Biophysical Mass Spectrometry for Biopharmaceutical Process Development: Focus on Hydrogen/Deuterium Exchange

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Summary

Mass spectrometry is an indispensable method to study biopharmaceuticals. It has been widely used to investigate protein primary structure but can also be instrumental in understanding higher-order structure. Among the tools to assess higher-order protein structure and structural dynamics, solution-phase hydrogen/deuterium exchange monitored by mass spectrometry has emerged as a powerful technique to study protein-protein interactions, folding/unfolding pathways, conformational changes induced by ligand binding, etc. On incubation in deuterated buffer, the labile protein backbone amide hydrogens exchange for deuterium if they are readily exposed to the solvent and are not engaged in secondary-structure hydrogen bonding. Quench of the exchange reaction and subsequent proteolytic digestion generates dozens of peptide fragments for which mass increase as a function of incubation period is monitored by mass spectrometry. Comparison of deuterium incorporation between two states of the protein provides insight into conformational stability and solvent accessibility which are in turn dictated by the secondary, tertiary, and/or quaternary structure of the protein. In this review, we briefly discuss direct mass measurment and covalent labeling techniques as other ways of assessing protein higher-order structure. We provide an overview of the molecular mechanism of H/D exchange and highlight the technique developments at every stage of the experiment. We also describe several applications for current and future use in the biopharmaceutical environment.

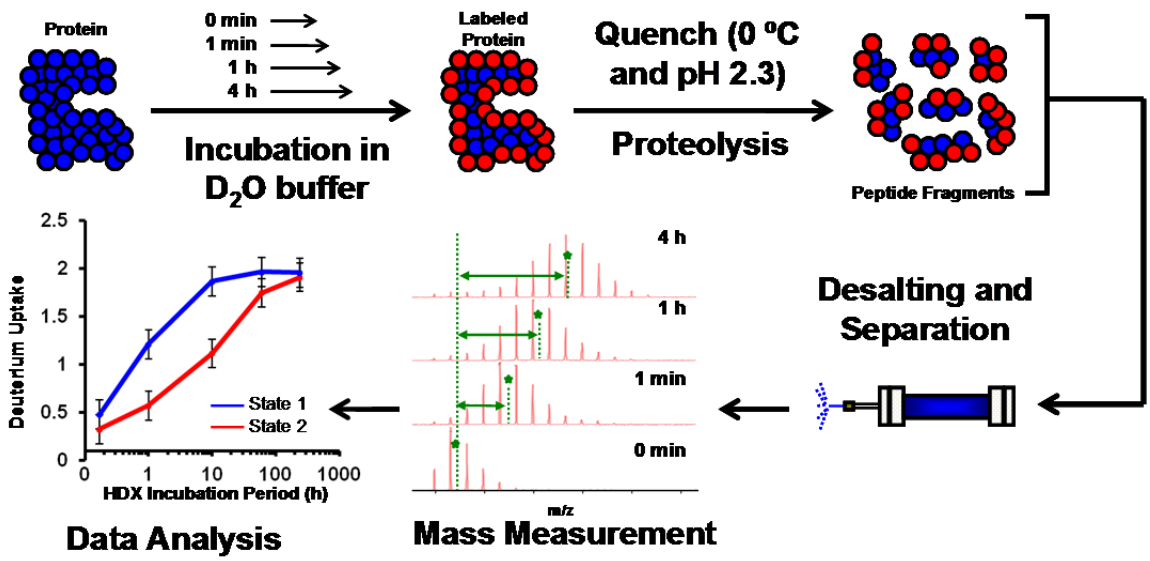

Fig. 1 Schematic diagram of the successive steps in a typical HDX experiment. Incubation of the protein in deuterated buffer for each of several periods of time is followed by quench (0 ºC, pH ~2.3) and proteolysis. The proteolytic peptides are then loaded onto a liquid chromatography column for fast separation and desalting prior to introduction into a mass spectrometer for mass measurement. Deuterium uptake for each peptide fragment is calculated by computing the difference in average mass between the deuterated fragment at each incubation period and the undeuterated control. Comparison of deuterium uptake profiles under two sets of conditions provides insight into the protein conformational dynamics.

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References

[1] Bou-Assaf, G. M. *et al.*, Biophysical Characterization of Proteins in Developing Biopharmaceuticals (Elsevier, Chenna, India), Chapter 12, pp. 307-339, (2014).