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

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ABSTRACT

The gas phase structures of sodium cationized 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. The IRMPD spectra of all six sodium cationized complexes exhibit both characteristic and unique spectral features over the range from ~1000 to 1900 cm^{-1} such that the complexes are easily differentiated. The intense band at ~1800 cm^{-1} in the IRMPD action spectrum of Na^+(U) indicates that, as expected, a free carbonyl group is present in this complex. Absence of an intense band at ~1800 cm^{-1} in the IRMPD action spectra for Na^+(2SU), Na^+(5Me2SU), Na^+(6Me2SU), and Na^+(4SU) complexes suggests that either sodium cationization preferentially stabilizes a minor tautomer of the nucleobase, or that the sodium cation binds to the keto group in these complexes, such that no free carbonyl stretch is observed. 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. Based on these comparisons and the energetic predictions from theory, sodium cations preferentially bind at the 4-keto position of the canonical 2,4-diketo or 2-thioketo-4-keto tautomer in the Na^+(U), Na^+(2SU), Na^+(5Me2SU), and Na^+(6Me2SU) complexes. In contrast, sodium cationization results in preferential stabilization of a minor tautomer of the nucleobase in the Na^+(4SU) and Na^+(24dSU) complexes, where proton transfer from the N3H group to the 4-thioketo group, facilitates strong binding of the sodium cation via chelation with the O2(S2) and N3 atoms.

1. Introduction

Sodium cations are of great importance due to their large abundance in biological systems and participation in many biological pathways. For example, sodium and potassium ions are well known for their use in creating an ion gradient by interacting with proteins such as voltage-gated ion channels and Na^+/K^+-ATPase to generate an electrical potential difference between the membranes of neurons, such that the electrical signal can be passed from neuron to neuron. This ion gradient can also be used for secondary active transport where many essential amino acids, sugars, nucleotides, and other substance are transported across cell membranes to carry out their various functions [1]. Another well known function of the sodium cation is its participation in nucleic acid chemistry. Sodium, potassium, and magnesium ions have long been known to participate in the stabilization of DNA double helixes [2,3] and RNA [4] structures by charge neutralization and noncovalent interactions with the phosphate backbone. Therefore, a high concentration of metal cation and binding of metal cations to the nucleobases can cause conformational changes to the DNA and RNA structure [3–8]. One of the best examples is the formation of G-quadruplexes [9], where a sodium or potassium cation interacts with the carbonyl groups of four guanine residues in a guanosine rich DNA or RNA strand. The metal cation stabilizes the four guanine residues into an orientation where they hydrogen bond to each other in a Hoogsteen fashion and form a planar tetrad with the metal cation in the center. Additional G-quadruplex may also be formed and stacked on top of each other through base π-stacking interactions, similar to the DNA double helix structure.

Thiouracils are an important class of modified nucleobases derived from uracil, one of the four natural RNA bases, and have been identified as minor components of t-RNA and peptide nucleic acids [10]. The replacement of uracil by thiouracils can cause improper translation of genetic information [11]. Furthermore, thiouracils and a series of derivatives of thiouracils have also found

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their way into many pharmacological applications for antithyroid [10,12], anticancer [13,14], anti-HIV [15], and heart diseases treatments [16,17].

Previously, in an effort to better characterize the binding interactions between alkali metal cations and uracil, and the effects of thioketo substitution on these complexes, energy-resolved collision-induced dissociation (CID) experiments were carried out using a custom built guided ion beam tandem mass spectrometer (GIBMS) in our laboratory, along with theoretical calculations to complement those results [18]. However, GIBMS techniques do not provide direct information regarding the gas-phase structures probed in the experiments. Instead, structural information can only be inferred by performing theoretical calculations and comparing the measured bond dissociation energies (BDEs) to theoretical estimates for these values. Advances in IRMPD action spectroscopy techniques where the high resolution and high trapping efficiency of a Penning trap [19] in a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) combined with a free electron laser (FEL) [20] allow the IR signatures of mass-selected ions to be probed in the IR fingerprint region [21,22]. This innovative experimental setup, along with the aforementioned importance of the alkali metal cations has sparked many studies to investigate the complexes of alkali metal cations with amino acids [23–33], peptides [34,35], sugars [36–38], and phosphate esters [39,40].

In our previous IRMPD action spectroscopy studies of protonated uracil and thiouracils [41], it was found that the binding of a proton preferentially stabilizes alternative tautomers rather than direct binding of the proton to the most favorable site on the canonical neutral tautomers, in contrast to similar studies [18,42,43] done by other experimental and theoretical methods. Therefore, by understanding the level of covalent and noncovalent interactions between protons and sodium cations with the analogous molecules, the underlying driving force for facilitated tautomerization may be revealed. 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 a sodium cation. 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 sodium cationized forms of these nucleobases, the IRMPD action spectra of these complexes are investigated and compared to the linear IR spectra of stable low-lying tautomeric conformations of these sodium cationized complexes derived from theoretical electronic structure calculations performed at the B3LYP/6-31G(d) level of theory.

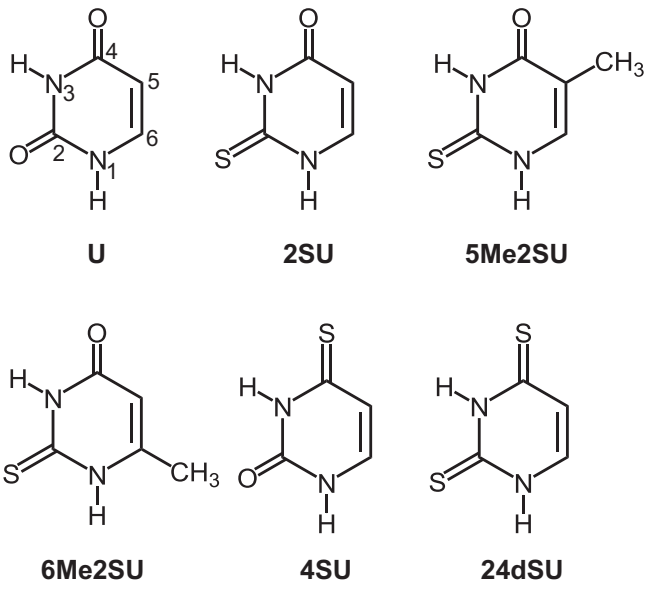

Fig. 1. Structures of uracil (U) and the thiouracils (*x*SU), where *x*SU = 2-thiouracil (2SU), 5-methyl-2-thiouracil (5Me2SU), 6-methyl-2-thiouracil (6Me2SU), 4-thiouracil (4SU), and 2,4-dithiouracil (24dSU).

2. Experimental and computational Section

2.1. Mass spectrometry and photodissociation

IRMPD action spectra of Na^+(U) and five Na^+(*x*SU) complexes were measured using a 4.7 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) 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 [20,44,45]. All of the nucleobase samples were obtained from Sigma Aldrich. The sodiated complexes of each nucleobase were generated using a Micromass "Z-spray" electrospray ionization (ESI) source from solutions containing 1 mM nucleobase and 1 mM of sodium chloride in an approximately 50%:50% MeOH/H_2O mixture. A solution flow rate of 10 μL/min was used and the electrospray needle was held at a voltage of ~3 kV. Ions emanating from the ESI source were accumulated in a hexapole trap for 5 s 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 used, 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 [46]. 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 10.5 μm (950 cm^{-1}) to 5.2 μm (1923 cm^{-1}).

2.2. Computational details

In the present work, all possible tautomers of neutral and sodium cationized uracil and 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 [47]. Single point energy calculations carried out at the B3LYP/6-311 + G(2d,2p) level of theory were used to determine the relative enthalpies and Gibbs free energies at 298 K of each conformer including zero-point vibrational energy (ZPE) and thermal corrections. Theoretical linear IR spectra were generated using the calculated harmonic vibrational frequencies (scaled by a factor of 0.96) and their 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

In all cases, IRMPD of the Na^+(U) and Na^+(*x*SU) complexes results in loss of the intact nucleobase and detection of Na^+ at $m/z = 23$. No other dissociation pathways were observed for these systems. An IRMPD yield was determined for each complex from the measured intensities of the reactant complex, ($I_{Na^+(SU)}$), and the sodium cation

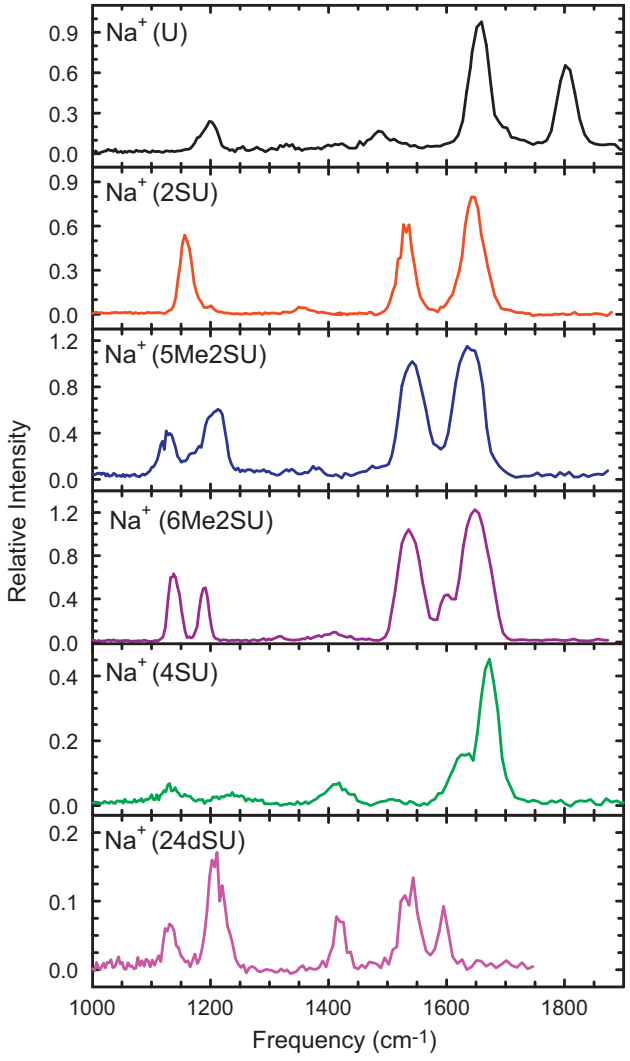

Fig. 2. Infrared multiple photon dissociation action spectra of Na^+(U) and Na^+(*x*SU) complexes, where *x*SU = 2-thiouracil (2SU), 5-methyl-2-thiouracil (5Me2SU), 6-methyl-2-thiouracil (6Me2SU), 4-thiouracil (4SU), and 2,4-dithiouracil (24dSU).

product, (I_{Na+}), after laser irradiation at each frequency as shown in Eq. (1).

$$\text{IRMPD yield} = \frac{I_{Na+}}{(I_{Na+(xSU)} + I_{Na+})} \quad (1)$$

The IRMPD yield was normalized linearly with laser power to correct for the variation in the laser power as a function of photon energy, i.e., the wavelength of the FEL. IRMPD spectra were obtained for Na^+(U), Na^+(2SU), Na^+(5Me2SU), Na^+(6Me2SU), and Na^+(4SU) over the range from ~1000 to 1900 cm^{-1}, while the spectrum for Na^+(24dSU) was measured from ~1000 to 1750 cm^{-1}. The IRMPD spectra of all six complexes examined here are compared in Fig. 2. As can be seen in the figure, unique spectral features are observed in each spectrum that allow these complexes to be differentiated from one another. The intense band at ~1800 cm^{-1} in the IRMPD spectrum of Na^+(U) is characteristic of a free carbonyl stretch. This band is absent in the IRMPD spectra of all of the other complexes investigated here. The intense band extending from ~1630 to 1675 cm^{-1} found in the spectra of Na^+(U), Na^+(2SU), Na^+(5Me2SU), Na^+(6Me2SU), and Na^+(4SU), but absent in the spectrum of Na^+(24dSU), likely arises from the interaction of the sodium cation with the carbonyl group. This interaction weakens the C=O bond, decreasing the frequency of the C=O stretch, and leads to a red shifting of this feature. Besides the absence of the unperturbed carbonyl stretch at ~1800 cm^{-1}, the peaks in the spectrum of Na^+(2SU) between 1125 and 1580 cm^{-1} are more intense and slightly blue shifted (1530 cm^{-1}) and red shifted (1155 cm^{-1}) as compared to the analogous features in the IRMPD spectrum of Na^+(U). The similarities in these IRMPD spectra may suggest that the conformation of Na^+(2SU) is very similar to that of Na^+(U), but that the strength of the noncovalent interaction with the sodium cation differs somewhat. The IRMPD spectrum of Na^+(5Me2SU) retains all of the spectral features observed in the Na^+(2SU) spectrum, while several features are enhanced. The peaks at 1540 and 1640 cm^{-1} are similar to, but broader than, the corresponding bands observed in the Na^+(2SU) spectrum. The very minor peak at 1200 cm^{-1} has increased in intensity and broadened, while the peak at 1155 cm^{-1} is red shifted to 1130 cm^{-1} in the Na^+(5Me2SU) spectrum. Methylation at the 6-position of Na^+(2SU) also results in a very similar IRMPD spectrum for Na^+(6Me2SU) as found for the Na^+(2SU) and Na^+(5Me2SU) complexes. The intensity of the peak at ~1200 cm^{-1} is intermediate between that observed for Na^+(2SU) and Na^+(5Me2SU), and the peak at 1140 cm^{-1} is less red shifted. In addition, a new distinct feature arises at ~1600 cm^{-1}, between the peaks at 1540 and 1640 cm^{-1}. In contrast, the IRMPD spectrum of Na^+(4SU) is markedly different from the Na^+(U) and Na^+(2SU) spectra except for the perturbed carbonyl stretch at 1670 cm^{-1}. The difference in these IRMPD spectra suggest that the nature of the binding of a sodium cation is very different to 4SU than to U and 2SU, or that binding of a sodium cation results in preferential stabilization of an alternative tautomer of 4SU as previously observed for protonated uracil and thiouracils [41,48]. As expected, the IRMPD spectrum of Na^+(24dSU) exhibits distinct features that differ significantly from the other five IRMPD spectra due to the substitution of both oxygen atoms by sulfur atoms. Unlike the carbonyl group, the thioketo moiety does not exhibit a strong IR absorption band. Therefore, it may be more difficult to extract accurate structural information regarding the thioketo group directly from the IRMPD action spectrum alone.

Comparison of the IRMPD yields for these six complexes may provide information regarding the relative stability and/or density of states available to these nucleobase complexes. The IRMPD yields increase in the order of Na^+(24dSU) < Na^+(4SU) < Na^+(2SU) < Na^+(U) < Na^+(5Me2SU) < Na^+(6Me2SU). IRMPD yields for the most intense peaks in the spectra differ by about a factor of six across these complexes. A nearly reverse trend was found for the sodium cation binding affinities of these nucleobases determined from energy-resolved collision-induced dissociation (CID) experiments carried out in a guided ion beam tandem mass spectrometer (GIBMS) [18]. The sodium cation binding affinities were found to decrease in the following order, Na^+(6Me2SU) > Na^+(5Me2SU) > Na^+(2SU) > Na^+(U) > Na^+(4SU) > Na^+(24dSU). The measured sodium cation binding affinities follow the expected trend as sodium cations are known to bind more strongly to a "hard" oxygen atom than the "softer" sulfur atom. Thus, the trend in the IRMPD yields is likely the result of differences in the density of states for these complexes [49]. The IRMPD yield should decrease upon thioketo substitution as a result of the reduced IR activity of the thioketo moiety. In contrast, the IRMPD yield should increase upon methylation as a result of the increase in the number of ro-vibrational modes of the system. Thus, the variation in the IRMPD yields is consistent with the trends in the density of states of these complexes.

3.2. Theoretical results

In our previous investigation of the gas-phase structures of the protonated complexes of uracil and the five thiouracils examined here, all possible tautomers of neutral uracil and the five

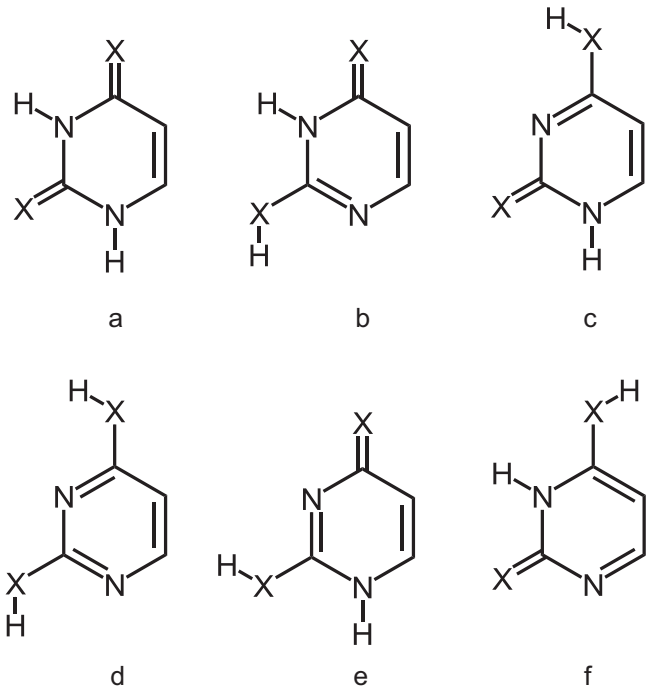

Fig. 3. Structures of the six most stable tautomeric forms of neutral uracil (U) and the thiouracils (*x*SU), where *x*SU = 2-thiouracil (2SU), 5-methyl-2-thiouracil (5Me2SU), 6-methyl-2-thiouracil (6Me2SU), 4-thiouracil (4SU), and 2,4-dithiouracil (24dSU), based on theoretical calculations at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d)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

thiouracils were calculated using the same protocol as followed in the present work for the sodium cationized complexes. Structures were optimized and frequency analyses were performed at the B3LYP/6-31G(d) level of theory, while single point energies were determined at the B3LYP/6-311+G(2d,2p) level [41]. The structures of all stable tautomeric conformations and their relative Gibbs free energies at 298 K were included in the Supplementary information in that paper. The six most stable tautomeric forms of neutral uracil and the thiouracils found in that work, and designated **a**, **b**, **c**, **d**, **e**, and **f**, are shown in Fig. 3. All of the sodium cationized complexes calculated here are determined at the same level of theory. All favorable sodium cation binding sites to each of the stable tautomeric conformations of each nucleobase were investigated. Enthalpies and Gibbs free energies relative to the ground-state tautomeric conformation calculated at the B3LYP/6-311+G(2d,2p) level of theory, including zero point energy (ZPE) and thermal corrections at 298 K for the twelve most stable tautomeric forms of Na^+(U) and the five Na^+(*x*SU) complexes are listed in Table 1. Structures and Gibbs free energies for all sodium cationized tautomeric conformations of uracil and each of the thiouracils computed in this study are compiled in Fig. 1S of the Supplementary data. All stable conformers within 20 kJ/mol of the ground-state conformation of each sodium cationized complex, i.e., those most likely to be accessed in the experiments along with their calculated linear IR spectra are compared to the measured IRMPD action spectra in Figs. 4–9. For all of the nucleobases possessing a carbonyl group at the 4-position, i.e., U, 2SU, 5Me2SU, and 6Me2SU, the ground-state conformer involves sodium cation binding at the O4 position in a linear fashion to the corresponding ground-state canonical **a** conformer, resulting in the **A** conformer. These structures are consistent with the ground-state conformations computed at the MP2(full)/6-311+G(2d,2p)//MP2(full)/6-31G(d) level of theory reported in the GIBMS study [18,50]. The most stable tautomeric conformations of sodium cationized 4-thiouracil and 2,4-dithiouracil determined here, however, differ from those

Table 1
Relative enthalpies and Gibbs free energies of the twelve most stable tautomeric forms of sodium cationized uracil and thiouracils calculated at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d) level of theory.[a]

Complex	Tautomer	ΔH_{298} (kJ/mol)	ΔG_{298} (kJ/mol)
Na^+(U)	A	0.0	0.0
	B	10.1	13.6
	C	11.0	15.2
	D	16.6	16.2
	E	26.3	30.4
	F	30.0	32.8
	G	35.9	36.5
	H	40.4	43.9
	I	45.9	48.4
	J	45.8	49.7
	K	56.6	60.2
	L	59.8	63.6
Na^+(2SU)	A	0.0	0.0
	I	15.4	15.6
	F	16.6	16.6
	C	12.9	16.8
	B	17.6	20.7
	G	25.2	24.1
	E	29.1	33.0
	O	34.4	33.1
	DD	41.3	38.5
	H	42.1	45.5
	J	51.7	53.1
	M	52.5	54.2
Na^+(5Me2SU)	A	0.0	0.0
	I	6.1	5.4
	F	7.8	7.8
	C	8.5	12.3
	B	12.0	14.7
	G	23.4	21.8
	DD	31.3	28.6
	E	26.4	29.0
	O	32.7	30.4
	H	38.4	40.7
	J	43.8	44.8
	M	48.6	49.3
Na^+(6Me2SU)	A	0.0	0.0
	C	9.3	12.6
	I	15.3	16.2
	B	13.8	16.4
	F	16.2	16.9
	G	25.5	24.2
	O	34.1	32.0
	E	30.8	32.4
	DD	42.0	39.3
	H	43.4	44.8
	M	51.4	51.0
	J	56.0	56.9
Na^+(4SU)	C	0.0	0.0
	B	4.2	3.9
	E	13.2	13.2
	H	15.9	15.1
	D	21.9	19.4
	AA	35.5	30.2
	AB	39.1	32.6
	J	40.1	40.3
	L	42.7	42.9
	K	46.7	46.2
	F	46.7	46.7
	N	55.1	54.0
Na^+(24dSU)	C	0.0	0.0
	B	7.1	6.6
	E	12.2	12.1
	H	14.3	13.6
	I	24.9	20.4
	F	29.3	26.0
	AA	32.0	27.8
	AB	34.6	29.3
	DD	42.6	37.9
	J	43.6	41.7
	G	48.8	43.2
	L	47.0	45.3

[a] Conformer designations are based on the structures from Fig. 1S of the Supplementary information.

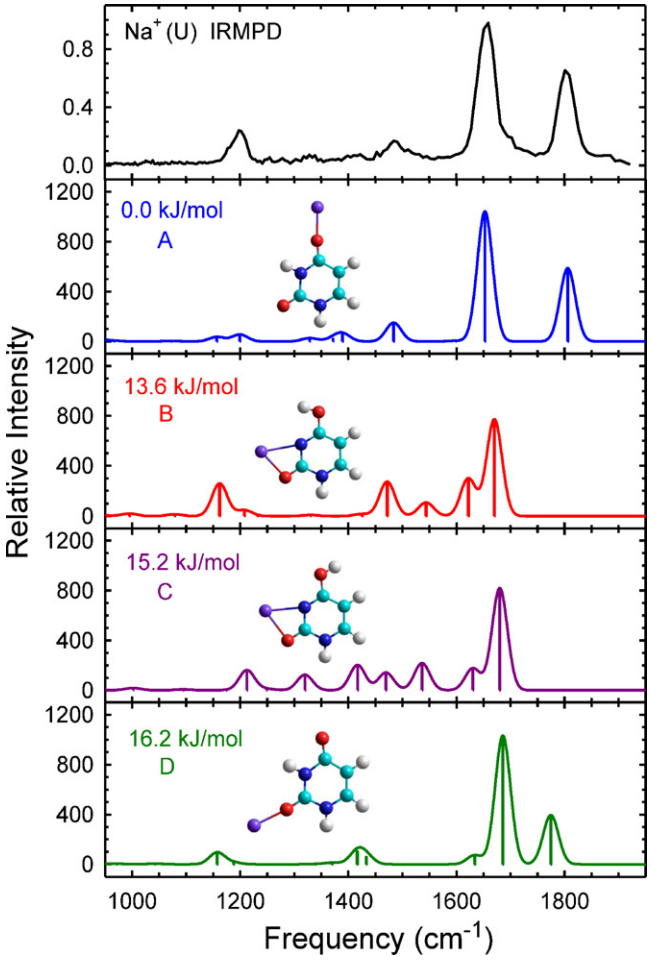

Fig. 4. Comparison of the measured IRMPD action spectrum of Na^+(U) with the linear IR spectra predicted for the four most stable conformers of Na^+(U) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energies of each conformer are also shown.

previously reported [18]. Alternative tautomers were not investigated in that work and binding of a sodium cation is found to preferentially stabilize an alternative tautomer of 4SU and 24dSU. In the ground-state tautomeric conformations of the Na^+(4SU) and Na^+(24dSU) complexes, the nucleobases are S4 rotamers of the **c** tautomer (Fig. 3), where the N3 hydrogen atom migrates to the neighboring S4 position and is oriented away from the N3 group. For the free ligands, these conformers lie 48.7 and 50.1 kJ/mol higher in free energy than the corresponding ground-state canonical neutral **a** tautomers, respectively, but provide more favorable binding to the sodium cation via bidentate interaction at the O2 (S2) and N3 atoms.

For the Na^+(U), Na^+(2SU), and Na^+(24dSU) complexes, three low-energy conformers were found that lie within 20 kJ/mol in free energy of the corresponding ground-state conformer. In contrast, four low-energy conformers were found within 20 kJ/mol of the ground-state conformation for the Na^+(5Me2SU), Na^+(6Me2SU), and Na^+(4SU) complexes. The theoretical linear IR spectra of these conformers are compared to the experimental IRMPD action spectra to determine the conformation(s) of the sodium cationized nucleobase complexes accessed in the experiments. The ground-state conformations of the sodium cationized nucleobases and all stable low-energy conformers within 20 kJ/mol, their relative Gibbs free energies, and calculated linear IR spectra are shown in Figs. 4–9. Structures and relative Gibbs free energies for all tautomeric conformations of sodium cationized complexes of uracil and each of the five thiouracils computed in this study are provided in Fig. 1S of the Supplementary data.

3.2.1. Na^+(U)

As discussed above, the ground-state structure of Na^+(U) is the **A** conformer shown in Fig. 4 in which the sodium cation binds to the O4 carbonyl oxygen atom of the canonical **a** diketo tautomer of U. The first-excited conformer of Na^+(U), the **B** conformer, lies 13.6 kJ/mol in free energy above the ground-state conformation. In this conformer, the 2-keto-4-hydroxyl tautomer of uracil produced by transfer of the N3 hydrogen atom of the canonical neutral diketo tautomer to the O4 position, facilitating bidentate binding of the sodium cation to the O2 and N3 atoms. Rotation of the 4-hydroxyl hydrogen atom of this conformer produces the **C** conformer, which lies 1.6 kJ/mol higher in free energy, or 15.2 kJ/mol above the ground-state conformation. Binding of a sodium cation at the O2 position of the canonical diketo tautomer produces the **D** conformer, which is 16.2 kJ/mol less favorable in free energy as compared to the ground-state **A** conformer.

3.2.2. Na^+(2SU)

The ground-state structure of Na^+(2SU) is again the **A** conformer shown in Fig. 5 in which the sodium cation binds to the O4 carbonyl oxygen atom of the canonical **a** 2-thioketo-4-keto tautomer of 2SU. The first-excited conformer of Na^+(2SU), the **I** conformer, lies 15.6 kJ/mol in free energy above the ground-state conformation and is a minor tautomer of 2-thiouracil in which the N3 hydrogen

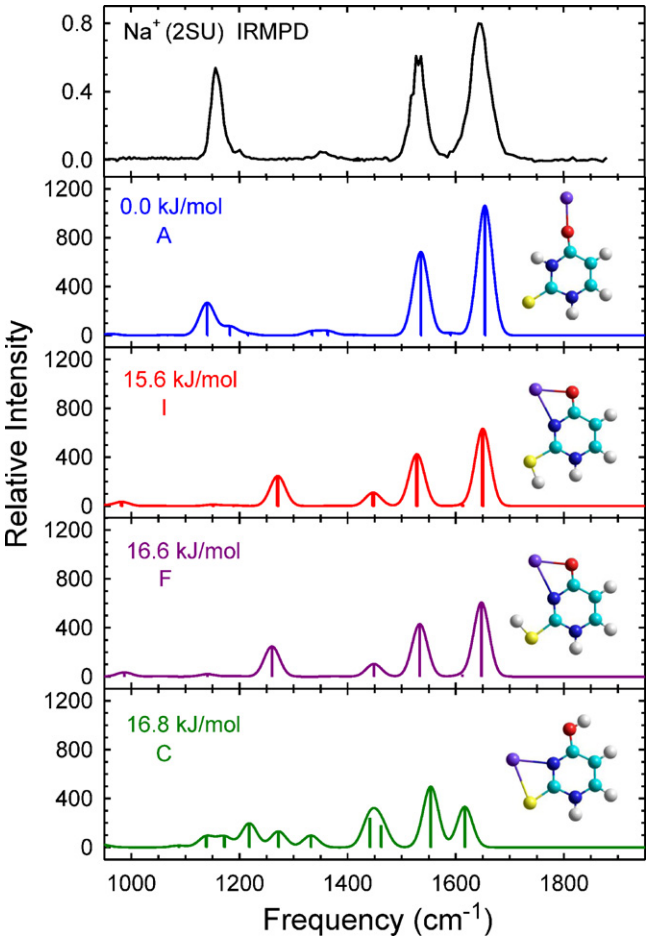

Fig. 5. Comparison of the measured IRMPD action spectrum of Na^+(2SU) with the linear IR spectra predicted for the four most stable conformers of Na^+(2SU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energies of each conformer are also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

atom of the canonical neutral **a** tautomer shifts to the S2 position, facilitating bidentate binding of the sodium cation to the N3 and O4 atoms. Rotation of the 2-sulfhydryl hydrogen atom of this conformer towards the N3 atom and sodium cation results in the **F** conformer. The **F** conformer lies only 1.0 kJ/mol higher in free energy, or 16.6 kJ/mol above the ground-state **A** conformer. The third-excited conformer, the **C** conformer, is also a minor tautomer where the N3 hydrogen atom is transferred to the O4 position and oriented away from the N3 atom, facilitating bidentate binding of the sodium cation to the N3 and S2 atoms. This tautomer lies just 1.2 kJ/mol above the **I** conformer, and 16.8 kJ/mol higher in free energy than the ground-state conformer. Binding of the sodium cation at the S2 position of the canonical 2-thioketo-4-keto tautomer is 38.5 kJ/mol less favorable, and therefore unlikely to be accessed under the experimental conditions employed.

3.2.3. Na^+(5Me2SU)

The ground-state structure of Na^+(5Me2SU) is again the **A** conformer as shown in Fig. 6 in which the sodium cation binds to the O4 carbonyl oxygen atom of the canonical **a** tautomer of 5Me2SU. The three most stable conformers of Na^+(5Me2SU) are exactly parallel to those of Na^+(2SU), however, 5-methylation stabilizes the first three excited conformers relative to the ground-state conformer by 4.5–10.2 kJ/mol as compared to the analogous Na^+(2SU) complexes. The relative Gibbs free energy of the fourth-excited conformer, **B**, the 4-hydroxyl rotamer of **C**, is also stabilized by 5-methyl substitution and lies 14.7 kJ/mol above the ground-state conformer. The binding of a sodium cation at the S2 position of the canonical 2-thioketo-4-keto tautomer also becomes more favorable upon 5-methyl substitution, but is still 28.6 kJ/mol less favorable than binding at the O4 position, the ground-state conformation.

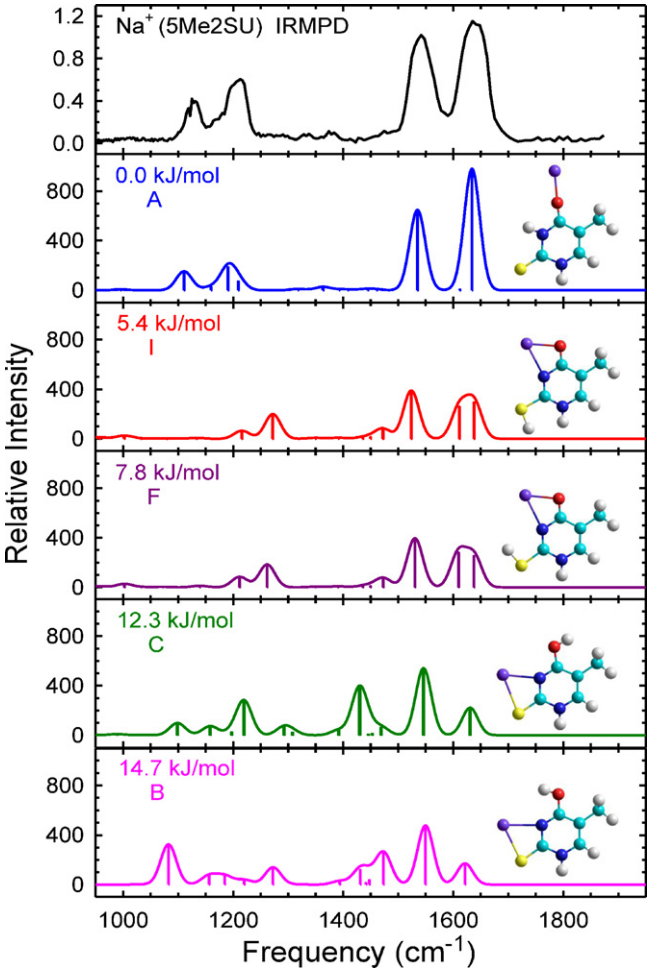

Fig. 6. Comparison of the measured IRMPD action spectrum of Na^+(5Me2SU) with the linear IR spectra predicted for the four most stable conformers of Na^+(5Me2SU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energies of each conformer are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

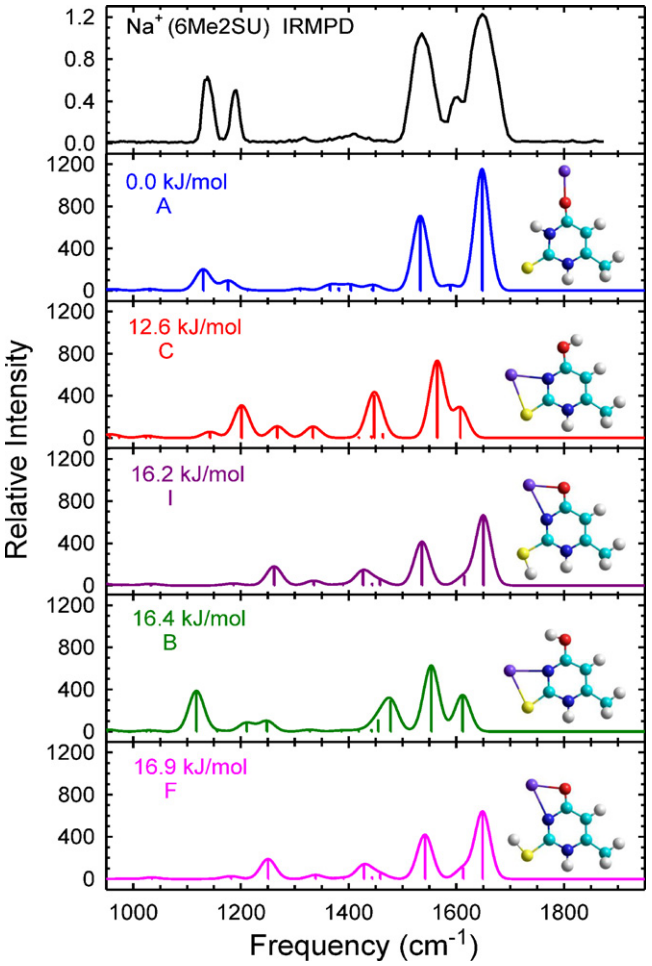

Fig. 7. Comparison of the measured IRMPD action spectrum of Na^+(6Me2SU) with the linear IR spectra predicted for the four most stable conformers of Na^+(6Me2SU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energies of each conformer are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

3.2.4. Na^+(6Me2SU)

As for the other complexes already discussed, the ground-state structure of Na^+(6Me2SU) is the **A** conformer shown in Fig. 7 in which the sodium cation binds to the O4 carbonyl oxygen atom of the canonical **a** tautomer of 6Me2SU. The relative stabilities of the low-energy excited conformers of Na^+(6Me2SU) differ drastically from those of Na^+(2SU) and Na^+(5Me2SU). Binding of the sodium cation at the S2 and N3 positions of the 2-thioketo-4-hydroxyl tautomer (**C**) of 6Me2SU is preferred over the binding at the N3 and O4 position of the 2-sulfhydryl-4-keto tautomer (**I**). The **C** and **I** conformers lie 12.6 and 16.2 kJ/mol above the ground-state **A** conformer, respectively. The 4-hydroxyl rotamer of **C**, conformer **B**, lies 16.4 kJ/mol, while the 2-sulfhydryl rotamer of **I**, conformer **F**, lies 16.9 kJ/mol higher in free energy than the ground-state **A** conformer.

3.2.5. Na^+(4SU)

The ground-state structure of Na^+(4SU) is the **C** conformer shown in Fig. 8 in which the sodium cation binds to the O2 and

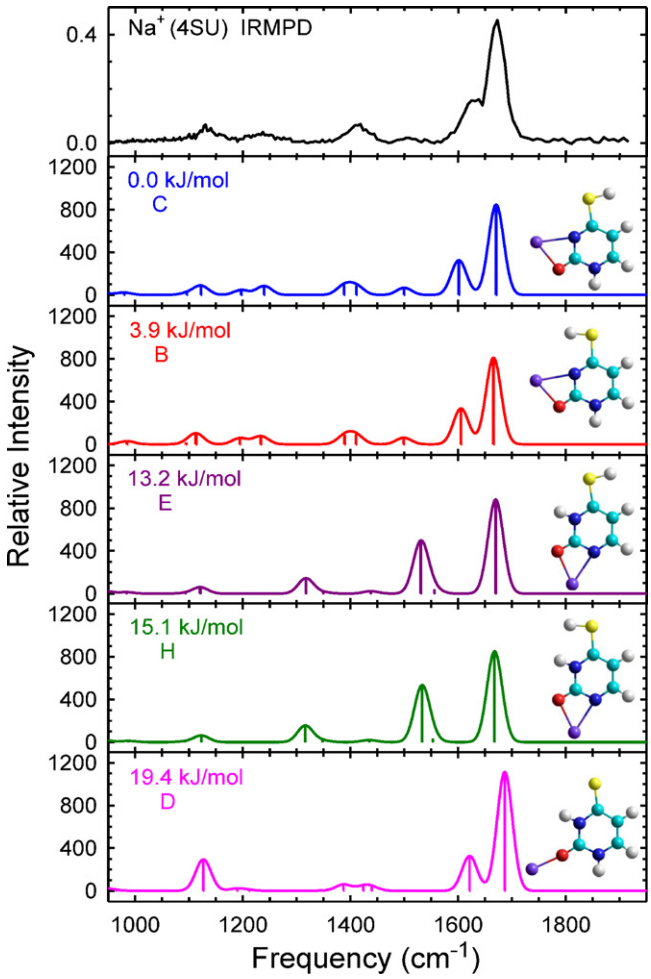

Fig. 8. Comparison of the measured IRMPD action spectrum of Na^+(4SU) with the linear IR spectra predicted for the four most stable conformers of Na^+(4SU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energies of each conformer are also shown.

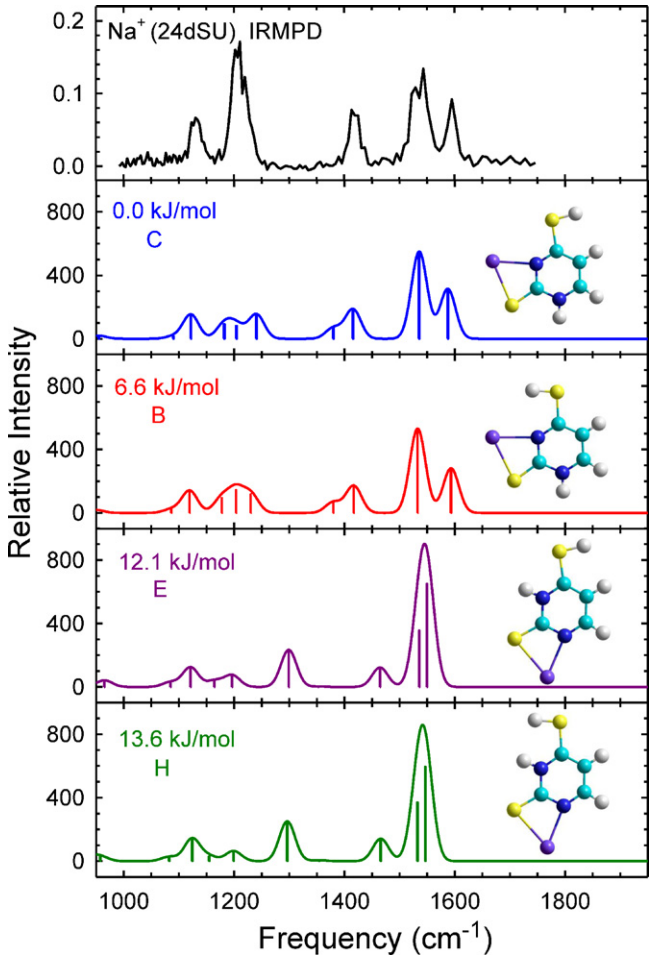

Fig. 9. Comparison of the measured IRMPD action spectrum of Na^+(24dSU) with the linear IR spectra predicted for the four most stable conformers of Na^+(24dSU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energies of each conformer are also shown.

N3 atoms of the 2-keto-4-sulfhydryl tautomer of 4SU, where the 4-sulfhydryl hydrogen atom is directed away from the adjacent N3 group. The first-excited conformer of Na^+(4SU), the **B** conformer, lies 3.9 kJ/mol higher in free energy and is the 4-sulfhydryl rotamer of the ground-state **C** conformer, where the hydrogen atom is oriented toward the adjacent N3 atom. The second-excited conformer, **E**, involves the binding of Na^+ to the N1 and O2 atoms of the **f** tautomer, where the N1 hydrogen atom has migrated to the S4 position and is oriented away from the adjacent N3H group. Binding of Na^+ to this tautomer is less favorable by 13.2 kJ/mol free energy compared to the ground-state **C** conformer. The 4-sulfhydryl rotamer of conformer **E**, the **H** conformer, lies 1.9 kJ/mol higher in free energy or 15.1 kJ/mol above the ground-state **C** conformer. Binding of Na^+ to the O2 position of the canonical 2-keto-4-thioketo tautomer, the **D** conformer, is less favorable than the **C** conformer by 19.4 kJ/mol. In contrast to the previous two cases, binding of Na^+ to the S4 position of the canonical 2-keto-4-thioketo tautomer can adopt two different conformations, the **AA** and **AB** conformers as shown in Fig. S1, where the sodium cation binds to the 4-thioketo group from the side rather than the end on manner, with ∠C=S–Na^+ bond angles of 121.6° and 126.6° when the sodium cation is oriented away and toward the adjacent N3H group, and are less favorable than the ground-state conformation by 30.2 and 32.6 kJ/mol in free energy, respectively.

3.2.6. Na^+(24dSU)

As for Na^+(4SU), the ground-state structure of Na^+(24dSU) is the **C** conformer shown in Figs. 9 and S1 in which the sodium cation binds to the S2 and N3 atoms of the 2-thioketo-4-sulfhydryl tautomer of 4SU, where the 4-sulfhydryl hydrogen atom is directed away from the adjacent N3 atom. The first three excited low-energy conformers of Na^+(24dSU) exactly parallel those of Na^+(4SU). The first-excited conformer, **B**, the 4-sulfhydryl rotamer of **C** lies 6.6 kJ/mol above the ground-state **C** conformer. The second-excited low-energy conformer, **E**, where Na^+ binds to the N1 and S2 atoms of the **f** conformer of the neutral nucleobase lies 12.1 kJ/mol, while the 4-sulfhydryl rotamer, the third-excited conformer, **H**, lies 13.6 kJ/mol higher in free energy than the ground-state tautomeric conformation. Binding of Na^+ to the S4 position of the canonical 2,4-dithioketo tautomer may adopt two different conformations, the **AA** and **AB** conformers, with ∠C=S–Na^+ bond angles of 122.2° and 126.5°, respectively, when the sodium cation is oriented away and toward the adjacent N3H group, and are less favorable than the ground-state conformer by 27.8 and 29.3 kJ/mol, respectively. Similar to the Na^+(2SU) complex, binding of the sodium cation to the S2 atom of the canonical 2,4-dithioketo **a** tautomer is 37.9 kJ/mol less stable than the ground-state conformer, and binds with a ∠C=S–Na^+ bond angle of 119.9° towards the adjacent N3H group and a ∠N1C2S2Na^+ dihedral angle of 143.6°, the **DD** conformer shown in Fig. S1.

4. Discussion

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

The experimental IRMPD action spectrum along with the calculated linear IR spectra and structures of the four most stable tautomeric conformations found for the Na^+(U) complex are compared in Fig. 4. The IRMPD action spectrum exhibits best agreement with the calculated IR spectrum of the ground-state conformer **A**. In particular, the strong sharp bands at 1652 and 1805 cm^{-1}, which correspond to the C4=O and C2=O carbonyl stretches, respectively, and the minor peaks at 1200 and 1485 cm^{-1}, which are the result of asymmetric in-plane C5–H and C6–H bending and in-plane N1–H bending, match almost perfectly. These results confirm that the ground-state conformer is the dominant conformer accessed in the experiments. It is, however, unclear whether the minor peaks at 1330 and 1387 cm^{-1} of the calculated spectrum are present in the experimental spectrum or just noise at baseline. It should be mentioned that this comparison is an excellent example demonstrating the resolving power of IRMPD action spectroscopy to distinguish different binding sites of the sodium cation to the same ligand, as the bands in the calculated spectrum of conformer **D** are red shifted (1156, 1422, and 1775 cm^{-1}) and blue shifted (1685 cm^{-1}) compared to the experimental IRMPD action spectrum. Comparison of the calculated linear IR spectra of the **B** conformer and its 4-hydroxyl rotamer, **C**, to the IRMPD action spectrum clearly indicates that these two conformers are not accessed in the experiments, as the presence of these conformers would sufficiently broaden the experimental spectrum at ~1658 cm^{-1}, in addition, many of the bands in the region from 1200 to 1620 cm^{-1} are not observed in the IRMPD action spectrum.

4.2. Comparison of experimental and theoretical IR spectra of $Na^+(2SU)$

The experimental IRMPD action spectrum along with the calculated linear IR spectra and structures of the four most stable tautomeric conformations found for the Na^+(2SU) complex are compared in Fig. 5. Similar to that found for the Na^+(U) complex, the calculated IR spectrum of the ground-state conformer, where the sodium cation binds to the O4 position of the canonical tautomer, provides the best agreement with the experimental IRMPD action spectrum. The two most intense peaks at 1530 and 1645 cm^{-1}, which correspond to asymmetric N1–H and N3–H bending and the C4=O carbonyl stretch, match the experimental spectrum very well. The very minor peaks at 1335 and 1363 cm^{-1} in the theoretical spectrum are also observed experimentally, and arise from the mixed character mode comprised of in-plane N3–H bending, symmetric C5–H and C6–H bending, and in-plane symmetric N1–H and N3–H bending. In contrast, the modest peak and small shoulder at 1155 and 1200 cm^{-1} in the experimental IRMPD spectrum are slightly blue shifted as compared to the linear IR spectrum indicating that theory underestimates the frequency of these modes. These vibrational modes correspond to the C2=S stretch and the mixed character mode associated with in-plane N1–H bending, in-plane C6–H bending, and the asymmetric stretching of the C2–N3–C4 moiety. The calculated IR spectra of the first-excited conformer, **I**, and its 2-sulfhydryl rotamer, **F**, exhibit two modest IR absorption peaks that are in very close agreement with the experimental IRMPD spectrum and the calculated linear IR spectrum of the ground-state conformer (~528 and 1650 cm^{-1}). However, the absence of the two minor peaks at ~1270 and 1448 cm^{-1} in the experimental IRMPD spectrum suggest that these two conformers are not accessed in the experiments. The linear IR spectrum calculated for the **C** conformer is markedly different from the measured IRMPD spectrum, indicating that this conformation was not accessed in the experiments.

4.3. Comparison of experimental and theoretical IR spectra of $Na^+(5Me2SU)$

The experimental IRMPD action spectrum along with the calculated linear IR spectra and structures of the five most stable tautomeric conformations found for the Na^+(5Me2SU) complex are compared in Fig. 6. In this comparison, the theoretical IR spectrum of the ground-state conformer where the sodium cation binds to the canonical 2-thioketo-4-keto tautomer at the O4 position of 5Me2SU, the **A** conformer, provides the best agreement with the experimental IRMPD spectrum. The intense peaks at 1540 and 1640 cm^{-1} again correspond to the asymmetric N1–H and N3–H bending and the C4=O carbonyl stretch observed in the Na^+(2SU) spectrum, as well as the C2=S stretching mode at 1130 cm^{-1} whose frequency is again underestimated by density functional theory. As mentioned previously, the broad and moderately intense peak at 1200 cm^{-1} of the Na^+(5Me2SU) spectrum has evolved markedly from the very minor shoulder observed in the Na^+(2SU) spectrum due to methyl substitution at the 5-position. This band arises from coupling of the C2–N1–C6 asymmetric stretching mode and the same mixed character mode associated with N1–H bending, in plane C6–H bending and the asymmetric stretching of the C2–N3–C4 moiety observed for Na^+(2SU). Vibrational modes in the range between 1300 and 1400 cm^{-1} correspond to in-plane C6–H bending, in-plane symmetric N1–H and N3–H bending, and the wagging of the methyl group that are nearly IR inactive in the theoretical linear IR spectrum, but are slightly active and visible in the IRMPD action spectrum due to the multiple photon absorption process. Similar to Na^+(2SU), the calculated IR spectra of the first two excited 2-sulfhydryl rotamers of Na^+(5Me2SU), conformers **I** and **F**, exhibit two IR absorption bands (1525 and 1630 cm^{-1}) that correspond well but are slightly more red shifted than the experimental IRMPD action spectrum. The very minor peak at 1471 cm^{-1} of the theoretical spectra appears as a small shoulder to the red of the band at 1525 cm^{-1} in the measured IRMPD spectrum. However, the absence of a minor peak at 1275 cm^{-1} in the experimental spectrum suggests that these two conformers are not significantly populated in the experiments. Interestingly, the theoretical IR spectrum of the third-excited conformer, where the sodium cation binds to the S2 and N3 atoms of the 2-thioketo-4-hydroxyl tautomer, conformer **C**, has many IR features that fit well with the measured IRMPD spectrum. In particular, the bands below 1300 cm^{-1} and above 1500 cm^{-1} exhibit reasonable agreement. However, the absence of a spectral feature associated with C4–OH stretching at ~1430 cm^{-1} in the IRMPD action spectrum, as well as the free energy (12.3 kJ/mol) relative to the ground-state conformer suggests that the **C** conformer is most likely not accessed in the experiments. This conclusion along with the poor agreement between the theoretical spectrum and the experimental IRMPD spectrum for the 4-hydroxyl rotamer of **C**, conformer **B**, indicate that this conformer is also not present in the experiments.

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

The experimental IRMPD action spectrum along with the calculated linear IR spectra and structures of the five most stable tautomeric conformations found for the Na^+(6Me2SU) complex are compared in Fig. 7. The IRMPD spectrum of Na^+(6Me2SU) exhibits many of the IR features observed for the Na^+(5Me2SU) complex, but also exhibits an additional feature. Therefore, the IRMPD action

spectra of Na^+(2SU), Na^+(5Me2SU), and Na^+(6Me2SU) can be easily distinguished from each other. This is particularly important for the two methylated complexes, as they occur at the same mass-to-charge ratio, and the IRMPD of these two complexes both result in the loss of the nucleobase and detection of the sodium cation at $m/z = 23$. The calculated linear IR spectrum of the ground-state **A** conformer, where the sodium cation binds to the 4-position of the canonical 2-thioketo-4-keto tautomer of 6Me2SU, provides the best match to the measured IRMPD spectrum, confirming the gas-phase structure of the Na^+(6Me2SU) complex. The intense peaks at 1140, 1540, and 1650 cm^{-1} in the measured IRMPD spectrum of Na^+(6Me2SU) correspond to the same C2=S stretching, asymmetric N1–H and N3–H bending, and the C4=O carbonyl stretching mode observed in the spectra of the Na^+(2SU) and Na^+(5Me2SU) complexes, respectively. The sharp peak at 1190 cm^{-1} is due to the mixed character mode involving in-plane C5–H bending and a very modest C2=S stretching motion that differs from the previous two complexes. The new distinct feature at 1600 cm^{-1} corresponds to the mixed character mode involving C5=C6 stretching and in-plane N1–H bending. The low intensity peaks in the range between 1300 and 1450 cm^{-1} are observed in the experimental IRMPD spectrum, and arise from various N–H bending motions and wagging of the methyl group, as well as the mixed character mode comprised of these motions. The theoretical IR spectra of the two low-lying conformational rotamers, **C** and **B**, are markedly different than the IRMPD action spectrum. Therefore, these conformers are not accessed in the experiments. The indistinguishable calculated IR spectra of the 2-sulfhydryl-4-keto tautomers of Na^+(6Me2SU), conformational rotamers **I** and **F**, also exhibit several features above 1500 cm^{-1} that are similar to the experimental IRMPD spectrum. However, the absence of the peaks at ~1260 and 1430 cm^{-1} in the measured spectrum once again suggests that both the **I** and **F** conformers are not accessed in the experiments.

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

The experimental IRMPD action spectrum along with the calculated linear IR spectra and structures of the five most stable tautomeric conformations found for the Na^+(4SU) complex are compared in Fig. 8. In this overall comparison, the theoretical spectra computed for the ground-state **C** conformer and the first-excited 4-sulfhydryl rotamer, **B**, are in best agreement with the experimental IRMPD spectrum. The carbonyl stretch predicted theoretically at 1670 cm^{-1} corresponds well with the experimental spectrum, while the features below 1630 cm^{-1} are slightly red shifted as compared to the experimental spectrum. The unresolved peak or shoulder to the red of the carbonyl stretch at 1631 cm^{-1} corresponds to the C5=C6 stretch. The three very low intensity IR absorption bands at 1120, 1240, and 1500 cm^{-1} are the result of C4–S stretching, C2–N3 stretching, and a mixed character mode associated with N1–H bending and aromatic ring stretching. The minor peak at 1415 cm^{-1} is due to the coupling of two very closely spaced IR absorption peaks that are assigned to the in-plane N1–H bending and a mixed character mode associated with N3=C4 stretching and symmetric C5–H and C6–H bending. In our previous study of the analogous protonated thiouracil complexes [41], it was found that the theoretical IR spectra calculated at the B3LYP/6-31G(d) level of theory do not allow sulfhydryl rotamers to be differentiated because they produce virtually identical IR spectra in the mid IR (fingerprint region) and in some cases in the N–H stretching region [51]. In some cases, the spectral lines are slightly shifted, but these shifts tend to be on the order of 1 to 5 cm^{-1}, much less than typical experimental broadening, and therefore cannot be resolved by the IRMPD action spectroscopy technique. The same behavior is observed again here for the Na^+(4SU) complex as can be seen in Fig. 8, making it impossible to determine whether or not the first-excited conformer, **B**, is accessed in the experiments. The transition state located between the **C** and **B** rotamers using the synchronous transit-guided quasi-Newton (STQN) method by Peng et al. [52] indicates a barrier height of 31.5 kJ/mol above the ground-state conformer. With the average internal energy available to the ground-state **C** conformer calculated at 298 K, 22.4 kJ/mol, it is unlikely that the first-excited conformer can be accessed in the gas phase. However, one must also consider the fact that all complexes were first formed in a 50:50 water–methanol mixture, and subsequently ionized by electrospray ionization. The polar solvent could significantly lower this activation barrier and stabilize various species and/or facilitate tautomerization necessary to produce a room temperature distribution, as observed previously for the protonated uracil and thiouracil complexes [41]. Based on the computed relative free energies of these conformers at 298 K, a Boltzmann distribution suggests that both would be present at a relative population of 83:17. However, even in the N–H stretching region, conformers B and C cannot be differentiated. The experimental IRMPD action spectrum and the theoretical IR spectra for the second and third-excited conformers, **E** and its 4-sulfhydryl rotamer **H**, exhibit good agreement in the carbonyl stretching region (1670 cm^{-1}). However, the peak at 1530 cm^{-1} and the minor peak at 1316 cm^{-1} are not observed in the experimental IRMPD spectrum, suggesting that these conformers are not populated in the experiments. The calculated spectrum for the sodium cation bound to the canonical keto-thioketo tautomer at the O2 position, conformer **D**, exhibits peaks at 1125 and 1620 cm^{-1} that are well matched to the IRMPD action spectrum, while the carbonyl stretch is just slightly blue shifted and the peaks around 1400 cm^{-1} are slightly less intense and better resolved. These well matched features make the differentiation of conformers **C** and **D** challenging. However, upon closer examination, the absence of two very weak bands at 1240 and 1500 cm^{-1} in the theoretical spectrum and the relatively high free energy computed for the **D** conformer, 19.4 kJ/mol, suggests that it is not accessed in the experiments.

4.6. Comparison of experimental and theoretical IR spectra of Na^+(24dSU)

The experimental IRMPD action spectrum along with the calculated linear IR spectra and structures of the four most stable tautomeric conformations found for the Na^+(24dSU) complex are compared in Fig. 9. The IRMPD action spectrum of Na^+(24dSU) is noisier than that measured for the other complexes as a result of the very low intensity of the reactant ion produced in the experiments. The minor dips observed in several of the major features in the IRMPD spectrum likely arise from the low reactant ion signal combined with minor instabilities in the intensity of the FEL source. However, it cannot be ruled out that they arise from slightly resolved distinct IR features. The calculated linear IR spectra of the ground-state conformer, **C**, and the first-excited 4-sulfhydryl rotamer, **B**, are in good agreement with the measured IRMPD action spectrum, similar to that found for the Na^+(4SU) complex. Upon closer examination, the theoretical IR spectrum of the **C** conformer exhibits a slightly better match to the experimental IRMPD action spectrum than the **B** conformer in the region around 1210 cm^{-1}. The peaks at 1130, 1540, and 1600 cm^{-1} correspond to the mixed character mode associated with N1–C2, C4–S, and C4–C5 stretching; the mixed character mode involving N1–H bending and aromatic ring stretching, and C5=C6 stretching, respectively. The unresolved IR features near ~1210 cm^{-1} in the IRMPD spectrum closely resemble the coupling of the three IR absorption peaks at 1180, 1205, and 1240 cm^{-1} in the calculated linear IR spectra, and arise from the mixed character mode involving C2=S and C4–S stretching, the mixed character mode associated with N1–H

bending and asymmetric C5–H and C6–H bending, and the C2–N3 stretching modes, respectively. The peak observed at 1420 cm^{-1} is predicted to arise from the coupling between two neighboring peaks in the theoretical spectrum calculated for **C** conformer. These features are associated with N3=C4 stretching and N1–H bending mode coupling to the in-plane symmetric C5–H and C6–H bending. Absence of the diagnostic band at ~1290 cm^{-1} observed in the theoretical spectra of the **E** and **H** conformers in the Na^+(24dSU) IRMPD spectrum clearly indicates that these two rotamers were not present in the experiments.

4.7. Comparison of proton and sodium cation binding to uracil and thiouracils

The gas-phase structures of the protonated forms of uracil and the five thiouracils examined here were previously investigated using the same methods employed here, IRMPD action spectroscopy and theoretical electronic structure calculations [41]. The ground-state conformers of the protonated and sodium cationized complexes of these systems are compared in Fig. 2S of the Supplementary information. In all cases, the ground-state tautomeric conformation of the nucleobase differs between the protonated and sodium cationized complexes except for 4SU. Protonation of U, 2SU, 5Me2SU, and 6Me2SU preferentially stabilizes a minor tautomer of the nucleobase, whereas sodium cationization of the canonical tautomer is most favorable for all of these bases. In contrast, protonation of the canonical tautomer of 4SU produces the most stable conformation, whereas sodium cationization preferentially stabilizes a minor tautomer of the nucleobase. However, in both cases, these complexes can be thought of as being derived from the same tautomeric conformation of the nucleobase because protonation of the canonical 2-keto-4-thioketo tautomer at the S4 position is equivalent to protonation of the 2-keto-4-sulfhydryl tautomer (the tautomeric conformation in the sodium complex) at the N3 position. In the case of 24dSU, both protonation and sodium cationization stabilize a minor tautomer of the nucleobase. However, the tautomeric conformations differ in the orientation of the 4-sulfhydryl hydrogen atom.

The IRMPD action spectra of the protonated and sodium cationized complexes of these systems are compared in Fig. 3S of the Supplementary information. Differences in the binding of a proton and sodium cation and the resulting preferred tautomeric conformations of the nucleobases in these complexes are reflected in their IRMPD action spectra, such that they can be easily distinguished. Sodium cationization results in less broadening of the spectral features than observed for protonation. Similar behavior was observed in the IRMPD action spectra of protonated and sodium cationized complexes of methionine [32]. However, the cause of the increased broadening in these systems is not well understood. In other systems significant broadening of spectral features arises from hydrogen bonding interactions in which the proton is nearly equally shared such as in carbohydrates [37] and proton bound dimers [53]. In these latter cases the mobility of the proton appears to play a major role in inducing broadening of the spectral features. The carbonyl stretching region is sufficient for differentiation of the effects of proton and sodium cation binding to all of the nucleobases except 24dSU. The presence of an unperturbed carbonyl stretch at ~1800 cm^{-1} and the extent of red shifting of the other carbonyl stretch that is weakened by interaction with a proton at ~1625 cm^{-1} or sodium cation at ~1650 cm^{-1} readily allow differentiation of the protonated and sodium cationized complexes. Clearly the interaction with the proton is stronger as the carbonyl stretches are red-shifted to a greater extent in the protonated complexes than in the sodium complexes, as expected.

4.8. Influence of the alkali metal cation size on the binding to uracil and thiouracils

In previous work, the effects of alkali metal cation size on the binding to uracil and the thiouracils were examined by energy-resolved collision-induced dissociation experiments and theoretical electronic structure calculations [18,50]. Results of that work confirm that the strength of the M^+–U interaction decreases monotonically with increasing size of the alkali metal cation. Reasonably good agreement between theory and experiment was found for all of the Na^+ and K^+ complexes examined in that work. However, the calculated values for the Li^+ complexes were systematically lower than the measured values. In that work, the canonical tautomer of the nucleobase was assumed to be accessed in the complexes generated in the source and upon CID, however, no direct evidence for the tautomeric conformations accessed was determined. Present experimental and theoretical results confirm that the sodium cationized complexes of U, 2SU, 5Me2SU, and 6Me2SU indeed involve Na^+ binding to the O4 carbonyl oxygen atom of the canonical tautomeric conformation of the nucleobase as previously reported. However, the present results suggest that the ground-state conformations of the Na^+(4SU) and Na^+(24dSU) complexes involve minor tautomers. At the B3LYP/6-311+G(2d,2p) level of theory, these tautomeric conformers are 19.4 and 27.8 kJ/mol more stable (free energy) than the structures previously calculated in the CID study. However, the Na^+–4SU and Na^+–24dSU bond dissociation energies (BDEs) measured (and calculated at the MP2(full)/6-311+G(2d,2p)//B3LYP/6-31G* level of theory) in the previous work, 125.8 ± 4.7 (122.1) and 100.2 ± 5.8 (98.9) kJ/mol, respectively, exhibit excellent agreement [18]. In contrast, the agreement between the measured and calculate BDEs is much poorer based on the new ground-state conformers found here. The calculated BDEs become 138.9 and 131.4 kJ/mol, respectively, based on MP2(full) single point energies. The agreement is even worse for energetics based on B3LYP single point energies, where the computed BDEs are 151.1 and 144.4 kJ/mol, respectively. This suggests that the complexes accessed in the CID study indeed involve Na^+ binding at O2 and S4 of the canonical tautomer of the nucleobase, respectively. The differences in the tautomeric conformations of the Na^+(4SU) and Na^+(24dSU) complexes accessed in that work and in the present study likely arise as a result of the differences in the way in which the ions were generated. In the CID study, the complexes were formed by three-body associative reactions and collisionally stabilized in a flow tube ion source, where the sodium cation was generated by dc discharge and the nucleobase was introduced by gentle heating in a thermal probe. The excellent agreement between theory and experiment found in that work suggests that collisional stabilization of the initially formed complex before tautomerization to form the ground-state conformer results in kinetic trapping of an excited conformer of the complex and that the measured BDE is a diabatic value. In contrast, these complexes were generated by ESI in the present work and spectroscopic results suggest that the ground-state conformers were accessed in all cases. Thus, it would be fruitful to re-examine the CID behavior of the Na^+(SU) and Na^+(24dSU) complexes generated by ESI.

To further investigate the effects of the size of the alkali metal cation on the structures and IR spectra of uracil and the thiouracils, we also calculated the low-energy tautomeric conformations of the analogous M^+(U) and M^+(*x*SU) complexes, where M^+ = Li^+, K^+, Rb^+, and Cs^+, and *x*SU = 2SU, 5Me2SU, 6Me2SU, 4SU, and 24dSU. The calculations were carried out at the same level of theory as used for the protonated and sodium cationized complexes, i.e., B3LYP/6-311+G(2d,2p)//B3LYP/6-31G(d). However, these basis sets are not parameterized for Rb and Cs. Thus, we used a hybrid basis set for the complexes involving these metal cations, where the effec-

tive core potentials and valence basis sets of Hay and Wadt [54] with an additional polarization (*d*) function added with exponents of 0.24 and 0.19 as suggested by Glendening et al. [55], respectively, were used to describe the Rb^+ and Cs^+, and the standard 6-31G(d) and 6-311+G(2d,2p) basis sets for all other atoms. The ground-state tautomeric conformations found for these complexes as well as the analogous complexes to Na^+ are compared in the Supplementary information in Fig. 4S. In all cases, the bond distances between the alkali metal cation and the neutral ligands increase as the atomic radius of the metal cation increases from Li^+ to Cs^+ in both monodentate (U, 2SU, 5Me2SU, and 6Me2SU) and bidentate (4SU and 24dSU) binding interactions. In the complexes to U, 2SU, 5Me2SU, and 6Me2SU, the ground-state tautomeric conformation of the nucleobase remains the same for all five alkali metal cation complexes. In contrast, in the complexes to 4SU and 24dSU, the ground-state tautomeric conformation of the nucleobase remains the same for Li^+ through Rb^+, but changes for the Cs^+ complexes. In addition, the larger radius of the sulfur atom results in a larger M^+–S2 bond distance in the M^+(2,4dSU) complexes as compared to the M^+–O2 bond distance in the M^+(4SU) complexes. Overall comparison of the relative free energies of the low-energy tautomeric conformers found suggests that the smaller, more strongly bound metal cations are more effective at stabilizing minor tautomers of the nucleobase.

Although the Li^+ and K^+ complexes were not examined in the present study, similar comparisons based on the theory can be made. At the B3LYP/6-311+G(2d,2p) level of theory, the ground-state tautomeric conformations of the Li^+(4SU), Li^+(24dSU), K^+(4SU), and K^+(24dSU) complexes are 23.9, 44.6, 8.3, and 13.6 kJ/mol more stable (free energy) than the structures previously calculated in the CID study, respectively. However the agreement between the BDEs measured (and calculated) in that work for these complexes, 213.1 ± 5.0 (180.1), 175.1 ± 4.5 (144.9), 97.3 ± 5.5 (91.7), and 80.8 ± 2.8 (69.7) kJ/mol, respectively, is not sufficient to rule out the presence of the ground-state tautomeric conformations for these complexes [18]. In contrast to the complexes to Na^+, the BDEs computed based on the new ground-state conformers found here for the complexes to Li^+ and K^+, 202.3, 195.6, 100.9, and 90.7 kJ/mol (MP2(full) results) and 217.1, 210.2, 103.4, and 92.3 kJ/mol (B3LYP results), respectively, exhibit better agreement with the measured values. Thus, it would also be fruitful to re-examine the CID behavior of the Li^+(4SU), Li^+(24dSU), K^+(4SU), and K^+(24dSU) complexes generated by ESI to more definitively establish the thermochemistry associated with these systems.

In addition to examining the structures and energetics of the alkali metal cation–nucleobase complexes, we also computed the linear IR spectra for the ground-state tautomeric conformations of these systems. The IRMPD action spectra of the Na^+(*x*SU) complexes are compared to the calculated linear IR spectra for the M^+(*x*SU) complexes for all five alkali metal cations in Fig. 5S of the Supplementary Information. For all complexes except those to 24dSU, a band associated with an alkali metal cation bound carbonyl stretch is found near 1650 cm^{-1}. This band becomes increasingly blue-shifted as the size of the alkali metal cation increases, or equivalently as the strength of the M^+–*x*SU interaction becomes weaker. Thus, once again, the carbonyl stretching region may be sufficient for characterizing these complexes.

Other bands in the IR spectra of these complexes exhibit systematic changes with the size of the alkali metal cation as well that can be used to further understand the effects of the size of the alkali metal cation on the binding interaction. In the M^+(U) complexes, the bands associated with the free carbonyl stretch at ~1800 cm^{-1} and in-plane N1–H bending at ~1485 cm^{-1} become increasingly red-shifted as the size of alkali metal cation increases. For the M^+(2SU) complexes, the bands associated with the C=S2 stretch at ~1155 cm^{-1} and asymmetric N1–H and N3–H bending at ~1530 cm^{-1} become increasingly red shifted as the size of the alkali metal cation increases. Likewise, for the M^+(5Me2SU) complexes, the bands associated with C=S2 stretching at ~1130 cm^{-1}, C2–N1–C6 stretching at ~1200 cm^{-1}, and asymmetric N1–H and N3–H bending at ~1530 cm^{-1} become increasingly red shifted as the size of the alkali metal cation increases. For the M^+(6Me2SU) complexes, the bands associated with C=S2 stretching at ~1140 cm^{-1} and asymmetric N1–H and N3–H bending at ~1540 cm^{-1} become increasingly red shifted, while the mixed character mode associated with C5=C6 stretching and in-plane N1–H bending at ~1600 cm^{-1} becomes increasingly blue shifted as the size of the alkali metal cation increases. For the M^+(4SU) complexes, the minor bands associated with C=S2 stretching at ~1120 cm^{-1} and C2–N3 stretching at ~1240 cm^{-1} become increasingly red shifted as the size of the alkali metal cation increases. Thus, the alkali metal cation complexes to U, 2SU, 5Me2SU, 6Me2SU, and 4SU all exhibit systematic changes in their IR spectra that allow the various complexes to be differentiated. The effects of the size of the alkali metal cation on the IR spectra of the M^+(24dSU) complexes are much less significant. Only the minor band at ~1130 cm^{-1} associated with the mixed character mode comprised of N1–C2, C4–S, and C4–C5 stretching is red shifted as the size of the alkali metal cation increases, whereas the positions of all other bands remain virtually unchanged as the size of the cation changes. Thus, these M^+(24dSU) complexes would be very challenging to differentiate based on their IRMPD action spectroscopy. As discussed above, the ground-state tautomeric conformation of 4SU and 24dSU changes for the complexes to Cs^+ as compared to the smaller alkali metal cations. These results indicate that the larger atomic radius of Cs^+ leads to longer metal-ligand distances and weaker interactions such that the metal cation is not as efficient at facilitating the tautomerization of the N3 hydrogen atom, and thus, the ground-state tautomeric conformations accessed in these complexes are the canonical tautomers of 4SU and 24dSU. As a result of the difference in the preferred binding mode the Cs^+(4SU) complex provides a diagnostic band at ~1120 cm^{-1}, while the Cs^+(24dSU) complex exhibits several diagnostic bands at ~1080, 1180, and 1460 cm^{-1}. Thus, the Cs^+ complexes of 4SU and 24dSU should be readily differentiated from the other alkali metal cationized complexes.

5. Conclusions

The IRMPD action spectra of the sodium cationized complexes of uracil and five thiouracils in the region ~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 the primary species accessed in the experiments. The ground-state structures calculated for Na^+(U), Na^+(2SU), Na^+(5Me2SU), and Na^+(6Me2SU) at the B3LYP/6-31G(d) level of theory reveal that the sodium cation preferentially binds to the 4-keto group of the canonical 2,4-diketo or 2-thioketo-4-keto tautomer in these complexes. In contrast, binding of a sodium cation in the Na^+(4SU) and Na^+(24dSU) complexes preferentially stabilizes an alternative 2-keto-4-sulfhydryl or 2-thioketo-4-sulfhydryl tautomer. In all cases, no evidence for additional low-energy conformers was observed in the measured IRMPD action spectra suggesting that only one tautomeric conformation was accessed for each complex. How-

ever, low-energy sulfhydryl rotamers of Na^+(4SU) and Na^+(24dSU) produce theoretical spectra that are nearly identical to that of the ground-state **C** conformers, making it impossible to definitively establish the presence or absence of these low-energy conformers in the IRMPD spectra. Comparison of the protonated and other alkali metal cationized complexes of these nucleobases suggest that the stronger the cation–nucleobase interaction, the more effective the cation is at stabilizing minor tautomers and thus the stability and hydrogen bonding characteristics of these systems.

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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.2011.06.019.

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