Hydrogen/deuterium exchange mass spectrometry (HXMS) has proven to be a useful analytical method for the study of protein dynamics and changes to protein confirmation. In order to maximize deuterium signal recovery during LC/MS analysis, rapid chromatographic separations at RT must be utilized. The addition of a gas phase ion mobility separation (IMS) into the HXMS workflow inserts an orthogonal separation that occurs on the millisecond timescale requiring no additional gas phase ion mobility separation (IMS) into the HXMS workflow. Gas phase ion mobility separation allows analysis of this otherwise unresolved isotope from the cluster of interest, each other isotope overlaps in the mobility dimension. With the exception of the monoisotopic reaction, IMS has no detrimental effect on HDX data collection.

METHODS

Liquid Chromatography
Waters nanoACQUITY UPLC® with HDX Technology
Shan Chen, Leap HDX Manager
Waters Corporation, Milford MA; Northeastern University, Boston MA

Chromatography
Analytical column was an ACQUITY UPLC® BEH C18 1.7 µm 2.1 x 5 mm. A linear trap was an ACQUITY VanGuard® Pre-column, BEH C18 1.7 µm 2.1 x 5 mm. Dipeptide injection was performed using a 2.1 x 30 mm immobilized peptic column (Applied Biosystems).

MS Data Collection and Processing
HDX data were collected for all analyses. Undeuterated peptide data were plotted as negative values. The measured differences are near zero for most peptide assignments. Each position along the horizontal axis represents a single peptide. The colored points represent the average of triplicate measurements. Error bars represent the intensity weighted standard deviation of three replicate measurements. Data indicates that the IMS has no detrimental effect on HDX data collection and processing. The measured differences are near zero for most peptide assignments. The plot shows a complex spectra resulting from the superimposition the target +1 isotope cluster (indicated by blue overhead) with two other +3 clusters. A three dimensional plot (bottom) reveals that each of the three clusters are baseline resolved spectrally with an overlap from one of the other clusters. The relative uptake analysis allows evaluation of this otherwise unresolved peptide.

RESULTS

Peptides analyzed: 112
Peptides with interferring ions: 39
Peptides with mobility resolving interfering ions: 21
Peptides with unresolved interfering ions: 5

On-line Fleet C18 2.1 x 30 mm (Applied Biosystems) digestion was performed using a 2.1 x 30 mm immobilized pepsin column (Applied Biosystems).

CONCLUSION

• IMS has no detrimental effect on HDX data
• IMS resolves overlapping isotopic clusters
• IMS demonstrated IMS software that takes advantage of IMS has been developed

References
2. Kmalı et al. (2014) J. Proteome Res. 13, 2194-2202
3. Engen, Mitchell, Protein Analysis with HD exchange MS in Comprehensive Analytical Chemistry, Vol 2086

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