嘉南藥理科技大學專題研究計畫成果報告

計畫編號：CNPH9505
計畫名稱：以甲醇作為沖提劑之LC/MS的蛋白質胺基酸定序偵測方法之探討

執行期間：95年1月1日至95年12月31日

□整合型計畫　　□個別型計畫

計畫總主持人：

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子計畫主持人：

中華民國96年□2月27日

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

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Background

- Proteomics can be viewed as an experimental approach to explain the genomic sequences and control of biological processes and pathways
- Some parameter 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

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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 elevated the resolution and sensitivity in LC/MS proteome method

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Experiment model

Target protein: (BSA , 1μg)

↓

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

↓

In-gel digestion

↓

Comparison of the effect of the pre-concentration column

Comparison of the different flow rate

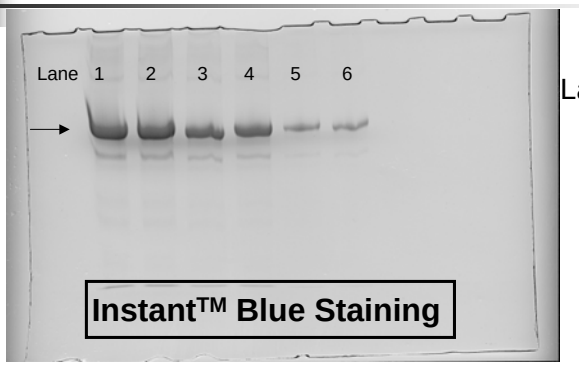
Comparison of the different modifier

↓

Real sample

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

Results



Lane 1,2: BSA10μg
3,4: BSA5μg
5,6: BSA1μg

Fig.1 Effects of BSA proteins expression in different concentration

LC/ESI-IT MS detection

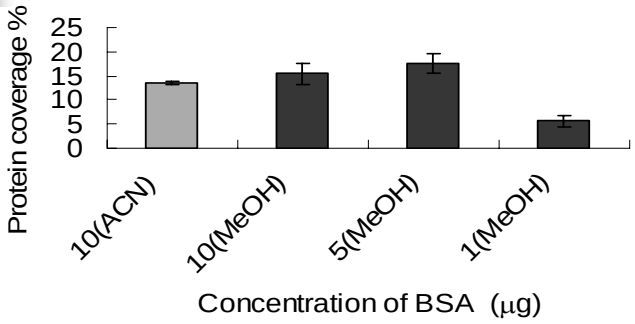


Fig. 2 Comparison of the different BSA concentration for HPLC 7100 pump (without pre-concentration column)

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

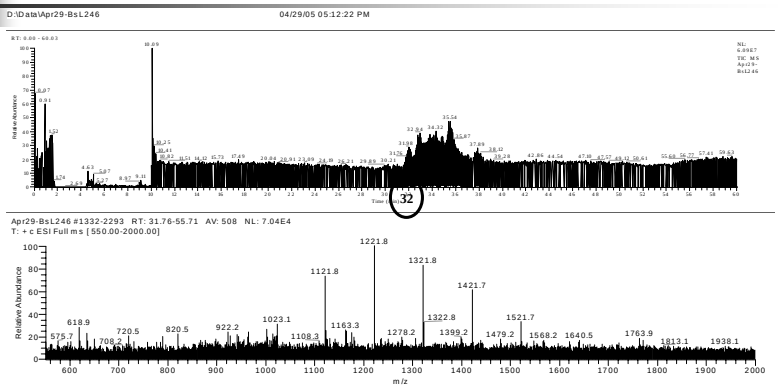

Fig. 3 The LC/ESI-IT MS detection in ACN solvent

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

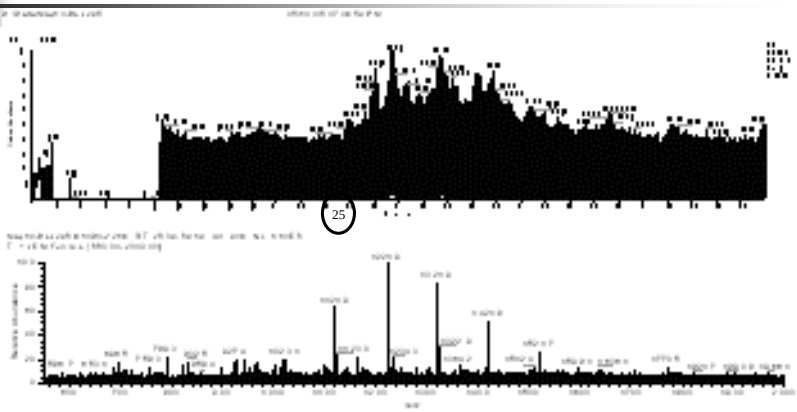

Fig. 4 The LC/ESI-IT MS detection in MeOH solvent

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LC/ESI-IT MS detection for different pump

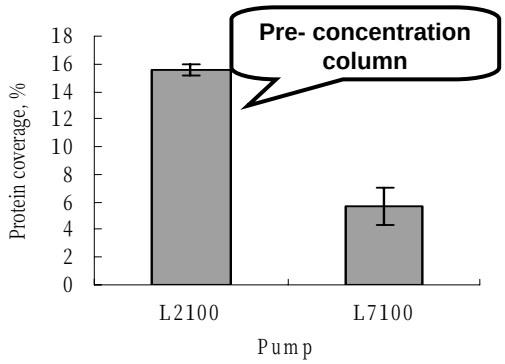

Fig.5 The effect of the different pump for LC/ESI-IT MS in BSA

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Comparison of the different flow rate

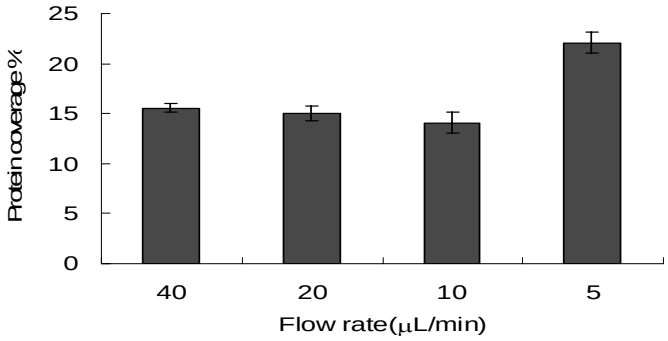

Fig.6 The effect of the different flow rate for LC/ESI-IT MS in BSA

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Comparison of the different modifier

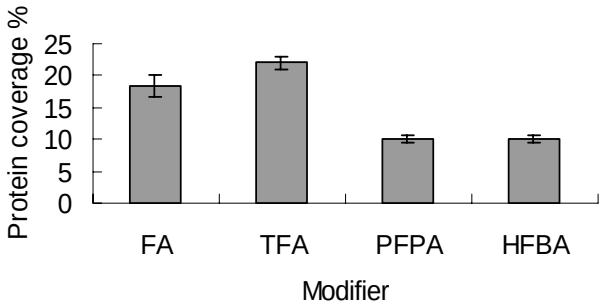

Fig. 7 The effect of the different modifier for LC/ESI-IT MS. (a)Formic acid (b)Trifluoroacetic acid (c)Pentafluoropropnionic acid (d)Heptafluorobutanoic acid

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Detection limits

(a) Instant™ Blue Staining **(b)Silver Staining**

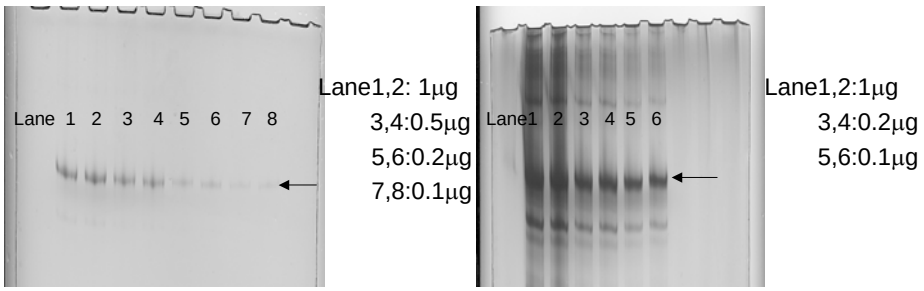

Fig.8 The detection limits of BSA which used different staining method: (a)Instant™ Blue Staining (b)Silver Staining

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Detection limits

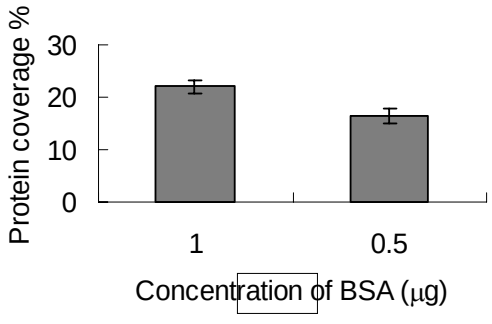

Fig.9 The detection limits of BSA which used Instant™ Blue staining method

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Detection limits

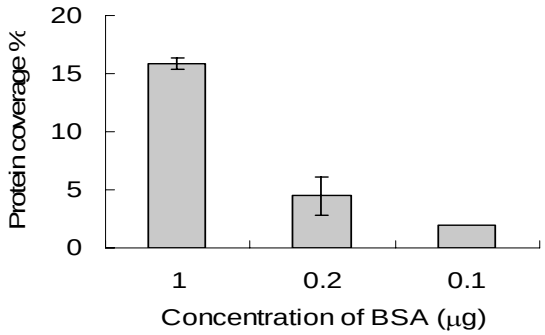

Fig.10 The detection limits of BSA which used silver staining method

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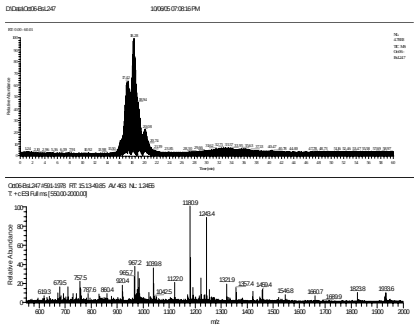
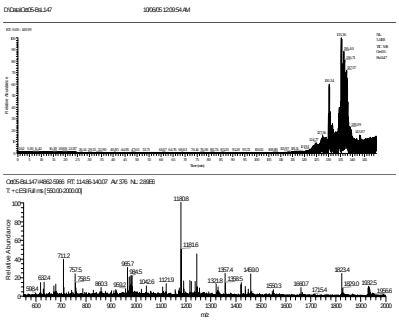
LC/MS detection of real sample: PDGF

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



(b) The MS chromatogram of PDGF-5μL/min



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PDGF

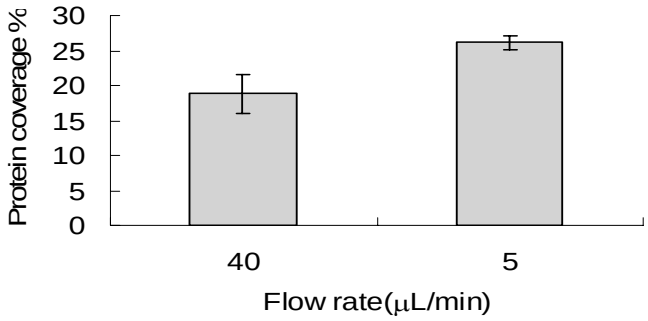

Fig.11 The comparison of the different flow rate

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Human saliva

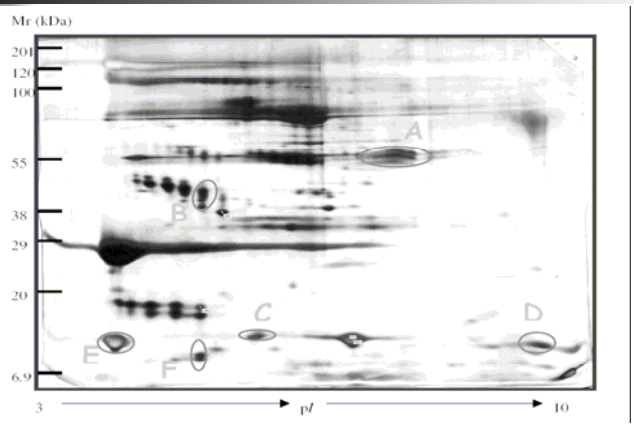

Fig.12 Isolation of human saliva by two-dimensional gel electrophoresis
(本電泳圖由嘉藥生科所葉東柏教授實驗室所提供)

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Human saliva

Spot	Protein description	p/	Mr (kDa)	Protein coverage%	SWISS Prot Accession No.
A	Salivary α-amylase	6.67	57674	12.9%	P04745
B	Parotide secretory protein	5.25	27075	17.0%	P07743
C	Cystatin SN	7.01	16378	19.8%	P01037
D	Cystatin C	8.94	15790	23.29%	P01034
E	Cystatin S	4.80	16205	50.7%	P01037
F	Prolactin-induced protein	8.07	16561	10.96%	P12273

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

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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.

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Conclusions

2. The best condition as follows:
   ①chose C18 pre-concentration column
   ②TFA as modifier
   ③optimized flow rate as 5 μL/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

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Thanks for your attention

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The Study and Applications of a Modified LC/MS Proteome Method

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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.