The study and the applications of a novel LC/MS proteome method

Speaker: 廖成仁
Adviser: 方嘉德 博士
Proteomics can be viewed as an experimental approach to explain the genomic sequences and control of biological processes and pathways.

Some parameters influence the result:
1. The properties of Mobile phases
   ① the slope of the gradient
   ② the percent of organic modifier
   ③ the pH of the buffer solution
   ④ the flow rate
2. The type of stationary phase of reverse phase column

The study of the aim

1. To construct the detection platform for real sample that can be applied the LC/MS proteome method
2. Replacing ACN with MeOH as the LC/MS solvent for decreasing environmental contamination
3. To elevate the resolution and sensitivity in LC/MS proteome method
In-gel digestion

Comparison of the different modifier

Comparison of the effect of the pre-concentration column

Comparison of the different flow rate

Real sample

1. PDGF (Platelet derived growth factor)
2. The 2-D gel detection of the band of Human Saliva

Target protein: (BSA, 1µg)

10% SDS-PAGE electrophoresis
(Comparison of the different staining Methods)

Results

Lane 1, 2: BSA 10µg
3, 4: BSA 5µg
5, 6: BSA 1µg

Instant™ Blue Staining

Fig. 1 Effects of BSA proteins expression in different concentration
Fig. 2 Comparison of the different BSA concentration for HPLC 7100 pump (without pre-concentration column)

The MS chromatogram of BSA using L7100 pump (without pre-concentration column) in ACN solvent

Fig. 3 The LC/ESI-IT MS detection in ACN solvent
Fig. 4 The LC/ESI-IT MS detection in MeOH solvent

LC/ESI-IT MS detection for different pump

Fig. 5 The effect of the different pump for LC/ESI-IT MS in BSA
Fig. 6 The effect of the different flow rate for LC/ESI-IT MS in BSA

Fig. 7 The effect of the different modifier for LC/ESI-IT MS. (a)Formic acid (b)Trifluoroacetic acid (c)Pentafluoropropanionic acid (d)Heptafluorobutanoic acid
Detection limits

(a) Instant™ Blue Staining

(b) Silver Staining

Fig. 8 The detection limits of BSA which used different staining method: (a) Instant™ Blue Staining (b) Silver Staining

Detection limits

Fig. 9 The detection limits of BSA which used Instant™ Blue staining method
Detection limits

![Bar chart showing protein coverage percentage at concentrations of 1, 0.2, and 0.1 µg of BSA.]

Fig. 10 The detection limits of BSA which used silver staining method.

LC/MS detection of real sample: PDGF

(a) The MS chromatogram of PDGF-40µL/min

(b) The MS chromatogram of PDGF-5µL/min
Fig. 11 The comparison of the different flow rate

PDGF

Protein coverage %

Flow rate (µL/min)

Fig. 12 Isolation of human saliva by two-dimensional gel electrophoresis

Human saliva

Fig. 12 Isolation of human saliva by two-dimensional gel electrophoresis
Human saliva

<table>
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<tr>
<th>Spot</th>
<th>Protein description</th>
<th>pI</th>
<th>Mr (kDa)</th>
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Note: MS and MS/MS data analysis was performed using the Xcalibur Software, which utilizes the TurboSEQUEST peptide mass fingerprinting and MS/MS ion search Software.

Conclusions

1. In terms of reversed-phase elution strength, MeOH is a weaker solvent than ACN. In our study, faster gradients and hence shorter analysis times were possible with MeOH versus ACN without any decrease in chromatographic performance.
Conclusions

2. The best condition as follows:
   ① chose C18 pre-concentration column
   ② TFA as modifier
   ③ optimized flow rate as 5 $\mu$L/min
3. This LC/MS proteome method can successfully replace the old LC/MS proteome method and can be applied to the protein identification of the true sample

Thanks for your attention
In this research work, we used the methanol to replace the common solvent, acetonitrile, in order to develop a modified LC/MS proteome method. We used a C18 pre-concentration column; and we expected to promote the resolution and sensitivity of LC/MS proteome method. The best chromatographic condition which we have found was by using following parameters: a C18 pre-concentration column, a C18 reverse phase column, flow rate of 5µL/min, chosen TFA as modifier. This condition has been applied in the study of detection limit and the real sample analysis. When we used BSA as the target protein and 0.2µg of BSA was used, and we got 3.6±0.5% protein coverage with 2 peptides. And the condition of 0.1µg of BSA, there was 2.0% protein coverage with 1 peptide too, but could not be reproducible. In this research work, we has applied this modified LC/MS proteome method to the detection of the real sample, the growth factor of human blood platelet produced by E. Coli, bronchoalveolar lavage fluid, and saliva of human of the 2-D gel detection of the band, too. From the results we can understand that this proteome method can identify these real samples. And this modified LC/MS proteome method can successfully replace the old LC/MS proteome method that has environmental injury, and can be successfully applied to the protein identification of the real sample.