Characterization of protein biotinylation sites by peptide-based immunoaffinity enrichment

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Introduction

Biotinylation in combination with LC-MS/MS has been widely applied in large-scale analyses of protein post-translational modifications. In the literature, biotinylated proteins can now be directly characterized. Direct characterization of biotinylated proteins has been a challenging task due primarily to the lack of reliable tools and reagents for the recovery of biotinylated proteins using conventional immunoprecipitation-based purification methods. It has been found that anti-biotin antibody is a better capture reagent for biotinylated peptides compared to streptavidin. In this study, we established an immunoaffinity enrichment method using a novel anti-biotin antibody and we compared to approaches from two published papers using monoclonal antibodies from different sources. We then demonstrated our enrichment method by applying it to characterization of protein biotinylation sites from APEX directly labeling in live cells.

Methods

Trypsin-digested mouse liver peptides were labeled with EZ-Link NHS-biotin, desalted, and finally analyzed on Thermo Scientific Q Exactive. An average of 3425(±227) unique MS/MS peptides were identified from mouse liver peptides using different technical analyses (including immuno-precipitation and LC-MS/MS). Compared to conventional methods such as co-immunoprecipitation, bioaffinity enrichment method utilizing streptavidin to enrich biotinylated peptides was highly specific. Compared to conventional methods such as conventional antibody enrichment method utilizing streptavidin to enrich biotinylated peptides was highly sensitive.

Results

The method comparison experiment is composed of two technical analyses (including immunoprecipitation and LC-MS/MS) of both labeled mouse liver peptides using different technical analyses and antibodies. As an average of 2302±272 peptides were identified from the anti-biotin (A7C2A) antibody enrichment method, compared to 234±68, 124±73, and 116±10 biotinylated peptides in the LC-MS/MS experiment. The table lists the corresponding abundance of biotinylated peptides in house.

Novel aspect

We describe a robust and well-performing immuno-affinity method for biotinylated peptide enrichment.

Conclusions

Proximity labeling is a long-term study such as APEX in cells. We are designing a platform allowing users to interrogate the protein interactome using a platform of protein-protein interactions. Compared to conventional methods such as co-immunoprecipitation, the immunoaffinity enrichment method utilizing streptavidin to enrich biotinylated peptides was highly specific. Compared to conventional methods such as conventional antibody enrichment method utilizing streptavidin to enrich biotinylated peptides was highly sensitive.

References


Acknowledgement

We thank the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School for providing us with APEX and APEX ligand.

Figure 1. Overview of APEX and immunoaffinity enrichment method. Step 1: Preparation of cell lines expressing APEX and proteins of interest. Step 2: Labeling of cells with biotinphenol and H2O2. Step 3: Enrichment of biotinylated peptides using anti-biotin antibody. Step 4: MS analysis. Step 5: Data analysis using SEQUEST.

Figure 2. Examples of peptide enrichment using immunoaffinity enrichment method. APEX is co-expressed with antibody (A7C2A) in HEK293T cells. Biotinylated peptides were enriched using anti-biotin antibody and subsequently analyzed by LC-MS/MS. Emerging method focuses on identifying biotinylated peptides in combination with LC-MS/MS has been widely applied in large-scale analysis of protein post-translational modification in cells.

Table 1. Abundance of biotinylated peptides in house.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Antibody (A7C2A)</th>
<th>Antibody (Monoclonal)</th>
<th>Antibody (Polyclonal)</th>
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<tbody>
<tr>
<td>MYO1B</td>
<td>Y618</td>
<td>23.4</td>
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<tr>
<td>ARF6</td>
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<td>11</td>
<td>25000</td>
</tr>
<tr>
<td>YBX1</td>
<td>Y208</td>
<td>23.4</td>
<td>200</td>
</tr>
</tbody>
</table>

Figure 3. Flow diagram showing enrichment of biotinylated peptides using novel anti-biotin antibody. This method is a platform allowing users to interrogate the protein interactome using a platform of protein-protein interactions. Compared to conventional methods such as co-immunoprecipitation, the immunoaffinity enrichment method utilizing streptavidin to enrich biotinylated peptides was highly specific. Compared to conventional methods such as conventional antibody enrichment method utilizing streptavidin to enrich biotinylated peptides was highly sensitive.