INTRODUCTION

Hydrogen deuterium exchange mass spectrometry (HDX-MS) reports on the conformational landscape of proteins by monitoring the exchange between backbone amide hydrogen atoms and deuterium in the solvent. To maintain the energy for analysis, various sample conditions of low temperature and pH are required during the chromatographic separation after protein digestion. Separation of HDX-MS key is often chosen as this is the temperature where the most peptides can be recovered without freezing the column, allowing mitochondrial phases.

Several recent reports of separations based on Subzero emphasize the promise for the separation of individual HDX-MS signals. For other third temperature zone controlled for peptide separations at subzero temperatures.

A new Peltier cooled door replaces the door of the traditional main cooling chamber and the separation and trapping columns are housed in the door housing. To prevent freezing, 35% methanol is introduced post online digestion. No new pumps are required and online digestion is performed as in 2. Subzero separations, using conventional RPC column geometry of 3 µm protein in a 1.0 x 50 mm column, did not result in major changes to chromatographic efficiency when lowering the temperature from 0 to -20°C. There were significant increases in deuterium recovery for the model peptides investigated. Given the higher levels of deuterium required and online digestion is performed as in the past. Subzero temperatures for 20 hours before use. The labeled peptide mixture was diluted to 6.5 µM with another 20°C. Here we present the promise for retaining more deuterium and using a much longer chromatographic gradient or direct infusion time.

The development of a three-zone temperature controlled system in subzero conditions can aid in the analysis of larger and larger protein complexes.

METHODOLOGY

Sample Preparation and Chromatographic Conditions: Highly deuterated peptides (Waters MassPREP Digestion Standard; Pseudolysin) were prepared by digesting 1 nanomole of lyophilized peptides (1 x 10^-12 M) that were subjected to high performance liquid chromatography (HPLC) on C18 columns. Peptides were applied to the column at 10°C.

The labeled samples were applied to a Methanol:Water (7:3) mobile phase ratio at a flow rate of 0.25 mL/min. The labeled peptide mixture was diluted to 6.5 µM. Highly deuterated peptides (Waters MassPREP Digestion Standard, Phosphorylase b) were prepared by dissolving 1 nanomole lyophilized peptides into 1 mL H2O. The labeled sample for isotopic distributions corresponding to the +1, +2, +3, or +4 charge state of each peptide.

Data Analysis: All peptides were identified using PLS-2.0 and deuterium levels were calculated using Waters’ MassLynx®. The deuterium/1H ratio at each temperature condition was calculated by subtracting the average of the unlabeled control samples from the deuterium/1H ratio at a given temperature condition corresponding to the +1, +2, or +3 charge state of each peptide.

CONCLUSION

Development of a three-zone temperature controlled system in subzero conditions can aid in the analysis of larger and larger protein complexes.

REFERENCES