table 1, the hydrogen atom is assumed to bond at the original site of deprotonation to form the parent nucleobase molecule. The reactions of deprotonated adenine, guanine, thymine, and uracil all occur at approximately the same rate within the precision of the experiment. Notably, the cytosine reaction occurs almost five times faster, perhaps because the electron binding energy of N1 deprotonated cytosine is lower than for the other nucleobases by ~0.2–0.3 eV.35 Our previous studies have demonstrated a correlation between rate constants of associative electron detachment processes and the electron binding energy of the anion,14,36 as well as with the overall reaction thermochemistry.35

All of the deprotonated nucleobases show moderately rapid reactivity with O atoms, and they generate a variety of remarkable products, as shown in Table 1. Previous studies characterized the O atoms formed by the gas-phase titration as the triplet state, O(3P). Therefore, the spin state of the reactants and products must be considered for the reaction X− + O. To generate ΔH theory values, two spin states were considered for each of the polyatomic products in this study, i.e., singlet and triplet (for species with an even number of electrons) or doublet and quartet (for species with an odd number of electrons). The lower-energy electronic state was always found to be the species of lower spin, i.e., the singlet or doublet; in Table 1, doublets are designated with a superscript “2”, while all other species are singlets. The optimized geometries for all reactants and products in Table 1 are provided in the Supporting Information (SI). In SIFT experiments, only the mass-to-charge ratios of the ions are measured, and the neutral products are not observed. Therefore, the neutral products of the O-atom reactions in Table 1 were proposed assuming reasonable, stable species; in all cases, these reactions were computed to be exothermic. The occurrence of spin conversion, as observed in many of these pathways, is common for reactions involving high-spin atoms such as O(3P).37

Several trends emerge for the X− + O reactions. First, associative electron detachment occurs for all reactions, representing from 20 to 70% of the reaction pathways. Second, a product ion 15 amu higher than the mass of X− is observed for each reaction. This ion corresponds to addition of O and loss of H [X− + O → X(=O−H)+ + H2]. Our previous study that investigated deprotonated organic heterocycles + O observed similar product channels.19 This substitution process conserves spin and is exothermic for each reaction system investigated. For the +15 amu anionic products, two or more isomer geometries are optimized to determine ΔH theory. The lowest-energy isomer is formed when O replaces H on an unsaturated ring carbon atom, suggesting that O preferentially attacks the nucleophilic regions of the molecule. For deprotonated adenine, cytosine, guanine, thymine, and uracil, these lowest-energy isomers correspond to replacement of H by an O-atom at positions 2, 6, 8, 6, and 5, respectively.

A third process common to all X− + O reactions is the formation of the cyano anion, OCN−, at m/z 42. This resonance stabilized anion possesses high thermodynamic stability and was the first anion detected in the interstellar medium.35,36 While OCN− is readily generated for each X− + O reaction, the mechanisms of formation may differ because each system contains multiple O−C−N structural motifs. When O binds to a molecule, the newly formed adduct can be excited by 75–150 kcal/mol. These highly excited transient species can undergo complex reaction mechanisms to generate the rich product distributions that are observed. Several of these additional pathways are discussed below.

Neutral adenine, a purine nucleobase, is a hydrogen cyanide pentamer (HCN)5. Our recent study of the fragmentation of deprotonated adenine by collision-induced dissociation revealed sequential loss of HCN moieties. In analogy with this decomposition pattern, the formation of OCN− may be accompanied by loss of four HCN molecules. Other product channels correspond to loss of fewer HCN molecules and/or loss of isocyanic acid (HCON) in which OCN− has been protonated before fragmentation. Guanine, also a purine nucleobase, shows similar product pathways with loss of multiple HCN and HNCO molecules. Both deprotonated adenine and guanine have six ionic product channels and exhibit extensive decomposition upon reaction with O-atoms.

In contrast to the purine nucleobases, the reactions of the deprotonated pyrimidine nucleobases with O-atoms generate carbonyl products. Carbon monoxide is produced by all of the pyrimidine reactions; in addition, formaldehyde is formed from cytosine, and ketene is formed from uracil. Extrusion of HCN...
and HNCO molecules occurs in several of the product channels. Quantum mechanical calculations to explore the detailed mechanisms of these processes would be extremely valuable; however, these computational studies will be challenging due to the relatively large size of the nucleobases, their structural complexity, and the occurrence of multiple reaction pathways.

In summary, the anions formed by deprotonation of the nucleobases (adenine, cytosine, guanine, thymine, and uracil) react readily with H-atoms by associative electron detachment. The anions are unreactive with N-atoms but exhibit a rich chemistry in reactions with O-atoms; associative electron detachment, exchange of hydrogen by oxygen, and formation of the cyanate anion are exhibited by all nucleobases. Many of the proposed neutral molecule products, including HCN, HNCO, H2CO, and CO, have been detected in the interstellar medium, as have the product anions OCN− and CN−. In addition to providing fundamental understanding of the reactivity of nucleobase ions, these studies provide insight into astrochemical environments where biomolecules, their ions, and their prebiotic building blocks exist. In particular, these studies reveal the chemical reactions that may occur at the boundaries between diffuse clouds, where atomic species are abundant, and the dense molecular clouds, where complex ions and molecules have been detected.

**REFERENCES**

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