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My group uses mass spectrometry combined with hydrogen/deuterium (H/D) exchange and computational chemistry to characterize the molecular interactions and dynamics in macromolecules (specifically amino acids, peptides and proteins). Mass spectrometry is an emerging as an effective probe macromolecule structure in both solution and the gas phase. The long-term goal of these projects is to take advantage of these techniques to develop a greater understanding of non-covalent interactions and molecular dynamics of large biological molecules in both solution and the gas phase. *This summer I will have projects focused on 1) extending our knowledge of the mechanism of H/D exchange of peptides in the gas phase using mass spectrometry 2) fundamental studies of the multiply charged peptides and different excitation techniques 3) using LC-MS to probe solution structures of proteins. In addition a project looking at environmental contaminants (pesticides and pharmaceutical products) may be available. Our instrumentation is well suited for this latter project, and interested students should consult Professor Marzluff for more information.*

Two different mass spectrometers will be used for these studies. An electrospray ionization Ion trap mass spectrometry will be used to study the gas phase hydrogen/deuterium exchange of small peptides. The LC-Q-TOF instrument will be utilized to probe solution structure, ligand binding and protein dynamics by hydrogen/deuterium exchange mass spectrometry, to examine the fundamental behavior of multiply charged peptides, and explore different excitation techniques.

Gas phase hydrogen exchange This project focuses on the fundamental structures of biological molecules in the absence of solvent.¹ Characterization of the gas phase structure of proteins is important for understanding the degree to which interactions with water play a role in determining three dimensional protein structures. This builds on work from the early 1990's when Fred McLafferty showed that proteins appear to adopt compact structures in the gas phase.² Accompanying commentary by Peter Wolynes discusses how in the absence of solvent "the stability of the native protein is not compromised... the classical secondary structures should be even more thermodynamically stable."³ This summer we will focus on hydrogen deuterium exchange of peptides, as understanding the exchange of simple systems is necessary for complete analysis of proteins. The mass spectrometry part of these studies is carried out using a Finnigan Electrospray Ionization Ion Trap Mass spectrometer.⁴ (ESI-ITMS). Electrospray ionization allows the volatilization of large molecules for study by mass spectrometry. H/D exchange of labile protons in a molecule (typically the hydrogen attached to nitrogen and oxygen) results in a mass shift by 1 amu for each hydrogen exchanged and is easily followed by mass spectrometry.

Hydrogen/Deuterium exchange mass spectrometry studies have not been limited to solution but have also been used to probe gas phase structures of proteins and peptides.^{5,6} To assist analysis of protein H/D exchange, a thorough understanding of the mechanisms and intrinsic rates of exchange are necessary. In past summers we have investigated the rates of exchange of small di and tri peptides with exchangeable side chains, primarily focusing on peptides with aromatic and acidic side chains, and recently introducing alkali metals in place of the proton. We use solid state synthetic techniques to create custom peptides, and electronic structure calculations to determine the low energy conformations of the molecules and to map out the potential energy surface for exchange.

These combinations of techniques allow us to start to investigate the subtle effects of amino acid position on exchange. This summer we will continue these investigations continue to branch out in complexity of peptides and side chains. These studies will combine synthesis, mass spectrometry, kinetic fitting and computational chemistry. In addition we will expand these studies of structure using the new Xevo Q-ToF mass spectrometer to investigate the collision induced dissociation of these peptides. No previous experience is necessary; students will learn techniques involved in studying structures of large and small molecules using mass spectrometry. Interested students might also be able to apply these techniques in solution as a comparison to the gas phase data. Students interested in these projects should start by consulting references 4c and 4d and 5b as background reading (readily available through the Grinnell College Library) as well as posters outside my office, prior to coming in for discussion.

Probing solution structure, ligand binding and protein dynamics by collision induced dissociation and hydrogen/deuterium exchange mass spectrometry Solution phase H/D exchange coupled with mass spectrometry can also be used to probe protein structure and interactions,⁷ and two projects exist in this area. An existing project is focused on determining protein ligand binding sites by chemically attaching the ligand to the protein and using mass spectrometry to determine the site of attachment.⁸ This project is being conducted in collaboration with the Levandoski group. A new project this summer is to also couple solution H/D exchange with protein digestion and using the LC-Q-TOF system to study the extent and locale of ligand binding in proteins. The rate of exchange of amide and amino acid side chain protons in solution is a strong function of pH and has been well characterized.⁹ By using mass spectrometry to determine the extent of deuterium incorporation into a protein as a function of time, inferences about structural features of a protein have been made.^{10,11} This technique can be augmented by performing rapid solution proteolysis prior to mass spectrometry to determine specific regions of the protein protected from exchange.¹² One example system of study for protein ligand interactions in the protein calmodulin. Calmodulin is an intracellular calcium binding protein which mediates the calcium dependent activation of a large number of intracellular proteins.¹³ Intermolecular interactions will be studied by investigating the binding of calmodulin with a variety of target molecules. Calmodulin is an ideal protein on which to focus these studies because it is symmetric, binds four calcium ions and a wide variety of small ligands, both in its apo (absence of calcium) and calcium saturated forms. In solution it typically undergoes a large conformation change upon ligand binding. This provides within a single system a variety of solution conformations.

Fundamental studies of multiply charged peptides and different excitation techniques Low energy collision induced dissociation experiments on ubiquitin indicate that with multiply charged large proteins there is a certain propensity for dissociation to occur adjacent to aspartic acid and or proline residues. This has also been noted for singly charged peptides and proteins.¹⁴ For example, the +7 charge state of ubiquitin is dissociated easily in the ion trap using CID to give one primary fragment. This fragment had been further isolated and dissociated into smaller fragments which can be used to map the protein in 10-20 amino acid lengths. These results will be compared to similar results using the collision cell in the Q-TOF on the intact protein and compared to those for carrying out digestion of the protein with trypsin to assess the accuracy of the techniques for identifying the main sites of exchange. An application of this is the potential determination of the protected sites which slowly undergo H/D exchange.

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