

“Subfemtomole level protein sequencing by Edman degradation carried out in a microfluidic chip”

Experimental details:

Chemicals and Materials

Synthetic peptide Angiotensin II was obtained from GLBiochem (Shanghai, China); synthetic peptide P14R, trifluoroacetic acid (TFA), phenyl isothiocyanate (PITC), α -cyano-4-hydroxycinnamic acid (CHCA), The stationary phase used to pack the microcolumn was Nucleosil ODS1 (Stacroma, Reinach, Switzerland), which is 3 μ m porous modified C18 based particles. Gel-separated protein sample of human amnion epithelial cells was obtained from department of pathology and pathophysiology, Zhejiang University, School of Medicine. MALDI matrix solution was made of saturated CHCA solution in 1:1 mixed 50% ACN/0.1% TFA and 80% ACN/0.1% TFA. Ultra-pure water (Millipore, Bedford, MA, USA) was used in the experiment. Other reagents were of analytical grade and from local suppliers.

Mass Spectrometry and Database Searches

Mass spectra were obtained using a DE-Star instrument (Applied Biosystems, Framingham, MA, USA) with delayed extraction. Peptide mass mapping was performed in the positive ion reflector mode, using the Data Explorer MS software. Two hundred single-shot spectra were accumulated. External calibration was carried out using ACTH18-39 and P14R and the internal calibration was performed using the autolytic peaks of trypsin.

Proteins were identified using the Mascot software (<http://www.matrixscience.com>) and nrNCBI database based on the peptide mass fingerprint. [The sequence information was submitted to Sequence Query searching software offered by MASCOT for protein identification.](#) The search parameters were defined as the following: cysteine as carbamidomethylated, methionine as oxidized, maximal mass tolerance of 60 ppm and up to 1 missed enzymatic cleavage, [Homo sapiens was specified.](#)

Table S1. The peptide sequences matched to the tryptic peptide mass fingerprint of Enolase 1

Enolase 1 [Homo sapiens]; Sequence Coverage: 12%; Score: 51		
No.	MW	Matched native peptide sequence
1	806.47	YNQLLR
2	1540.77	VVIGMDVAASEFFR
3	1804.94	AAVPSGASTGIYEALRL
4	1908.00	LAMQEFMILPVGAANFR

Table S2. The peptide sequences matched to the tryptic peptide mass fingerprint of T-cell receptor beta chain

T-cell receptor beta chain V region (V-beta 26, germline) precursor - human (fragment); Sequence Coverage: 32% ; Score: 51		
No.	MW	Peptide-matched sequence
1	1804.94	IHPAEPDRAVYLYSS
2	1540.77	HKVTNMGQEVILR
3	1046.56	QNLREQEMK