Infrared multiple photon dissociation spectroscopy of protonated histidine and 4-phenyl imidazole

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The gas-phase structures of protonated histidine (His) and the side-chain model, protonated 4-phenyl imidazole (PhIm), are examined by infrared multiple photon dissociation (IRMPD) action spectroscopy utilizing light generated by the free electron laser FELIX. To identify the structures present in the experimental studies, the measured IRMPD spectra are compared to spectra calculated at a B3LYP/6–311+G(d,p) level of theory. Relative energies of various conformers are provided by single point energy calculations carried out at the B3LYP, B3P86, and MP2(full) levels using the 6–311+G(2d,2p) basis set. On the basis of these experiments and calculations, the IRMPD action spectrum for H+(His) is characterized by a mixture of [N2 N3] and [N2 CO] conformers, with the former dominating. These conformers have the protonated nitrogen atom of imidazole adjacent to the side-chain (N4) hydrogen bonding to the backbone amino nitrogen (N6) and to the backbone carbonyl oxygen, respectively. Comparison of the present results to recent IRMPD studies of protonated histamine, the radical His+, cation, H+(HisArg), H22+(HisArg), and M+(His), where M+ = Li+, Na+, K+, Rb+, and Cs+, allows evaluation of the vibrational motions associated with the observed bands.

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1. Introduction

Histidine (His) is chemically one of the most flexible protein residues because the imidazole side chain can function as both an acid and base near neutral pH [1]. The histidine molecule presents three potential coordination sites in aqueous solution. The carboxyl group (pKₐ = 1.9), the imidazole nitrogen (pKₐ = 6.1), and the amino nitrogen (pKₐ = 9.1) become available for complexation as pH increases. The acid–base properties of various conformers are provided by single point energy calculations carried out at the B3LYP, B3P86, and MP2(full) levels using the 6–311+G(2d,2p) basis set. On the basis of these experiments and calculations, the IRMPD action spectrum for H+(His) is characterized by a mixture of [N2 N3] and [N2 CO] conformers, with the former dominating. These conformers have the protonated nitrogen atom of imidazole adjacent to the side-chain (N4) hydrogen bonding to the backbone amino nitrogen (N6) and to the backbone carbonyl oxygen, respectively. Comparison of the present results to recent IRMPD studies of protonated histamine, the radical His+ cation, H+(HisArg), H22+(HisArg), and M+(His), where M+ = Li+, Na+, K+, Rb+, and Cs+, allows evaluation of the vibrational motions associated with the observed bands.

In recent years, the structures of many proteins have been studied by X-ray crystallography; however, because of the poor sensitivity of X-ray crystallography in detecting hydrogen atoms, the protonation structures and hydrogen-bonding interactions of amino acid side chains are not well resolved in many cases. To obtain such information, vibrational spectroscopy is a powerful method as it is more sensitive to chemical bonds and molecular interactions. In the case of histidine, its protonation state, metal binding, and hydrogen bonding interactions have been investigated using both Raman [6–18] and Fourier transform infrared (FTIR) [19] spectroscopy. Huang et al. [20] investigated the neutral, protonated, and deprotonated histidine conformers in the gas phase using time-dependent density functional theory (TDDFT) to calculate the electronic spectra and charge-transfer processes. Also, Hasegawa et al. [21] studied 4-methylimidazole (a simple model compound of the histidine side chain) and its different protonation forms using FTIR and Raman spectra for systematic investigation of vibrational markers of the protonation state of histidine.

Infrared multiple photon dissociation (IRMPD) spectroscopy has been used to probe the structures of ionized complexes in the gas phase and can be a powerful tool for understanding ion–protein interactions. An important advantage of this technique is the ability to investigate the structures of biomolecules in isolation, where complicating structural effects of solvent and counter-ion...
are absent. Recently, gas-phase structures of protonated histamine [22], histidine radical cation (His\(^{+}\)) [23], H\(^+(\text{HisArg})\) and H\(_2\text{His}^+(\text{HisArg})\) [24], and M\(^+(\text{His})\) [25], where M\(^+\) = Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), and Cs\(^+\), have all been investigated by IRMPD spectroscopy utilizing light generated by a free electron laser. In the present study, we measure the IRMPD action spectra for photodissociation of protonated His and 4-phenyl imidazole (PhIm), where the latter provides a model for assessing the vibrations of the imidazole side-chain ring. Conformations of these molecules are identified by comparing the experimental spectra to IR spectra of the low-lying structures of the cationized His and PhIm complexes predicted by quantum chemical calculations at the B3LYP/6–311+G(d,p) level of theory. IRMPD action spectra for H\(^+(\text{His})\) and H\(^+(\text{PhIm})\) are also compared to the previous results for H\(^+(\text{histamine})\), His\(^{+}\), H\(_2\text{His}^+(\text{HisArg})\), and M\(^+(\text{His})\), where M\(^+\) = Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), and Cs\(^+\).

2. Experimental and computational

2.1. Mass spectrometry and photodissociation

Experiments were performed using the Free Electron Laser for Infrared eXperiments (FELIX) [26] in combination with a home-built Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, which has been described in detail elsewhere [27–29]. Protonated histidine and protonated phenyl imidazole complexes were generated using a Z-spray (Micromass UK Ltd.) electrospray ionization source. Solutions used were 1.0–3.0 mM His and 1.0 mM phenyl imidazole acidified with acetic acid in 58% MeOH and 42% H2O for H\(^+(\text{PhIm})\). Solution flow rates were about 10 \(\mu\)l/min and the electrospray needle was held at a voltage of ~3.2 kV. Ions were accumulated in a hexapole trap for about 4 s followed by pulsed extraction through a quadrupole bender prior to being injected into the ICR cell via a radio frequency (rf) octopole ion guide. Electrostatic pulsing of the dc bias of the ion transfer octopole allows ions to be captured in the ICR cell without the use of a gas pulse [28]. In contrast to the conventional gas pulsing method to stop the ions, this technique does not cause collisional heating of the ions. The precursor ions were mass selected using stored and deformed inverse Fourier transform (SWIFT) techniques and irradiated by the FEL at pulse energies of 50 mJ per macropulse of 5 \(\mu\)s duration, although they fell off to about 20 mJ toward the blue edge of the scan range. Complexes were irradiated for 2–4 s, corresponding to interaction with 10–20 macropulses. The fwhm Gaussian line shape for comparison with experimental spectra.

At the request of a reviewer, we also calculated geometries and single point energies adding a diffuse function for hydrogen, i.e., R/6–311+G(2d,2p)//B3LYP/6–311+G(d,p), where R indicates B3LYP, B3P86, and MP2(full) levels. This is intended to provide a better description of hydrogen bonding, even though previous explorations in our group have indicated that hydrogen difference functions have negligible effects on geometries and relative energies [46–49]. In the present case, six conformers of three different types were examined. Structures were essentially unchanged with hydrogen bonds (a total of 16 interactions) changing by –0.002s to +0.0008 Å, with the average absolute change being 0.0006 ± 0.0007 Å. Relative energies at this level of theory agreed with those presented below within 0.1kJ/mol in all cases. Furthermore, vibrational frequencies are the same within 0.01%. Because the addition of diffuse functions to the hydrogen atoms introduces little distortions, energy changes, or vibrational shifts, such calculations were not pursued further for any other conformations.

3. Results and discussion

3.1. IRMPD action spectroscopy

The photodissociation spectra of protonated (m/z 156) His both as depletion of the parent ion and the yield for loss of H\(_2\text{O} + \text{CO}\) (m/z 110) (corrected for laser power and uncorrected) are shown in Fig. S1 of the Supporting Information. Very small amounts (>10 times smaller) of just H\(_2\text{O}\) loss were also detected. These decompositions pathways match the lowest energy products observed in the collision-induced dissociation (CID) spectrum of H\(^+(\text{His})\), as observed previously [50,51]. Parent and product ion intensities were monitored as a function of the laser frequency, and the IRMPD yield shown in Fig. S1 and the figures below was calculated as the integrated intensity ratio \(I_{110}/(I_{110} + I_{156})\). This was normalized linearly with laser power to roughly account for the change in laser power as a function of photon energy. As shown in Fig. S1, the depletion spectrum of the m/z 156 parent ion is similar to the appearance spectrum of the m/z 110 product ion. The depletion of the parent ion signal exceeds 50% at the most intense resonances. Because the IRMPD yield is normalized for parent ion fluctuations, it exhibits better signal-to-noise ratio than the depletion signal and will be compared to the calculated spectra.
To provide a spectrum characteristic of the imidazole (Im) side-chain of His, we also tried acquiring the IRMPD spectrum of protonated Im. Unfortunately, this molecule is sufficiently small that dissociation is inefficient and no spectrum could be collected. To overcome this size limitation, it would be useful to examine Im substituted at the 4-position to mimic the His side-chain. Simple alkyl-substituted Im are unstable or not readily available, but 4-phenyl imidazole (PhlM) is commercially available. For protonated PhlM, two IR photodissociation pathways were observed corresponding to the loss of HCN (m/z 118) and 2 HCN (m/z 91). The sum of these two decomposition pathways is shown as the IRMPD action spectrum below.

3.2. Theoretical results (structures)

The nomenclature used here to identify different structural iso-

cmers of H+(His) is similar to that described previously for the IRMPD study of M+(His) [25]; however, here the atoms of His are named according to the IUPAC Compendium of Chemical Terminology (Gold Book), in which positions of the nitrogen atoms of the imi-
dazole ring relative to the side chain are denoted by pros (near’), abbreviated π and also referred to as N1) and telc (far’, abbrevi-

ated τ or N3) [52], Fig. 1. In addition, conformations of protonated His are identified by their proton binding sites in brackets, fol-

lowed by a description of the histidine orientation, named by a series of dihedral angles starting from the carboxylic acid hydro-
gen of the backbone and going to the imidazole side-chain nitrogen (Nτ)/OCCC, OCCC, CCCC, and CCCN, respectively). Dihedral angles are distinguished as cis (c, for angles between 0° and 45°), gauche (g, 45°-135°), or trans (t, 135°-180°), and + or − indicating their sign when necessary to distinguish similar structures. In some cases, these four dihedral angles are insufficient to distinguish similar conformers and a fifth dihedral angle is added in parentheses to define the electron lone pair orientation of the NH2 group. In most conformations, this cis with respect to the adjacent backbone CC bond, such that only alternate orientations (gauche or trans) are indicated by this fifth dihedral angle.

Eight low-lying and representative higher energy conforma-
tions of H+(His) are illustrated in Fig. 1, with a total of 24 structures shown in Fig. S2 in the Supporting Information. The protonated histidine complex, H+(His), has a set of low energy structures, [Nα-Nτ], in which the protonated imidazole side-chain hydrogen bonds to the backbone amino nitrogen ([Nα-H···Nτ]), Fig. 1. If the proton shifts to Nα, retaining a hydrogen bond to Nτ, the [Nα-Nτ] conformer is formed. These structures are not shown in Fig. 1 as the only signif-

icant distinction from the analogous [Nα-Nτ] structures is the position of the bridging proton. Other possible binding motifs are [Nα-CO] and [Nα-OH], in which the protonated imidazole side-chain hydrogen bonds to the backbone carbonyl oxygen ([Nα-H···OC) or hydroxyl oxygen ([Nα-H···OH). The latter are not shown in Fig. 1 because they resemble the [Nα-CO] structures with the carboxylic group rotated by 180°. Relative Gibbs free energies at 298 K and rel-

ative energies at 0 K including zero-point energy (ZPE) corrections with respect to the ground state calculated at three different levels of theory are given in Table 1 for H+(His). Because the relative Gibbs free energies may be more relevant in describing the experimental distributions, these values are used throughout the discussion below.

At all levels of theory, the ground state (GS) structure for H+(His) is [Nα-Nτ]-ttgc, Fig. 1. In addition, there are five other stable geometries having this binding motif. Only slightly higher in energy, 1–5 kJ/mol, is the ttgc conformer. Both the tgcc and ttgc structures have similar Nα-H···Nτ hydrogen bond lengths (1.916 and 1.902 Å, respectively), but the former appears to be stabilized by a stronger Nα-H···OC interaction, as suggested by bond lengths of 2.272 and 2.420 Å, respectively. [Nα-Nτ]-ttgc and ttgc conformers are found at slightly higher energies, 4–5 and 5–10 kJ/mol above the GS, respectively, and have slightly longer Nα-H···Nτ hydrogen bond lengths (1.967 and 1.932 Å, respectively). These conformers differ from the lower energy structures by the ∠dihedral angle (1.673 Å) among the [Nα-H···OCCO] variants being lowest in energy. In contrast, MP2 calculations find that the [Nα-H···OC] conformer being lowest in energy than their [Nα-Nτ] analogs, with the tgcc conformer being lowest of the [Nα-Nτ] conformers. These [Nα-Nτ] conformers have relatively short Nα-H···Nτ hydrogen bond lengths of 1.63–1.66 Å, indicating that the proton generally prefers to be on the Nα nitro-

gen.

Eight different [Nα-CO] conformers were located with the ctg-g structure being the lowest at the DFT levels of theory, 2–4 kJ/mol above the GS. This structure has the shortest Nα-H···OC hydrogen bond length (1.673 Å) among the [Nα-CO] conformers and is stabi-
lized by a OH···Nα hydrogen bond, Fig. 1. The next four conformers in energy are all tggc with Nα-H···OC hydrogen bond lengths of 1.78–1.83 Å, Figs. 1 and S2. In these conformers, the hydroxyl group has rotated, losing the OH···Nα hydrogen bond and replacing it with

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*All values calculated at the level of theory indicated using the 6–311+G(d,p) basis set with structures and zero-point energies calculated at the B3LYP/6–311+G(d,p) level of theory. Ground state species are identified in bold. Italics indicate species that collapse because the TS energy is lower.
an OH···OC interaction augmented by various N₉H-H-O(H) interactions depending on the orientation of the N₉H₂ group, (g) or (t).

DFT calculations find that these species lie 5–11 kJ/mol above the GS and 1–9 kJ/mol above the [N₉,CO]-ctg,g- structure, whereas MP2 calculations find that the lowest energy [N₉,CO] conformer is ctg,g(t) with the ctg,g- variant lying 5 kJ/mol higher. Finally, there are three higher-lying conformers, ctg,g-, ctg,g(t), and cg,gg, having N₉H-H-OC hydrogen bond lengths of 1.74–1.85 Ǻ.

The power-corrected experimental spectrum for H⁺(His) is shown in Fig. 3 and exhibits major peaks at 1778, 1609, 1399, 1306, 1132, 822, 676, and 609 cm⁻¹. Additional weaker bands are observed at 1747, 1433, 1340, ~990, ~923, and ~742 cm⁻¹, with an unresolved shoulder at 1079 cm⁻¹. Fig. 3 compares the experimental spectrum with those calculated for the most stable conformers of [N₉,N₉₁], [N₉,N₉₂], and [N₉,CO], which are the most likely conformers to be populated along with [N₉,N₉₁]-ttgc and [N₉,CO]-ctg,g- on the basis of visualizing the molecular motions. Fig. S3 shows that the four lowest structures for [N₉,N₉₁], all txxc where x = g or t, have

Fig. 2 shows the potential energy surfaces along the N₉-H-N₉₁ coordinate linking all six [N₉,N₉₁] and [N₉,N₉₂] conformers calculated at the B3LYP/6-311+G(d,p) level by explicitly controlling the N₉-H bond distance and allowing all other degrees of freedom to optimize. For comparison, the potential for protonated histidine, which is His without the carboxylic acid on the α-carbon, is also included. It can be seen that in all cases two minima in an asymmetric double-well potential exist. Therefore the relative stabilities and instabilities noted above are the result of differences in zero point energies. This is partially indicated by the zero of the scale in Fig. 2, which is set to the zero point energy of the harmonically calculated proton motion of the [N₉,N₉₁] conformers (scaled frequencies ranging from 3025 to 3170 cm⁻¹). Anharmonic frequency calculations for the [N₉,N₉₁]-ttgc conformer find a modest shift from 3085 cm⁻¹ (harmonic, 0.975 scaling) to 2937 cm⁻¹ (anharmonic, unscaled). Despite this, the use of harmonic frequencies on such an anharmonic potential is not exact and use of the harmonic frequency for proton motion of the [N₉,N₉₁] conformers would place the zero differently for each conformer. These frequencies range from 2338 to 2465 cm⁻¹ (scaled), which places the zero point level for these conformers roughly 14 kJ/mol above the bottom of the [N₉,N₉₁] potential wells.

3.3. Comparison of experimental and theoretical IR spectra: H⁺(His)

The power-corrected experimental spectrum for H⁺(His) is shown in Fig. 3 and exhibits major peaks at 1778, 1609, 1399, 1306, 1132, 822, 676, and 609 cm⁻¹. Additional weaker bands are observed at 1747, 1433, 1340, ~990, ~923, and ~742 cm⁻¹, with an unresolved shoulder at 1079 cm⁻¹. Fig. 3 compares the experimental spectrum with those calculated for the most stable conformers of [N₉,N₉₁], [N₉,N₉₂], and [N₉,CO], which are the most likely conformers to be populated along with [N₉,N₉₁]-ttgc and [N₉,CO]-ctg,g- on the basis of visualizing the molecular motions. Fig. S3 shows that the four lowest structures for [N₉,N₉₁], all txxc where x = g or t, have

![Fig. 2](image-url)
similar spectra, as do the four analogous structures for \([\text{N}_2\text{N}_8\text{g}_8]\). In contrast, the cis orientation of \(\angle\text{HOC}\) in the higher-energy \(\text{cxgc}\) conformers of both \([\text{N}_8\text{N}_8\text{g}_8]\) and \([\text{N}_8\text{N}_8\text{g}_8]\) removes the OH···OC interaction such that the intense bands associated with the CO–OH stretch and COH bend motions shift from \(\sim1140\) to \(\sim1240\) cm\(^{-1}\) and those for the CO stretch shift from \(\sim1780\) to \(\sim1800\) cm\(^{-1}\). The four \([\text{N}_8\text{CO}]\text{-cxgg}\) conformers show similar spectra although the intense band associated with the C–OH stretch and COH bend motions is located at \(\sim1380\) cm\(^{-1}\) (shifted because of the OH···N\(_8\) interaction) for ctg\(_{g}\) and ctg\(_{g}\) and at \(\sim1290\) cm\(^{-1}\) for ctg\(_{g}\)(t) and cggg, which no longer have the OH···N\(_8\) interaction. In these four cases, the CO stretch is red-shifted to \(\sim1745\) cm\(^{-1}\).
We also note that the extremely intense band at 1387 cm\(^{-1}\) in the [N\(_\pi\),CO]-tggc spectrum, which corresponds to a COH bend and C–OH stretching motion, shifts to 1342 cm\(^{-1}\) when an anharmonic frequency calculation is performed, potentially explaining the unassigned band observed at 1340 cm\(^{-1}\). The results of the anharmonic frequency calculation are shown for all the bands in this frequency range in Fig. 5A, along with a comparable calculation for the [N\(_\pi\),N\(_\pi\)]-tggc conformer. Few bands shift appreciably between the scaled harmonic and unscaled anharmonic calculation, so the comparison shown in Fig. 3 remains reasonable.

Although much of the spectrum shown for the [N\(_\pi\),N\(_\pi\)]-tggc conformer can agree with the experimental spectrum, Fig. 3, there is little if any intensity observed near 1500 cm\(^{-1}\), where the predicted spectra for all six [N\(_\pi\),N\(_\pi\)] conformers have intense bands associated with the umbrella motion of the N\(_H_3\) group. Likewise, there is no evidence in the experimental spectrum for a carbonyl stretch near 1800 cm\(^{-1}\), suggesting that the [N\(_\pi\),N\(_\pi\)]-cgxc, [N\(_\pi\),N\(_\pi\)]-cgxg, and [N\(_\pi\),OH] conformers are not present experimentally. Both conclusions are generally consistent with the relative energetics of these species, Table 1.

On the basis of calculated spectra and thermodynamic data, [N\(_\pi\),N\(_\pi\)]-txgc conformers are identified as the major carrier of the measured IRMPD spectrum with total predicted populations of 68–75% and the tggc and tgtc conformers accounting for most of this intensity. [N\(_\pi\),CO] conformers are predicted to account for 3–20%, consistent with the observation of the peak at 1747 cm\(^{-1}\) and perhaps that at 1340 cm\(^{-1}\). MP2 theory suggests that the [N\(_\pi\),N\(_\pi\)] conformers have a population of 25%, whereas the DFT calculations put this below 3%. The latter prediction seems more in line with the experimental observations, although the complex coupling between the potential wells associated with these conformations seen in Fig. 2 may complicate this conclusion.

### 3.4. Comparison of experimental and theoretical IR spectra: H\(^+\)(4-phenyl imidazole)

Fig. 4 shows the experimental IRMPD action spectra of H\(^+\)(PhIm) and H\(^+\)(His) compared with calculated IR spectra for the lowest energy conformer of H\(^+\)(PhIm). This structure has the proton on the imidazole nitrogen with the two rings twisted out of planarity by 35\(^\circ\). Fig. 4. Clearly, protonation anywhere else will be much higher in energy and the rigidity of the phenyl side-chain restricts the number of possible structures. The predicted spectrum for H\(^+\)(PhIm) shows a close correspondence to the observed spectrum, in terms of both band positions and relative intensities. The primary exception is the band at 1340 cm\(^{-1}\), which is not predicted accurately, either because the band predicted at 1281 cm\(^{-1}\) is shifted or the band at 1326 cm\(^{-1}\) should be more intense. (An anharmonic frequency calculation for H\(^+\)(PhIm) is very similar to the scaled harmonic spectrum in Fig. 4, with no large shifts in any band having noticeable intensity.) Notably, the band at 1340 cm\(^{-1}\) in the H\(^+\)(His) spectrum was also not accurately reproduced, a comparison that might suggest that this corresponds to a motion of the imidazole side chain (in contrast to the assignment based on the anharmonic frequency calculation). Given these provisos, the experimental IRMPD action spectrum can be explained adequately by the calculated spectrum of the ground state conformer.

Detailed analysis of band positions and vibrational assignments for H\(^+\)(PhIm) are given in Table 3 on the basis of visualizing the molecular motions. Although the majority of the bands in the IRMPD spectrum of H\(^+\)(PhIm) correspond to motions throughout the molecule, the most intense bands generally involve vibrations of the imidazole ring. Only the band at 752 cm\(^{-1}\) is primarily centered in the phenyl ring, an out-of-plane CH bend. Given the non-polar nature of the phenyl ring, this seems reasonable.
Comparison of the H⁺(Phlm) spectrum with that of H⁺(His) shows many similar features, Fig. 4. Obvious differences are that H⁺(Phlm) no longer exhibits the carbonyl stretches at 1750–1800 cm⁻¹ nor the band at 1139 cm⁻¹ corresponding to the COH bend and C–OH stretch, which gives much of the intensity to the 1132 cm⁻¹ peak in the H⁺(His) spectrum. Likewise, the band at about 700 cm⁻¹ in H⁺(Phlm) is enhanced by contributions from the phenyl group. One interesting difference in the two spectra is the apparent shift in the bands at 1399 and 1433 cm⁻¹ for H⁺(Phlm) because its predicted intensity has dropped by a factor of 33. Overall, this comparison demonstrates the contributions that the imidazole side-chain make to the spectrum of H⁺(His).

3.5. Comparison of experimental IR spectra: H⁺(His) versus H⁺(histamine)

In a recent study, Lagutschenkov et al. [22] published a detailed analysis of the IRMPD spectrum of protonated histamine, a neurotransmitter, which is His without the carb oxylic acid on the α-carbon. They compared the experimental IRMPD spectrum of protonated histamine with predicted IR spectra of several low-energy structures, which were characterized by quantum-chemical calculations at the B3LYP and MP2 levels of theory using the cc-pVDZ basis set. These calculations predict the most stable conformer is the imidazolium-type conformer with protonation at the imidazole ring and gauche conformation of the ethylamine side chain, which is significantly stabilized by an intramolecular ionic N⁺H⋯Nα hydrogen bond. This structure is equivalent to the [Nα,Nα⁺] conformers for H⁺(His), Fig. 1, which are all the same once H replaces the COOH group. Close in energy is the ammonium-type Nα⁺ conformer, equivalent to [Nα,Nα⁺] conformers of H⁺(His). All other conformations found for H⁺(histamine) no longer contain the Nα⁺⋯H⋯Nα hydrogen bond and are therefore much higher in energy (>32 kJ/mol). Similar to the findings discussed above for the analogous H⁺(His) complexes, B3LYP calculations prefer H⁺(histamine) [Nα,Nα⁺], the imidazolium ion structure, by 6.3 kJ/mol, whereas MP2 calculations stabilize the [Nα,Nα⁺], the ammonium ion structure, actually finding that it lies lower in energy by 1.7 kJ/mol. Larger basis sets shift the balance back to the [Nα,Nα⁺] structure, with the relative differences between B3LYP and MP2 remaining. These results are confirmed here where, for the three levels of theory used above, the [Nα,Nα⁺]-type is the ground state by 7.8 (B3LYP), 5.2 (B3P86), and ~0.1 (MP2) kJ/mol, respectively.

Good agreement was found between and the experimental spectrum and the predicted spectrum for H⁺(histamine) [Nα,Nα⁺], whereas the [Nα,Nα⁺] structure was concluded to contribute only a minor amount to the observed spectrum. Interestingly, as Lagutschenkov et al. point out, all other related protonated neurotransmitter ions studied to date prefer protonation at the
alkylamino side chain, such that histamine is unique in its preference for imidazole protonation. The IRMPD action spectra of H\(^+(\text{His})\) and H\(^+(\text{histamine})\) have many similar features. Bands at 1609, 1433, 1399, 1340, 1306, 1132, 1079, ~990, ~923, 822, 798, 676, and 609 cm\(^{-1}\) in the spectrum of H\(^+(\text{His})\) are observed at 1598, 1453, 1389, 1359, 1293, 1107, 1075, 997, 909, 830, 788, 685, and 607 cm\(^{-1}\) in the protonated histamine spectrum. This can be seen by a comparison of the B3LYP/6–311+G(d,p) calculated spectra in Fig. 5. Carbonyl stretches (1778 and 1747 cm\(^{-1}\)) and the OCO bend (742 cm\(^{-1}\)) in the H\(^+(\text{His})\) spectrum are not observed for protonated histamine for obvious reasons. Likewise, the COH bend and C–OH stretch contributes to the experimental band in H\(^+(\text{His})\) at 1132 cm\(^{-1}\), thereby blue shifting it from the 1107 cm\(^{-1}\) observed for H\(^+(\text{histamine})\), assigned to mainly an in-plane \(\text{N}_x\text{H}\) bend. Overall, this comparison is consistent with the assignment above, namely, that the \([\text{N}_x\text{N}_y,\text{N}_z]\) form of H\(^+(\text{His})\) dominates the experimental spectrum, with additional contributions from the \([\text{N}_x\text{N}_y,\text{CO}]\) form, which is not available to H\(^+(\text{histamine})\).

In making this comparison, we did note one interesting anomaly in comparing IR spectra for the most stable conformer of H\(^+(\text{histamine})\) calculated at the B3LYP/6–311+G(d,p) level compared with that reported by Lagutschenkov et al. [22]. They agree well over the experimental range examined in most respects, with average deviations between band positions of \(6 \pm 6\) cm\(^{-1}\) except for the band associated with the out-of-plane \(\text{N}_x\text{H}\) bend, i.e., a proton motion perpendicular to the \(\text{N}_x\text{H}\)···\(\text{H}\)···\(\text{N}_z\) hydrogen bond. Here, our predicted frequency (1016 cm\(^{-1}\), 0.975 scaling) differs by 62 cm\(^{-1}\) (after scaling), with that for the B3LYP/cc-pVDZ calculations (1078 cm\(^{-1}\), 0.98 scaling), where the latter agree better with experiment (1075 cm\(^{-1}\)). This difference appears to be exclusively one related to the size of the basis set as B3LYP/aug-cc-pVDZ, B3LYP/cc-pVTZ, and B3LYP/aug-cc-pVTZ calculations yield frequencies for this motion of 1036, 1026, and 1018 cm\(^{-1}\) (0.98 scaling); however, this frequency is nearly unique in this regard. For example, the in-plane bend of the same \(\text{N}_x\text{H}\) has calculated frequencies of 1117, 1114, 1121, 1118 (0.98 scaling), and 1113 cm\(^{-1}\) (0.975 scaling) for cc-pVDZ, aug-cc-pVDZ, cc-pVTZ, aug-cc-pVTZ, and 6–311+G(d,p) basis sets. All other frequencies in the experimental range of interest agree with one another with similarly small variations.

3.6. Comparison of experimental IR spectra: H\(^+(\text{His})\) and His**

Recently, Steill et al. [23] used IRMPD spectroscopy to study the histidine radical cation. They compared the experimental IRMPD spectrum of His** with predicted IR spectra of several low energy structures, finding a good match with the predicted IR spectrum of the global minimum structure, a captodative radical ion that has a structure essentially the same as \([\text{N}_x\text{CO}]-\text{ttg}···\text{g}···\text{t}\) shown in Fig. 1 but without the hydrogen atom on the \(\alpha\)-carbon. Notably this radical center allows the \(\text{H}_2\text{N}···\text{C}–\text{COOH}\) atoms to be nearly coplanar, allowing resonant stabilization along the NCCO atoms and a shorter \(\text{N}_x\text{H}···\text{OC}\) hydrogen bond, 1.68 Å compared to 1.78 Å for \([\text{N}_x\text{CO}]-\text{ttg}···\text{g}···\text{t}\) as calculated at the B3LYP/6–311+G(d,p) level. The IRMPD action spectra of H\(^+(\text{His})\) and His** have many similar features. Bands comparable to those at 1609, 1399, 1132, 822, 742, 676, and 609 cm\(^{-1}\) in the spectrum of H\(^+(\text{His})\) are also observed in the His* spectrum. Key differences in the spectra are shifts in the observed carbonyl stretches for H\(^+(\text{Hist})\) at 1778 and 1747 cm\(^{-1}\) to 1666 cm\(^{-1}\) for His**, and the presence of a band at about 1490 cm\(^{-1}\) for His**, which corresponds to stretches along the \(\text{N}_x\text{C}_x\text{CO}\) backbone. The latter band clearly shifts because of the resonant delocalization associated with the radical center. Comparison of the \([\text{N}_x\text{N}_y,\text{N}_z]\) and \([\text{N}_x\text{N}_y,\text{CO}]\) spectra of Fig. 3 also shows a red shift in the carbonyl stretch resulting from the stronger \(\text{N}_x\text{H}···\text{OC}\) hydrogen bond in the latter structure (and His**), but not as large a shift as for His**. This demonstrates that the resonant delocalization from the \(\text{C}_x\) radical center of His** removes electron density from the carbonyl \(\pi\) bond, weakening it further.

It is also interesting to consider why an \([\text{N}_x\text{N}_y,\text{N}_z]\) binding motif is no longer energetically favorable for His**, as found for H\(^+(\text{His})\). Although an exhaustive search for conformations was not conducted, calculations for His** at the present levels of theory find the captodative radical analog of \([\text{N}_x\text{N}_y,\text{N}_z]-\text{ttgc}\) to lie 42–43 kJ/mol higher in energy than the \([\text{N}_x\text{N}_y,\text{CO}]\) ground state. These calculations show that the NH\(_2\) amino group in the \([\text{N}_x\text{N}_y,\text{N}_z]\) conformer is no longer nearly planar, becoming pyramidal in order to form the \(\text{N}_x\text{H}···\text{N}_z\) hydrogen bond, thereby losing the resonant stabilization of the radical noted above.

3.7. Comparison of experimental IR spectra: H\(^+(\text{His})\) versus H\(^+(\text{HisArg})\) and H\(_2\text{H}^+\)(\text{HisArg})

The Williams group has examined H\(^+(\text{HisArg})\) and H\(_2\text{H}^+\)(\text{HisArg}) with IRMPD spectroscopy and theory [53]. The IRMPD action spectra of H\(^+(\text{His})\) and H\(^+(\text{HisArg})\), the latter protonated on the Arg side chain, have some similar features. The observed carbonyl stretch (1788 cm\(^{-1}\)), NH bends (~1650 cm\(^{-1}\)), and in-plane hydroxyl bend (1150 cm\(^{-1}\)) for the H\(^+(\text{HisArg})\) spectrum are also seen for H\(^+(\text{His})\) at 1778, 1609, 1132 cm\(^{-1}\), respectively. A sharp, intense peak at 1080 cm\(^{-1}\) is observed for H\(^+(\text{HisArg})\) that is attributed to the neutral histidine side chain on the basis of a comparison to the IR spectra of condensed phase imidazole. Consistent with this assignment is the observation that protonation of the imidazole side chain in the IRMPD spectrum of H\(_2\text{H}^+\)(\text{HisArg}) decreases the intensity of this peak appreciably, becoming a shoulder on the intense 1150 cm\(^{-1}\) band. This is comparable to the shoulder observed at 1079 cm\(^{-1}\) in the H\(^+(\text{His})\) spectrum, Fig. 3, consistent with protonation of the imidazole ring.
3.8. Comparison of experimental IR spectra: $H^+(\text{His})$ versus $M^+(\text{His})$

We can also compare the experimental IRMPD spectrum for $H^+(\text{His})$ with that for alkali metal cation complexes, $M^+(\text{His})$, where $M^+ = \text{Li}^+$, $\text{Na}^+$, $\text{K}^+$, $\text{Rb}^+$, and $\text{Cs}^+$ [25]. There are several similarities although most bands are broader in the protonated species. The highest frequency band corresponding to the carbonyl stretch at 1778 cm$^{-1}$ for $H^+(\text{His})$ is compared to 1732–1753 cm$^{-1}$ for the alkali metal complexes. Clearly, the hydrogen bond between the protonated imidazole side-chain nitrogen and backbone amino nitrogen perturbs the carbonyl stretch less than direct binding to the heavier alkali cations. An intense band at 1125–1157 cm$^{-1}$ is observed in all spectra, consistent with the fact that the COH bending and $C$–$OH$ stretching motions of the $H^+(\text{His})$ [N$_a$N$_h$] and $M^+(\text{His})$ [CO,N$_a$N$_h$] conformers are similar. The latter complex is a tridentate structure in which the metal cation binds to the carbonyl and amino group of the backbone along with the N$_h$ nitrogen of the side-chain. The shoulder at 1079 cm$^{-1}$ in the $H^+(\text{His})$ spectrum becomes a sharp, isolated band in the spectra of all $M^+$ (His) complexes. This is consistent with its identification as a signature peak associated with a neutral imidazole side chain at 1080 cm$^{-1}$ [53,54] and attributed to CN stretches and in-plane CH and NH bends of the side chain ring. Observed bands at 1580 cm$^{-1}$ in the $K^+$ (His)–$Cs^+$ (His) spectra correspond to an NH$_2$ bend in the [CO,N$_a$N$_h$] spectra. The comparable band in the $H^+(\text{His})$ spectrum is found at 1609 cm$^{-1}$, again consistent with more perturbation induced by direct metal binding to the NH$_2$ site.

The theoretical results for $K^+(\text{His})$–$Cs^+(\text{His})$ indicate that the bidentate [CO,N$_a$N$_h$] conformer, in which the metal cation binds to the backbone carbonyl oxygen and nitrogen atom of the imidazole side chain, has an intense band near 1400 cm$^{-1}$, corresponding to the COH bending motion. This motion is largely unaffected by the increasing cation size, being observed at $\sim$1390, $\sim$1385, and $\sim$1394 cm$^{-1}$ for $K^+(\text{His})$–$Cs^+(\text{His})$. This is also true for $H^+(\text{His})$ [N$_a$CO], being predicted at 1387 cm$^{-1}$ and observed at 1399 cm$^{-1}$. Similarly, weak bands at 742, 822, and 1433 cm$^{-1}$ are observed in all cationized spectra, from Li$^+(\text{His})$ to Cs$^+(\text{His})$, which seems reasonable as these frequencies correspond to motions that mainly involve groups not directly attached to the ion. Interestingly, the former two frequencies become more prominent as the cation size increases, which is consistent with the increasing populations of [CO,N$_a$N$_h$] and [COOH] conformations for which these modes gain intensity compared to [CO,N$_a$N$_h$]. The weak band observed at $\sim$990 cm$^{-1}$ in $H^+(\text{His})$, largely the NH$_2$ wag, can be found in all the $M^+(\text{His})$ spectra but has appreciable intensity only in the Li$^+(\text{His})$ spectrum, suggesting that the higher charge density may enhance the intensity of this mode. The band observed at 676 cm$^{-1}$ in $H^+(\text{His})$ is absent in all the $M^+(\text{His})$ spectra, consistent with the predicted IR intensities for these motions, which are greatly reduced for the metal systems.

4. Conclusions

The gas-phase structures of protonated histidine (His) and the side-chain model, protonated 4-phenyl imidazole (PhIm), are examined by infrared multiple photon dissociation (IRMPD) action spectroscopy utilizing light generated by the free electron laser FELIX. Comparison of the measured IRMPD spectra to single photon absorption results calculated at a B3LYP(6–311+G(d,p)) level of theory show that $H^+(\text{His})$ is characterized by a [N$_a$N$_h$CO] conformer along with some [N$_a$CO]. These conformers have the protonated nitrogen atom of imidazole adjacent to the side-chain (N$_h$) hydrogen bonding to the backbone amino nitrogen (N$_a$) and to the backbone carbonyl oxygen, respectively. Comparison of the spectra for $H^+(\text{PhIm})$ and $H^+(\text{His})$ allows identification of the vibrational contributions from the protonated imidazole side-chain. Likewise, the IRMPD action spectra of $H^+(\text{His})$ and $H^+(\text{histamine})$ [22], which is His without the carboxylic acid on the $\alpha$-carbon, are shared to find many similar features. Comparison of the IRMPD spectra of $H^+(\text{His})$ with that of the $His^{++}$ radical cation provides further evidence for the resonant electron delocalization from the C$_\alpha$ radical center of His$^{+}$ that is characteristic of the captodative radical ion identified by Steill et al. [23]. When compared to results for $H^+(\text{HisArg})$, $H_2^2+(\text{HisArg})$ [53], and $M^+(\text{His})$ [25], the IRMPD action spectrum of $H^+(\text{His})$ is consistent with protonation of the imidazole ring in only $H^+(\text{His})$ and $H_2^2+(\text{HisArg})$. Trends in the frequencies observed for $M^+(\text{His})$, where $M^+=H^+, Li^+, Na^+, K^+, Rb^+$, and $Cs^+$, are also explained.

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Appendix A. Supplementary data

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References