Hydrogen Deuterium Exchange Mass Spectrometry And Its

Hydrogen deuterium exchange mass spectrometry has emerged as an important technique to probe protein structure and conformational dynamics. The rate of exchange of hydrogen with deuterium by the peptide backbone is dependent on the solvent accessibility, extent of hydrogen bonding in secondary structural elements and protein dynamics. The extent and the rate of deuterium incorporation are affected by changes in protein structure, interaction with ligand, protein-protein interaction and environmental factors such as pH and temperature. These conformational changes can be global and/or local. The increase in the mass is used to localize the deuterium incorporation after pepsin digestion of the protein and analysis by electrospray ionization mass spectrometry. In this dissertation traditional HDX-MS and a new deuterium trapping assay were used to probe the interaction sites between E. coli cysteine desulfurase SufS and acceptor protein SufE. SufS and SufE form an important part of the SUF pathway, essential for the biosynthesis of Fe-S clusters under oxidative stress and iron depletion conditions. In addition, SufE is known to stimulate SufS cysteine desulfurase activity, but the mechanism is unknown. The HDX-MS results show that the regions affected by the SufS-SufE interaction are dependent on the catalytic intermediate states of the two proteins. HDX-MS was also used to probe the conformational changes resulting upon persulfuration of SufS of Cys364 in the active site. The persulfuration of SufS not only affected regions in the active site cavity, but also had other conformational changes in more distal regions. Based on our findings a model for the interaction SufS and SufE was proposed. A mechanism for the enhancement of SufS cysteine desulfurase activity upon interaction with SufE was also postulated. In all this work demonstrates that hydrogen deuterium exchange mass spectrometry and the deuterium trapping methodology optimized for this system can be easily and effectively used to study the protein-protein interactions and the accompanying changes in structural dynamics for other proteins. Deuterium trapping was demonstrated to be fast, sensitive and reliable method to deduce the changes in solvent accessibility between two or more states of a protein. Both techniques can easily be applied to large number of protein complexes to determine the regions of interaction as well as gain mechanistic information not available through traditional methods such as X-ray crystallography and NMR.

Thorough insight into a protein's structure is necessary to understand how it functions and what goes wrong when it malfunctions. The structure of proteins, however, is not easily analyzed. The analysis must take place under a narrow range of conditions or risk perturbing the very structure being probed. Furthermore, the wide diversity in size and chemistry possible in proteins significantly complicates this analysis. Despite this numerous methods have been developed in order to analyze protein structure. In this work, we demonstrate that mass spectrometry (MS)-based techniques are capable of characterizing the structure of particularly challenging proteins. This is done through the study of two model systems: (1) the amyloid forming protein [beta]2-microglobulin and (2) the protein therapeutics human growth hormone and immunoglobulin G1. [beta]-2-microglobulin ([beta]2m) is an amyloidogenic protein and is the major constituent of fibrils in the disease dialysis related amyloidosis (DRA). Stoichiometric concentrations of Cu(II) have been used in vitro to induce the amyloid formation of [beta]2m, but the structural changes caused by Cu(II) have not been fully elucidated. Other transition metals, such as Zn(II) and Ni(II), do not cause [beta]2m amyloid formation, yet a comparison of the structural changes caused by these metals and those caused by Cu(II) could reveal essential structural changes necessary for amyloid formation. To probe these different structural changes, we have used a combination of hydrogen-deuterium exchange (HDX) and covalent labeling together with MS. Results from these measurements reveal that Cu(II) alone is capable of inducing the cis-trans isomerization of the X-Pro bond of Pro32 and the other necessary conformational changes that allow [beta]2m to form an amyloid competent state, even though Ni(II) binds the protein at the same site. We also find that Zn(II) binding leads to increased dynamics, indicating increase structural instability, which is consistent with the amorphous aggregation observed in the presence of this metal. The second part of this dissertation investigates the use of diethylpyrocarbonate (DEPC) - based covalent labeling to detect three-dimensional structural changes in immunoglobulin G1 and human growth hormone after they have been exposed to degrading conditions. We demonstrate that DEPC labeling can identify both specific protein regions that mediate aggregation and those regions that undergo more subtle structural changes upon mishandling of these proteins. Importantly, DEPC labeling is able to provide information for up to 30% of the surface residues in a given protein, thereby providing excellent structural resolution. Given the simplicity of the DEPC labeling chemistry and the relatively straightforward mass spectral analysis of DEPC-labeled proteins, we expect this method should be amenable to a wide range of protein therapeutics and their different formulations. In the final section of this dissertation, we demonstrate that, in certain instances, scrambling of the DEPC label from one residue to another can occur during collision-induced dissociation (CID) of labeled peptide ions, resulting in ambiguity in label site identity. From a preliminary study of over 30 labeled peptides, we find that scrambling occurs in about 25% of the peptides and most commonly occurs when histidine residues are labeled. Moreover, this scrambling appears to occur more readily under non-mobile proton conditions, meaning that low-charge state peptide ions are more prone to this reaction. For all peptides, we find that scrambling does not occur during electron transfer dissociation, which suggests that this dissociation technique is a safe alternative to CID for correct label site identification.

The aim of this book is to present a range of analytical methods that can be used in formulation design and development and focus on how these systems can be applied to understand formulation components and the dosage form these build. To effectively design and exploit drug delivery systems, the underlying characteristic of a dosage form must be understood--from the characteristics of the individual formulation components, to how they act and interact within the formulation, and finally, to how this formulation responds in different biological environments. To achieve this, there is a wide range of analytical techniques that can be adopted to understand and elucidate the mechanics of drug delivery and drug formulation. Such methods include e.g. spectroscopic analysis, diffractometric analysis, thermal investigations, surface analytical techniques, particle size analysis, rheological techniques, methods to characterize drug stability and release, and biological analysis in appropriate cell and animal models. Whilst each of these methods can encompass a full research area in their own right, formulation scientists must be able to effectively apply these methods to the delivery system they are considering. The information in this book is designed to support researchers in their ability to fully characterize and analyze a range of delivery systems, using an appropriate selection of analytical techniques. Due to its consideration of regulatory approval, this book will also be suitable for industrial researchers both at early stage up to pre-clinical research.

Measuring Histone Protein Dynamics by Hydrogen-deuterium Exchange Mass Spectrometry

Hydrogen/deuterium Exchange Mass Spectrometry as a Technology Platform for Studying Conformational Dynamics in Large Protein Complexes

Development and Application of Ultrahigh Resolution Mass Spectrometry Monitored Hydrogen/deuterium Exchange

Probing the conformational dynamics of integral membrane proteins by hydrogen/deuterium exchange mass spectrometry

Characterizing Protein Dynamics of Protein-Ligand Interactions by Hydrogen-Deuterium Exchange Mass Spectrometry

This volume details the importance of multiple experimental techniques and computational methods needed to obtain the comprehensive picture of protein complex structure, dynamics and assembly afforded by the emerging field of integrative structural biology. Chapters guide readers through the broad spectrum of approaches required for a complete representation of protein complexes, including expression and purification, experimental characterization of structure and assembly, and computational methods for identifying protein complexes and modelling their assembly. Written in the highly successful Methods in Molecular Biology series format, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and tips on troubleshooting and avoiding known pitfalls. Authoritative and cutting-edge, Protein Complex Assembly: Methods and Protocols aims to ensure successful results in the further study of this vital field.

13.2.1 Protein Dynamics of SA WT and S1 Mutant DHFR in Apo and Bound States

Proteins are essential to all biological systems. Proteins participate in numerous cellular processes by interacting with other proteins, other metabolites and membranes in a dynamic environment. Studying the structural and conformational properties of proteins in the solution phase is necessary to understand their protein folding and interaction dynamics. This research project focused on the development and application of hydrogen deuterium exchange mass spectrometry (HDX-MS) technology for studying the conformational dynamics of large multi-subunit protein systems. HDX-MS studies were conducted on representative proteins of two much researched protein families, namely Peroxiredoxins (Prxs) and Cullin Ring Ligases (CRLs). As part of this research we implemented tandem mass spectrometry in the data independent acquisition (MS[supserscript E]) mode for the HDX-MS analysis. We also used ion mobility as a second and orthogonal dimension of separation to overcome the spectral crowdedness. Peroxiredoxins are ubiquitous antioxidant enzymes present in many organisms. Their catalytic activity is regulated by redox dependent oligomerization and their sensitivity to overoxidation is related to the flexibility of the active site loop to undergo partial unfolding. In this research we conducted HDX-MS experiments for determining to what extent the flexibility of the active site loop governs the sensitivity of peroxiredoxins to overoxidation. As example of a robust peroxiredoxin we studied initially the conformational properties of Salmonella typhimurium AhpC wild-type protein by HDX-MS. Subsequently, we conducted comparative HDX-MS analysis on the reduced form of the wild-type protein, and two single point mutants, T77V, and T77I, with the objective to decipher to what extent the stability of the dimer-dimer (A)interface affects the conformational dynamics of the active site loop. Differential HDX-MS results of the wild-type, disulfide reduced wild-type protein have exhibited a decrease in the motility of the active site loop and the C-terminal end of the protein upon disulfide reduction. The Thr77 single point mutation by valine enhanced the dimer-dimer interaction thereby stabilizing the decamer interface and increasing the motility of the active site loop. Whereas, the substitution of T77 by isoleucine increased the motility of the interfacial region which forms the dimer-dimer interface thereby promoting the dissociation of the decamer to dimers. A technically more advanced HDX-MS experimental setup was used to study the exchange-in properties of two robust peroxiredoxins, namely the wild-type StAhpC and the C46S mutant of StAhpC, which mimicks the reduced wild-type StAhpC, in comparison to human Prx2, a peroxiredoxin which is considered as sensitive to overoxidation. When differential deuterium uptake of wild-type StAhpC, C46S mutant StAhpC were compared, increased conformational rigidity was observed in the C46S mutant protein compared to the wild-type Prx. The peptide with highest deuterium incorporation levels in the human Prx2 is much lower compared to the bacterial wild type and C46S mutant Prxs. These comparative HDX-MS studies have fostered our understanding of the underlying conformational dynamics that lead to robust and sensitive Prxs. The second protein system that was studied was a representative of the Cullin Ring Ligases (CRLs), the largest family of RING-type E3 ligases that catalyze ubiquitylation of substrates. Protein ubiquitination is a post-translational modification that regulates several important biological processes in eukaryotic cells. It involves a three enzyme enzymatic cascade consisting of an ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligases (E3). In this study focus was directed toward the Cullin scaffold protein, which adopts an elongated structure that allows substrate receptor binding at the N-terminal domain (NTD) via adaptor proteins. Its C-terminal domain (CTD) binds to E2-ubiquitin through the RBX ring subdomain. Covalent attachment of the ubiquitin-like protein Nedd8 to the conserved lysine residue of the CTD stimulates the transfer of ubiquitin to substrate proteins thereby promoting ubiquitination. The HDX-MS studies of CUL1-RBX1 protein and its neddylated form highlighted that neddylation induces significant flexibility in the conformational dynamics of the CUL1 and RBX1 protein. The HDX-MS results support a mechanistic model in which conformational flexibility in the C-terminal domain of CUL1 and a concomitant opening of the RBX1 protein is necessary to allow the ubiquitin-bound E2 to be placed in close proximity to the protein substrates thereby facilitating the CRL activity.

Fundamentals, Methods, and Applications

A Method for Obtaining Conformational Structures of Proteins in Solution

Dynamical Effects of Incorporating Site-specific Crosslinks in Peptides Examined by Hydrogen-deuterium Exchange Mass Spectrometry

Mass Spectrometry of Biological Materials, Second Edition

Studies of the Oxidative Refolding of Cystinyl Proteins by Hydrogen/deuterium Exchange-mass Spectrometry

In aqueous solutions with high concentrations of methanol at low pH, the global conformation of ubiquitin denatures to a structure of increased helical character at 50% methanol to a highly helical denatured structure at 90% methanol. Circular dichroism analysis and hydrogen/deuterium exchange mass spectrometry experiments have been reported for the monitoring of alcohol-induced conformational transitions of ubiquitin upon exposure to increasing concentrations of methanol. A bottom-up analysis of deuterium labeled ubiquitin was used in this study to examine the local conformational transitions of ubiquitin upon exposure to solutions of varying concentrations of methanol. Analysis of ubiquitin and nine peptide fragments, produced from a ubiquitin-pepsin digest, were monitored through the use of a Varian 500-LCMS ion trap. The bottom-up approach to hydrogen/deuterium exchange mass spectrometry isolated most deuterium exchange to the [beta] B-strands of ubiquitin, suggesting the methanol-induced transitions of ubiquitin were highly characterized by the unfolding of the native-state [beta] B-sheets.

This volume explores strategies and detailed protocols for the preparation of macromolecular complexes and their characterization in view of structural analysis. The chapters in this book are separated into three parts: Part One focuses on sample preparation, and covers strategies for recombinant expression of multiprotein complexes in prokaryotic and eukaryotic hosts, for genome engineering using the CRISPR/Cas9 system and for production of specific binders such as reformatted antibodies and artificial binding proteins. Part Two looks at the biophysical methods that can provide useful indicators for sample optimization, and often complement structural information obtained with core technologies for structure determination--x-ray crystallography and cryo-electron microscopy--by quantitative solution data. Part Three discusses the characterization of multiprotein complexes in a cellular environment using the latest technologies and in vivo approaches. Written in the highly successful Methods in Molecular Biology series format, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and tips on troubleshooting and avoiding known pitfalls. Cutting-edge and authoritative, Multiprotein Complexes: Methods and Protocols is a valuable resource for structural and molecular biologists who need to prepare multi-components for their applications, and for other scientists working on macromolecular assemblies from other angles that need to know the latest approaches that the field has to offer.

Biophysical Characterization of Proteins in Developing Biopharmaceuticals, Second Edition, presents the latest on the analysis and characterization of the higher-order structure (HOS) or conformation of protein based drugs. Starting from the very basics of protein structure, this book explains the best way to achieve this goal using key methods commonly employed in the biopharmaceutical industry. This book will help today’s industrial scientists plan a career in this industry and successfully implement these biophysical methodologies. This updated edition has been fully revised, with new chapters focusing on the use of chromatography and electrophoresis and the biophysical characterization of very large biopharmaceuticals. In addition, best practices of applying statistical analysis to biophysical characterization data is included, along with practical issues associated with the concept of a biopharmaceutical’s developability and the technical decision-making process needed when dealing with biophysical characterization data. Presents basic protein characterization methods and tools applicable to (bio)pharmaceutical research and development Highlights the capabilities and limitations of each technique Discusses the underlining science of each tool Empowers industrial biophysical chemists by providing a roadmap for applying biophysical tools Outlines the needs for new characterization and analytical tools in the biopharmaceutical industry

Development of Hydrogen/deuterium Exchange Mass Spectrometry Simulations to Enable Accurate Protein Structure and Complex Selection

Protein Complex Assembly

Analytical Techniques in the Pharmaceutical Sciences

Hydrogen Deuterium Exchange Mass Spectrometry for Protein-protein Interaction and Structural Dynamics

Hydrogen/deuterium Exchange Mass Spectrometry for the Study of Methanol-induced Conformational Transitions in Ubiquitin

Lafora Disease (LD) is a fatal neurodegenerative disease that is correlated with mutation of the human phosphatase, Laforin. While Laforin has been shown to dephosphorylate phosphoglucans, little is known about the mechanism of dephosphorylation and Laforin's structure. Laforin consists of two domains: a carbohydrate binding module (CBM) and a dual specificity phosphatase (DSP) domain. The absence of Laforin function results in hyperphosphorylated and poorly branched sugar accumulations. It is hypothesized that these insoluble sugar deposits lead to neurodegeneration and pre-mature death in LD patients. We utilized hydrogen/deuterium exchange mass spectrometry (DXMS) to define Laforin's structural components and to probe Laforin's substrate interactions. DXMS analysis was performed on wild-type (WT) Laforin and LD mutants : W32G, G240S, and Y294N. The analysis of WT Laforin revealed the strongest substrate interaction with glycogen, when compared to interactions with the carbohydrates amylopectin and [Beta]-cyclodextrin. WT data revealed that regions of the CBM were protected from deuteration when bound to glucans. Similarly, structurally important regions of the DSP showed deuteration protection. W32G data confirmed the lack of protection from deuteration in the absence of substrate binding, whereas G240S data confirmed deuteration protection due to substrate binding. Lastly, the Y294N data revealed surprisingly strong substrate interactions in the DSP mutation region, despite weak CBM interaction. Our results confirm the role of Laforin's CBM in glucan binding and highlight the significance of structural DSP elements in the dephosphorylation of phosphoglucans.

Hydrogen exchange mass spectrometry is widely recognized for its ability to probe the structure and dynamics of proteins. The application of this technique is becoming widespread due to its versatility for providing structural information about challenging biological macromolecules such as antibodies, flexible proteins and glycoproteins. Although the technique has been around for 25 years, this is the first definitive book devoted entirely to the topic. Hydrogen Exchange Mass Spectrometry of Proteins: Fundamentals, Methods and Applications brings into one comprehensive volume the theory, instrumentation and applications of Hydrogen Exchange Mass Spectrometry (HX-MS) - a technique relevant to bioanalytical chemistry, protein science and pharmaceuticals. The book provides a solid foundation in the basics of the technique and data interpretation to inform readers of current research in the method, and provides illustrative examples of its use in bio- and pharmaceutical chemistry and biophysics In-depth chapters on the fundamental theory of hydrogen exchange, and tutorial chapters on measurement and data analysis provide the essential background for those ready to adopt HX-MS. Expert users may advance their current understanding through chapters on methods including membrane protein analysis, alternative proteases, millisecond hydrogen exchange, top-down mass spectrometry, histidine exchange and method validation. All readers can explore the diversity of HX-MS applications in areas such as ligand binding, membrane proteins, drug discovery, therapeutic protein formulation, biocomparability, and intrinsically disordered proteins.

Isotope Labeling of Biomolecules: Applications, the latest in the Methods in Enzymology series, focuses on stable isotope labeling methods and applications for biomolecules. This practical guide to biomolecular labeling looks at new techniques that are becoming widely used. Continues the legacy of this premier serial with quality chapters authored by leaders in the field Focuses on stable isotope labeling of biomolecules, which is important for structural studies of proteins and nucleic acids

Hydrogen-deuterium Exchange Studies by Mass Spectrometry

Multiprotein Complexes

Elucidating the Mechanism of Myristolylated Amp-activated Protein Kinase by Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS)

Mass Spectrometry Handbook

Obtaining Structural Insights on Bacterial Protein Complexes Using Time-Resolved Hydrogen-Deuterium Exchange Mass Spectrometry

Mass Spectrometry in the Biological Sciences covers the most recent technological and applied developments in the area, including both ionization techniques and ion analysis. It introduces and reviews some of the newer ionization methods, describes the major instrumentation involved in mass analysis, and presents the scope of the technology in biology, medicine, and environmental science. Specific examples are given for a number of topics. It also deals with recent achievements in the on-line combination of separation techniques such as gas chromatography, liquid chromatography, and supercritical fluid technology.

Second Edition provides up-to-the-minute discussions on the application of mass spectrometry to the biological sciences. Shows how and why experiments are performed and furnishes details to facilitate duplication of results.

Epigenetics fine-tunes the life processes dictated by DNA sequences, but also kick-starts pathophysiological processes including diabetes, AIDS and cancer. This volume tracks the latest research on epigenetics, including work on new-generation therapeutics.

Protein Conformational Studies by Hydrogen/deuterium Exchange Mass Spectrometry

Mass Spectrometry in Biomolecular Sciences

Characterization of Impurities and Degradants Using Mass Spectrometry

Hydrogen/Deuterium Exchange Mass Spectrometry (DXMS) Analysis of the Human Carbohydrate Phosphatase, Laforin

Exploring Functional and Folding Energy Landscapes by Hydrogen-deuterium Exchange Mass Spectrometry

Proteins are biological macromolecules responsible for the majority of all physiological processes. In order to properly function proteins are required to adopt highly ordered structures. These structural aspects may be found within a single protein or arise from multi-protein complexes. Here hydrogen/deuterium exchange mass spectrometry (HDX-MS) is employed as a tool to determine the extent of protein higher order structure. Exposure to D2O-based solvent causes the heavier isotope to exchange with amide hydrogens in the polypeptide backbone. This exchange is mainly dependent on protein conformation because the presence of stable hydrogen-bonded secondary structure will impede the incorporation of deuterium when compared to regions that are unstructured. In this work HDX-MS is used to study denaturant-induced unfolding of oxidized and reduced cytochrome c as well as ATP binding to the subunit of FOF1-ATP synthase. This work also lays the foundation to use this technique to study larger, more complex systems.

This book collects up-to-date advanced protocols and advice from leading experts in the area of membrane protein biology that can be applied to structural and functional studies of any membrane protein system. The contents explore methods for cloning and expression of membrane proteins and membrane protein complexes in prokaryotic and eukaryotic systems, approaches for protein purification, nanobody applications, as well as biophysical characterization and much more. Written for the highly successful Methods in Molecular Biology series, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and tips on troubleshooting and avoiding known pitfalls. Authoritative and thorough, Expression, Purification, and Structure Biology of Membrane Proteins serves to guide and encourage young researchers and newcomers to the field to tackle bold new studies on membrane proteins. Chapter 11 is available open access under a CC-BY 4.0 license via link.springer.com.

Proteins are not static objects. They have a great variety of internal motions with different amplitudes and different timescales. These internal motions play an important role in catalytic processes. Therefore, the existence of an intimate relationship between protein dynamics and protein function is widely accepted. Due to the significance of protein dynamics, techniques have been developed to study protein dynamics including nuclear magnetic resonance (NMR) spectroscopy, electron paramagnetic resonance (EPR) spectroscopy, and mass spectrometry (MS). Compared with NMR and EPR spectroscopy, MS has less stringent sample requirements, including protein concentration and protein size. Moreover, the mass accuracy, sensitivity, and faster data analysis also have contributed to the rapid growth of MS based techniques. Hydrogen-deuterium exchange mass spectrometry (HDX-MS), a combination of HPLC and MS, has become a common and sensitive tool to probe protein structural flexibility and solution dynamics. In this dissertation, HDX-MS was applied to study dynamic changes of proteins due to substrate binding and protein-protein interactions. The GT-A glycosyltransferase glucosyl-3-phosphoglycerate synthase from Mycobacterium tuberculosis (MtGpgS) catalyzes the first step of biosynthesis of 6-O-methylglucose lipopolysaccharides (MGLPs), which are essential to growth and existence of mycobacterium. The HDX-MS data revealed that the two substrates UDP-glucose (UDPG) and 3-phosphoglycerate (3PGA) can bind to

MtGpgS independently, disagreeing with the previous proposal that 3PGA can only bind to MtGpgS after UDPG. Moreover, 3PGA was found to bind to or allosterically affect the UDPG binding site. Furthermore, the HDX-MS data revealed that MtGpgS may provide a necessary conformation for UDPG binding, while it goes through a large conformational change on 3PGA binding. The GT-B glycosyltransferase MshA from Corynebacterium glutamicum (CgMshA) catalyzes the initial step of mycothiol biosynthesis. A large conformational change was observed in CgMshA on nucleotide binding by superimposing APO structure of CgMshA and complex structure with UDP. HDX-MS was utilized to study conformational changes of CgMshA on substrate binding from the aspect of dynamics, providing a complementary to static structures. The HDX-MS data showed that both substrates uridine diphosphate glucose-N-acetylglucosamine (UDP-GlcNAc) and 1-L-myo-inositol-1-phosphate (I1P) can bind to CgMshA independently, but the I1P binding is not productive since it binds to an uncorrect site. Moreover, the I1P binding can lead to dynamic changes of CgMshA, while only UDP-GlcNAc can induce the major conformational change of CgMshA. Furthermore, the 3PGA binding cannot induce further dynamic changes of CgMshA in the presence of UDP. HDX-MS was also employed to study dynamic changes of protein complex SufBC2D from Escherichia coli on ADP/Mg2+ binding. This complex is responsible for Fe-S cluster assembly under oxidative stress. The crystal structure of SufBC2D complex has been determined, while little dynamic information is known. So HDX-MS was applied to study dynamic changes of the SufBC2D complex. The HDX-MS data revealed that SufC has a significant conformational change, which may be required by ATP binding and hydrolysis. Moreover, SufB and SufD are detected to have dynamic changes due to SufC conformational changes. These dynamic changes suggest that SufB-SufD protomer may have a conformational change in order to provide a suitable conformation for Fe-S cluster assembly. This work demonstrates that HDX-MS can be effectively used to study protein-ligand and protein-protein interactions, as well as the accompanying changes in structural dynamics. HDX-MS data detects substrate binding mechanism and conformational changes that are not available through x-ray crystallography. With these advantages, HDX-MS has been applied in study of protein structure and dynamics, studying protein-ligand and protein-protein interactions, protein folding, as well as protein therapeutics discovery and development.

Methods and Protocols

Hydrogen Exchange Mass Spectrometry of Proteins

Structural Investigation of Membrane Proteins by Hydrogen/deuterium Exchange Mass Spectrometry

Development and Application of Histidine Hydrogen Deuterium Exchange Mass Spectrometry

Application of Hydrogen Deuterium Exchange Mass Spectrometry in Protein-ligand and Protein-protein Interactions

Hydrogen Exchange Mass Spectrometry of ProteinsFundamentals, Methods, and ApplicationsJohn Wiley & Sons

The application of hydrogen/deuterium exchange mass spectrometry (HDX-MS) to investigating protein-carbohydrate interactions is described. Proteins from three bacterial toxins, the B subunit homopentamers of Cholera toxin (CTB5) and Shiga toxin type 1 (Stx1B5) and a fragment of Clostridium difficile toxin A (TcdA-A2), and their interactions with native carbohydrate receptors, GM1 pentasaccharide (GM1-os), Pk trisaccharide and CD-grease, respectively, were first served as model systems for this study. The results suggested that HDX-MS can serve as a useful tool for localizing the ligand binding sites in carbohydrate-binding proteins. Following this, HDX-MS measurements were applied to explore the existence of distinct HMOs binding sites on toxins. Altogether, two toxins were studied, CTB5 and TcdA-A2, and their interactions with HMOs, 2'-fucosyllactose (2'-FL) and lacto-N-tetraose (LNT), respectively. For CTB5 and its interaction with 2'-FL, a novel binding site was localized for 2'-FL, different from the one for native receptor GM1-os. For TcdA-A2 and its interaction with LNT, however, the localized binding site was the same as its native carbohydrate receptor CD-grease. A HDX-MS based titration method Protein-Ligand Interactions in solution by Mass Spectrometry, Titration and hydrogen/deuterium Exchange (PLIMSTEX), was also applied to CTB5 and its interactions GM1-os, to test the reliability of using peptides as indicators to obtain the protein-carbohydrate binding affinities. The average apparent association constant measured for the addition of GM1-os to CTB at pH 7.0 and 20 °C was found to be (1.6 ± 0.2) * 106 M-1. This is in reasonable agreement with the reported value of (3.2 ± 0.2) * 106 M-1, which was measured using direct ESI-MS assay at pH 6.9 and room temperature.

Due to its enormous sensitivity and ease of use, mass spectrometry has grown into the analytical tool of choice in most industries and areas of research. This unique reference provides an extensive library of methods used in mass spectrometry, covering applications of mass spectrometry in fields as diverse as drug discovery, environmental science, forensic science, clinical analysis, polymers, oil composition, doping, cellular research, semiconductor, ceramics, metals and alloys, and homeland security. The book provides the reader with a protocol for the technique described (including sampling methods) and explains why to use a particular method and not others. Essential for MS specialists working in industrial, environmental, and clinical fields.

Epigenetics: Development and Disease

Hydrogen-deuterium Exchange/esi Mass Spectrometry for Determining Conformation of Human Lens [alpha]-crystallins

Investigating Protein-carbohydrate Interactions with Hydrogen/deuterium Exchange Mass Spectrometry (HDX-MS)

The Application of Hydrogen/deuterium Exchange and Covalent Labeling Coupled with Mass Spectrometry to Examine Protein Structure

Applied Biophysics for Drug Discovery

The book highlights the current practices and future trends in structural characterization of impurities and degradants. It begins with an overview of mass spectrometry techniques as related to the analysis of impurities and degradants, followed by studies involving characterization of process related impurities (including potential genotoxic impurities), and excipient related impurities in formulated products. Both general practitioners in pharmaceutical research and specialists in analytical chemistry field will benefit from this book that will detail step-by-step approaches and new strategies to solve challenging problems related to pharmaceutical research.

Application of Hydrogen-Deuterium Exchange/Mass Spectrometry for Determination of Cytochrome C Orientation on SAMs

Development of Hydrogen Deuterium Exchange Mass Spectrometry Methodologies for the Analysis of Bacterial Nano-compartments

Expression, Purification, and Structural Biology of Membrane Proteins

Conformational Dynamics and Function of Proteins Studied by Hydrogen/deuterium Exchange Mass Spectrometry