Protein structure and protein function are directly related: understanding protein function means understanding protein structure, and vice versa. In addition, protein structures are dynamic by nature and many proteins undergo conformational changes to perform their biological function. This includes the structural changes of enzymes during their catalytic cycle, and the operation of motor proteins. The full characterization of a protein requires analysis of the interplay between dynamics, function and structure.

How can mass spectrometry be used to address these issues? By using selective labeling processes, the incorporation of labels becomes a function of the solution conformation. All that remains is to find the labels and this can be done by measuring the mass of the protein, or by cutting the protein into pieces and finding the mass of all the pieces. This special focus issue on Hydrogen Exchange Mass Spectrometry describes one particular type of labeling strategy that has become widespread in recent years.

Labeling methods for biological molecules have been around for a long time, for both proteins and nucleic acids. In the 1950s, the director of the Carlsberg Laboratory in Copenhagen, Kaj Lindstrom-Lang, discovered the natural phenomenon called protein hydrogen exchange. This is a process whereby hydrogens attached to N, O and S are in continuous exchange with the hydrogens of water. This phenomenon occurs all the time for all proteins but mostly goes unnoticed in H₂O since hydrogen is just replacing hydrogen. If instead of an all H₂O solution, the solvent is changed to all D₂O (or T₂O as was done in some work), an isotope of hydrogen gets incorporated into the protein. A number of spectroscopic methods are sensitive to the difference between hydrogen and deuterium and can be used to detect and locate the incorporated hydrogen isotope.

In many ways, labeling a protein with deuterium is the most subtle and “natural” of labels. While tritium is significantly different from hydrogen in many regards, the difference between hydrogen and deuterium is quite small, apart from the mass difference. While there are isotope effects, the amount of disturbance caused by changing from an H to a D is magnitudes of order less than say incorporating larger covalent label containing tens of atoms. For this reason, hydrogen/deuterium exchange is attractive as it has the least potential to alter the protein itself during the course of the labeling. Labeling a protein with deuterium could be considered a quasi-covalent label, or one that is covalent but still labile. The label can change back to hydrogen if the conditions are not controlled, and this can be particularly easy given that the main solvent for proteins is H₂O, and that H₂O is present in the air around us.

Exchange of backbone amide hydrogens is the exchange reaction that is most often monitored. In folded proteins, both solvent occlusion and hydrogen bonding protect backbone amide hydrogens from exchange. In unstructured peptides exchange is rapid. For example, the exchange rate for an unprotected amide hydrogen at pH 7 and 25 °C in polyalanine is ~14 s⁻¹. In folded proteins however, the exchange can be many orders of magnitude slower.

Hydrogen exchange has been extensively measured by NMR and was the primary detection method for many years (see Ref. [1] for a brief history). Not long after it became possible to do mass spectrometry of proteins and peptides, hydrogen exchange measured by MS arrived [2]. These early experiments were at the intact protein level. A further improvement was the incorporation of digestion [3], based on the earlier work of Rosa and Richards [4]. Other improvements came with time, but for the first 5–10 years, only a few labs were really working with this method in earnest. Although other studies that utilized LC/MS were much more popular as early as 1994–1995, the popularity of HX MS probably waned for a few reasons. One is that it requires careful control of the experimental conditions otherwise all the deuterium is lost during analysis. Another was the amount of data processing that had to be done. A final, and perhaps the most important reason, was that for the experiments to find their way into the hands of the people most interested in proteins, protein folding, molecular biology and biochemistry, there needed to be people trained in both mass spectrometry and molecular biology/biochemistry to bridge the gap between the method and the application. Sure it was nice to have this great mass spectrometry method available but if interesting things were not found to work on, it was not going to go very far. Protein mass spectrometry did not really exist until the early 1990s so there was an unavoidable lag period between the first use of the method and the production of lots of skilled people that could bridge the gap effectively.

But those days are behind us now. The field is to the point that a wide variety and number of labs both use the method to study interesting proteins and proteins systems, and to continue to make improvements in the technology. To our knowledge, this is the third special focus issue of a mass spectrometry journal that is devoted to HX MS, the first being in 1999 in JASMS (vol. 10, no. 8, August 1999), the second in 2006 in JASMS (vol. 17, no. 11, November 2006). With this special issue, we highlight the international nature of where the field of HX MS is now. There are 21 contributions from 10 countries. Experiments are faster, more robust, there is software for data processing, there is better chromatography in the front, automated digestion, much improved mass spectrometers for the actual mass analysis. There has been a desire to locate deuterium at the single amino-acid level, and after almost 10 years of work, the community is coalescing around the right way to do just that. The breadth of
applications is wide, ranging the entire spectrum of proteins from enzymes in action, to membrane proteins, to structural proteins, to adapter molecules. Binding can be observed, allosteric communication revealed, unfolding monitored, stability calculated and the list goes on. A much larger number of people see the value of HX MS experiments and with the evergrowing importance of proteins as drugs, the method is expected to continue to expand.

References


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