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Mini Review

Usage of Hydrogen/Deuterium Exchange Mass Spectrometry to Bridge Protein Structure, Dynamics, and Function

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Abstract

The analysis of the structural characteristics and dynamic properties of proteins is being done using a technology called hydrogen/deuterium exchange mass spectrometry, or HDX-MS. It might stand alone or work in conjunction with structural biology techniques such as cryo-electron microscopy (EM). Both small protein complexes and big protein aggregates may be studied using HDX-MS. Due to recent methodological developments and expanding equipment availability, HDX-MS is increasingly being used on a regular basis for various applications. Conformation and ligand interaction analyses by HDX-MS are already practically standard procedures when working with samples of low to medium complexity and sizes of less than 150 kDa. The quick development of the computational (software) backdrop, which makes it easier to analyse the data from the experiments, is another strong argument in favour of this. Analytes that are challenging to analyse with any other method can occasionally be handled using HDX-MS. This approach can also be used to analyse large complexes like viral capsids and disordered proteins. Given that it can now also analyse membrane proteins and post-translational changes, HDX-MS has lately established itself as a reliable tool in the drug discovery and biopharmaceutical development processes (Hjortland et al., 1976).

Static high resolution structures have contributed significantly to our understanding of protein structure and mechanistic function. As structural biology progressed, it became clear that highresolution structures alone could not fully capture the mechanistic basis for protein structure and function in solution. Hydrogen/Deuteriumexchange Mass Spectrometry (HDXMS) has recently emerged as a powerful and versatile tool for structural biologists, providing novel insights into protein structure and function. HDXMS allows for direct monitoring of a protein's structural fluctuations and conformational changes in solution while it is performing its functions. Static high-resolution structures have played a significant role in the development of our knowledge of protein structure and molecular function. High-resolution structures by themselves are unable to adequately reflect the molecular underpinnings of protein structure and function in solution; it has become abundantly obvious as structural biology has progressed. For structural biologists, Hydrogen/Deuterium-exchange Mass Spectrometry (HDX-MS) has recently grown into a potent and adaptable technology that offers fresh perspectives on the structure and function of proteins. When a protein is performing its functions in solution under native conditions, HDX-MS provides direct observation of structural fluctuations and conformational changes (Chaudhuri et al., 2015).

Keywords: Conformational switching, Conformational transitions, Hydrogen/deuterium-exchange mass spectrometry (HDX-MS), Intrinsic disorder, Structural dynamics, Structural rearrangements

INTRODUCTION

The development of hydrogen-deuterium exchange began in the middle of the 20th century, when it was used for the first time to examine molecular dynamics in the area of NMR spectroscopy. In the 1990s, when it became practical to investigate big proteins and their complexes, mass spectrometry was introduced to this subject. Deuterium-Hydrogen Exchange Mass spectrometry (HDX-MS) has emerged as a potent tool for structural protein research, offering in-depth knowledge of protein structure as well as conformational dynamics and function. In the area of protein structure, this methodology is already well-known and frequently used. A group of top experts in the industry developed concise guidelines for carrying out and deciphering HDX-MS investigations last year, paving the path for genuinely standardised tests. Studies of protein complexes, ligand binding, dynamic properties like conformational changes and folding, unfolding, and refolding, conformational changes resulting from allosteric effects, the structure and stability of biopharmaceuticals and epitopes, and other applications are just a few of the many that can be used with HDX-MS. HDX-MS has the benefit of having no or a very high size limit, which makes it superior to many other biophysical methods. It can be used to study both small and large protein complexes (Chmielecki et al., 2014). This is mostly because proteolysis and radical-induced fragmentation (also known as electron transfer dissociation, or ETD, and electron capture dissociation, or ECD, respectively) were used throughout the research. The use of HDX-MS as a standard analytical method in drug development and life science research is on the rise. The use of HDX-MS is encouraged more by the apparent rise in biopharmaceutical products available globally. As the need for such measurements has been steadily growing, a better commercial availability is also now readily apparent. The technique's popularity is also demonstrated by the rising number of pertinent applications reported each year. Even though HDX-MS is a very adaptable approach, understanding this methodology can be quite difficult due to the wide variety of applications and their unique reporting requirements. Furthermore, interpretation of the processes behind the measured experimental HDX-MS data is rarely simple, mostly because of problems with the spatial resolution of the mass spectrometry signal. Therefore, a thorough understanding of the used experimental design is not sufficient; software-assisted data interpretation is also required (Clarke et al., 2009).

Proteins are dynamic molecules that are always in motion, but high-resolution three-dimensional structures of macromolecular complexes have improved our understanding of their assembly and production. Small changes in local structure to large-scale conformational rearrangements between various structural states are all examples of these motions. Although these dynamic motions and structural reorganisations are essential for protein function, we still do not fully comprehend how they affect the structure and operation of proteins. High-resolution models are typically only accessible for the outcomes of dynamic processes and what happens during protein mobility and structural changes can only be hypothesised. Understanding the dynamic structural changes that result in functionally different conformations is essential to comprehending how a protein works in its entirety. Recent years have seen the development of Hydrogen/Deuterium-exchange Mass Spectrometry (HDX-MS) into a potent solution-state structural method that permits the investigation of macromolecular protein complexes in their natural environments. By directly observing backbone amide solvent accessibility in solution, which is sensitive to protein structural conformation and dynamics, HDX-MS enables one to probe protein structure and function (Onaga 2014).

The complexity and scale of the systems that can be analysed by HDX-MS have substantially increased because to developments in mass spectrometry and liquid chromatography. In contrast to other high-resolution structural methods, HDX-MS can offer information on protein structure for samples of various sizes and complexity, as long as the analyte of interest has a homogeneous composition and behaves well in solution. While HDX can also be investigated by NMR at the residue level, this method is typically only applicable to tiny soluble proteins that can be studied in the solution form. Mass spectrometers, in contrast, have been utilised to examine systems as huge and complicated as entire ribosome complexes since they have no inherent size restriction (Anger et al., 1977).

DISCUSSIONS

Protein mobility is constant and ranges from quick, transitory variations in local structure to large-scale, slower structural rearrangements. Although essential to protein function, these intrinsic, frequently modest, dynamic motions across the protein backbone cannot be easily explored by standard structural tools. Temperature, pH, and the specific kind of amino acid all have major roles in the inherent chemical rate of deuterium exchange of an amide group in a residue. Exchange rates for fully exposed amide hydrogens range from 10^1 to 10^3 s^{-1} under healthy conditions. Furthermore, HDX-MS applied to proteins is responsive to protein motion and structural dynamics and sensitive to the accessibility, or exposure, of backbone amide hydrogens coming from a protein's shape. Backbone amide hydrogen's accessibility in native proteins is substantially influenced by hydrogen bonding, regional secondary structure, and solvent accessibility. More exposed backbone amide hydrogens will exchange more quickly than those blocked in the protein core. Similar to this, amide hydrogens involved in stable hydrogen bonds will exchange more slowly because they are shielded from deuterium. Backbone amide hydrogens are momentarily exposed to deuterium due to local changes in protein structure. A peptide segment's exposed amide

hydrogens' tendency to exchange with deuterium depends on how often and how long, in relation to the chemical rate of exchange, they are in an exposed, exchange-competent condition. During continuous labelling HDX-MS investigations, these transitions are observed in equilibrium. In such an experiment, a protein is diluted into a deuterated solution over a range of times, from a few seconds to several hours or days. Dropping the solution pH to 2.5 and 0°C, when the labelling rate is at its lowest, then quenches the exchange reaction (Kelley et al., 2010).

Under physiological circumstances, the majority of local protein mobility is quicker than the rate of HDX labelling, which causes the deuterium to gradually bind to the peptide backbone. When a peptide transitions between its shielded, exchange-incompetent form and its exposed, exchange-competent state at a rate that is quicker than the labelling rate, this is known as so-called EX2 kinetics. In this instance, only a portion of the collectively exposed amide hydrogens can exchange during any given transition to the exchange competent state due to fast opening and closure events. Unimodal mass spectrum envelopes that gradually move to higher masses over time define these quick oscillations (Salmela et al., 2011). After regularly sampling the exposed, exchange-competent condition, the full peptide segment will eventually get deuterated. A peptide's deuterium uptake rate and extent under EX2 kinetics can be understood in terms of the local structural dynamics, mobility, and flexibility. Furthermore, one can start to understand and analyse the structural underpinnings of protein function and behaviour by quantifying how this exchange profile varies in response to the environment, to ligand interaction, or to homologous proteins (Chen et al., 2014).

CONCLUSION

HDX-MS will soon be a typical technique in structural biology as it advances. Because automation and commercial HDX equipment are widely accessible, it is a fantastic choice for even the high-throughput screening needed by the pharmaceutical industry. The method is uniquely suited to study the conformational dynamics of proteins in solution for both small and large systems, and it is complementary to many biophysical approaches, such as cryo-EM. The most recent developments broaden the scope of problems that HDX-MS can tackle and shine a light on this method's inherent potential. HDX-MS has evolved over time into a potent and flexible solution state biophysical and structural approach that can analyse both the structure and motion of native proteins as well as that of complex assemblies. Recent advancements have made it possible to characterise transitory structure in IDPs, observe large integral membrane proteins in real time while catalysing reactions, and study protein folding intermediates (Chen et al., 2017). These studies demonstrate how HDX-MS is a useful tool for analysing protein structure dynamics in complex, large-scale systems, where other approaches have encountered challenges. As the field matures, the method's power and accessibility increase, enabling greater scale and complexity of systems that may be studied. For instance, HDX-MS has recently been used to study the structural dynamics of proteins on intact enveloped viruses like the influenza virus and dengue virus. Similar to this, structural characterization of integral membrane proteins in their native membrane contexts using HDX-MS has become standard practise. Structural biology can now achieve goals that were previously impossible. As researchers use HDXMS more often, significant efforts have been undertaken within the field to standardise experimental procedures as well as data processing and reporting procedures.

REFERENCES

1. Hjortland MC, McNamara PM, Kannel WB (1976). Some atherogenic concomitants of menopause: the Framingham study. Am J Epidemiol. 103: 304-311.
2. Chaudhuri A, Manjushree R, Kumar S, Somenath H, Ghosh S (2015). To Study Correlation of Body Fat and Blood Lipids with Autonomic Nervous System Activity in Postmenopausal Indian Women. J Basic Clin Reprod Sc. 4: 59-65
3. Chmielecki J, Meyerson M (2014). DNA sequencing of cancer: what have we learned? Annu Rev Med. 65: 63-79.
4. Clarke J, Wu HC, Jayasinghe L, Patel A, Reid S, et al (2009). Continuous base identification for single-molecule nanopore DNA sequencing. Nat Nanotechnol. 4: 265-270.
5. Onaga LA (2014). Ray Wu as Fifth Business: Demonstrating Collective Memory in the History of DNA Sequencing. Stud His Philos Biol Biomed Sci. 46: 1-14.
6. Anger F, Nicklen S, Coulson AR (1977). DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 74: 5463-5477.
7. Kelley DR, Schatz MC, Salzberg SL (2010). Quake: Quality-aware detection and correction of sequencing errors. Genome Biol. 11: R116.
8. Salmela L, Schroder J (2011). Correcting errors in short reads by multiple alignments. Bioinformatics. 27: 1455-1461.
9. Chen D, Hwu WM, Heo Y, Ma J, Wu XL (2014). BLESS: Bloom filter-based error correction solution for high-throughput sequencing reads. Bioinformatics. 30: 1354-1362.
10. Chen Q, Jiang P, Li W, Li J, Wong L, et al (2017). MapReduce for accurate error correction of next generation sequencing data. Bioinformatics. 33: 3844-3851.