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Infrared multiple photon dissociation action spectroscopy of sodium cationized halouracils: Effects of sodium cationization and halogenation on gas-phase conformation

C.M. Kaczan [a], A.I. Rathur [a], R.R. Wu [a], Y. Chen [a], C.A. Austin [a], G. Berden [b], J. Oomens [b,c], M.T. Rodgers [a,*]

[a] Department of Chemistry, Wayne State University, Detroit, MI 48202, USA
[b] Radboud University Nijmegen, Institute for Molecules and Materials, FELIX Facility, Toernooiveld 7, 6525 ED Nijmegen, The Netherlands
[c] van't Hoff Institute for Molecular Sciences, University of Amsterdam, Amsterdam, The Netherlands

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In honor of Veronica M. Bierbaum on the occasion of her 65th birthday, in thanks for her numerous contributions to gas-phase ion thermochemistry, and in appreciation for her friendship, mentoring and support throughout my professional career.

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ABSTRACT

The gas-phase structures of sodium cationized complexes of 5- and 6-halo-substituted uracils are examined via infrared multiple photon dissociation (IRMPD) action spectroscopy and theoretical electronic structure calculations. The halouracils examined in this investigation include: 5-flourouracil, 5-chlorouracil, 5-bromouracil, 5-iodouracil, and 6-chlorouracil. Experimental IRMPD action spectra of the sodium cationized halouracil complexes are measured using a 4.7 T Fourier transform ion cyclotron resonance mass spectrometer coupled to the FELIX free electron laser (FEL). Irradiation of the mass selected sodium cationized halouracil complexes by the FEL was carried out over the range of frequencies extending from 950 to 1900 cm^{-1}. Theoretical linear IR spectra predicted for the stable low-energy conformations of the sodium cationized halouracils, calculated at B3LYP/6-31G(d) level of theory, are compared with the measured IRMPD action spectra to identify the structures accessed in the experiments. Relative stabilities of the low-energy conformations are determined from single-point energy calculations performed at the B3LYP/6-311+G(2d,2p) level of theory. The evolution of IRMPD spectral features as a function of the size (F, Cl, Br, and I) and position (5 versus 6) of the halogen substituent are examined to elucidate the effects of the halogen substituent and noncovalent interactions with sodium cations on the structure of the nucleobase. Present results are compared with results from energy-resolved collision-induced dissociation and IRMPD action spectroscopy studies previously reported for the protonated and sodium cationized forms of uracil, and halo-, methyl-, and thioketo-substituted uracils. The present results suggest that only a single conformer is accessed for all of the 5-halouracil complexes, whereas multiple conformers are accessed for the Na^+(6ClU) complex. In all cases, the experimental IRMPD action spectra confirm that the sodium cation binds to the O4 carbonyl oxygen atom of the canonical diketo tautomer in the ground-state conformers, and gains additional stabilization via chelation interactions with the halogen substituent in the complexes to the 5-halouracils as predicted by theory.

1. Introduction

The 5- and 6-halouracils have been shown to exhibit antitumor and antiviral activities [1–18]. The ability of 5- and 6-halouracils to control damage to healthy tissues during radiation therapy has been evaluated [1]. 5-Chlorouracil and 5-bromouracil have been studied for their use in the treatment of inflammatory tissue [2]. 5-Fluorouracil is currently employed in colorectal, breast, and head and neck cancer treatments [3–5] due to its ability to inhibit thymine synthase, and thus prevent the conversion of uracil to thymine [4,5]. 5-Fluorouracil is incorporated into DNA by DNA polymerase and excised by DNA glycosylase leading to toxic abasic sites and subsequent DNA strand breaks [5]. Fluorine is similar in size to a hydrogen atom, and like uracil 5-fluorouracil is excised by DNA glycosylase, whereas chlorine and bromine are similar in size to a methyl group such that 5-chlorouracil and 5-bromouracil, like thymine, are not excised by DNA glycosylase. Similar mechanisms for 5-chlorouracil and 5-bromouracil utilizing thymine DNA glycosylase have been suggested [19]. However, the mechanisms of antitumor and antiviral actions of these nucleobases are not well understood. Thus, research into the structures and binding interactions of halouracils are of great importance in pharmaceutical research.

* Corresponding author. Tel.: +1 3135772431; fax: +1 3135778822.
E-mail address: mrodgers@chem.wayne.edu (M.T. Rodgers).

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Sodium cations are essential to biological systems as they play vital roles in heart activity, blood and fluid regulation, nerve impulse transmission, and metabolic functions. One of the most recognized functions of sodium cations is their role in creating ion gradients with potassium ions. Secondary active transport utilizes a Na^+/K^+ ion gradient to transport amino acids, nucleotides, and other biological molecules across cell membranes. Voltage-gated ion channels found in neurons also utilize a Na^+/K^+ ion gradient to pass electrical signals from neuron to neuron [20]. Sodium cations are also involved in nucleic acid chemistry exhibiting charge neutralization and noncovalent interactions that lead to the stabilization of DNA and RNA [21,22]. The presence of metal cations influences the function, stability, and conformational behavior of surrounding molecules. Metal cations binding to the nucleobase can produce more significant effects on DNA and RNA conformation than those binding to the phosphate backbone [23]. The formation and stabilization of minor nucleobase tautomers can be caused by the presence of metal cations [24]. These nucleobase modifications can potentially lead to base mispairing and cause errors in the transfer of genetic information [25].

The properties and characteristics of protonated and sodium cationized uracil and modified uracils have been extensively studied [25–31]. Previous infrared multiple photon dissociation (IRMPD) action spectroscopy studies by Crampton et al. of protonated halouracils [26], Salpin et al. of uracil [27], and Nei et al. of uracil, thioketo-, and methylthioketo-uracils [28] found that protonation preferentially stabilizes minor noncanonical tautomers of all of the nucleobases examined thus far except 4-thiouracil. In contrast, previous CID and theoretical studies by Rodgers and Armentrout of sodium cationized uracil [29] and by Yang et al. of sodium cationized methyl- [25], thioketo- [30], and halo-substituted uracils, [31] suggest that the ground-state structures involve the canonical tautomers and the dominant CID processes observed arise from loss of the intact neutral nucleobase. Similarly, the IRMPD study by Nei et al. of sodium cationized uracil, thioketo- and methylthioketo-uracils found the ground-state structures to involve the canonical tautomer of the nucleobase except in complexes of 4-thiouracil and 2,4-dithiouracil, where minor tautomers are preferentially stabilized [32].

Structural information regarding the sodium cationized halouracils in the previous CID study was inferred by comparison of measured and theoretically estimated bond dissociation energies [31]. In the current study, IRMPD action spectroscopy techniques are used to probe the structures of sodium cationized halouracils. The influences of halogenation and noncovalent interactions with sodium cations on the gas-phase tautomeric conformations of the halouracils are also examined. The structures and relative stabilities at 298 K of all possible tautomers of the sodium cationized halouracils are calculated. The halouracils investigated in this study include: 5-fluorouracil (5FU), 5-chlorouracil (5ClU), 5-bromouracil (5BrU), 5-iodouracil (5IU), and 6-chlorouracil (6ClU), allowing the influence of the size and position of the halogen substituent on the structure of sodium cationized halouracils to be elucidated. The canonical structures of neutral uracil and the halouracils investigated here are shown in Fig. 1. In order to definitively determine the ground-state and low-energy tautomeric conformations of the sodium cationized forms of these nucleobases, IRMPD action spectra of these complexes are measured and compared to theoretical linear IR spectra of the stable low-energy structures of the sodium cationized halouracil complexes derived from electronic structure calculations performed at the B3LYP/6-31G(d) level of theory.

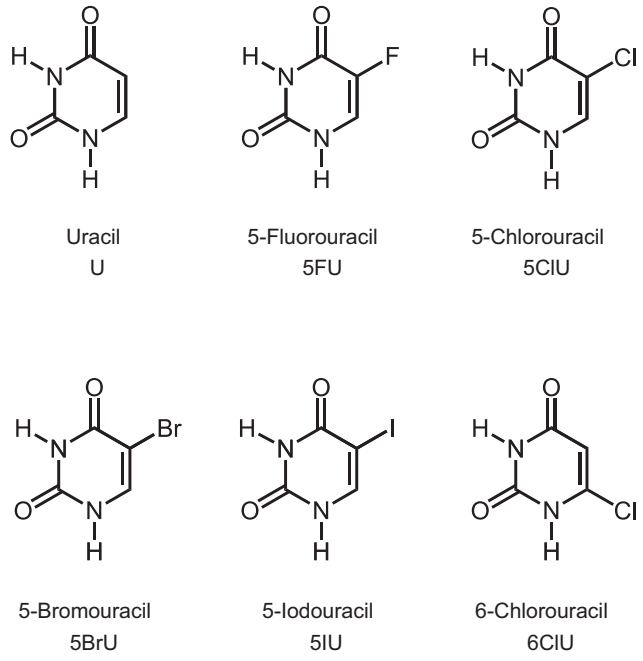

Fig. 1. Structures of uracil (U) and the halouracils (*x*U), where *x*U = 5FU, 5ClU, 5BrU, 5IU and 6ClU.

2. Experimental and computational

2.1. Mass spectrometry and photodissociation

Experimental studies were carried out at the FELIX facility using a 4.7 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) coupled to a free electron laser (FEL) [33–35]. IRMPD action spectra were obtained for five Na^+(*x*U) complexes, where *x*U represents 5FU, 5ClU, 5BrU, 5IU, and 6ClU. 5FU and 5BrU were obtained from Sigma–Aldrich, whereas 5ClU, 5IU, and 6ClU were obtained from TCL Tokyo Kasei. Solution conditions employed for the samples examined were 1–2 mM nucleobase with 1–2 mM NaCl in 50%:50% to 80%:20% MeOH:H_2O solutions.

Sample solutions were delivered via a syringe pump to a Micromass z-spray electrospray ionization (ESI) source at a flow rate of 10 μL/min and a needle voltage of 3.2–3.4 kV. Ions emanating from the ESI source were accumulated in a hexapole ion trap for several seconds, and then pulse extracted and sent through a quadrupole bender into an octopole ion guide. Ions were slowed within the octopole ion guide by traveling up a potential difference created by a negative dc bias with relative ground potential at the ICR cell to allow for easy capture by gated ion trapping [33]. Once ions were trapped within the ICR cell, stored waveform inverse Fourier transform (SWIFT) excitation was used to isolate the desired sodium cationized halouracil complex from unwanted ions formed in the ionization process. As chlorine and bromine exist naturally in two isotopes, complexes with both isotopes were isolated and used to produce greater precursor ion intensity and to properly identify the desired precursor ion. Selected ions were irradiated by the FEL (entering from the back of the ICR cell) at pulse energies of ~40 mJ per macropulse of 5 μs duration for 3 s, corresponding to interaction with 15 macropulses over the wavelength range extending from 10.5 μm (950 cm^{-1}) to 5.3 μm (1900 cm^{-1}) to induce photodissociation. Dissociation occurs when the wavelength of the radiation corresponds to a resonant absorption mode of the precursor ion. Precursor ions absorb photons and distribute the absorbed energy throughout the