Optimizing Electron Transfer Dissociation Conditions for Hydrogen/Deuterium Exchange Mass Spectrometry and Its Application to the Study of Protein Conformation

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ABSTRACT

Purpose: C5-HDX-ETD utilizing electron transfer dissociation (ETD) was developed to pinpoint protein conformation with near single amino acid and residue level resolution.

Results: A model peptide and protein were used to develop the designed workflow. Both an MS full scan and data dependent MS experiments were performed and compared to an ETD only experiment. More than 160 peptides were identified and 127 peptides were used for deuterium uptake measurement. The differences ranged from 0.2 to 4 (Figure 4 b). The selected peptide ETD MS2 data of the two labeling conditions were processed with HDExaminer software. The deuterium uptake for the peptide was calculated and the peptide fragments were used to identify the deuterium incorporation for the two labeling conditions as shown in Figure 5. After the deuterium content significant change area had been identified from the residual plot, the specific ETD MS2 spectra of the peptides in that region were investigated. Figure 6 presents the summary of the deuterium content differences. The selected peptide ETD MS2 data of the two labeling conditions were processed with HDExaminer software. The deuterium uptake for the peptide was calculated and the peptide fragments were used to identify the deuterium incorporation for the two labeling conditions as shown in Figure 6. The residual plot of the two conditions showed that the peptide under the HEPES labeling condition had more deuterium uptake than the same peptide under the buffer labeling condition. Furthermore, the difference slope was shallower for the 3600s measurements. There was more deuterium uptake for all the fragments under the HEPES labeling condition. For the different regions in cytochrome C, there was no significant difference in the deuterium content between the two labeling conditions.

CONCLUSIONS

- The HDX-ETD experimental conditions were identified and optimized.
- ETD/ETD bottom up top down workflows were developed and successfully applied to study protein C fragmentation.
- Both批量 amino acid and residue were identified for many numbers of peptides for the bottom-up approach. Near single amino acid and residue level resolution was obtained for the N, C terminal top-down approach.

REFERENCES


2. D. W. Weikart et al., Protein Exchange Mass Spectrometry of Proteins:  
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