HDX for Studying Effect of pH

1. Acid induced conformational refolding of membrane proteins

Many bacterial toxin proteins are secreted as water-soluble proteins, but once their target is reached they must undergo extensive conformational refolding to expose hydrophobic surfaces which can partition into membranes. The conformational change is often triggered by the change in pH of the aqueous environment and usually involves the protonation of basic amino acid residues. One example is diphtheria toxin T domain, a 22 kDa protein that is water-soluble at neutral pH, but undergoes a series of structural refoldings at acidic pH in endosomes, resulting in its insertion into a cell membrane. Although numerous biophysical studies have been devoted to the structural rearrangement of these toxin proteins, little is known about the conformational changes and dynamics at the molecular level. HDX is a powerful tool for probing protein structural perturbations induced by stimuli such as ligand binding and protein-protein interactions. When pH is constant, changes in protein dynamics are often directly related to specific protein conformational changes. However in the case of pH-dependent protein refolding, because the intrinsic rate of hydrogen deuterium exchange changes in response to pH, we need to deconvolve this effect by converting the on-exchange times.

2. Determining the pH dependence of amide hydrogen exchange rate.

Griffin and coworkers demonstrated the use of a time-window expansion method to deconvolve the pH effect according to Equation 1,

\[ k_{ch} \sim k_{OH}[OH^-] = A \exp \left( \frac{-E_a}{RT} \right) [OH^-] \]

(1)

where A is the frequency factor, and E_a is the activation energy of the dominant base-catalyzed amide hydrogen exchange reaction in the range of pH 5-10. When temperature is constant, a simple equation (Equation 2) can be derived from Equation 1 to calculate the ratio of the amide hydrogen exchange rates between the two pH conditions:

\[ \frac{k_{ch1}}{k_{ch2}} = \frac{[OH^-]_1}{[OH^-]_2} = \frac{k_w/[H^+]_1}{k_w/[H^+]_2} = \frac{10^{-pH_2}}{10^{-pH_1}} = 10^{pH_1-pH_2} \]

(2)

According to Equation 2, the intrinsic HDX rate decreases \(10^{7.5-x}\)-fold when decreasing pH from pH 7.5 to pH x. Thus, to have the same amount of amide hydrogen exchanged at all pH conditions, one needs to deconvolve this effect by converting the exchange times referenced to a standard condition (e.g., pH 7.5).

3. The case of diphtheria toxin T domain: pH dependent HDX study

The crystallographic structure of water-soluble T domain is only available for the water-soluble form. To date it has not been possible to obtain a high resolution structure for T domain in the course of pH-induced refolding, owing to the co-existence of multiple conformations. In our study, the conformational dynamics of water soluble T domain was investigated by HDX as a function of pH (7.5-5.0) in the absence of membrane. The changes in HDX dynamics from the neutral state (7.5) to low pH states (7.0, 6.5, 6.0, 5.5, and 5.0) provides information on the kinetics of pH-induced conformational transition as well as the structural rearrangements accompanying each conformational transition of T domain (Figure 1).
HDX revealed two major steps of histidine protonation. The initial structural transition occurs by pH 6, where the protonation of histidine residues triggers the unfolding of the N-terminal segment and the exposure of the hydrophobic hairpin (TH8-9). Further decrease of pH to 5.5, however, exposes more of the helical hairpin TH8 and TH9 to the aqueous environment, leading to the formation of oligomer via hydrophobic interaction in the absence of membrane.

This pH-dependent HDX deuterium exchange method can be readily applied to the analysis of other proteins that change conformation in a pH-dependent manner. Knowledge of how water-soluble protein refolds and crosses lipid bilayers may provide insight into the general principles of protein transportation across biological membranes.