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Review

Biological insights from hydrogen exchange mass spectrometry ☆

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ABSTRACT

Over the past two decades, hydrogen exchange mass spectrometry (HXMS) has achieved the status of a widespread and routine approach in the structural biology toolbox. The ability of hydrogen exchange to detect a range of protein dynamics coupled with the accessibility of mass spectrometry to mixtures and large complexes at low concentrations result in an unmatched tool for investigating proteins challenging to many other structural techniques. Recent advances in methodology and data analysis are helping HXMS deliver on its potential to uncover the connection between conformation, dynamics and the biological function of proteins and complexes. This review provides a brief overview of the HXMS method and focuses on four recent reports to highlight applications that monitor structure and dynamics of proteins and complexes, track protein folding, and map the thermodynamics and kinetics of protein unfolding at equilibrium. These case studies illustrate typical data, analysis and results for each application and demonstrate a range of biological systems for which the interpretation of HXMS in terms of structure and conformational parameters provides unique insights into function. This article is part of a Special Issue entitled: Mass spectrometry in structural biology.

1. Introduction

Since its first applications in the 1990s [1–4], hydrogen exchange mass spectrometry (HXMS) has emerged as a powerful technology to elucidate protein structure, dynamics and interactions in solution [5–20]. This tool capitalizes on the sensitivity of hydrogen exchange to conformational fluctuations spanning from small to large-scale and the broad applicability of mass spectrometry to protein samples that pose a challenge for other structural techniques [21–24]. Recent advances in automating HXMS data collection and data analysis have made this a routine approach accessible to a wider variety of scientists, including those in industry. Furthermore, the ability of HXMS to screen ligand binding and protein stability [25,26] in a high throughput manner will continue to improve, adding important dimensions to the development and monitoring of therapeutics [17,27,28].

Recent reviews cover the breadth of HXMS applications and highlight the potential and promise of HXMS. Konermann et al. provide a tutorial on HXMS fundamentals, explaining the kinds of information obtained from different labeling techniques, and discussing the localization of labels to specific residues through fragmentation approaches [13]. Brock focuses solely on fragmentation HXMS, showing how recent developments have overcome many earlier limitations, highlighting its role in biotherapeutics and broadly surveying other applications [17]. Chalmers et al. describe cutting edge methodology for differential HXMS to probe ligand interactions and discuss the challenges of applying the technique to growing datasets of increasingly larger protein complexes [5]. Wani and Udgaonkar survey HXMS applications in protein folding and unfolding [19]. Iacob and Engen argue that, despite early perceptions of HXMS as fraught with difficulties, it is now possible for an established lab to carry out a complete HXMS study of the structure and dynamics of a protein or protein–ligand complex in one week [15]. These reviews and others provide much more detail on HXMS and the ever-expanding number of studies exploiting HXMS than I am able to cover in this review. Here, after a brief overview of HXMS, I focus on four recent studies to show how HXMS can provide unique biological insights, and describe some of the challenges and opportunities in interpreting HXMS results in terms of structure and conformational parameters.

1.1. Overview of hydrogen exchange

1.1.1. Hydrogen exchange detects structure

As detailed in Baldwin's recent historical overview [29], Linderstrom-Lang developed hydrogen exchange (HX) in 1954 with the goal of identifying hydrogen-bonded structures in proteins. Using deuterated solvent, he observed the rapid exchange of amide protons for deuterium

Abbreviations: HXMS, Hydrogen exchange mass spectrometry; k_{op}, Rate constant for opening; k_{cl}, Rate constant for closing; k_{int}, Rate constant for intrinsic chemical exchange; k_{HX}, Experimentally observed rate constant of exchange; k_u, Rate constant for unfolding; K_u, Equilibrium constant for unfolding; P_f, Protection factor; ΔG_u, Free energy of unfolding; ESI, Electrospray ionization; MALDI, Matrix assisted laser desorption ionization; apoA-I, Apolipoprotein A1; HDL, High density lipoparticle; TPR, Tetratricopeptide repeat domain; DD, Hsp90 dimerization domain; MD, Hsp90 middle domain; NBD, Hsp90 nucleotide binding domain; α_1-AT, Serpin alpha-1 anti-trypsin; BR, Retinal-bound bacteriorhodopsin; BO, Retinal-free bacteriorhodopsin

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in short unstructured peptides. HX kinetics for insulin—a folded protein—were complex and took place slowly. The classic model Linderstrom-Lang developed to explain the slowed HX in proteins still holds today: A backbone amide proton in an HX incompetent state exists in equilibrium, determined by k_{op} and k_{cl}, with an HX competent state from which the proton can undergo exchange with the rapid exchange rate k_{int} (Eq. (1)) [30,31].

$$\text{HX incompetent(NH)} \underset{k_{cl}}{\overset{k_{op}}{\rightleftarrows}} \text{HX competent(NH)} \xrightarrow{k_{int}} \text{Exchanged(ND)} \quad (1)$$

Studies on model peptides [32–34] and unfolded proteins [35,36] have shown that k_{int} is determined solely by chemical considerations: temperature, pH and sequence neighbors.

Despite the development of HX as an important biophysical approach for understanding protein structure and dynamics since Linderstrom-Lang's discovery, the basis for the HX incompetency of the subset of sites protected from rapid exchange has remained a subject of debate [37]. Quench-flow HXMS [38,39] and specialized NMR methods [37,40,41] that enable measurement of the most rapidly exchanging sites demonstrate that the majority of sites at the protein surface do not exchange with the k_{int} measured for unstructured peptides. Focusing on surface sites only, Truhlar et al. found that the HX protection correlates strongly with total buried surface area for the nonglobular protein IκBα, but not as strongly for the globular protein thrombin [39]. Combining HX rate measurements on a stable *Staphlyococcal* nuclease variant (SNase) for fast exchanging sites (1–100 s^{-1}) detected through a Cleanex-PM NMR method, and for slower sites (10^{-3} to 10^{-7} s^{-1}) detected through 2D HSQC NMR, Englander and coworkers have recently conducted a systematic examination of HX behavior, neighbor relationships and structural context [37,42]. For this collection of sites (110 monitored/140 total) across SNase, participation in a hydrogen bond appears to be the stronger determinant of protection from HX than surface burial [37]. More data on HX across all types of sites for a larger collection of proteins will be required to sort out the relative importance of burial from solvent and hydrogen bond involvement, which may vary depending on the globularity or other aspects of the specific protein. In any case, as both surface burial and hydrogen bond formation are features characterizing structured elements of proteins, HX remains an ideal probe to distinguish structured from unstructured regions of a protein.

1.1.2. Hydrogen exchange detects conformational parameters

As Linderstrom-Lang observed for insulin, given enough labeling time, even amide protons that do not exchange with a rapid k_{int} will eventually exchange with solvent, with a constant rate of exchange k_{HX} that is slowed relative to k_{int} by up to eight orders of magnitude. Dynamic structural processes that lead to both hydrogen bond breakage and exposure to the solvent to enable HX at an initially protected site include random local fluctuations at independent sites in the context of the folded state, and cooperative unfolding events involving multiple sites. Englander et al. highlighted an example of the former as a simple "crankshaft" rotation of a peptide group around neighboring alpha carbons [42]. Unfolding events exposing protected sites to HX include partial unfolding to an intermediate, and global unfolding of the entire protein. HX through different processes can be distinguished through denaturant sensitivity [42–46]. The exposure of surface area leads to the denaturant dependence for HX of amides that exchange through unfolding events that expose multiple sites, with a steeper dependence for global compared to partial unfolding. In contrast, HX of a site exchanging through a local fluctuation demonstrates no dependence on denaturant, until reaching the concentration at which the structural element containing that site undergoes unfolding (partial or global) [43–45].

Observation of HX from protected sites through unfolding (whether partially to an intermediate ensemble, or completely to a globally unfolded ensemble) provides insight into the thermodynamic and kinetic parameters of a protein's conformational ensemble. Based on Linderstrom-Lang's model (Eq. (1)), for a stable protein ($k_{cl} \gg k_{op}$), the exchange rate constant k_{HX} for protected sites is determined in part by the chemistry (k_{int}) and in part by the conformational equilibrium governing the opening of structure that allows amide protons to exchange [47]:

$$k_{HX} = k_{op} \times k_{int}/(k_{cl} + k_{int}) \quad (2)$$

Importantly, k_{HX} only reports directly on conformational parameters at two limits of exchange, based on the relationship between k_{cl} and k_{int}. If $k_{cl} \ll k_{int}$ when a protein samples an open conformation with k_{op}, all amides newly exposed in that conformation undergo exchange with k_{int}. Under these limiting conditions, Eq. (2) reduces to a first order expression and HX is designated as occurring in the EX1 regime, with k_{HX} reporting directly on the unfolding rate constant (k_u) for accessing the open conformation, whether in the form of a partially unfolded intermediate or the fully unfolded state.

$$k_{HX,EX1} = k_{op} = k_u \quad (3)$$

$k_{HX,\ EX1}$ values determined through HX have matched other measurements of the global k_u for a number of proteins [48–53].

At the other limiting extreme, known as EX2, when $k_{cl} \gg k_{int}$, Eq. (2) reduces to the second order form:

$$k_{HX,EX2} = k_{op} \times k_{int}/k_{cl} \quad (4)$$

Host–guest peptide studies enable the calculation of k_{int} for a given amino acid sequence at any temperature and pH [34]. Thus, in EX2, the ratio of k_{HX}/k_{int}, reveals the equilibrium constant (K_u) for the local fluctuation, partial or global unfolding event underlying exchange:

$$k_{HX,EX2}/k_{int} = k_{op}/k_{cl} = K_u \quad (5)$$

To quantify and compare the extent of HX protection for amide sites the inverse ratio of calculated k_{int} to observed $k_{HX,EX2}$ is used to determine a protection factor (P_f) [54,55], which is simply the inverse of the equilibrium constant:

$$P_f = k_{int}/k_{HX,EX2} = 1/K_u \quad (6)$$

Thus, in the EX2 regime, either P_f or K_u can be transformed into a free energy using Eq. (7), enabling assignment of thermodynamic stability for each amide site, or for a range of sites, depending on the resolution of the method for deuterium readout.

$$\Delta G_u = -\mathrm{RTln}K_u = +\mathrm{RTln}P_f \quad (7)$$

ΔG_u values determined using equations (5) and (7) from $k_{HX,EX2}$ measurements have demonstrated a good correlation with values determined from other approaches [56].

HX provides an equilibrium approach to detect the sampling of non-native conformations from the folded state, distinguishing conformations through different levels of protection, and, in the limiting regimes, extracting the parameters k_u and K_u (in EX1 and EX2, respectively) [57–59]. It is important to note that significant changes in pH, temperature and/or denaturant are often required to shift the k_{cl}/k_{int} ratio to achieve one or both of the limiting exchange regimes. Indeed, for a significant fraction of proteins with folding characterized under standard conditions of 25 °C, pH 7 and 150 mM ionic strength [60], HX through global unfolding is predicted to occur between EX1 and EX2 as their folding rate constants (k_{cl}) are on the same order as the mean k_{int} calculated for their sequences [23]. Furthermore, as k_{int} values for sites within a given protein sequence may vary up by up to two to three orders of magnitude [34], mixed EX1 and EX2 behavior may be observed for sites exchanging through the same unfolding event [53,61–63]. Therefore, extraction of conformational parameters