A Novel Approach for Quantitation of Hydrogen Deuterium Exchanged Peptides to Reveal Full Distribution of the Exchanged Species at Various Time Points

Yongdong Wang, Leo Xu, and Ming Gu Cerno Bioscience, Norwalk, CT

Overview

➢Peptides undergoing HDX at different time points were analyzed by LC/MS as doubly charged ions. The MS spectra were calibrated by not only m/z but also peak shape to allow for exact isotope modeling and quantification of many mutually overlapping peptide species.

➢Full distribution of HDX peptides at any given time point and individual peptides undergoing HDX during a time course are presented.

Introduction

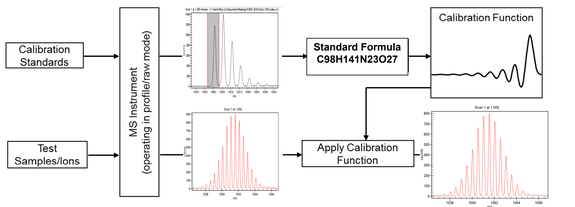
The biological activity of proteins is largely determined by their high order structure – conformation. The conformational change can be detected by hydrogen deuterium exchange mass spectrometry (HDX-MS). In HDX-MS study, the deuterium level is determined by the mass shift using mass spectrometry. Current methodologies for such determination employ centroid mass spectral data, which may not lead to a correct conclusion as demonstrated in a HDX MS investigation on the changes induced by Ca^{2+} binding to an N-terminus truncated downstream regulatory element antagonist modulator (1). Typically these methods monitor only averaged mass shift on peptides during HDX process and do not produce detail distribution of individual peptides under HDX. Although a few methods (2, 3) reportedly were capable of computing the distribution, they used centroid spectra and inevitably were lack of desired accuracy (4). Here we report a novel approach for quantitation of HDX peptides utilizing calibrated profile/raw data. Based on MS peak shape calibration technology and exact isotope modeling, this approach provides accurate measurements of the exchange and a detailed distribution of various deuterium-exchanged ion species.

Methods

All the data used in this presentation was kindly provided by Prof. David Weis at the Univ. of Kansas. Details on sample preparation and LC/MS analysis were described previously in his published paper (5). On HDX MS experiments, he and his workers briefly described following. "8–10 nmol of Lck SH3 were mixed with a 10-fold molar excess of Tip LBD1 or angiotensin II and allowed to equilibrate for 1–3 h in TSD buffer at ambient temperature. Small aliquots (1–2 mL) containing 350 pmol of Lck SH3 and 3500 pmol of peptide were rapidly diluted 20-fold with deuterium-labeling buffer (25 mM Tris, 100 mM NaCl, 3 mM DTT, D_2O at pH 7.5, 21°C) to initiate H/D exchange. Undeuterated samples were prepared by substitution of TSD buffer for the deuterium-labeling buffer. After time intervals of 3–1000 sec, the H/D exchange reaction was quenched by addition of an equal volume of quench buffer (200 mM potassium phosphate, H_2O at pH 2.6). Each sample was immediately frozen on liquid nitrogen and stored at –80°C until analysis. To minimize variability, two independent sets of samples were prepared using the same peptide stock solutions and buffers. All samples in a given set were prepared concurrently and analyzed in a single day as described below. Each hydrogen exchange sample was rapidly thawed at 0°C, desalted, and concentrated by HPLC prior to mass analysis. HPLC was carried out with a Shimadzu HPLC system with water and acetonitrile (both containing 0.05% TFA) as the mobile phases. The protein was desalted and concentrated on a 5 mL protein trap (Michrom BioResources) using 15% B at 200 mL/min for 3 min and 50 mL/min for 30 sec (this step also removed the Tip LBD1 or angiotensin II peptide). The desalted protein was directed at 50 mL/min with a 2 min 15%–65% B gradient into a Waters QTOF2 mass spectrometer for mass analysis" (5).

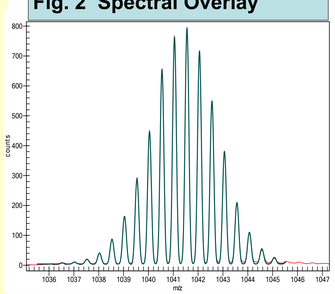
Results and Discussion

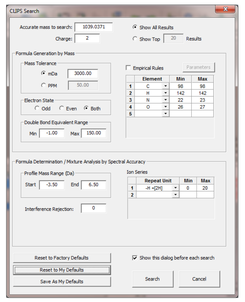
Fig. 1 MassWorks Calibration and HDX MS Quantitation



All the HDX MS spectra were processed first through peak shape calibration and CLIPS search (Calibrated Lineshape Isotope Profile Search). With the peak shape calibrated MS, spectral accuracy can be calculated and utilized to perform exact mixture analysis between calibrated spectra and theoretically calculated spectra for the purpose of quantitation of all HDX peptides. For example, the HDX MS spectra of a sample undergoing 10 min HDX were processed with spectral accuracy of 98.6%, showing great match between calibrated spectra (red) and theoretically calculated spectra (green) as demonstrated by spectral overlay in Fig. 2. As a results, relative concentration of individual HDX peptides were calculated. At this 10 min time point, the top three of the most dominant HDX peptides have 8, 9, 10 hydrogen atoms exchanged with deuterium atoms, at relative concentrations of 18%, 15%, and 15% respectively (Fig. 3); while at time point of 3 hours, the top three of the most dominant HDX peptides changed to 12, 13, and 11 deuterium exchanged peptides, at relative concentrations of 25%, 23%, and 20% respectively (Fig. 4). We have calculated the distribution of individual HDX peptides at 9 different time points which are summarized in Table 1 with the most dominant HDX peptide highlighted in blue. In addition to the HDX distribution at a given time point, it is also possible to show the changes of the relative concentration of individual HDX peptides during a time course. For example, HDX peptide, $C_{98}H_{134}N_{23}O_{27}[^2H]_8$, appeared to be about 4% initially, reached to its maximum of 18% at 10 min, and almost disappeared at 480 min; while $C_{98}H_{130}N_{23}O_{27}[^2H]_{12}$ could not reach to about 5% till 30 min and arrived its maximum of 25% at 180 min (Fig. 5).

Fig. 2 Spectral Overlay





- Calibrating for high spectral accuracy
- Exact mixture deconvolution
- Quantitative analysis

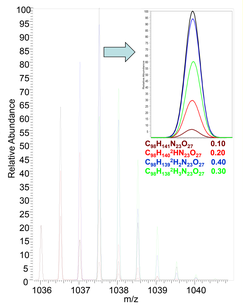


Fig. 3 HDX Distribution (10 min)

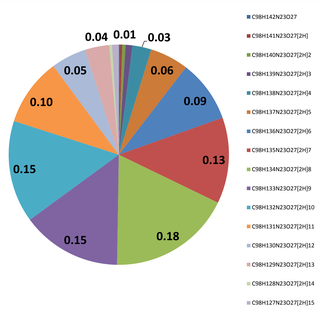


Fig. 4 HDX Distribution (3 hr.)

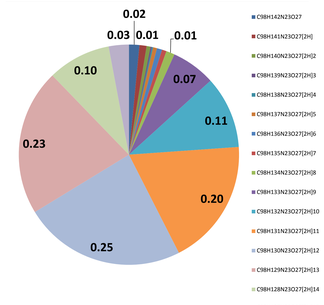


Fig. 5 Time Profiles of Individual HDX Peptides Peptides

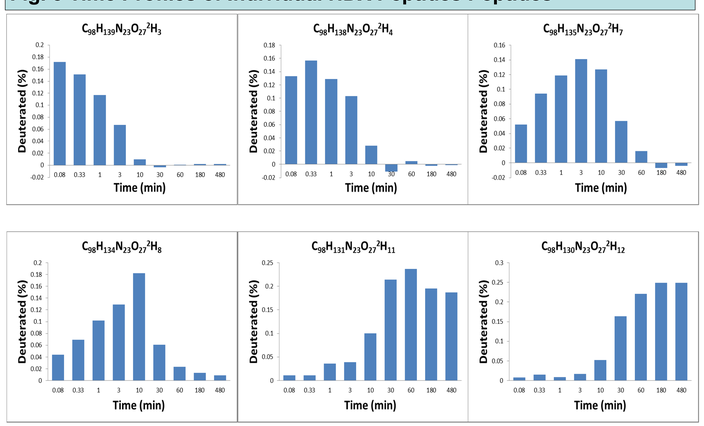


Table 1. Summary of HDX Quantitation

# of HDX	5 sec	20 sec	1 min	3 min	10 min	30 min	1 hr	3 hr	8 hr
0	0.05	0.02	0.00	0.01	0.00	0.03	-0.01	0.02	0.01
1	0.11	0.05	0.02	0.01	0.00	0.02	-0.01	0.01	0.01
2	0.19	0.11	0.06	0.03	0.01	0.01	0.00	0.01	0.01
3	0.17	0.15	0.12	0.07	0.01	0.00	0.00	0.00	0.00
4	0.13	0.16	0.13	0.10	0.03	-0.01	0.01	0.00	0.00
5	0.13	0.15	0.16	0.14	0.06	-0.01	0.01	-0.01	0.00
6	0.06	0.11	0.13	0.14	0.09	0.00	0.01	-0.01	-0.01
7	0.05	0.09	0.12	0.14	0.13	0.06	0.02	-0.01	0.00
8	0.04	0.07	0.10	0.13	0.18	0.06	0.02	0.01	0.01
9	0.01	0.03	0.05	0.08	0.15	0.15	0.08	0.07	0.05
10	0.02	0.03	0.04	0.06	0.15	0.20	0.15	0.11	0.09
11	0.01	0.01	0.04	0.04	0.10	0.21	0.24	0.20	0.19
12	0.01	0.02	0.01	0.02	0.05	0.16	0.22	0.25	0.25
13	0.01	0.01	0.01	0.02	0.04	0.10	0.18	0.23	0.22
14	0.00	0.00	0.01	0.00	0.00	0.03	0.07	0.10	0.15
15	0.00	0.00	0.00	0.01	0.01	0.01	0.03	0.03	0.03

Conclusions

➢ Through innovative peak shape calibration technology, spectral accuracy calculation makes it possible to accurately determine relative concentration levels for many HDX peptides.

➢ This approach allows to show not only individual HDX peptide distribution at a given time point, but also individual HDX peptide profile during a time course.

➢ Employing profile spectra and exact isotope modeling, this approach generates accurate quantitation results by avoiding errors associated with centroiding process and should correctly identify HDX peptides from proteins with bimodal or multimodal exchange time profiles.

References

(1) Jun Zhang, Pradeep Ramachandran, Rajiv Kumar, Michael L. Gross; H/D Exchange Centroid Monitoring is Insufficient to Show Differences in the Behavior of Protein States ; *J. Am. Soc. Mass Spectrom.* (*2013*) **24**, *450-453*

(2) John K. Chik, Jaclyn L. Vande Graaf, and David C. Schriemer. Quantitating the Statistical Distribution of Deuterium Incorporation To Extend the Utility of H/D Exchange MS Data. *Anal. Chem.* (*2006*), **78**, 207-214

(3) Robert Lindner, Xinghua Lou, Jochen Reinstein, Robert L Shoeman, Fred A Hamprecht, Andreas Winkler; Hexicon 2: Automated Processing of Hydrogen-Deuterium Exchange Mass Spectrometry Data with Improved Deuteration Distribution Estimation. *Am. Soc. Mass Spectrom.* (*2014*) **24**, 450-453

(4) Yongdong Wang and Ming Gu, The Concept of Spectral Accuracy for MS. *Anal. Chem. (2010)*, **82**, 7055–7062

(5) David D. Weis, Peter Kjellen, Bartholomew M. Sefton and John R. Engen. Altered dynamics in Lck SH3 upon binding to the LBD1 domain of Herpesvirus saimiri Tip. *Protein Sci.* (2006) **15**, 2402-2410

Acknowledgement

The authors gratefully acknowledge the TOF data provided by Prof. David Weis from Univ. of Kansas.