FT-IR Study of Histidine Model Compounds: Key Vibrational Bands of Imidazole Group

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Abstract: L-Histidine is an important amino acid because it is a key part of the active site of many proteins. The aim of the study was to develop a higher understanding of the structure of protein vibrations.. By measuring the absorption spectra of L-Histidine at low pH and high pH, we can identify infrared signals revealing if the histidine is protonated. The spectra of the L-Histidine side chain, ethylimidazole, the polymer of 8 His residues(8-mer), and 15N-His, were measured to identify key infrared signals from the His side chain. A catalog of absorption signals that correspond to a specific protonation site will provide a tool to analyze FTIR spectra of many proteins. A set of vibrational modes were found to be sensitive to protonation and 15N-labeling, providing tools to identify and analyze His side chains in proteins. The identification of these vibrational bands will lead to a better understanding of the structure and function of proteins.

Keywords: Fourier Transform Infrared Spectroscopy, Histidine, Molecular Vibration

Introduction

Understanding the structure of a protein is the first step towards understanding the function of that protein. In the past, the primary method of finding the structure was X-ray crystallography. However, X-ray crystallography lacks the speed of FTIR spectroscopy and does not provide the location of protons, nor does it provide the solution structure for proteins that normally exists in an aqueous solution (Xie et al. 2001). The infrared absorption spectrum is specific to each amino acid of each protein due to the structure of the amino acids in that protein (Barth 2000). Proteins are built upon a carbon/nitrogen backbone with the individual amino acids of the protein being defined by the chains which extend from the carbon spine. Each atom and bond of this chain absorbs different frequencies of infrared that are directly correlated to the vibrations of those bonds (Barth 2000). Strong bonds and light atoms tend to cause vibrations at higher frequencies than weak bonds and heavy atoms (Liang et al. 2011). By understanding the absorption spectrum of an amino acid, we gain a better understanding of the chemistry of that amino acid and the protein which comprises it.

Due to of L-Histidine's primary function in the active site of many proteins, we chose L-His to be the sample of our project. L-His is the most frequently used amino acid catalyst in the human body largely due to its neutral pKa, allowing it to act as an acid-base catalyst (Gutteridge and Thornton 2005). For L-His to function properly, it must be in the right tautomeric state between either its protonated Nδ or Nε

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Table 1 – Chemicals and instruments used

Chemicals Needed:	**Instruments Used:**
L-Histidine (Sigma-Aldrich)	Bruker Vertex 80v FTIR Spectrometer
$^{15}N_3$ L-Histidine (Cambridge Isotope Laboratories)	Mettler Toledo FiveEasy digital pH meter
4(5)-Ethlyl-Imidazole (Tokyo Chemical Industry Co.)	Mettler Toledo (Columbus, OH) AG204 analytic balance)
8mer L-Histidine peptides (Genemed Synthesis Inc.)	Centrifuge Eppendorf (Hamburg, Germany)
8mer L-Histidine peptides (Genemed Synthesis Inc.)	OPUS software
NaOH and NaOD	
D_2O (Cambridge Isotope Laboratories) & pure H_2O	

(Gutteridge and Thornton 2005). Because the tautomeric states of L-His are important, identifying IR peaks that correspond with the protonation of the imidazole would lead to a better understanding of the structure and function of proteins. L-His is composed of a carboxylic acid group, an amino group, and its unique side chain ethyl-imidazole group. The infrared absorption spectrum for L-His was measured using a Bruker infrared spectrometer with an AquaSpec cell, displaying peaks unique to the characteristics of L-His. Because the spectrum shown for L-His is congested with absorption peaks resulting from the carboxylic acid group, amino group, and protein backbone, we also measured the side chain ethyl-imidazole. This spectrum provides a refined view of the spectrum unique to L-His. Because protein FTIR

Table 2 – pH of each sample

Samples:	L-Histidine:	Ethyl Imidazole:	$^{15}N_3$ L-Histidine:	8mer L-His Peptide:
LpH	3.8	3.8	3.9	3.8
HpH	10.9	10.8	11.2	11.4
LpD	4.2	3.8	4.1	3.8
HpD	10.8	10.8	11.0	11.4

spectra are often measured using protein dissolved in D2O, we measured FTIR spectra of Histidine compounds both in H_2O and D_2O. The L-His samples were prepared in low pH's & pD's around 4 and high pH's & pD's around 11. The low pH caused the Nitrogen found in the imidazole side chain to be protonated whereas the high pH samples caused the Nitrogen to be deprotonated.

Isotopes were used to cause shifts, due to the difference in mass, in the spectra of L-Histidine allowing the protonation states to be assigned. Samples were prepared in H_2O as well as D_2O, heavy/deuterium water, allowing different peaks to be observed due to the shift that were otherwise concealed by the H_2O or D_2O absorption spectrum. L-His samples were prepared with both ^{14}N and ^{15}N causing peak shifts in His vibrational modes, allowing those peaks to be assigned. The ^{15}N isotopic L-His could be inserted in a protein allowing those unique His absorption peaks to shift and be recognized, helping understand the structure of that protein.

Because proteins fold when in a hydrogen-deuterium solution, the observed protein infrared absorption spectrum's peaks would show shifts in the peaks as the folding occurs. Understanding the peaks that correlate with protonation would aid in predicting the pathways available for protein folding by measuring hydrogen exchanges (Miranker et al. 1993). This difference would show that the protein had folded causing some amino acids to be in an inner, hydrophobic region of the protein. The changing peaks in infrared absorption spectrums could hypothetically be used to understand the structure of the newly folded

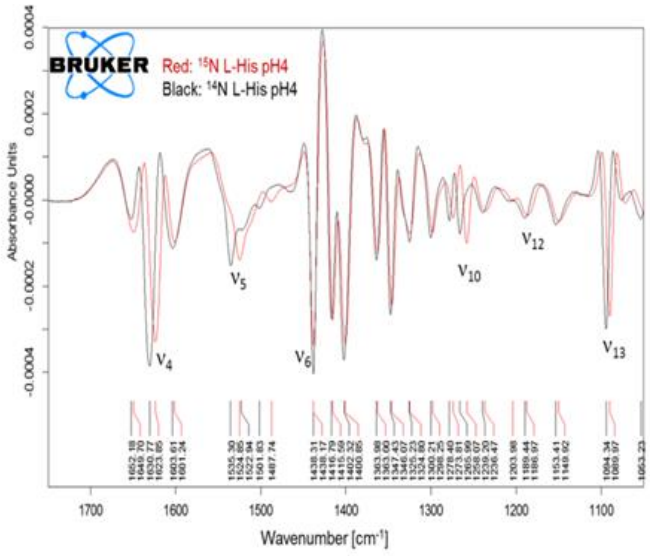

Figure 1 - L-His N15 LpH vs L-His N14 LpH (second derivative) imidazole

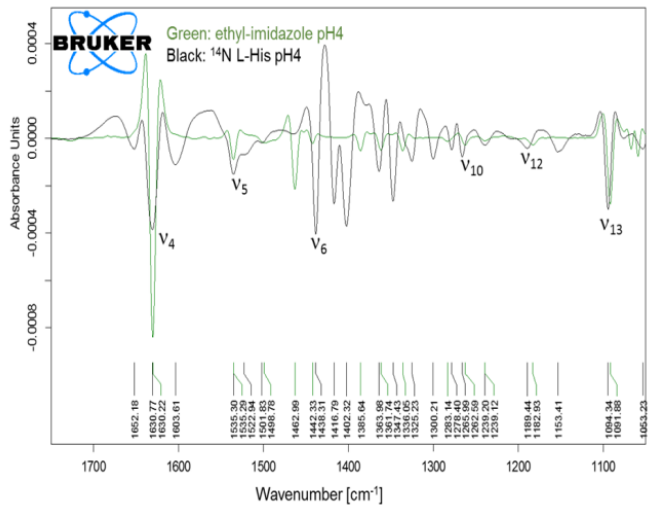

Figure 2 - LpH vs. L-His 14N LpH (second derivative)

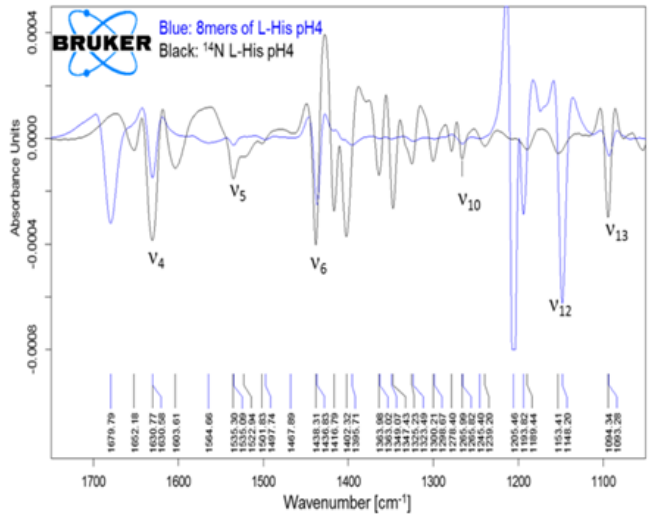

Figure 3 - 8-mer LpH vs. L-Histidine 14N LpH (second derivative)

protein. Because the Bruker infrared spectrometer can rapidly measure high-quality infrared absorption spectra, we can record the change in the absorption spectrum, as the protein changes its shape during folding or function. People could later interpret this information to show how the protein folded, therefore helping explain its function.

Methods

Sample preparation

Using the chemicals and instruments (Table 1): four samples of L-Histidine, Ethyl Imidazole, $^{15}N_3$ L-Histidine, and 8mer L-Histidine Peptides were prepared for analyzation- two of each were dissolved in H_2O and the other two in D_2O, except for 8mer L-Histidine Peptide, which was dissolved at 62.5 mM. Each chemical was observed as four different labeled samples: LpH (symbolizing low pH dissolved in H_2O), HpH (symbolizing high pH dissolved in H_2O), LpD (symbolizing low pH dissolved in D_2O) and HpD (symbolizing high pH dissolved in D_2O) Then, a set amount of the chemicals were weighed and then dissolved in either H_2O or D_2O. After dissolving, 6 M HCl was added to LpH and LpD samples to lower the samples pH to 4.0 (+/- 0.2). HpH samples received 6 M NaOH and HpD samples were given 6 M NaOD to acquire pH 11.0 (+/- 0.2) for each. With these continuation of these ordered steps, two independent batches of this sample preparation were conducted, then centrifuged for three minutes to remove air bubbles. The samples were then ready for data analysis through FTIR spectroscopy. The total volume of each sample fully prepared sample- L-His: 95 microliters, Ethyl Imidazole: 193.5 microliters, $^{15}N_3$ L-Histidine: 92.9 microliters, 8mer L-His: 273.6 microliters (Table 2).

FTIR Data collection:

For data collection, the Vertex 80v FTIR Spectrometer instrument observes infrared at scanner velocity of 240 kHz at resolution 2 cm^{-1}. The sample cell was held at constant temperature of 20 degrees C. For

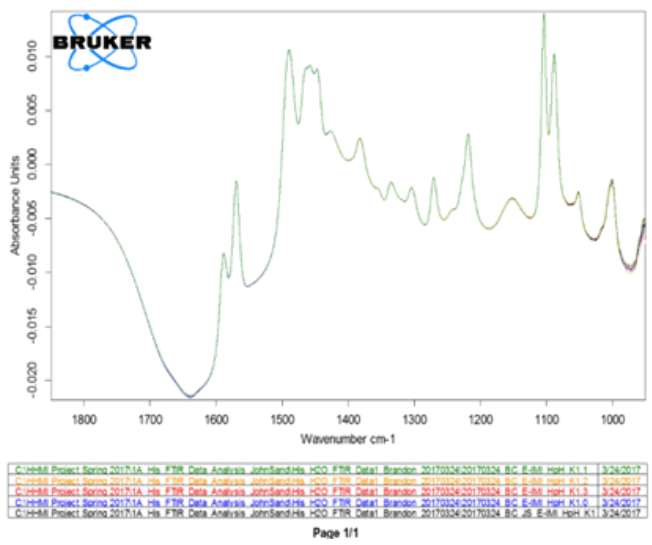

Figure 5 - E-IMI HpH K1 Av4 Dv2

each H_2O sample, flush cell with 40 microL and read background. Then, load 10 microL of sample, collect data and repeat until clean spectra had sufficiently high signal to noise ratio. Repeat the same steps as above for all D_2O samples, but use the N_2 gas purge to keep the D_2O from becoming DOH

Multiple sets of data were collected for each sample. The average of these sets was then taken to create a clean single spectra . The second derivative was then taken from these sample sets to help identify peak positions and their averages were calculated. We used peak picking and smoothing to create a clean spectra that would make finding bands easier for the naked eye (Figures 1-6).

Results and Discussion

The data collection process allowed the opportunity to identify vibrations of the L-His side chain in infrared spectra that are structurally informative for protein science. Using the equation 3N – 6, we can determine y, for Histidine, the number of vibrational modes is 42, exhibiting 42 different absorption bands. Of these 42, we identified five L-His vibrational modes sensitive to ^{15}N labeling (n_4, n_5, n_6, n_{10}, n_{12}, and n_{13}), allowing their assignment in FT-IR spectra of proteins. Previous computational studies on the vibrational modes of His allowed the identification of which atoms move in which way during each of these vibrational modes. We can also see two signature modes, n_4 and n_5, can be used to detect the protonation state of histidine imidazole group. Finally, based on previous computational work, we were able to identify one signature band's, n_6, as being sensitive to hydrogen bonding interactions of the histidine side chains. From our data, we are able to determine not only the structure of the amino acid simply through infrared absorbance, but also to outline the mechanics and functions of specific areas on the amino acid, unique to any other previous method of protein structure analysis. With

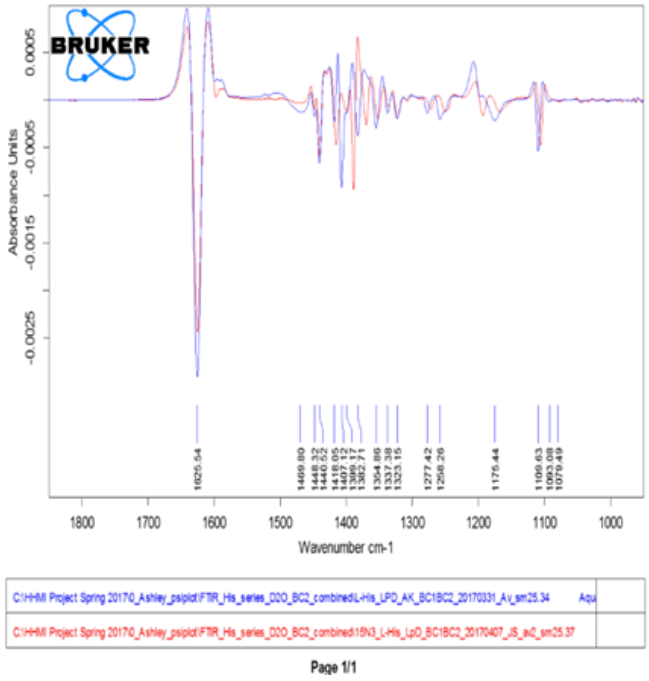

Figure 4 - Comparison of 14N & 15N L-His

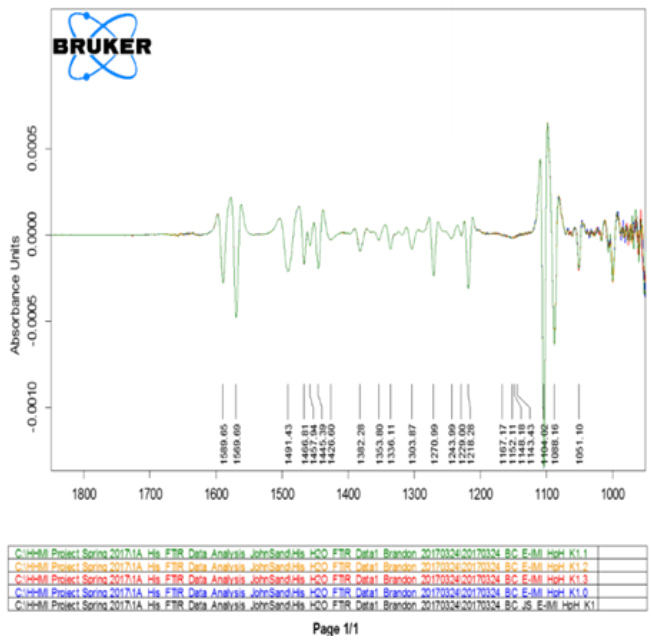

Figure 6 - E-IMI HpH K1 av4 dv2 after AV2 at LPD and Smooth 25

continued effort, a catalog of amino acid absorbance can aid in improving FTIR spectroscopy and the science of protein structure analysis as a whole, as well as contribute to the development of Infrared structural biology.

Acknowledgments

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