Comparison of CID, ETD, and HCD for Top-down Characterization of Histones

Zhixin(Michael) Tian
Nikola Tolić, Rui Zhao, Shawna, Hengel, Si Wu, Ronald J. Moore, Errol W. Robinson, Richard D. Smith, Ljiljana Paša-Tolić

Environmental Molecular Sciences Laboratory at Pacific Northwest National Laboratory
Histones and post-translational modifications (PTMs)

Core histones: H4, H2B, H2A, H3

Histones are heavily modified and very complex

Human H3.1 (UniProt)

A=Acetylation, M=Methylation, M2=asymmetrical dimethylation
MM=symmetrical dimethylation, M3=trimethylation, P=Phosphorylation

RPLC-MS/MS of HeLa core histones
Online RPLC/WCX-MS/MS
HeLa core histones

RPLC separation of histones

WCX-MS/MS of H4

Methods | IDs
---|---
RPLC-MS/MS | 127
WCX-MS/MS | 135
RPLC-WCX-MS/MS | 708
Unambiguous localization of K12ac in histone H4

CID

ETD

Ambiguous identifications:
H4_S1acK{5, 8, 12}acK16acK20me2

Unique identification:
H4_S1acK12acK16acK20me2
Optimizing fragmentation for histone characterization

HeLa core histones (3.75 µg)

<table>
<thead>
<tr>
<th></th>
<th>NCE (%)</th>
<th>AT (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCD_45_30</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>HCD_45_60</td>
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<tr>
<td>HCD_60_60</td>
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<td>CID_35_30</td>
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## Different fragmentation conditions

<table>
<thead>
<tr>
<th></th>
<th>NCE (%)</th>
<th>RT (ms)</th>
<th>IDs</th>
<th>Avg [−log(P_Score)]</th>
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<td>136</td>
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<tr>
<td>HCD_60_60</td>
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<td>60</td>
<td>144 ±8</td>
<td>20.4 ±0.3</td>
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<td>ETD_20</td>
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<td>20</td>
<td>138</td>
<td>28.9</td>
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</tbody>
</table>
HCD conditions identification of H4_S1acK16ack20me2

HCD_45_30: Matching fragments=27, P_Score=6.5E-31

HCD_45_60: Matching fragments=38, P_Score=5.9E-39

HCD_60_60: Matching fragments=42, P_Score=1.3E-50
## Optimal CID, ETD, and HCD

<table>
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<th>Avg [–log(P_Score)]</th>
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<td><strong>CID_60_60</strong></td>
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<tr>
<td><strong>ETD_15</strong></td>
<td>/</td>
<td>15</td>
<td>160</td>
<td>34.5</td>
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</table>

The Venn diagram illustrates the overlap and unique components of CID, ETD, and HCD at different conditions. Each circle represents a method, and the numbers within the circles indicate the unique and overlapping features of each method.
ETD, HCD, and CID: H4_S1acK16ack20me2

ETD: Matching fragments=56, P_Score=1.1E-76

HCD: Matching fragments=46, P_Score=3.3E-56

CID: Matching fragments=31, P_Score=1.1E-40
H2AX_S1acS139p uniquely identified only by ETD

Top-down only! Not with bottom-up or middle-down!
Advantage of top-down fragmentation

**HCD_60_60:** H31_K9me2K27me2K36meK79me

y106
y76
y46
y16
- b121: P-K-D-I-Q-L-A-R-R-I-R-G-R-E-R-
y1

**CID_60_60:** H2B1O_K46meK57me2

y96
y66
y36
y6
- b121: Y-T-S-S-K-
y1
Fragmentation efficiency of CID, ETD, HCD

\[
\% \text{ of unique IDs} = \frac{\text{unique IDs}}{\text{unique IDs} + \text{ambiguous IDs}} \times 100
\]
Conclusions

• An online 2D LC-FTMS platform dramatically improved throughput and sensitivity compared to more traditional platforms, and resulted in identification of hundreds of histone isoforms from microgram levels of protein, including phosphorylation.

• For a single fragmentation method, ETD provides the most confident identifications on average.

• Total number of identifications more than doubled when CID was switched to ETD or HCD.

• Combinatorial modifications across the entire protein sequence could only be identified using top-down proteomics.
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