

Infrared Spectroscopy of Amino Acid Side Chains

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Abstract

Infrared structural biology is a novel and promising approach for detecting structure and functional processes of proteins. However, the process of assignment of infrared signals to specific amino acid side chains in a protein requires further development. For our experiment, we examined individual amino acids to help understand how they contribute to infrared signals of intact proteins. Specifically, we worked with arginine, histidine, and lysine. The long-term goal of this experiment is to create a definitive map of the FTIR spectra for each of these amino acids. We measured the infrared absorbance of these three amino acid side chains. Measuring and comparing the results of the different amino acids and different volume sizes allowed us to define optimal experimental conditions for these measurements.

Keywords: Fourier transform infrared spectroscopy, Amino acids, Polymers, Time-resolved spectroscopy, Infrared absorbance

Introduction

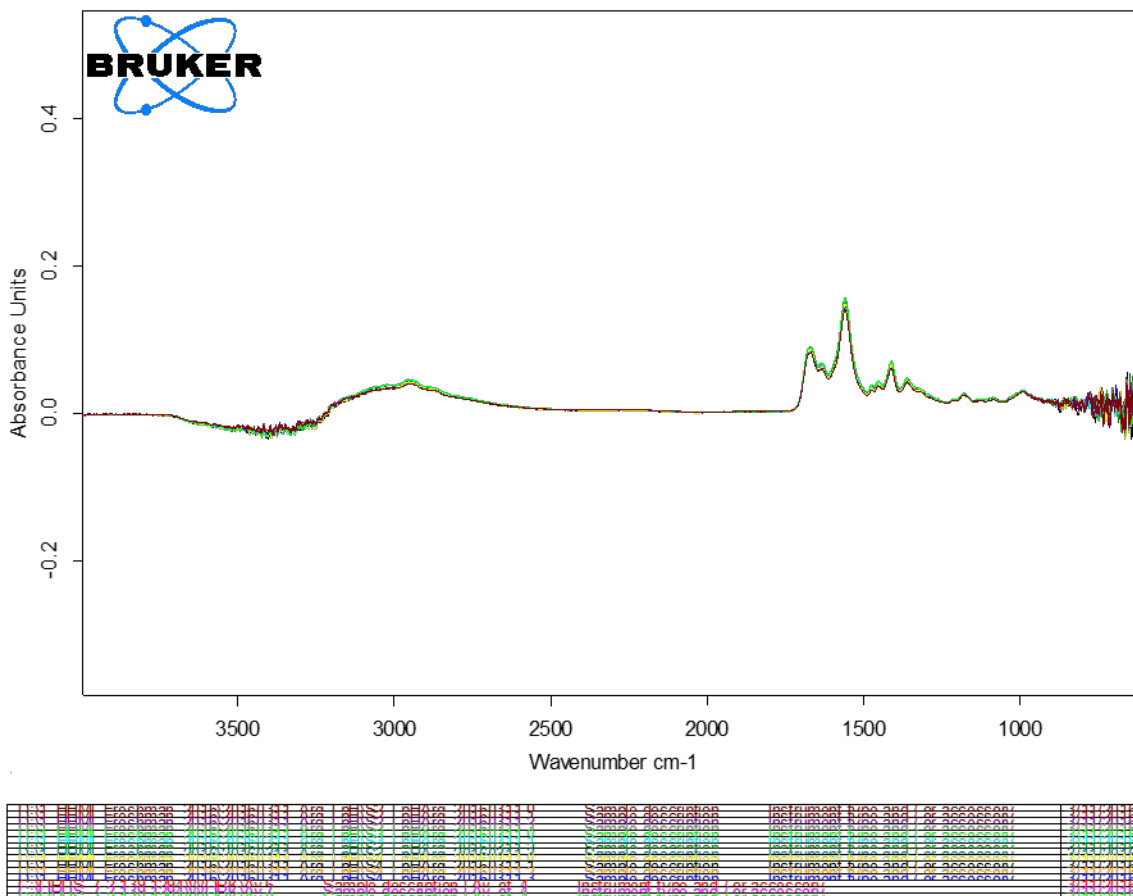
Infrared structural biology can be used to detect structure and functional processes of proteins. Proteins have an evolutionary history and many relatives and ancestors. A protein family is a group of evolutionarily-related proteins, and in many cases, these families have corresponding gene families. From a chemical point of view, the side chains of the protein are connected to a central carbon, with the structure side chain defining the amino acid. In this project, our team worked with three amino acids – all of which have positive side chain charges. Namely, we will be working with arginine, histidine, and lysine as they occur in proteins. They all include an amino acid group and a carboxylic acid group, but all have different positively charged side chains.

Our research team measured the FTIR (Fourier transform infrared spectroscopy) of each of the three amino acids. Fourier transform infrared spectroscopy is a technique used to obtain an infrared absorption spectrum of a solid, liquid, or gas. An infrared spectrum represents a fingerprint of the sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. No two compounds produce the exact same infrared spectrum, because each different material is a unique combination of atoms, chemical bonds, and weak interactions. In general, the stronger the covalent bond between atoms, the higher the vibration frequency; the heavier the mass of the vibrating atoms, the lower the vibration frequency. In current protein FTIR spectroscopy, there is no definitive map of the free amino acid side chains. This poses a

problem, because without this knowledge, it is difficult to study how these amino acids contribute to the FTIR spectrum of, and therefore the function of, any of the proteins in which these amino acids are present. This hampers progress in the advancement of the new field of infrared structural biology, which has great promise and limitless possibilities.

The goal of this project is to create a definitive map of each of these amino acids with the FTIR spectra. This research will ultimately allow experts in FTIR spectroscopy to use the technology to understand how proteins vibrate and change their structure and ultimately perform their function. The use of stable isotopes also opens up different opportunities for mapping out proteins in the case that data between two amino acids is similar and peaks in the same area. The isotope data allows researchers to accurately tell which amino acid is creating an observed infrared signal.

Previous studies in the concerning time resolved FTIR testing shows that “Time-resolved infrared difference spectroscopy is a powerful method to probe and characterize the structural dynamics of proteins” (Brudler *et al.* 2001; Friedrich *et al.* 2002; Gerwert 1999; Heberle 2000; Xie *et al.* 2001). Many of the problems faced are the lack of solid framework for these projects to stand on. Our technology really stands on the ground work that Nie *et al.* (2005) have laid, showing that the stretching vibration frequency can show the type of hydrogen bond. This directly shows how our research can be used to further examine intermediate functional states



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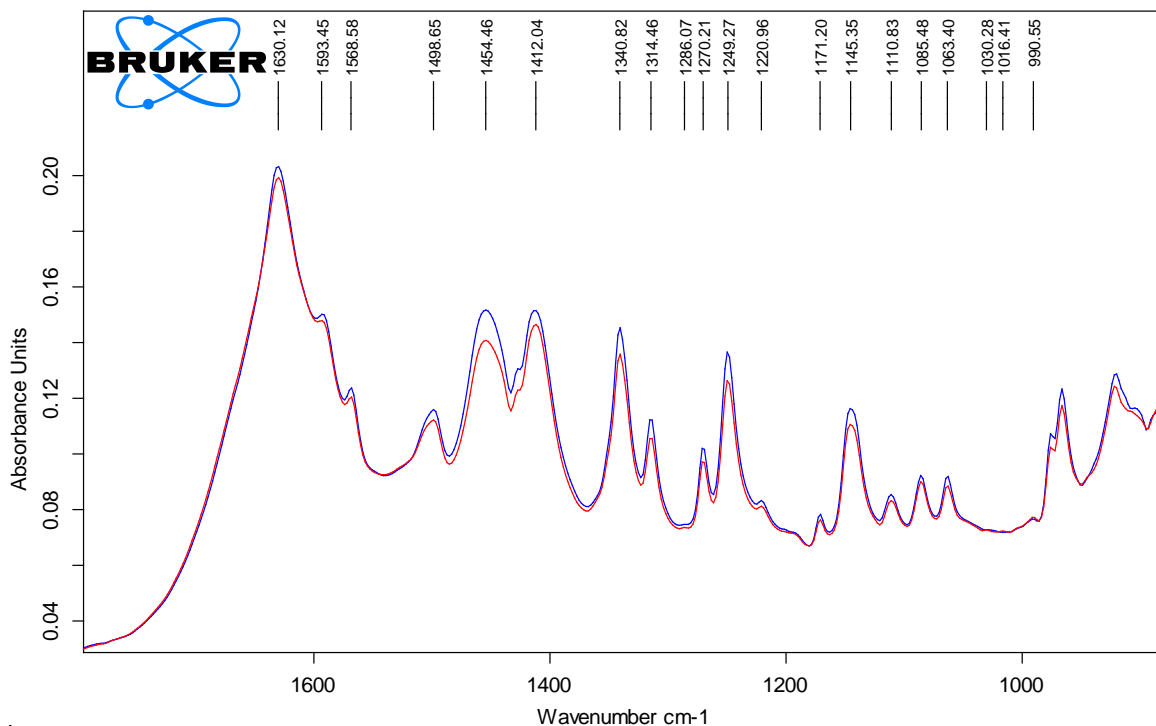
Figure 1 - Vibration peaks for LpH Arginine

of proteins. This approach is particularly powerful since time-resolved FTIR instruments have been developed. Time-resolved spectroscopy helps scientists study dynamic processes in materials or chemicals. Time-resolved infrared spectroscopy allows us to get down to the picoseconds and surpasses the structural information that can be obtained from transient absorption and emission spectroscopy. This technology allows us to see the contents and bending formation of polymers more accurately than previous technology such as x-ray crystallography. We will be able to see all of this in real time using far easier methods.

This will finally be possible with the basic knowledge of the spectra of each amino acid. Therefore, our project is not hypothesis driven, but more driven by data and developing a method. The overall hypothesis for the project is that because there is a large amount of unused information regarding the structure of proteins contained in their infrared spectra, knowing the infrared spectra of each 20 amino acid side chains dissolved in water will help retrieve the valuable information on protein structure.

Methods

We measured the infrared absorbance of amino acid side chains. In order to do this, our research team measured the FTIR of each of the three amino acids: arginine, histidine, and lysine. We conducted four different experiments to help conjure results in our hopes of advancing scientific knowledge of the amino acid behavior in proteins. In the first experiment, we used an eight piece homopolymer, or mock proteins. The second experiment used a monomer of each amino acid, the third with a nitrogen isotope of each amino acid, and the last will be conducted using the isolated amino acid side chain. We are conducting these four related measurements in order to get the most conclusive results to get a comprehensive data on the infrared absorbance spectrum of each amino acid side chain. To find the frequencies of vibrations between the bonds of these atoms, we will be using an infrared spectroscopy instrument. In a sense, our approach is to subtract the amino groups and the acid groups



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Figure 2 - Vibration peaks for MHPH Histidine

from the side chains. However, it is not as simple as mere subtraction but studying the three types of samples separately. After getting the absorption spectra, we take both spectra and compare them to each other. After comparing them to one another, we will further compare them to the data from the side chains and monomer version to see how the amino acid changes when being put into a polymer type form.

Results

Starting off, we measured arginine, lysine, and histidine to get a look at how their vibration sequences would turn out. We did this by using the Bruker BioATRII infrared measurement cell. Performing such measurements gave us an idea of what the peaks of these amino acids looked like and how they differed. We used low pH arginine, medium to high pH histidine, and low to medium pH lysine. The results for these measurements are presented below:

After studying and repeating these tests, we began to question how low of a sample volume can

be used, to extend the usage of samples and save money, and still conclude with accurate and viable results. Using the same BioATRII machine, and aiming for the smallest usable volume, we measure LMpH Lysine at volumes of 30 μ L, 20 μ L, 10 μ L, 8 μ L, and 5 μ L:

Discussion

The results of our experiment are just the beginning of what we have planned. Measuring and observing the absorbance peaks of the three amino acids we used makes way for our main experiment goal, because it helps us become more familiar with identify said amino acids and their peak behaviors. Measuring and comparing the results of the different sample sizes allowed us to be better prepared when we begin our goal experiment. We will now be able to save samples for longer use as well as save money because our results showed us that 5 μ L produces viable and even better results than the samples measure with higher volumes. The BioATRII manual told us that the lowest volume that could be used was around 10 μ L and that 20-25 μ L was the ideal

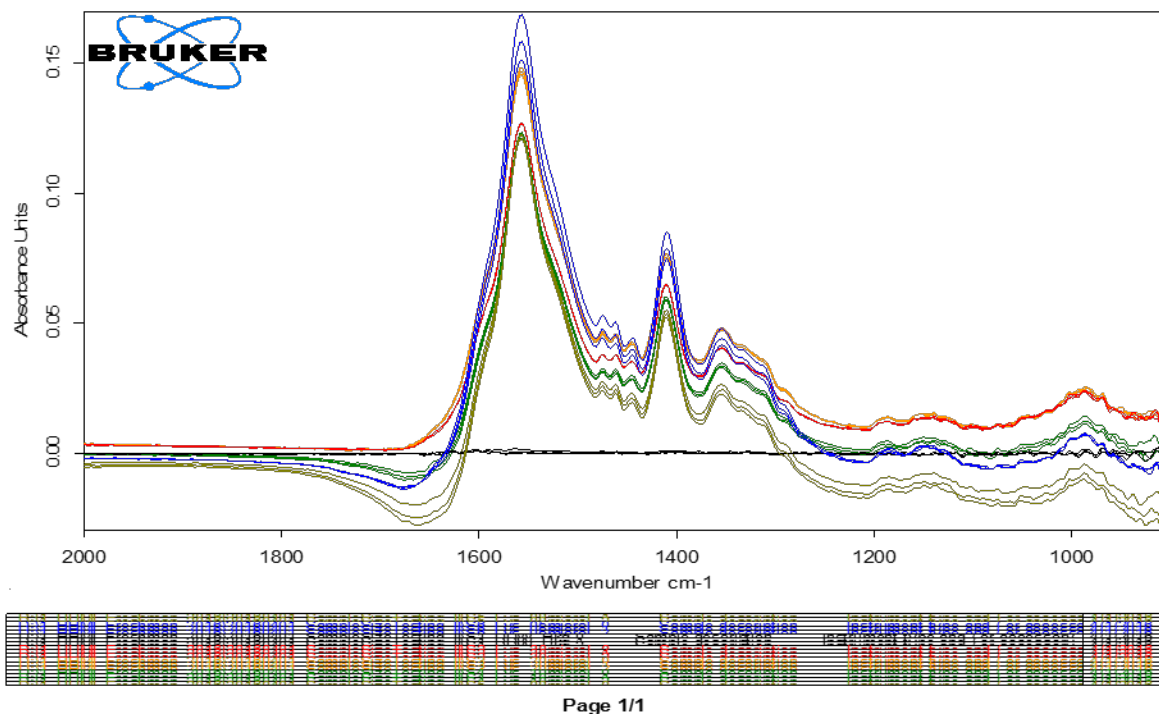


Figure 3 - Vibration peaks from sample tests of LMPH Lysine at volumes of 30 μ L, 20 μ L, 10 μ L, 8 μ L, and 5 μ L

volume size, but our results have improved upon this statement.

The experiments that we conducted this spring have opened way for better results and better success for our main experiment. Again, our experimental goal is to officially create a map of each of these amino acids with the FTIR spectra to accurately tell which amino acid is creating an infrared peak; ultimately allowing us to obtain results in our hopes of advancing scientific knowledge of the amino acid behavior in proteins. Our plans are to continue our research in the fall of 2016.

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