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Conformational insight into multi-protein signaling assemblies by hydrogen–deuterium exchange mass spectrometry

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Hydrogen–deuterium exchange (HDX) mass spectrometry (MS) can provide information about proteins that can be challenging to obtain by other means. Structure/function relationships, binding interactions, and the effects of modification have all been measured with HDX MS for a diverse and growing array of signaling proteins and multiprotein signaling complexes. As a result of hardware and software improvements, receptors and complexes involved in cellular signaling — including those associated with membranes — can now be studied. The growing body of HDX MS studies of signaling complexes at membranes is particularly exciting. Recent examples are presented to illustrate what can be learned about signaling proteins with this technique.

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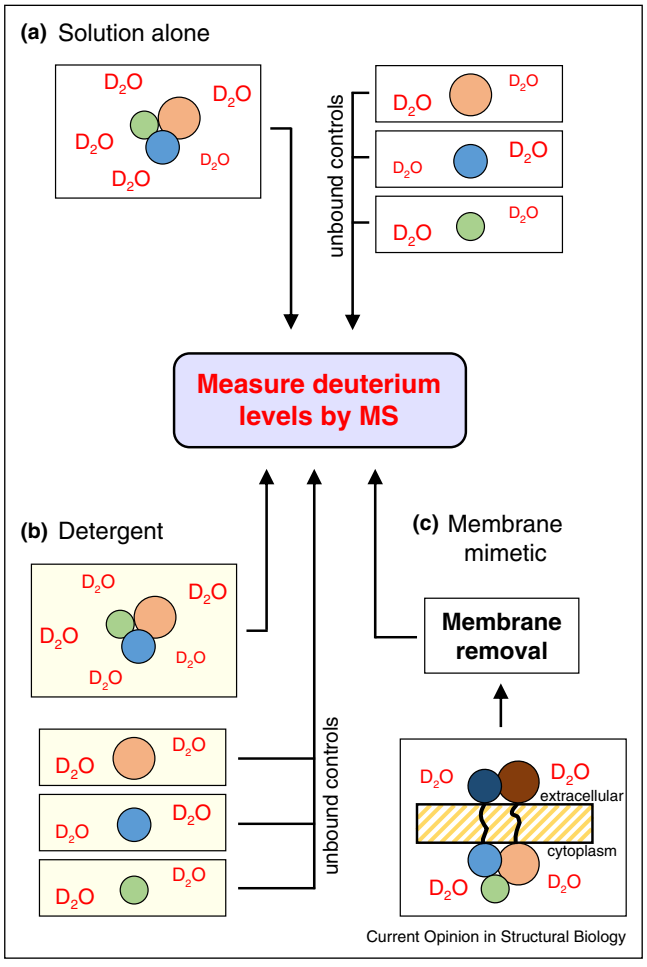
Introduction

Few would debate that at present it can be challenging to determine the structure of multi-protein signaling assemblies, let alone to determine conformational changes important for function. Size, complexity, and/or proximity to membranes of many signaling assemblies can often complicate or completely inhibit analysis. Mass spectrometry (MS) methods have a role to play in conformational studies and can be used not only to characterize the quaternary structure of assemblies, but also to obtain details about conformation and conformational changes [1–3]. Selective protein labeling using covalent labels or hydrogen–deuterium exchange (HDX), with subsequent MS detection, may provide insight into conformational features, including for many proteins intractable by other methods. This review will cover the use of HDX MS to study signaling complexes, especially including the analysis of assemblies in detergent and in the presence of membranes (Figures 1 and 2).

The theory and practice of HDX MS have been reviewed extensively (recent examples: [4,5••,6]). In short, backbone amide hydrogens in the protein exchange with deuterons in solution. The rate of this exchange is dependent on pH, temperature, hydrogen bonding, and solvent accessibility. With proper control of pH and temperature, deuteration reports on conformation and conformational changes affected by hydrogen bonding and solvent accessibility. Generally, poorly hydrogen bonded and/or solvent exposed regions will incorporate deuterium more quickly than those that are strongly hydrogen bonded and/or solvent protected. A common approach is to compare two (or more) different conformational states of the same proteins(s), and report where differences in exchange, and hence conformation, exist between the states (Figure 3). In a typical implementation, a protein-containing solution (solution A) of a given composition at physiological pH (in H_2O) is diluted with a solution (solution B) of the same composition and pH (labeling pH) made with all D_2O. The only difference between solution A and B is the presence of deuterium. Once the deuterated solution is added, the labeling reaction proceeds for various amounts of time before being slowed 4–5 orders of magnitude by reduction of the pH to 2.5 and the temperature to 0 °C (quench conditions). Labeled protein in quench conditions can be digested into smaller fragments with an aspartic protease, such as pepsin, and the resulting peptide fragments — which bear a signature of deuterium that reports on the conformation at the labeling pH — are then separated by liquid chromatography (LC). The LC is coupled to a mass spectrometer and the mass of each labeled peptide is measured. HDX MS as a technique is 25 years old and has become much more accessible in recent years due to advances in methodology, hardware, and data processing software [7,8]. Single proteins involved in signal transduction have been studied by HDX MS for many years, while analyses of multi-protein complexes and assemblies have recently become technically feasible and more common. HDX MS has become more amenable for the analysis of large proteins and complexes due largely to advances in chromatographic peak capacity by utilization of UPLC rather than HPLC, more sensitive mass spectrometers, and the development of powerful data processing software (described in more detail in [4,7,8]).

Figure 1



Summary of the ways in which comparative HDX MS can be used to study multi-protein complexes. **(a)** Solution alone. Each protein (green, orange, blue circles) is labeled individually in an unbound state (right panel), and the results compared to labeling of each protein when part of a complex (left panel). **(b)** In detergent. Labeling and comparisons are similar to solution alone except detergent (yellow background) is present to aid in solubilizing the hydrophobic regions of certain proteins. **(c)** Membrane mimetics. Protein complexes are assembled in a membrane mimetic (e.g., bicelles, monolayers, lipid vesicles, nanodiscs). Deuterium labeling is measured after membrane component removal by chromatography and compared with the labeling of each component alone (not shown).

HDX MS of complexes that do not involve a membrane

Some of the earlier examples of how HDX MS can be used to study multi-protein signaling assemblies are also the simplest — they were performed in solution alone without any detergents or membrane mimetics (Figure 1a). In 2012, Choi *et al.* [9•] used HDX MS to investigate signaling by notch receptors, type I transmembrane proteins that communicate signals in response to transmembrane ligands on neighboring cells and regulate a variety of cellular events during development and in normal tissue homeostasis. Changes in protein dynamics and interactions were assessed upon stepwise assembly of a complex composed of a transcriptional co-activator of the Mastermind family (MAML1), the Notch intracellular domain (NICD), and DNA-binding factor CSL. MAML stabilized the ankyrin (ANK) domain facilitating a stronger interaction with CSL, explaining why MAML is essential for the cooperative dimerization of NTCs on paired-site DNA. At the time, this was a rather large multiprotein complex for HDX MS.

While there are numerous examples using HDX MS to follow signaling pathways in animals, West *et al.* [10••] used HDX MS to study the abscisic acid (ABA) receptor in plant biochemistry. The means by which plants regulate growth and respond to environmental cues can involve ABA pathways, initiated by the PYR/PYL/RCAR class of receptors, which promote signaling by disrupting phosphatase inhibition of Snf1-related kinases (SnRKs). The abscisic acid receptor PYL2, HAB1 phosphatase, and SnRK2.3 and 2.6 were probed by HDX MS and the results revealed that in the presence of binding partners, the phosphatase adopts receptor-specific conformations involving a Trp385 'lock' required for signaling, and showed that kinase activity is linked to a more stable conformation.

The most studied signaling pathways by HDX MS are those including G-proteins and G-protein coupled receptors (GPCRs). Exchange has been monitored for G-proteins and GPCRs in all types of conditions, including alone in solution, in detergents, and with membrane mimetics (Figure 1). A recent illustrative example [11••] of HDX MS in solution (Figure 1a) involved a regulator of G protein signaling (RGS) which binds both active and inactive Gα proteins using two separate binding motifs. The N-terminal RGS motif binds to active Gα-GTP, and the C-terminal G protein regulatory (GPR) motif binds inactive Gα-GDP. Communication between these two G protein binding motifs was probed by HDX MS to determine whether regulator of G protein signaling 14 (RGS14) could interact with both forms of Gα at the same time. The results showed that RGS14 was a dynamic protein that underwent allosteric conformational changes when bound to Gα-GDP. When RGS14 formed a complex with Gα-GDP, there was stimulation of GTPase activity in Gα-GTP, meaning that RGS14 does interact with both Gα subunits simultaneously, thereby clarifying our understanding of how RGS14 integrates signaling by G protein subunits.

HDX MS in the presence of detergents

Although performing HDX MS experiments in solution alone can be valuable, many proteins, particularly those involved in signaling, may contain hydrophobic regions meant to interact with membranes and therefore may require a detergent to improve solubility. Several detergents [e.g., n-dodecyl β-D-maltoside (DDM) [14•,15•],