Interpretation of Hydrogen-Deuterium Exchange Mass Spectrometry Structural Proteomics Data using Molecular Dynamics Simulations

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Introduction

Hydrogen-deuterium exchange (HDX) combined with mass spectrometry (MS) is a useful technique for studying protein structure. Peptide-bond amide hydrogen-deuterium exchange reflects the hydrogen bonding status and therefore the presence of secondary structure in proteins. We have recently shown that disordered proteins may undergo disorder-order transitions characterized by dramatic change in HDX protection values (PMID 31683063). We found that disordered proteins exist in rather compact globular states, and that the transition occurs while preserving overall protein structure without a dramatic change in distances between hydrogen-bond-forming donor-acceptor atom pairs. Here, we show that the simplified empirical approach of determining the opening frequencies of the backbone amide hydrogen-bond-forming donor-acceptor pairs in molecular dynamics (MD) simulations can be used to correctly interpret mass spectrometry-derived HDX values..

Methods

Top-down ECD- or ETD-HDX was performed using a Bruker 12 T Apex-Qe hybrid Fourier transform (Bruker Daltonics) or an Orbitrap Lumos (Thermo) mass spectrometer, respectively. Protein samples were dialyzed into 10 mM ammonium acetate, mixed with D_2O online at 1:4 ratio (2.5 s exchange time), mixed at a 1:1 ratio online with a quenching buffer containing 0.4% formic acid (FA) and acetonitrile/H_2O/D_2O in a 2:1.6:6.4 v:v:v ratio, and directly infused into a mass spectrometer. Deuteration values were determined using our HDX Match software (PMID 26162650) by fitting the experimental isotopic envelopes of the intact proteins. MD simulations were conducted using GROMACS 2018 software using CHARMM36 force field. Real-mean-square-fluctuation values calculated along the trajectory were used as a measure of the conformational flexibility for each protein.

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Results

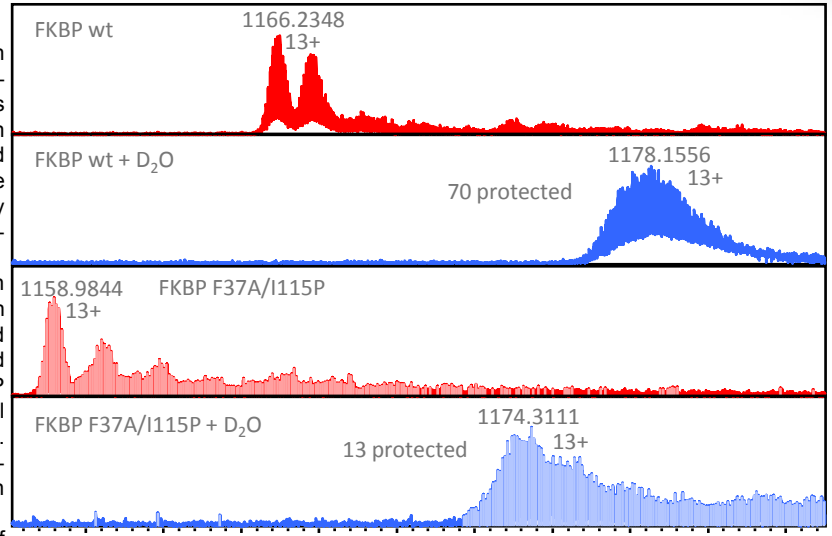
Measuring the mass of the intact deuterated protein provides data on the total number of protected peptide-bond amide protons in the whole protein. Using this technique, we previously obtained good correlation between the observed total number of protected peptide bond amide protons for a 2.5-s exchange time and the number of residues involved in secondary structure elements for proteins with known three-dimensional structures (PMID 28695211).

While characterizing the structural differences between the disordered and ordered states of the FKBP domain by molecular dynamics simulations, we did not find dramatic changes in overall structure between the wild type (wt, ordered) and the double mutant F37A/I115P (dm, disordered) forms of the protein, but the total exchange HDX values were dramatically different. Overall distribution of average distances for hydrogen-bond-forming atom pairs also were similar between these two states of the protein.

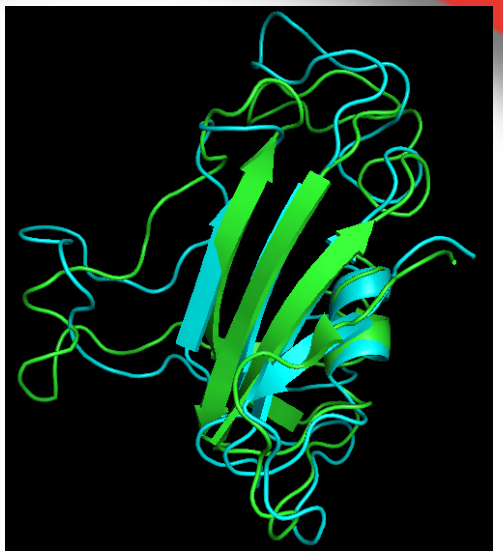
To explain this paradox, we performed a comparison of the main-chain fluctuations along the MD trajectory between the ordered and disordered structures. Here, we noticed substantial differences between states. To relate the observed differences to the HDX experimental data, we calculated the frequencies of opening along the MD trajectory of the backbone-amide donor-acceptor pairs which constitute the hydrogen bonds measurable by HDX. We were able to obtain a fairly good correlation of the calculated frequency values with the observed HDX data. For 72 pairs (70 protected hydrogens in wt), 61 pairs exhibited higher frequencies of opening for dm vs. wt, and 11 pairs stayed unchanged (13 protected hydrogens in the dm).

Conclusions

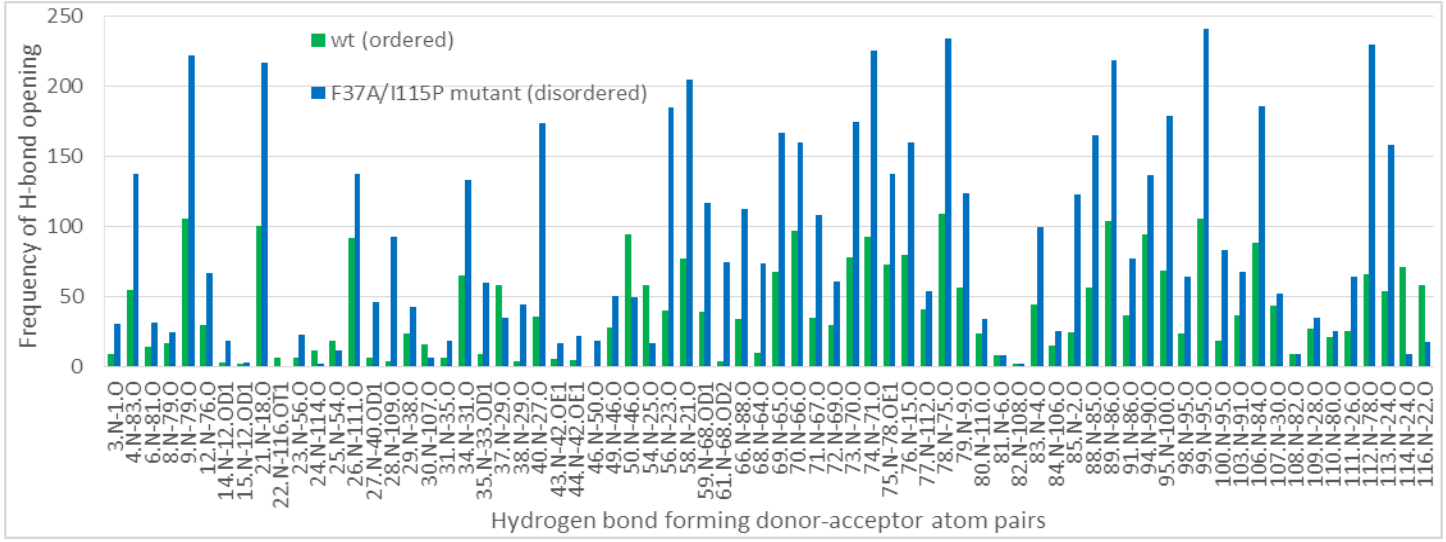
We have shown that this simplified empirical approach of determining the opening frequencies of the backbone amide hydrogen-bond-forming donor-acceptor pairs in MD simulations provides a better interpretion/prediction of MS-derived HDX values than does the average hydrogen-bond-forming atom pair distances, and also provides the correct estimation of the secondary-structure content in the proteins.



Total HDX of intact wt (ordered) and F37A/I115P dm (disordered) proteins.



Molecular dynamics simulation structures of wt (ordered) (green) and F37A/I115P dm (disordered) (cyan) proteins.



Frequencies of H-bonds opening for intact wt (ordered) and F37A/I115P dm (disordered) proteins correlate well with total HDX values.