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Infrared multiple photon dissociation action spectroscopy of protonated uracil and thiouracils: Effects of thioketo-substitution on gas-phase conformation

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ABSTRACT

The gas-phase structures of protonated complexes of uracil and five thiouracils including 2-thiouracil (2SU), 5-methyl-2-thiouracil (5Me2SU), 6-methyl-2-thiouracil (6Me2SU), 4-thiouracil (4SU), and 2,4-dithiouracil (24dSU) are examined via infrared multiple photon dissociation (IRMPD) action spectroscopy and theoretical electronic structure calculations. IRMPD action spectra of all six protonated complexes exhibit both similar and distinct spectral features over the range of ~1000–1900 cm^{-1}, such that the complexes are easily differentiated by their IRMPD action spectra. Absence of the carbonyl stretch at ~1825 cm^{-1} in the IRMPD spectra for the H$^+$(U), H$^+$(2SU), H$^+$(5Me2SU), and H$^+$(6Me2SU) complexes suggests that the binding of a proton preferentially stabilizes alternative tautomers of the nucleobases in these complexes such that no free carbonyl stretch is observed. In contrast, the intense band at ~1825 cm^{-1} in the IRMPD action spectrum of H$^+$(4SU) indicates that a free carbonyl group is still present in this complex. Measured IRMPD action spectra are compared to linear IR spectra calculated at the B3LYP/6-31G(d) level of theory to identify the structures accessed in the experimental studies. On the basis of these comparisons and energetics derived from the calculations, protonation results in preferential stabilization of a minor tautomer of the nucleobase in the H$^+$(U), H$^+$(2SU), H$^+$(5Me2SU), H$^+$(6Me2SU), and H$^+$(24dSU) complexes, where both keto (thioketo) groups are converted to hydroxy (sulfhydryl) groups by proton binding and proton transfer from the neighboring N3H group. In contrast, the proton preferentially binds at the 4-thioketo position to the canonical keto–thioketo tautomer in the H$^+$(4SU) complex. Additional band(s) are present in the IRMPD action spectra of the H$^+$(U) and H$^+$(4SU) complexes that suggest that a small population of excited low-energy conformers are also accessed in those systems.

1. Introduction

Hydrogen bonding plays an important role in biological systems. Specifically, the base-pairing of purine and pyrimidine nucleobases in DNA and RNA requires the correct hydrogen bonding between the nucleobases, cytosine with guanine and adenine with thymine in DNA or uracil in RNA, in order to form stable double helices and correct encoding information [1]. Tautomeric shift of the nucleobases from their canonical tautomers may cause base-pairing mismatch and result in mutation [2–5]. Uracil has been the subject of many experimental [6–13] and theoretical [14–24] studies due to the possibility to form alternative enol tautomers through proton transfer reactions. However, all of these studies have come to the conclusion that the most stable conformation of neutral uracil in both the gas and solid phases is the canonical 2,4-diketo tautomer shown in Fig. 1. The activation barriers to form other tautomers of uracil are sufficiently high that these conformers are unlikely to be populated. Several experimental studies [25–27] have sought to determine the proton affinity of uracil, and with the aid of theoretical studies [28–31], to characterize its gas-phase structure. However, the structural information determined using high-pressure mass spectrometry (HPMS) and Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry experiments along with theoretical calculations are generally ambiguous. Recently, Salpin et al. [32] examined the structure of protonated uracil by infrared multiple photon dissociation (IRMPD) spectroscopy in a quadrupole ion trap. They concluded that the binding of a proton preferentially stabilizes an alternative tautomeric structure, while the tautomer corresponding to the binding of a proton to the canonical diketo tautomer of uracil was also present in minor abundance. This result was then further confirmed by an IRMPD spectroscopy study on the monohydrated protonated uracil complex [33].

Thiouracils are an important and interesting class of modified nucleobases due to their biological, pharmacological, and spectroscopic properties. 2-Thiouracil, 5-methyl-2-thiouracil, 4-thiouracil,

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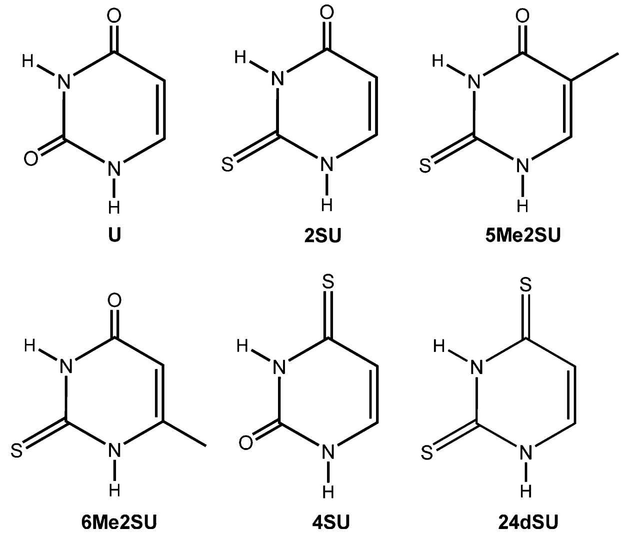

Fig. 1. Structures of uracil (U) and the thiouracils (*x*SU), where *x*SU = 2-thiouracil (2SU), 5-methyl-2-thiouracil (5Me2SU), 6-methyl-2-thiouracil (6Me2SU), 4-thiouracil (4SU), and 2,4-dithiouracil (24dSU).

and 2,4-dithiouracil have been identified as minor components of t-RNA and peptide nucleic acids [34–36]. The replacement of uracil by thiouracils in m-RNA can lead to misrecognition and results in mutation [37]. Interestingly, the substitution of oxygen by sulfur and further methyl-substitution of uracil results in modified nucleobases that have found use in many pharmacological applications [34,38–51]. 2-Thiouracil and 4-thiouracil are well known for their use in antithyroid, anticancer [34,38,39], and heart disease treatments [40,41]. Similarly, 6-methyl-2-thiouracil has also been demonstrated to be an effective antithyroid drug [42]. Due to these therapeutic activities, a series of derivatives of 2-thiouracil and 4-thiouracil can also be found in many pharmacological drugs for anticancer [43], antithyroid [46,47], and anti-HIV [48] treatments.

The thioketo group exhibits strong absorption bands in the visible region, which provide an ideal method for detection of thiouracils. Thus, the properties and characteristics of thiouracils have attracted many experimental [52–56] and theoretical [57–59] studies to characterize the different tautomeric forms that they take on in the gas and solid phases. Of most relevance to the present work are the gas-phase basicity and proton affinity measurements using Fourier transform ion cyclotron resonance mass spectrometry techniques and theoretical calculations by Lamsabhi et al. [60]. In this study, the proton affinities measured for 4-thiouracil and 2,4-dithiouracil correspond well with theoretical estimates for the binding of a proton at the S4 position of the canonical keto–thioketo tautomer of 4-thiouracil and dithioketo tautomer of 2,4-dithiouracil. In addition, the binding of a proton to 2-thiouracil was found to stabilize an alternative tautomeric structure, similar to that found in the IRMPD spectroscopy study of protonated uracil mentioned above. However, structural information regarding these protonated thiouracil complexes was only inferred from comparison with theory. No direct experimental characterization of the structures of the protonated complexes of these thiouracils has been reported.

In the present work, we examine the interactions of uracil (U) and five thiouracils (*x*SU), where *x*SU = 2-thiouracil (2SU), 5-methyl-2-thiouracil (5Me2SU), 6-methyl-2-thiouracil (6Me2SU), 4-thiouracil (4SU), and 2,4-dithiouracil (24dSU) with protons. The canonical structures of neutral uracil and the thiouracils investigated here are shown in Fig. 1. In order to definitively determine the ground-state and low-energy tautomeric conformations of the protonated forms of these nucleobases, the IRMPD action spectra of these complexes are investigated and compared to linear IR spectra of the low-energy structures of these protonated complexes derived from theoretical electronic structure calculations performed at the B3LYP/6-31G(d) level of theory.

2. Experimental and computational section

2.1. Mass spectrometry and photodissociation

IRMPD action spectra of H^+(U) and five H^+(*x*SU) complexes were measured using a 4.7 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer coupled to a free electron laser (FEL) source that is housed at the FOM Institute for Plasma Physics, “Rijnhuizen”, and has been described in detail elsewhere [61–63]. All of the nucleobase samples were obtained from Sigma–Aldrich. The protonated complexes of each nucleobase were generated using a Micromass “Z-spray” electrospray ionization (ESI) source from solutions containing 2 mM nucleobase and 1–4 mM of acetic acid in an approximately 50%:50% MeOH/H_2O mixture. A solution flow rate of 10 μL/min was used and the electrospray needle was generally held at a voltage of ~3 kV. Ions emanating from the ESI source were accumulated in a hexapole trap for several seconds followed by pulsed extraction through a quadrupole bender and injection into the ICR cell by an rf octopole ion guide. To avoid collisional heating of the ions by the gas pulse deceleration method, a negative dc bias was applied to the octopole with relative ground potential on the ICR cell so that the ions were slowed down by climbing the potential difference and easily captured by gated trapping in the ICR cell. This dc bias switching method is described in detail elsewhere [62]. The precursor ions were mass selected using stored waveform inverse Fourier transform (SWIFT) techniques and irradiated by the FEL 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 9.9 μm (1010 cm^{-1}) to 5.2 μm (1923 cm^{-1}). Each of the protonated nucleobases containing a carbonyl group, i.e., all complexes investigated here except H^+(24dSU), were also irradiated for 7 s (interaction with 35 macropulses) in the vicinity of the carbonyl stretching region; the wavelength range extending from 6.0 μm (1667 cm^{-1}) to 5.2 μm (1923 cm^{-1}).

2.2. Computational details

In the present work, all possible tautomers of neutral and protonated uracil and the five thiouracils are examined. All structures were optimized and harmonic vibrational frequencies calculated at the B3LYP/6-31G(d) level of theory using the Gaussian 03 suite of programs [64]. Single point energy calculations carried out at the B3LYP/6-311+G(2d,2p) and MP2(full)/6-311+G(2d,2p) levels of theory were used to determine the relative enthalpies and Gibbs free energies at 298 K of each conformer (B3LYP) and the 12 most stable conformers of the protonated complexes (MP2(full)) including zero-point energy (ZPE) and thermal corrections determined using harmonic vibrational frequencies calculated at the B3LYP/6-31G(d) level of theory. Theoretical linear IR spectra were generated using the calculated harmonic vibrational frequencies (scaled by a factor of 0.96, corresponding to the scale factor recommended for vibrational frequencies by Foresman and Frisch [65]) and IR intensities. Before comparison with the measured IRMPD spectra, the calculated vibrational frequencies are convoluted with a 20 cm^{-1} fwhm Gaussian line shape.

3. Results

3.1. IRMPD action spectroscopy

IRMPD of the H^+(U) and H^+(*x*SU) complexes results in the formation of a variety of product ions, which are listed in Table 1. The

Table 1
Reactant and product ions observed upon IRMPD.

Complex	Reactant ion *m*/*z*	Product ions *m*/*z*	Fragment ion or neutral loss
H^+(U)	113	96	NH_3
		70	HOCN
H^+(2SU)	129	112	NH_3
		84	NH_3 and CO
		70	HSCN
H^+(5Me2SU)	143	126	NH_3
H^+(6Me2SU)	143	126	NH_3
		98	NH_3 and CO
H^+(4SU)	129	112	NH_3
		102	C_2H_3
		86	HOCN
		69	C_3SH^+
		59	$CSNH^+$
H^+(24dSU)	145	128	NH_3
		86	HSCN
		69	C_3SH^+

loss of NH_3 was observed throughout the entire series. Sequential loss of CO from this primary fragment ion was observed only in the case of H^+(2SU) and H^+(6Me2SU). The loss of HOCN was observed for H^+(U) and H^+(4SU), while the loss of HSCN was observed for H^+(2SU) and H^+(24dSU). Although not definitive in several cases, these latter neutral losses appear to arise from C2 and N3 of the ring with their respective functional groups. Interestingly, the formation of C_3SH^+ is only observed for the H^+(4SU) and H^+(24dSU) complexes, suggesting that this fragment probably arises from the 4-thioketo moiety. IRMPD of H^+(4SU) also occurs via two additional fragmentation pathways, the loss of C_2H_3 and formation of $HSCN^+$, in addition to the products mentioned above. It is also worth mentioning that the IRMPD pathways observed for each system investigated here exhibit the same wavelength dependence.

An IRMPD yield was determined from the precursor intensity (I_p) and the sum of the fragment ion intensities (I_f) after laser irradiation at each frequency as shown in Eq. (1)

$$\text{IRMPD yield} = \frac{\sum I_f}{I_p + \sum I_f} \tag{1}$$

The IRMPD yield was normalized linearly with laser power to correct for the change in the laser power as a function of photon energy, i.e., the wavelength of the FEL. IRMPD spectra were obtained for H^+(U) and H^+(xSU), where xSU = 2SU, 5Me2SU, 6Me2SU, 4SU, and 24dSU, over the range from ~1000 to 1900 cm^{-1} and are shown in Fig. 2. As can be seen in the figure, these complexes exhibit both similar and distinct spectral features. The absence of an intense carbonyl stretch at ~1825 cm^{-1} for the H^+(U), H^+(2SU), H^+(5Me2SU), and H^+(6Me2SU) complexes is a clear indication that the binding of a proton results in preferential stabilization of an alternative tautomer where the keto, (thioketo) functional groups have been converted to hydroxyl (sulfhydryl) groups in these complexes. Upon thioketo-substitution at the 2-position of H^+(U), the spectrum becomes slightly more complex and features in the range from ~1450 to 1650 cm^{-1} are severely broadened. Methylation at the 5-position of H^+(2SU) results in slightly red shifted but better resolved spectral features between ~1450 and 1650 cm^{-1}, while new distinct features arise over the range from ~1100 to 1400 cm^{-1}. Methylation at the 6-position of H^+(2SU) results in similar behavior, red shifting but only slightly resolved peaks between ~1450 and 1650 cm^{-1} as compared to H^+(2SU). Interestingly, the new spectral features observed over the range between ~1100 and 1400 cm^{-1} upon 5-methylation are not observed when the methyl group is substituted at the 6-position, i.e., it is easy to distinguish the site of methylation in the IRMPD action spectra of these systems. The IRMPD action spectrum of H^+(4SU) is markedly different than the spectra for H^+(U) and H^+(2SU) and its methylated analogs. The H^+(4SU) complex is the only nucleobase examined here that exhibits a carbonyl stretch at ~1825 cm^{-1}. This suggests that the binding of a proton to 4-thiouracil is very different than to uracil and 2-thiouracil. Finally, as expected the IRMPD action spectrum of H^+(24dSU) exhibits distinct features that are very different from the other five IRMPD spectra due to the substitution of both oxygen atoms by sulfur atoms. Unfortunately, it is difficult to distinguish whether 2,4-dithiouracil retains a thioketo group upon binding of a proton from its IRMPD action spectrum, because unlike the carbonyl functional group, the thioketo moiety does not exhibit a strong IR absorption band. Because the IRMPD yield is obtained by calculating the fragmentation yield of each protonated complex, comparison of the IRMPD yields in Fig. 2 provides insight into the relative stability and reactivity of each protonated complex as a function of thioketo- and methyl-substitution of uracil. The IRMPD yield increases upon 2-thioketo-substitution suggesting that this substitution increases the reactivity (or decreases the stability) of uracil. Methylation at the 5- or 6-position of 2-thiouracil results in a decrease in the IRMPD yield, suggesting that methylation decreases the reactivity of 2-thiouracil. In contrast to that found for 2-thioketo-substitution, the IRMPD yield decreases upon 4-thioketo-substitution suggesting that this substitution decreases the reactivity, or stabilizes uracil. In accordance with these observations, the IRMPD yield for H^+(24dSU) is similar to that found for uracil as a result of the opposing tendencies of 2-thioketo and 4-thioketo-substitution.

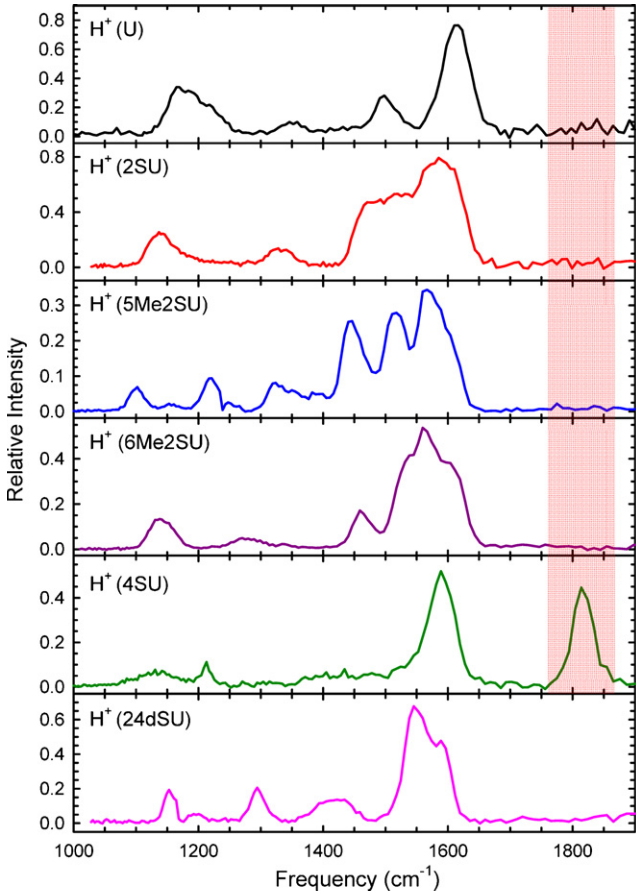

Fig. 2. Infrared multiple photon dissociation action spectra of H^+(U) and H^+(xSU) complexes.

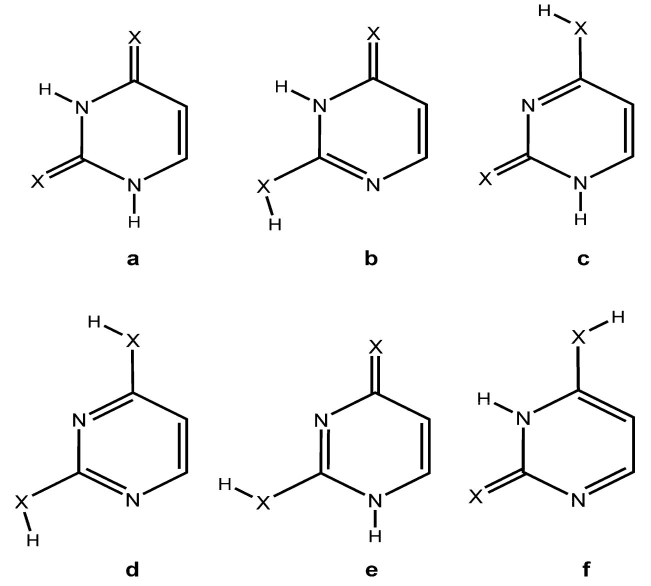

Fig. 3. Structures of the six most stable tautomeric forms of neutral uracil (U) and the thiouracils (*x*SU), where *x*SU = 2-thiouracil (2SU), 5-methyl-2-thiouracil (5Me2SU), 6-methyl-2-thiouracil (6Me2SU), 4-thiouracil (4SU), and 2,4-dithiouracil (24dSU), based on theoretical calculations at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level of theory. The oxygen and sulfur atoms are indicated by X.

3.2. Theoretical results

The six most stable tautomeric forms of neutral uracil and the thiouracils at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level of theory are shown in Fig. 3. Including the rotamers of each stable tautomer shown in Fig. 3 leads to a total of 13 possible low-energy tautomeric structures of uracil and each of the thiouracils, which are shown along with their relative Gibbs free energies in Fig. 1S of the Supplementary data. In all cases, the ground-state structure is the canonical tautomer, **a**, while the next most stable tautomers are 25.8–45.6 kJ/mol higher in free energy. Based on the relative stabilities of the neutral nucleobases and the results of previous theoretical studies, protonation of the canonical **a** tautomers is expected to occur preferentially at the O4 or S4 position [30,31,60,66,67]. However, it should be kept in mind that all complexes were produced by electrospray ionization from polar solutions, and the relative stabilities of the various tautomers can vary markedly in polar solvents and may be strongly influenced by protonation. A similar IRMPD spectroscopy and theoretical study of protonated uracil by Salpin et al. [32] found that the ground-state conformation of H^+(U) is a dihydroxy tautomer, corresponding to protonation of a keto–hydroxy tautomer rather than the canonical diketo tautomer of uracil.

The 12 most stable tautomeric forms of H^+(U) and the H^+(*x*SU) complexes based on theoretical calculations at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level of theory are shown in Fig. 4. Enthalpies and Gibbs free energies of these species relative to the ground-state tautomeric conformation calculated at the B3LYP/6-311+G(2d,2p) and MP2(full)/6-311+G(2d,2p) levels of theory, including zero-point energy (ZPE) and thermal corrections at 298 K, are listed in Table 2 for H^+(U) and the five H^+(*x*SU) complexes investigated here. As can be seen in Table 2, the absolute relative stabilities of the various tautomeric conformations of each of the protonated nucleobases differ slightly between the B3LYP and MP2(full) theories, but the trends in the relative stabilities are generally preserved. Further, discrepancies between the B3LYP and MP2(full) results are generally small and primarily occur for relatively high-lying tautomeric conformations, which are unlikely to be accessed in the IRMPD experiments. A significant exception to this agreement is found for the H^+(4SU) complex, where the relative stabilities of the three most stable tautomeric conformations differ. Because the B3LYP calculations are more exhaustive, and the MP2(full) results generally parallel the B3LYP results, the following discussion is based on the B3LYP results except as noted.

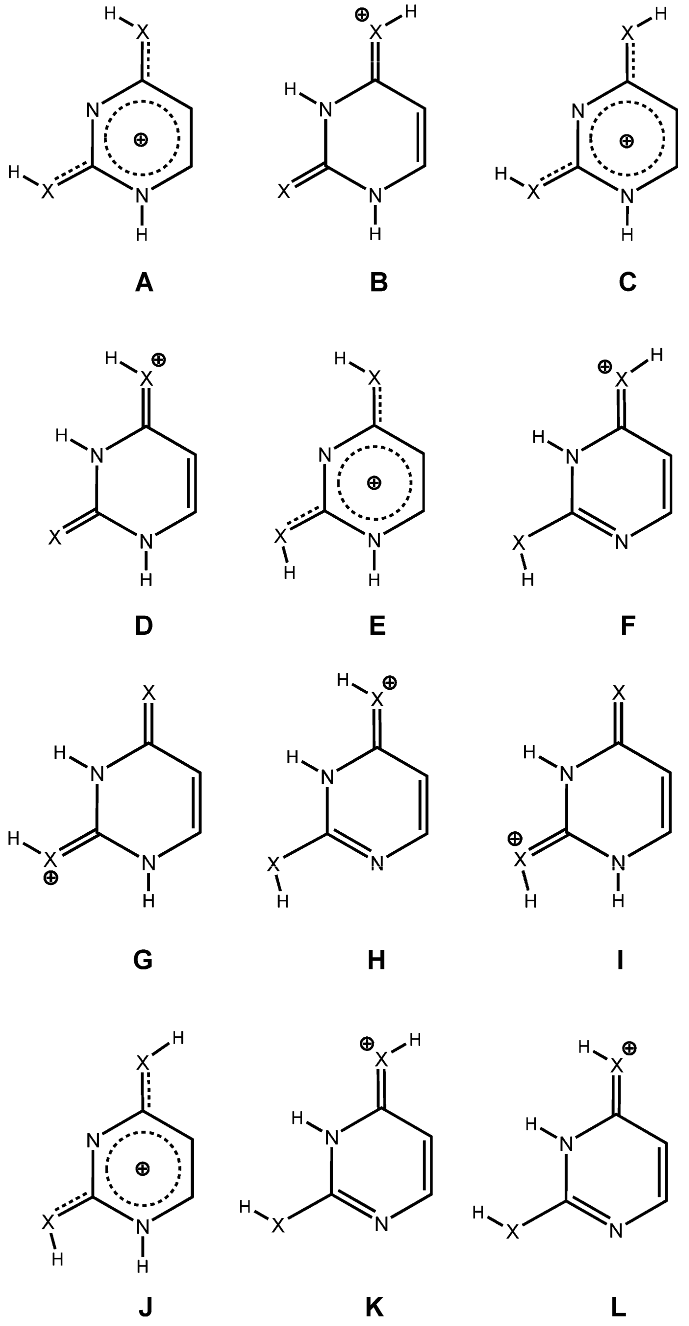

Fig. 4. Structures of the 12 most stable tautomeric forms of protonated uracil, H^+(U), and the protonated thiouracils, H^+(*x*SU), where *x*SU = 2-thiouracil (2SU), 5-methyl-2-thiouracil (5Me2SU), 6-methyl-2-thiouracil (6Me2SU), 4-thiouracil (4SU), and 2,4-dithiouracil (24dSU), based on theoretical calculations at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level of theory.

In all cases except for 4-thiouracil, the most stable conformation of the protonated nucleobase is the **A** conformer shown in Fig. 4, where the hydrogen atoms are bound to the N1, O2 (S2), and O4 (S4) positions, with both of the O2 (S2) and O4 (S4) hydrogen atoms oriented toward the adjacent N3 group. Consistent with the other nucleobases, the MP2(full) results suggest that A is also the ground-state tautomeric con-

Table 2
Relative enthalpies and Gibbs free energies of the twelve most stable tautomeric forms of protonated uracil and thiouracils.

Complex	Tautomer[a]	B3LYP		MP2(full)	
		ΔH_{298} (kJ/mol)[b]	ΔG_{298} (kJ/mol)[b]	ΔH_{298} (kJ/mol)[c]	ΔG_{298} (kJ/mol)[c]
H^+(U)	A	0.0	0.0	0.0	0.0
	B	6.5	5.6	10.9	10.1
	C	16.5	16.2	16.7	16.5
	D	17.7	16.5	22.3	21.2
	E	31.0	30.1	31.5	30.5
	F	31.3	31.0	32.5	32.2
	G	45.2	43.1	43.6	41.5
	H	48.0	47.0	49.4	48.4
	I	50.4	47.9	48.5	46.0
	J	51.7	50.4	52.5	51.2
	K	67.4	66.0	69.5	68.1
	L	88.2	85.5	90.8	88.1
H^+(2SU)	A	0.0	0.0	0.0	0.0
	E	6.9	6.8	7.6	7.5
	C	17.7	17.6	18.2	18.0
	B	19.6	20.5	20.4	21.2
	J	26.4	26.0	27.7	27.3
	G	30.0	27.0	29.6	26.6
	D	30.7	31.3	31.6	32.2
	F	31.7	31.5	32.7	32.4
	I	32.0	28.7	31.5	28.2
	K	40.4	40.0	42.3	41.8
	H	49.3	48.3	50.3	49.3
	L	59.5	58.0	61.5	60.0
H^+(5Me2SU)	A	0.0	0.0	0.0	0.0
	E	6.5	6.1	7.2	6.9
	C	19.8	20.2	20.1	20.4
	B	25.5	26.5	27.9	28.9
	G	26.1	27.0	27.7	22.1
	I	27.9	27.4	29.5	26.3
	J	28.1	28.0	29.2	29.1
	F	34.8	33.8	34.9	34.0
	D	36.0	36.3	38.2	38.5
	K	43.4	42.3	44.5	43.3
	H	51.3	49.3	51.1	49.1
	L	61.4	58.9	62.3	59.8
H^+(6Me2SU)	A	0.0	0.0	0.0	0.0
	E	7.3	7.4	8.0	8.0
	B	17.0	17.0	19.1	19.2
	C	18.0	17.9	18.4	18.4
	J	27.0	26.8	28.2	27.9
	D	27.8	27.8	30.0	30.0
	F	29.9	29.2	31.5	30.8
	G	32.4	29.0	31.4	28.0
	I	34.7	31.7	33.5	30.5
	K	37.9	36.6	40.5	39.2
	H	47.1	45.6	48.6	47.1
	L	56.4	54.0	59.1	56.7
H^+(4SU)	B	0.0	0.0	2.4	1.2
	D	1.5	1.6	4.4	3.3
	A	1.7	3.0	0.0	0.0
	C	5.4	6.6	4.0	4.0
	F	25.0	25.4	22.0	21.2
	H	28.6	28.5	26.1	24.9
	E	34.1	34.2	33.1	32.0
	J	39.5	39.5	38.9	37.8
	G	58.0	58.4	49.5	48.8
	K	62.3	61.2	60.0	57.7
	I	62.3	62.2	53.6	52.3
	L	67.3	65.4	65.7	62.6
H^+(24dSU)	A	0.0	0.0	0.0	0.0
	C	3.8	3.7	4.1	4.1
	E	7.0	6.7	7.9	7.6
	B	10.3	10.7	10.5	10.9
	J	11.6	11.3	13.0	12.7
	D	11.6	12.1	12.3	12.7
	F	23.6	22.5	22.0	20.9
	H	27.5	25.9	26.4	24.8
	K	32.7	31.2	31.9	30.5
	L	37.2	35.1	37.0	34.9
	G	39.5	36.7	33.8	31.0
	I	41.3	37.0	35.6	31.2

[a] Tautomer designations are based on the structures shown in Fig. 4.
[b] Calculated at the B3LYP/6-311+G(2d,2p) level of theory.
[c] Calculated at the MP2(full)/6-311+G(2d,2p) level of theory.

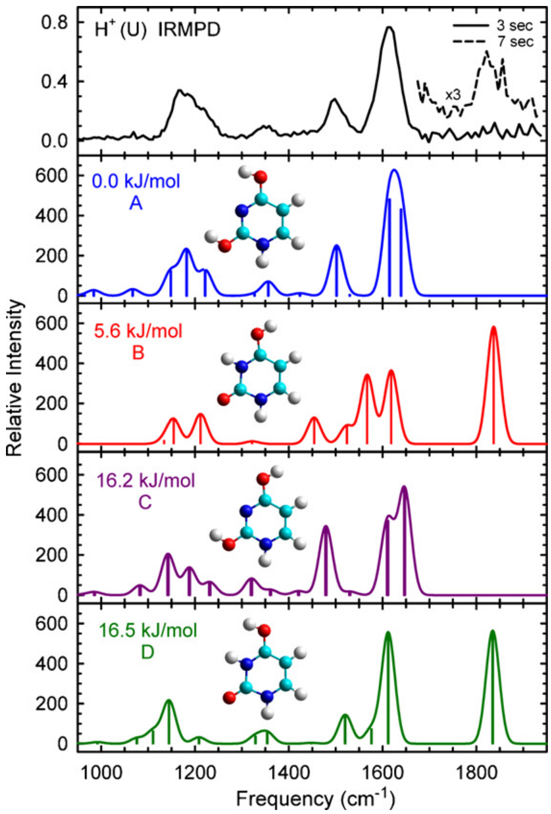

Fig. 5. Comparison of the measured IRMPD action spectrum of H$^+$(U) with the linear IR spectra predicted for the four most stable conformers of H$^+$(U) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative stabilities of each conformer are also shown.

formation of H$^+$(4SU). Compared to the tautomeric forms of the neutral nucleobases shown in Fig. 3, this conformer must arise from the binding of a proton to the **c** (keto–hydroxy, thioketo–hydroxy, or thioketo–sulfhydryl tautomer) or **e** (hydroxy–keto, sulfhydryl–keto, or sulfhydryl–thioketo tautomer) conformers, which lie 42.5–52.6 kJ/mol and 67.1–78.9 kJ/mol higher in free energy than the ground-state canonical tautomeric conformations, respectively. Alternatively, protonation could also occur to the canonical tautomer, **a,** at the 4-position, followed by proton transfer and rearrangement facilitated by the polar solvent or acid catalyzed reaction, resulting in the more stable protonated **A** conformer. In contrast, the ground-state structure of protonated 4-thiouracil corresponds to the **B** conformer, which results from the binding of a proton at the S4 position of the canonical keto–thioketo conformer of 4-thiouracil with the proton directed away from the adjacent N3H group. The ground-state structures determined here for H$^+$(U), H$^+$(2SU), H$^+$(4SU), and H$^+$(24dSU) are consistent with those previously identified [27–29,60] using different basis sets and level of theories. Based on the computed ground-state conformers of the protonated nucleobases, the absence of the carbonyl stretch at ~1825 cm^{-1} in the IRMPD action spectra for the H$^+$(U), H$^+$(2SU), H$^+$(5Me2SU), and H$^+$(6Me2SU) complexes is easily understood.

Only three low-energy conformers were found that lie within 20 kJ/mol of the corresponding ground-state conformer for all complexes except H$^+$(24dSU), where five such low-energy conformers were found. The theoretical linear IR spectra of these conformers are compared to the experimental IRMPD action spectra to determine the conformation(s) of the protonated complexes accessed in the experiments. The ground-state conformation and the three lowest-energy conformers of each protonated complex and their relative Gibbs free energies are shown in Figs. 5 through 10, along with their calculated linear IR spectra. Structures and relative Gibbs free energies for all tautomeric conformations of protonated uracil and each of the protonated thiouracils computed in this study are provided in Fig. 2S of the Supplementary data.

3.2.1. H$^+$(U)

As discussed the above, the ground-state structure of H$^+$(U) is the **A** conformer shown in Fig. 4, where the hydrogen atoms are bound to the N1, O2, and O4 positions, and with both the O2 and O4 hydrogen atoms oriented toward the adjacent N3 group. Proton binding at the O4 position of the canonical diketo conformer, **a**, of uracil with the proton directed away from the adjacent N3H group results in the **B** conformer, which is less favorable by 5.6 kJ/mol of free energy compared to the ground-state **A** conformer. The next most stable conformer, **C**, corresponds to a rotamer of the ground-state **A** conformer, where the hydrogen atom at the O4 position is directed away from the adjacent N3 group. Rotation of the hydrogen atom at the O4 position destabilizes the complex by 16.2 kJ/mol relative to the ground-state **A** conformer. The 4-hydroxyl rotamer of the first-excited canonical **B** conformer, where the hydrogen atom is directed toward the adjacent N3H group, the **D** conformer, lies 16.5 kJ/mol above the ground-state **A** conformer.

3.2.2. H$^+$(2SU)

As discussed the above, the ground-state structure of H$^+$(2SU) is the **A** conformer shown in Fig. 4, where the hydrogen atoms are bound to the N1, S2, and O4 positions, and with both the S2 and O4 hydrogen atoms oriented toward the adjacent N3 group. The first two excited low-energy conformers of H$^+$(2SU), the **E** and **C** conformers, are both rotamers of the ground-state conformer **A**. In the first excited-state **E** conformer, the hydrogen atom at the S2 position is flipped away from the adjacent N3 group, which destabilizes this conformer by 6.8 kJ/mol relative to the ground-state **A** conformer. The hydrogen atom at the O4 position is oriented away from the adjacent N3 moiety in the next most stable conformer, **C**, which results in destabilization by 17.6 kJ/mol relative to the ground-state **A** conformer. Proton binding to the canonical thioketo–keto **a** conformer at the O4 position with the proton directed away from the adjacent N3H group, the **B** conformer, is less favorable than the ground-state **A** conformer by 20.5 kJ/mol.

3.2.3. H$^+$(5Me2SU)

The low-energy structures of H$^+$(5Me2SU) are very similar to those of H$^+$(2SU). The ground-state structure of H$^+$(5Me2SU) is again the **A** conformer shown in Fig. 4, where the hydrogen atoms are bound to the N1, S2, and O4 positions, and with both the S2 and O4 hydrogen atoms oriented toward the adjacent N3 group. The first two excited conformers, **E** and **C**, are again rotamers of the ground-state **A** conformer. As previously found for H$^+$(2SU), rotation of the sulfhydryl group results in less destabilization than rotation of the hydroxyl group, producing conformers that lie 6.1 kJ/mol (**E**) and 20.2 kJ/mol (**C**) above the ground-state **A** conformation, respectively. Binding of a proton to the O4 position of the canonical thioketo–keto conformer, resulting in conformer **B**, is 26.5 kJ/mol less favorable than the ground-state **A** conformer.

3.2.4. H$^+$(6Me2SU)

The relative stabilities of the low-energy conformers of H$^+$(6Me2SU) differ slightly from those of H$^+$(2SU) and H$^+$(5Me2SU). The ground-state conformer is again the A conformer, while the first-excited state is again the sulfhydryl rotamer, **E**, which lies 7.4 kJ/mol above the ground-state **A** conformer. However, the hydroxyl rotamer, **C**, which lies 17.9 kJ/mol higher in free energy than the ground-state **A** conformer, is less favorable than the protonated canonical thioketo–keto conformer, **B**, by 0.9 kJ/mol.

3.2.5. H^+(4SU)

The ground-state structure of protonated 4-thiouracil corresponds to the **B** conformer shown in Fig. 4, where the hydrogen atoms are bound to the N1, N3, and S4 positions, and the S4 hydrogen atom is oriented away from the adjacent N3H group. Similar to the other protonated nucleobases, three conformers within 20 kJ/mol of the ground-state **B** conformer were found for H^+(4SU). Rotation of the sulfhydryl group towards the neighboring N3H moiety of the ground-state **B** conformer results in conformer **D**, which lies 1.6 kJ/mol higher in energy. Although the ground-state **B** conformer of H^+(4SU) differs from that found for all of the other protonated complexes, the conformer analogous to the ground-state **A** conformer of H^+(U), H^+(2SU), H^+(5Me2SU), H^+(6Me2SU), and H^+(24dSU) only lies 3.0 kJ/mol above the ground-state protonated canonical keto–thioketo tautomer, **B**. The sulfhydryl rotamer, conformer **C**, of this low-energy excited-state conformer is just slightly less favorable, and lies 6.6 kJ/mol above the ground-state **B** conformation. As discussed above, the MP2(full) results provide a different order of relative stabilities for the low-energy conformers of H^+(4SU). The MP2(full) results suggest that the **A** conformer is the ground-state tautomeric conformation, while the **B**, **D**, and **C** conformers lie 1.2, 3.3, and 4.0 kJ/mol higher in energy, respectively. The change in the order of relative stabilities of these tautomeric conformations is likely the result of their very similar stabilities. The preservation of the order of relative stabilities for the other protonated nucleobase complexes is achieved because the low-energy conformers differ more significantly in stability.

3.2.6. H^+(24dSU)

The ground-state structure of H^+(24dSU) is the **A** conformer shown in Fig. 4, where the hydrogen atoms are bound to the N1, S2, and S4 positions, and both the S2 and S4 hydrogen atoms are oriented toward the adjacent N3 group. In contrast to other protonated complexes, five conformers were found for the H^+(24dSU) complex that lie within 20 kJ/mol of the ground-state conformation. The first two excited conformers, **C** and **E**, are both sulfhydryl rotamers of the ground-state **A** conformation, where the conformer that results from rotation of the hydrogen atom at the S4 position away from the adjacent N3 group, conformer **C**, is slightly more stable than the conformer derived from rotation of the hydrogen atom at the S2 position, conformer **E**, which lie 3.7 and 6.7 kJ/mol above the ground-state conformer, respectively. Binding of a proton to the canonical dithioketo **a** tautomer at the S4 position with the proton oriented away from the adjacent N3H group, conformer **B**, lies 10.7 kJ/mol higher than the global minimum. Rotation of both sulfhydryl groups at the S2 and S4 positions of the ground-state conformer away from the neighboring N3 group results in a rotamer, conformer **J**, that lies 11.3 kJ/mol above the ground-state conformation (Fig. 2S). The sulfhydryl rotamer of the protonated canonical dithioketo tautomer, conformer **D**, lies 12.1 kJ/mol above the ground-state conformation (Fig. 2S).

4. Discussion

4.1. Comparison of experimental and theoretical IR spectra of H^+(U)

The experimental IRMPD action spectrum along with the calculated IR spectra and structures of the four most stable tautomeric conformations found for the H^+(U) complex are compared in Fig. 5. This comparison shows excellent agreement between the IRMPD action spectrum and the calculated linear IR spectrum for the ground-state conformer, **A**. In particular, the bands at 1130–1250, 1355, 1500, and 1625 cm^{-1} match almost perfectly, confirming that the ground-state dihydroxy tautomer is the dominant conformer accessed in the experiments. Comparison of the calculated IR spectrum of the first excited-state protonated canonical diketo conformer, **B**, to the IRMPD action spectrum shows no obvious similarity or contributions from IR features in the region of 1100–1670 cm^{-1} from this tautomer. However, the presence of the minor band in the IRMPD spectrum at ~1825 cm^{-1}, which appears when the irradiation time is extended from 3 to 7 s, corresponds to the carbonyl stretch, and the shoulder at ~1550–1600 cm^{-1} that corresponds well with the peak at ~1570 cm^{-1} of the first excited-state conformer, **B**, suggest that this tautomer is present in very minor abundance in the experiments. The calculated IR spectrum of the 4-hydroxyl rotamer of the ground-state conformer, conformer **C**, exhibits several bands that are red shifted compared to the ground-state structure, e.g., the bands at 1320, 1480, and 1610 cm^{-1}, and one band at 1649 cm^{-1} that is blue shifted. These red and blue shifted bands are not observed in the IRMPD action spectrum, as the presence of these bands would sufficiently broaden the experimental spectrum. Therefore, this rotamer is most likely not present in the experiments. This is a good example demonstrating the ability of DFT calculations and IRMPD action spectroscopy to facilitate differentiation of hydroxyl rotamers. Interestingly, the IR spectrum of the third excited-state conformer, **D**, exhibits many peaks that correspond well with the experimental spectrum, specifically the small peaks at ~1210 and 1350 cm^{-1}, and the large band at 1610 cm^{-1}. However, the peak at 1145 cm^{-1} is red shifted, and the peak at 1520 cm^{-1} is blue shifted compared to the experimental spectrum. These comparisons suggest that the **D** conformer is also not accessed in the experiments. A transition state between the first and third excited-state conformers, **B** and **D**, was located using the synchronous transit-guided quasi-Newton (STQN) method of Peng et al. [68] and lies 32.3 kJ/mol above the first excited-state **B** conformer (or 37.9 kJ/mol above the ground-state **A** conformer). Although a small abundance of the first excited-state **B** conformer may be accessed in the experiments, the excitation energy needed to overcome the barrier to access the third excited-state **D** conformer exceeds the energy available to this complex at room temperature. Therefore, the **D** conformer is most likely not populated in the experiments.

Compared to the IRMPD spectrum of H^+(U) collected by Salpin et al. [32] at the Free Electron Laser facility of the Centre Laser Infrarouge d'Orsay (CLIO), France, using an electrospray ionization quadrupole ion trap mass spectrometer, the IRMPD action spectrum measured here is slightly blue shifted and exhibits slightly better agreement with theory when the same scaling factor of 0.96 and 20 cm^{-1} fwhm Gaussian line shape is applied to the vibrational frequencies of conformer **A**. The peaks at ~1180 and 1615 cm^{-1} in our spectrum are slightly broader, and nicely match the coupling of the closely spaced peaks in these regions and the small abundance of the first excited-state conformer. In both IRMPD spectra, a weak absorption band is observed at ~1825 cm^{-1}, confirming that a small population of the protonated form of the canonical diketo tautomer of uracil is accessed in the experiments.

The broad spectral feature in the measured IRMPD action spectrum and the calculated spectrum for the ground-state **A** conformer of H^+(U) at ~1130–1250 cm^{-1} is composed of three absorption bands at 1150, 1185, and 1220 cm^{-1}. These bands correspond to the in-plane symmetric O–H bending of both hydroxyl groups, in-plane O–H bending of the 2-hydroxyl group, and the same bending motion by the 4-hydroxyl group, respectively. The small peak observed at 1355 cm^{-1} is due to the mixed character mode that arises from in-plane N1–H bending and asymmetric stretching of the C2=N3=C4 moiety. The IR absorption band at 1500 cm^{-1} corresponds to the C4–OH stretch. Finally, the two IR absorption bands that result in the most intense peak in the experimental spectrum

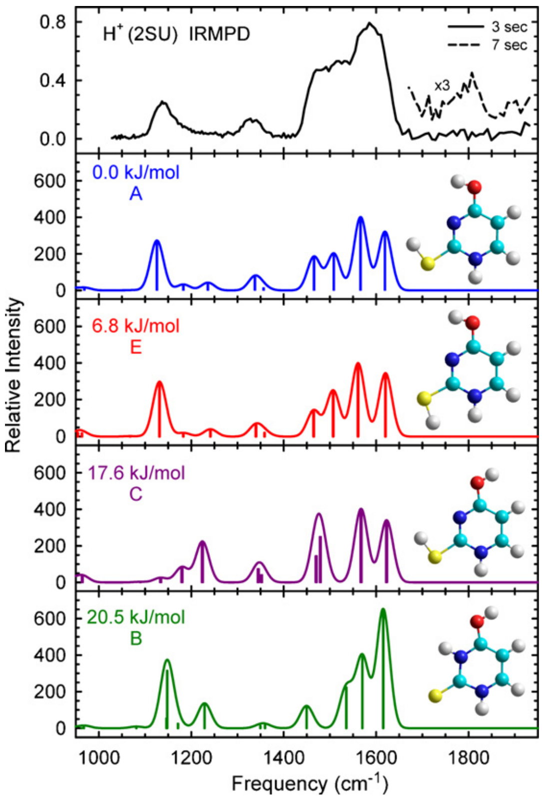

Fig. 6. Comparison of the measured IRMPD action spectrum of H⁺(2SU) with the linear IR spectra predicted for the four most stable conformers of H⁺(2SU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative stabilities of each conformer are also shown.

are the C2–OH stretch (1615 cm^{-1}), and a mixed character mode involving coupling of C5═C6 and C2═N3 stretching (1640 cm^{-1}).

4.2. Comparison of experimental and theoretical IR spectra of H⁺(2SU)

The experimental IRMPD action spectrum is compared to the calculated IR spectra and structures of the four most stable conformers found for the H⁺(2SU) complex in Fig. 6. The ground-state **A** conformer and the first-excited 2-sulfhydryl rotamer provide the best match to the experimental IRMPD action spectrum. Specifically the bands at 1125 and 1340 cm^{-1}, and the broad IR feature at ∼1440–1670 cm^{-1} in the measured IRMPD spectrum closely resemble the coupling of the four IR absorption bands at 1465, 1510, 1565, and 1620 cm^{-1} of the calculated linear IR spectra. It should be noted here that broadening of infrared absorption bands is often observed in IRMPD spectra as a result of anharmonicity of the vibrational potential, especially when two or more bands lie close to each other [69]. Although such broadening could potentially complicate the IRMPD spectrum, these spectral signatures may be useful in understanding the effects of anharmonicity in the potential that may not be easily predicted by theoretical calculations. Again, the calculated spectra do not allow differentiation of the sulfhydryl rotamers. Although the bands in the theoretical spectrum of the first excited-state **E** conformer at 1130 and 1240 cm^{-1} are slightly blue shifted, and the band at 1560 cm^{-1} is slightly red shifted compared to the theoretical spectrum of the ground-state **A** conformer, these shifts are sufficiently small relative to the broadening of spectral features in the measured IRMPD action spectrum that the presence or absence of the first excited-state **E** rotamer (6.8 kJ/mol) cannot be ruled out. The transition state between the **A** and **E** rotamers exhibits an activation energy of 27.9 kJ/mol, which just slightly exceeds the average internal energy of the ground-state **A** conformer at room temperature. Therefore, it is plausible that a small abundance of the first excited-state **E** rotamer contributes to the experimental spectrum. As seen for H⁺(U), the theoretical spectra permit differentiation of hydroxyl rotamers. Clearly, the hydroxyl rotamer of the ground-state conformer, conformer **C**, is not accessed in the experiments as the peaks at 1180 and 1225 cm^{-1} in the theoretical spectrum are not observed in the IRMPD action spectrum. The relative energies of these species at 298 K, and the barrier to interconversion of the **A** and **C** rotamers, suggest that the **C** rotamer is not accessed even though the broadening at ∼1440–1670 cm^{-1} makes the comparison in this region of the IR spectrum difficult. Interestingly, the theoretical IR spectrum of the third excited-state conformer, the thioketo–hydroxyl conformer, **B**, exhibits many IR features that fit well with the measured IRMPD spectrum. In particular, the bands at 1420–1650 cm^{-1} are very similar to the broadened region in the IRMPD spectrum, and the peak at 1150 cm^{-1} also corresponds well. However, the absence of the peak or small shoulder at ∼1230 cm^{-1} in the experimental IRMPD action spectrum indicates that this conformer is most likely not accessed in the experiments. Similar to that observed for the H⁺(U) complex, a very minor band at ∼1810 cm^{-1} is observed in the IRMPD spectrum of H⁺(2SU) when the irradiation time is extended from 3 to 7 s. The presence of this band may suggest that a very minor population of a conformer possessing a free keto group is also accessed under the experimental conditions employed. However, the intensity of this band does not differ appreciably from the noise level in this region. In addition, the most stable conformers possessing a free keto group, conformers **G** and **I**, are calculated to lie 27.0 and 28.7 kJ/mol above the ground-state conformer. Together these results suggest that either, the very minor band at ∼1810 cm^{-1} can be attributed to noise, or that theory underestimates the stability of these conformers.

Comparison of the IRMPD action spectrum to the theoretical IR spectrum of the ground-state **A** conformer of H⁺(2SU) suggests that the small absorption band at 1130 cm^{-1} corresponds to the in-plane bending of O4–H and a very mild stretching of the C2–S bond. The small shoulder at 1330 cm^{-1} corresponds to the mixed character modes associated with in-plane N1–H bending, in-plane C6–H bending, and the asymmetric stretching of C2═N3═C4. The four IR absorption bands that make up the broad spectral feature at ∼1440–1670 cm^{-1} in the IRMPD spectrum correspond to the symmetric stretching of C2═N3═C4 (1468 cm^{-1}); combined C4═C5 stretching and in-plane N1–H bending modes (1510 cm^{-1}); coupling of in-plane N1–H bending and aromatic ring stretches (1568 cm^{-1}); and the combination of C2═N3 stretching and C5═C6 stretching at 1620 cm^{-1}.

4.3. Comparison of experimental and theoretical IR spectra of H⁺(5Me2SU)

The experimental IRMPD action spectrum along with the calculated IR spectra and structures of the four most stable conformers found for the H⁺(5Me2SU) complex are compared in Fig. 7. Compared to the IRMPD action spectrum of H⁺(2SU), the H⁺(5Me2SU) spectrum exhibits more distinct IR absorption peaks over the range from 1080 to 1410 cm^{-1}, while the spectral features in the range from 1410 to 1660 cm^{-1} are better resolved. Thus, methylation at the 5-position not only enhances the IR absorption of certain vibrations, it also leads to improved resolution of the spectral features in the region between 1410 and 1660 cm^{-1}. The calculated IR spectra of the ground-state and first excited-state conformers, **A** and **E**, provide the best match to the experimental spectrum. However, several peaks are slightly red shifted as compared to the measured IRMPD action spectrum. This could simply be due to theory under-

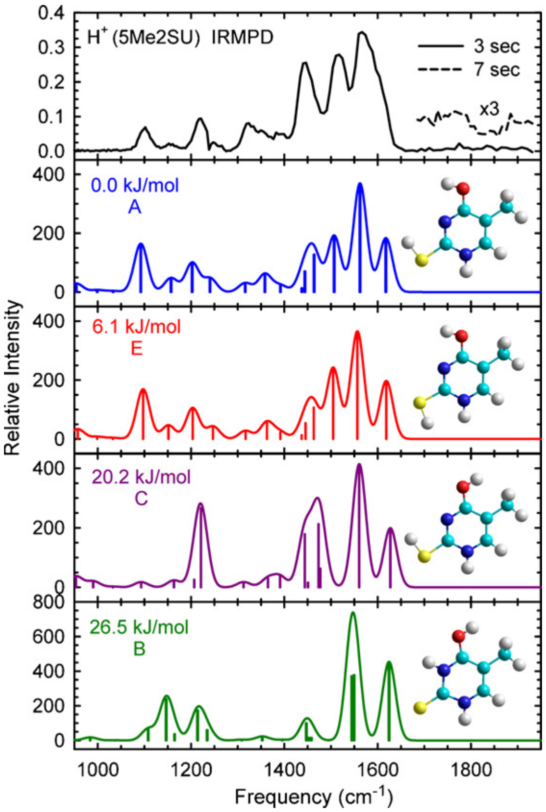

Fig. 7. Comparison of the measured IRMPD action spectrum of H^+(5Me2SU) with the linear IR spectra predicted for the four most stable conformers of H^+(5Me2SU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative stabilities of each conformer are also shown.

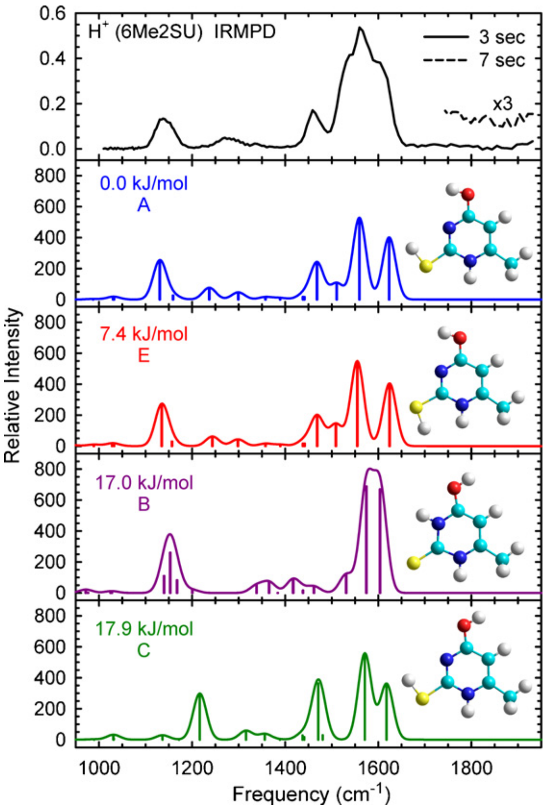

Fig. 8. Comparison of the measured IRMPD action spectrum of H^+(6Me2SU) with the linear IR spectra predicted for the four most stable conformers of H^+(6Me2SU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative stabilities of each conformer are also shown.

estimating the oscillator strength of these modes. Using the STQN method, an activation energy for interconversion of these rotamers was found to lie 24.8 kJ/mol higher in energy than the ground-state **A** conformer (and 18.7 kJ/mol higher than the first excited-state sulfhydryl rotamer, **E**). The ground-state **A** conformer should have sufficient internal energy at room temperature to overcome this relatively low barrier, making the first excited-state **E** rotamer readily accessible. A relatively high activation energy (48.8 kJ/mol) is found between the ground-state **A** conformer and the second excited-state hydroxyl rotamer, **C**. The high barrier is consistent with the measured IRMPD action spectrum, which suggests that the **C** conformer is most likely not accessed in the experiments. The absence of the 2-thioketo-4-sulfhydryl conformer, **B**, as supported by the comparison of the measured IRMPD action spectrum and the corresponding theoretical spectrum confirms that the binding of proton stabilizes an alternative tautomer of 5Me2SU rather than the canonical thioketo–keto tautomer.

Comparison of the vibrational modes found from the DFT calculations to the IRMPD action spectrum of H^+(5Me2SU) suggests that the in-plane O4–H bending motion is observed in the peaks at 1100 and 1220 cm^{-1}, and small shoulders at 1150 and 1250 cm^{-1} along with C2–S stretching, C6–N1 stretching, C4–C5 stretching, and C2–N1 stretching, respectively. The small broad feature at 1300–1410 cm^{-1} is composed of three weak absorption bands that correspond to in-plane C6-H bending, a mixed character mode involving C2=N3=C4 asymmetric stretching and in-plane N1–H bending, and the in-phase symmetric bending of the C–H bonds. The intense band observed at 1450 cm^{-1} is the result of two very closely spaced bands that are both assigned to the wagging of the methyl group along with C4–O stretching and the symmetric stretching of C2=N3=C4, respectively. The strong absorption band at 1520 cm^{-1} is slightly blue shifted compared to the peak in the theoretical spectrum that corresponds to the mixed character mode associated with C2=N3 stretching, C4=C5 stretching, and in-plane N1–H bending at 1510 cm^{-1}. The most intense band in the experimental spectrum at 1570 cm^{-1} appears to arise from two unresolved bands in the theoretical spectrum at 1560 and 1620 cm^{-1}, which correspond to a mixed character mode associated with in-plane N1–H bending and aromatic ring stretching, and the combination of C5=C6 and C2=N3 stretching.

4.4. Comparison of experimental and theoretical IR spectra of H^+(6Me2SU)

The experimental IRMPD action spectrum along with the calculated IR spectra and structures of the four most stable conformers found for the H^+(6Me2SU) complex are compared in Fig. 8. Interestingly, the IRMPD spectrum of H^+(6Me2SU) exhibits greater similarity to the spectrum of H^+(2SU) than that of H^+(5Me2SU). This suggests that methylation at the 6-position has a less pronounced effect on 2-thiouracil than methylation at the 5-position, i.e., no additional IR absorption peaks were observed in the range of 1100–1420 cm^{-1}, and the spectral features in the range of 1420–1660 cm^{-1} appear more congested. In this overall comparison, the theoretical spectra of the ground-state **A** conformer and the first excited-state sulfhydryl rotamer, **E**, are in best agreement with the experimental spectrum. The transition state located between the **A** and **E** rotamers confirms that the activation energy barrier (26.7 kJ/mol) is sufficiently low that with the internal energy available to the ground-state **A** conformer at room temperature, it is likely that a small abundance of the first excited-state sulfhydryl rotamer, **E**, also contributes to the experimental spectrum. Compar-

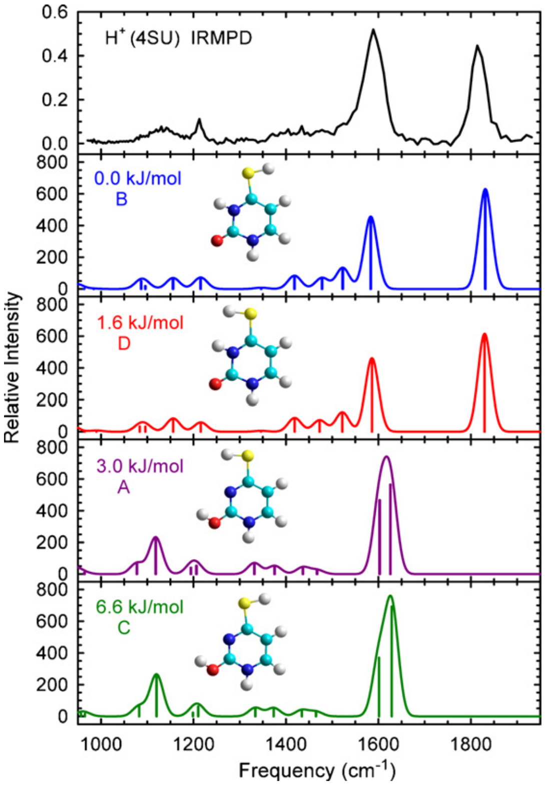

Fig. 9. Comparison of the measured IRMPD action spectrum of H$^+$(4SU) with the linear IR spectra predicted for the four most stable conformers of H$^+$(4SU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative stabilities of each conformer are also shown.

ison of the experimental spectrum and the calculated IR spectrum of the third excited-state hydroxyl rotamer, **C**, along with the high activation energy barrier found between the **A** and **C** rotamers (47.0 kJ/mol) again suggests that the **C** conformer is most likely not present in the experiments. The poor match between the experimental spectrum and the calculated IR spectrum of the thioketo–hydroxyl tautomer, **B**, is consistent with the behavior observed in all other complexes except for H$^+$(4SU), i.e., binding of a proton results in stabilization of an alternative tautomer rather than the canonical thioketo–keto tautomer.

The small peak at 1140 cm^{-1} of the IRMPD spectrum of H$^+$(6Me2SU) corresponds to in-plane O4–H bending and C2–S stretching, while the weak band at 1250–1300 cm^{-1} is most likely due to two adjacent IR absorptions that are calculated at 1235 and 1300 cm^{-1}, which are assigned to the mixed character modes associated with in-plane O4–H bending and C2═N3 stretching, and with C2═N3═C4 asymmetric stretching and C6═N1 stretching. The low-intensity peak at 1470 cm^{-1} is due to the symmetric stretching of C2═N3═C4 and the methyl group wagging mode. The broad IR absorption band at 1500–1660 cm^{-1} is due to three unresolved bands calculated at 1510, 1560, and 1620 cm^{-1} associated with mixed character modes involving: C2═N3 stretching, C4═C5 stretching, and in-plane N1–H bending; in-plane N1–H bending and aromatic ring stretching; and C5═C6 and C2–N3 stretching, respectively.

4.5. Comparison of experimental and theoretical IR spectra of H$^+$(4SU)

The experimental IRMPD action spectrum along with the calculated IR spectra and structures of the four most stable conformers found for the H$^+$(4SU) complex are compared in Fig. 9. The IRMPD action spectrum is in very good agreement with the calculated IR spectra of the ground-state, **B**, and first excited-state, **D**, conformers. These two conformers are merely rotamers of each other, and produce almost identical IR spectra in contrast to the previous case, where the hydroxyl rotamers exhibit distinct features in their IR spectra. Thus, the theoretical results suggest that sulfhydryl rotamers cannot be differentiated spectroscopically. Although it is impossible to determine whether or not the first excited-state **D** rotamer is accessed in the experiments by comparing the IRMPD action spectrum to the calculated IR spectra, the relative stabilities of these two conformers calculated at B3LYP/6-311+G(2d,2p) level of theory, as well as the transition state located between the ground-state **B** conformer and the first excited-state rotamer, **D** (30.2 kJ/mol), using the method mentioned previously, suggest that this complex should have sufficient internal energy at 298 K to interconvert the **B** and **D** rotamers. Therefore, both the ground-state **B** conformer and its sulfhydryl rotamer, **D**, are most likely accessed in the experiments. As mentioned in Section 3.2, binding of a proton to either the **c** or **e** tautomer of neutral 4-thiouracil results in the protonated **A** tautomer, shown in Fig. 4, which is only 3.0 kJ/mol less favorable than the ground-state **B** conformer. The calculated IR spectra of the **A** tautomer and its sulfhydryl rotamer, **C**, exhibit many similar spectral features that are present in the experimental spectrum; most notably the bands at 1120, 1200, and 1625 cm^{-1}. The appearance of these bands in the IRMPD spectrum may suggest that a small abundance of the second and third excited-state conformers, **A** and **C**, were accessed in the experiments. Theoretical IR spectra based on a statistically weighted averages of the calculated IR spectra of the **B**, **D**, **A**, and **C** low-energy conformers based on their relative Maxwell–Boltzmann populations at room temperature are compared with the experimental spectrum in Fig. 3S of the Supplementary data. Both theories exhibit very good agreement between the thermally weighted theoretical spectrum and the measured spectrum, confirming that multiple conformers were accessed in the experiments. However, the B3LYP spectrum provides a slightly better fit to the measured spectrum in terms of the band positions, and also exhibits better agreement with respect to the band intensities. This comparison suggests that the relative stabilities computed for these low-energy tautomeric conformations of H$^+$(4SU) at the B3LYP level of theory are probably more reliable than those determined at the MP2(full) level of theory.

In the calculated IR spectrum of the ground-state **B** conformer of H$^+$(4SU), the three weak IR absorption bands at 1090, 1155, and 1215 cm^{-1} correspond to the asymmetric stretching of N1═C2═N3, mixed character modes involving C2═N3 stretching and in-plane C5–H bending, and in-plane C6–H bending, respectively. While those at higher frequencies, 1420, 1480, and 1520 cm^{-1} are the result of in-plane N1–H bending, in-plane N3–H bending, and the coupling of C4═C5 and N1═C6 stretching. The most intense and characteristic peaks observed at 1585 and 1830 cm^{-1} are the C4═C5 stretch and the C2═O carbonyl stretch. In contrast, the IR absorption bands of the 2-hydroxy-4-sulfhydryl second excited-state **A** conformer are more complicated and involve many normal modes with mixed character. The low-intensity peaks at 1080, 1195, 1205, and 1440 cm^{-1} are all due to the combination of in-plane O2–H bending with the following modes: in-plane C5–H bending, in-plane asymmetric bending of the C5 and C6 hydrogen atoms, in-plane C6–H bending, and in-plane symmetric bending of the C5 and C6 hydrogen atoms, respectively. The modest peak at 1120 cm^{-1} corresponds to in-plane O2–H bending. Other low-intensity bands at 1330, 1375, and 1470 cm^{-1} correspond to the mixed character modes involving in-plane N1–H and C2═N3═C4 asymmetric stretching, coupling of the weak C2–OH stretch and in-plane symmetric bending of the C5 and C6 hydrogen atoms, and C2═N3═C4 symmetric stretching. The broad intense peak at 1570–1660 cm^{-1}

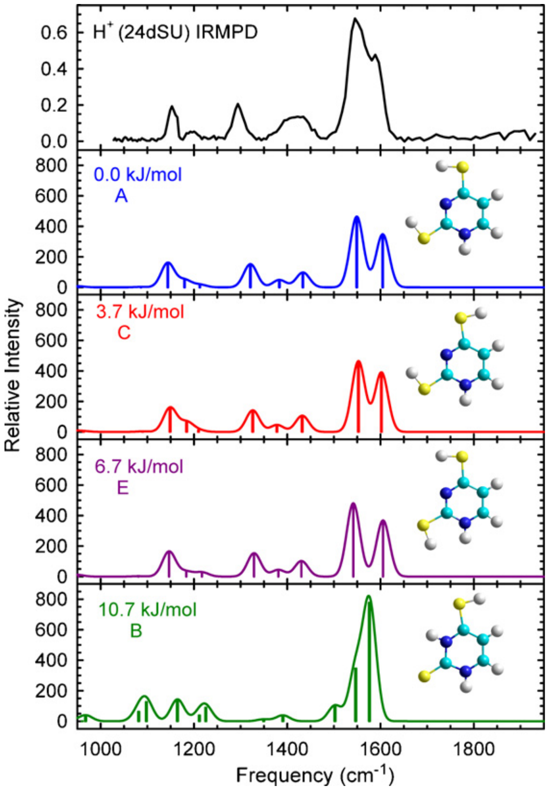

Fig. 10. Comparison of the measured IRMPD action spectrum of H$^+$(24dSU) with the linear IR spectra predicted for the four most stable conformers of H$^+$(24dSU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative stabilities of each conformer are also shown.

is the result of strong C2–OH stretching at 1600 cm^{-1}, and the combination of C2═N3 and C5═C6 stretching at 1625 cm^{-1}.

4.6. *Comparison of experimental and theoretical IR spectra of H$^+$(24dSU)*

The experimental IRMPD action spectrum is compared to the calculated IR spectra and structures of the four most stable conformers found for the H$^+$(24dSU) complex in Fig. 10. The IRMPD action spectrum is in good agreement with the calculated IR spectra of the ground-state, **A**, and first two excited-state, **C** and **E**, conformers as these structures are merely sulfhydryl rotamers. The two broad features between 1350 and 1470 cm^{-1} and between 1500 and 1630 cm^{-1} are less resolved, the peak at 1300 cm^{-1} is red shifted, and the peaks at 1150 and 1200 cm^{-1} are blue shifted and more resolved as compared to the theoretical spectra. Several peaks in the calculated IR spectra of all three rotamers are slightly red and blue shifted from each other, by ~1 cm^{-1}, however these shifts are too small to clearly establish the presence or absence of each rotamer in the experiments. In addition, the relative energies of these rotamers suggest that they should all be accessible at room temperature. The clear disagreement between the calculated IR spectrum of the 2-thioketo-4-sulfhydryl conformer, **B**, and the experimental spectrum confirms the absence of this tautomer and the stabilization of an alternative tautomer of 2,4-dithiouracil upon protonation.

Examining the vibrational information provided for H$^+$(24dSU) by DFT calculations, the peak at 1150 cm^{-1} appears to correspond to the mixed character mode associated with C2═N1 stretching, C4–S stretching, and C4═C5 stretching. The small shoulder at ~1200 cm^{-1} is possibly due to the coupling of two closely spaced IR absorption bands that correspond to the mixed character mode associated with C2–S stretching, C4–S stretching, in-plane asymmetric bending of the C5 and C6 hydrogen atoms, and the mixed character mode associated with in-plane C6–H and N1–H bending. The absorption band at 1295 cm^{-1} is the result of the C2═N3═C4 asymmetric stretching and in-plane N1–H bending. The small broadened feature in the region from 1350 to 1470 cm^{-1} is probably due to two unresolved IR absorption bands that correspond to the mixed character modes associated with symmetric stretching of C2═N3═C4 and in-plane C5–H bending, and C4═C5 stretching and in-plane symmetric bending of the C5 and C6 hydrogen atoms. The intense broad IR feature at ~1490–1630 cm^{-1} with a small dip at around 1585 cm^{-1} clearly indicates the merging of two closely spaced peaks, 1550 and 1605 cm^{-1}, in the calculated IR spectrum of the ground-state **A** conformer due to the anharmonic red shifting of the latter peak. The peak at 1550 cm^{-1} is the result of strong absorption of in-plane N1–H bending that also causes a mild absorption of the adjacent C2═N3═C4 asymmetric stretching, most likely due to the resonant character of the aromatic ring. The red shifted peak corresponds to the mixed character mode associated with C2═N3 and C5═C6 stretching.

4.7. *Validation of theoretical results*

As briefly discussed in Section 3.2, the absolute relative stabilities of the various tautomeric conformations of each of the protonated nucleobases differ slightly between the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) and MP2(full)/6-311+G(2d,2p)//B3LYP/6-31G(d) theories examined here, but the trends in the relative stabilities are generally preserved. The discrepancies between the B3LYP and MP2(full) results are generally small (within a few kJ/mol) and primarily occur for relatively high-lying tautomeric conformations, which are unlikely to be accessed in the IRMPD experiments. A significant exception to this agreement is found for the H$^+$(4SU) complex, where the relative stabilities of the three most stable tautomeric conformations differ. Comparison of the B3LYP and MP2(full) results for the H$^+$(4SU) system to the experimental results suggests that both theories do a good job, but that the B3LYP relative energetics are probably slightly more reliable.

Comparisons can also be made to the theoretical results previously reported in the literature [28,29,60]. Kryachko et al. [29] examined deprotonated, neutral, and protonated uracil to characterize the relative stabilities of the various tautomeric forms and determine the proton affinities and deprotonation enthalpies of uracil. They carried out calculations at the B3LYP/6-31+G(d,p) level of theory. Excellent agreement is found for the relative stabilities of the ground- and first three-excited state tautomeric conformers of H$^+$(U) with the B3LYP results determined here. Trends among the 12 most stable tautomeric conformations are highly parallel, but several minor differences are found as a result of the slightly different size basis sets employed for optimizations and energetics. Wolken and Tureček [28] examined the H$^+$(U) system in nearly as much detail as here, but performed more extensive calculations on the five most stable species including HF, B3LYP, MP2, QCISD, and QCISD(T) theories with several different sized basis sets for geometry optimizations and energetics including 6-31G(d,p), 6-31+G(d,p), 6-311+G(2df,p), and 6-311+G(3df,2p). In all cases, the trends in the relative stabilities of the tautomeric conformations examined parallel that found here. The relative stability of the **B** conformer was found to lie between 5 and 10 kJ/mol above the ground-state **A** conformer for all theories they examined, similar to that found here. However, in all cases the second and third excited-state conformers, **C** and **D**, are found to be less stable than found here by 15–25 kJ/mol. Based on all theories examined, the **C** and **D** conformers are not expected to be populated under the experimental conditions employed.

Lamsabhi et al. examined neutral and protonated thiouracils including: 2SU, 4SU, and 24dSU using the MP2/6-31G(d)//HF/6-31G(d) level of theory to determine their proton affinities and the nature of the protonation site. Both H$^+$(2SU) and H$^+$(24dSU) exhibit excellent agreement for the two most stable structures, but somewhat different trends than that found for the B3LYP results determined here for higher-lying tautomeric conformations. The results for these two complexes parallel the behavior determined here using MP2(full) theory. In contrast, the results for the H$^+$(4SU) complex exhibit better agreement with the B3LYP results, and in particular find the same order of relative stabilities for the four most stable tautomeric conformations, than the MP2(full) results determined here.

Overall, the comparisons between the experimental data and the theoretical results determined here suggest that in general both the B3LYP/6-311+G(2d,2p) and MP2(full)/6-311+G(2d,2p) perform well and provide reasonably accurate IR spectra and energetics for these systems. However, the reliability of the relative stabilities of the various tautomeric conformations is probably only of the order of $\sim$4 kJ/mol and thus caution should be exercised when interpreting results for structures calculated to be of very similar stability. The present results are also the most comprehensive, and except for the QCISD and QCISD(T) results reported by Wolken and Tureček [28], probably the most accurate reported to date.

5. Conclusions

The IRMPD action spectra of the protonated complexes of uracil and five thiouracils in the region $\sim$1000–1900 cm^{-1} have been measured. Comparison of the measured IRMPD spectra with the linear IR spectra calculated at the B3LYP/6-31G(d) level of theory allows the conformations accessed under the experimental conditions employed to be identified. In all cases, the measured IRMPD spectra are in excellent agreement with the theoretical linear IR spectra of the ground-state conformations computed, indicating that these conformers were accessed in the experiments. The ground-state structures calculated for H$^+$(U), H$^+$(2SU), H$^+$(5Me2SU), H$^+$(6Me2SU), and H$^+$(24dSU) at the B3LYP/6-31G(d) level of theory reveal that the binding of a proton stabilizes an alternative tautomer of the nucleobase, where the diketo, thioketo–keto, and dithioketo groups are both converted to hydroxyl (or sulfhydryl) groups, whereas the ground-state structure for H$^+$(4SU) corresponds to protonation of the canonical keto–thioketo tautomer at the 4-thioketo position. For the H$^+$(U) and H$^+$(4SU) complexes, evidence for additional low-energy conformers was also observed in the measured IRMPD spectra suggesting that these conformers were presented in low abundance in the experiments. However, low-energy sulfhydryl rotamers of H$^+$(2SU), H$^+$(5Me2SU), H$^+$(6Me2SU), and H$^+$(24dSU) produce theoretical spectra that are nearly identical to that of the ground-state **A** conformers, making it impossible to definitively establish the presence or absence of these low-energy conformers in the IRMPD spectra. Results from the measured IRMPD spectra and the theoretical linear IR spectra provide information about the most stable conformers, the relative stability, reactivity, and IR active vibrational modes of these protonated complexes, and in particular for the complexes of H$^+$(2SU), H$^+$(5Me2SU), and H$^+$(6Me2SU).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2010.08.005.

References

[1] J.D. Watson, F.H. Crick, Nature 171 (1953) 964.
[2] P.-O. Löwdin, Rev. Mod. Phys. 35 (1963) 724.
[3] M.D. Topal, J.R. Fresco, Nature 263 (1976) 285.
[4] P.O.P. Ts'o, Basic Principles in Nucleic Acids Chemistry, Academic, New York, 1974.
[5] H. Rüterjans, E. Kaun, W.E. Hull, H.H. Limbach, Nucleic Acid Res. 10 (1982) 7.
[6] D. Dougherty, K. Wittel, J. Meeks, S.P. McGlynn, J. Am. Chem. Soc. 98 (1976) 3815.
[7] Y. Tsuchiya, M. Fujii, M. Ito, J. Phys. Chem. 92 (1988) 1760.
[8] B.B. Brady, L.A. Peteanu, D.H. Levy, Chem. Phys. Lett. 147 (1988) 538.
[9] D. Shugar, K. Szczepaniak, Int. J. Quantum Chem. 20 (1981) 573.
[10] M. Szczesniak, M.J. Nowak, K. Szczepaniak, W.B. Person, D. Shugar, J. Am. Chem. Soc. 105 (1983) 5969.
[11] S. Chin, I. Scott, K. Szczepani, W.B. Person, J. Am. Chem. Soc. 106 (1984) 3415.
[12] R.D. Brown, P.D. Godfrey, D. McNaughton, A.P. Pierlot, J. Am. Chem. Soc. 110 (1988) 2329.
[13] P. Beak, J.M. White, J. Am. Chem. Soc. 104 (1982) 7073.
[14] J.S. Kwiatkowski, T.J. Zielinski, R. Rein, Adv. Quantum Chem. 18 (1986) 85.
[15] R. Czerminski, B. Lesying, A. Pohorille, Int. J. Quantum Chem. 16 (1979) 605.
[16] M.J. Scanlan, I.H. Hillier, J. Am. Chem. Soc. 106 (1984) 3737.
[17] U. Norinder, THEOCHEM 36 (1987) 259.
[18] I.R. Gould, I.H. Hiller, J. Chem. Soc. Perkin Trans. 2 (1990) 329.
[19] I.R. Gould, N.A. Burton, R.J. Hall, I.H. Hiller, THEOCHEM 331 (1995) 147.
[20] J. Leszczynski, J. Phys. Chem. 96 (1992) 1649.
[21] A. Leś, L. Adamowicz, J. Phys. Chem. 93 (1989) 7078.
[22] D.A. Estrin, L. Paglieri, G. Corongiu, J. Phys. Chem. 98 (1994) 5653.
[23] S.X. Tian, C.F. Zhang, Z.J. Zhang, X.J. Chen, K.Z. Xu, Chem. Phys. 242 (1999) 217.
[24] L. Paglieri, G. Corongiu, D.A. Estrin, Int. J. Quantum Chem. 56 (1995) 615.
[25] E.P. Hunter, S.G. Lias, Evaluated gas-phase basicities and proton affinities of molecules: an update, J. Phys. Chem. Ref. Data 27 (1998) 413.
[26] M.A. Kurinovich, L.M. Phillips, S. Sharma, J.K. Lee, Chem. Commun. 20 (2002) 2354.
[27] R. Wu, T.B. McMahon, J. Am. Chem. Soc. 129 (2007) 569.
[28] J.K. Wolken, F. Tureček, J. Am. Soc. Mass Spectrom. 11 (2000) 1065.
[29] E.S. Kryachko, M.T. Nguyen, T. Zeegers-Huyskens, J. Phys. Chem. A 105 (2001) 1288.
[30] M.T. Nguyen, A.K. Chandra, T. Zeegers-Huyskens, J. Chem. Soc. Faraday Trans. 94 (1998) 1277.
[31] Y. Podolyan, L. Gorb, J. Leszczynski, J. Phys. Chem. A 104 (2000) 7346.
[32] J.-Y. Salpin, S. Guillamont, J. Tortajada, L. MacAleese, J. Lemaire, P. Maitre, Chem. Phys. Chem. 8 (2007) 2235.
[33] J.M. Bakker, R.K. Sinha, T. Besson, M. Brugnara, P. Tosi, J.-Y. Salpin, P. Maitre, J. Phys. Chem. A 112 (2008) 12393.
[34] W. Saenger, Principles of Nucleic Acid Structure, Springer-Verlag, New York, Berlin, Heidelberg, Tokyo, 1984, Chapter 7.
[35] M. Yaniv, W.R. Folk, J. Biochem. 250 (1975) 3243.
[36] G.A. Jeffrey, W. Saenger, Hydrogen Bonding in Biological Structures, Springer, New York, 1991.
[37] C.F. Beck, G. Howlett, J. Mol. Biol. 111 (1977) 1.
[38] W.H. Miller, R.O. Robin, E.B. Astwood, J. Am. Chem. Soc. 67 (1945) 2201.
[39] R.H. Williams, G.W. Bissell, Science 98 (1943) 156.
[40] T.M. Ortiga-Carvalho, K. Hashimoto, C.C. Pazos-Moura, D. Greenen, R. Cohen, R.M. Lang, F.E. Wondisford, Endocrinology 145 (2004) 1625.
[41] R. Gredilla, G. Barja, M. Lopez-Torres, Free Radical Res. 35 (2001) 417.
[42] A. Palumbo, M. d'Ishia, Biochem. Biophys. Res. Commun. 282 (2001) 793.
[43] A. Sulkowska, J. Równicka, B. Bojko, W. Sulkowski, J. Mol. Struct. 651 (2003) 133.
[44] C. Theodossiou, P. Schwarzenberger, Am. J. Med. Sci. 319 (2000) 96.
[45] M. Macchia, S. Barontini, S. Bertini, V. Di Bussolo, S. Forli, E. Giovannetti, E. Grossi, F. Minutolo, R. Danesi, J. Med. Chem. 44 (2001) 3994.
[46] A.N. Elias, Med. Hypotheses 62 (2004) 431.
[47] C.D. Antoniadis, G.J. Corban, S.K. Hadjikakou, N. Hadjiliadis, M. Kubicki, S. Warner, I.S. Butler, Eur. J. Inorg. Chem. 8 (2003) 1635.
[48] D.R. Imam, A.A. El-barbary, C. Nielsen, E.B. Pedersen, Monatsh. Chem. 133 (2002) 723.
[49] A.A.H. Abdel-Rahman, A.E.S. Abdel-Megied, A.E.S. Goda, I.F. Zeid, E.S.H. El Ashry, Nucleos. Nucleot. 22 (2003) 2027.
[50] M. Inazumi, F. Kano, S. Sakata, Chem. Pharm. Bull. 40 (1992) 1808.
[51] J. Castillo, O. Benavente-Garcia, J. Lorente, M.J. Alcaraz, A. Redondo, A. Ortuno, J.A. Del Rio, J. Agric. Food Chem. 48 (2000) 1738.
[52] H. Rostkowska, A. Barski, K. Szczepaniak, M. Szczesniak, W.B. Person, J. Mol. Struct. 176 (1988) 137.

[53] H. Rostkowska, K. Szczepaniak, M.J. Nowak, J. Leszczynski, K. KuBulat, W.B. Person, J. Am. Chem. Soc. 112 (1990) 2147.
[54] M. Graindourze, T. Grootaers, J. Smets, T. Zeegers-Huyskens, G. Maes, J. Mol. Struct. 237 (1990) 389.
[55] L. Lapinski, H. Rostkowska, M.J. Nowak, J.S. Kwiatkowski, J. Leszczynski, Vib. Spectrosc. 13 (1996) 23.
[56] A.R. Katritzky, G. Baykut, S. Rachwal, M. Szafran, K.C. Caster, J. Eyler, J. Chem. Soc. Perkin Trans. 2 (1989) 1499.
[57] A.R. Katritzky, M. Szafran, J. Stevens, J. Chem. Soc. Perkin Trans. 2 (1989) 2194.
[58] A. Leś, L.J. Adamowicz, J. Am. Chem. Soc. 112 (1990) 1504.
[59] Y.V. Rubin, Y. Morozov, D. Venkateswarlu, J. Leszczynski, J. Phys. Chem. A 102 (1998) 2194.
[60] M. Lamsabhi, M. Alcamí, O. Mó, W. Bouab, M. Esseffar, J.L.-M. Abboud, M. Yáñez, J. Phys. Chem. A 104 (2000) 5122.
[61] J.J. Valle, J.R. Eyler, J. Oomens, D.T. Moore, A.F.G. van der Meer, G. von Heldon, G. Meijer, C.L. Hendrickson, A.G. Marshall, G.T. Blakney, Rev. Sci. Instrum. 76 (2005) 023103.
[62] N.C. Polfer, J. Oomens, D.T. Moore, G. von Helden, G. Meijer, R.C. Dunbar, J. Am. Chem. Soc. 128 (2006) 517.
[63] N.C. Polfer, J. Oomens, Phys. Chem. Chem. Phys. 9 (2007) 3804.
[64] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, Revision D.01, Gaussian, Inc., Pittsburgh, PA, 2005.
[65] J.B. Foresman, A.E. Frisch, Exploring Chemistry with Electronic Structure Methods, 2nd ed., Gaussian, Pittsburgh, PA, 1996, p. 64.
[66] Z. Yang, M.T. Rodgers, J. Phys. Chem. A 110 (2006) 1455.
[67] E. Kryachko, M.T. Nguyen, T. Zeegers-Huyskens, J. Phys. Chem. A 105 (2001) 3379.
[68] C.Y. Peng, H.B. Schlegel, Isr. J. Chem. 33 (1994) 449.
[69] J. Oomens, B.G. Sartakov, G. Meijer, G. von Helden, Int. J. Mass Spectrom. 254 (2006) 1.