

Purification of Serum Peptides by Ultrafiltration and Solid Phase Extraction

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Introduction

As the field of proteomics continues the exponential growth, the challenges that researchers must face to measure molecules responsible for specific biological effects, also continues to grow. These challenges explain the significant role that biomarkers play in the drug discovery and development process. A biomarker can be defined as a molecule that indicate abnormal physiology. Biomarkers provide powerful clues to genetic susceptibility, disease progression, and predisposition, as well as offer information on physiological and metabolic profiling of diseases and drug response. They can also provide valuable diagnostic and prognostic information that can facilitate personalized medicine. For diagnostic purpose, peptide biomarkers found in sera and other body fluids can be very useful. One of the major impediments to the discovery of new biomarkers is the fact that plasma or serum contains a significant number of salts, proteins, and lipids which make it difficult to detect and analyze peptides by mass spectrometry. Therefore, solutions are needed to reduce the complexity of samples. This poster describes a combination of ultrafiltration and solid-phase extraction (SPE) techniques that allow researchers to purify peptides from biological samples in a bench-top, high-throughput format, ready for MS and MS/MS analysis, using any available mass spectrometer and related laboratory equipment.

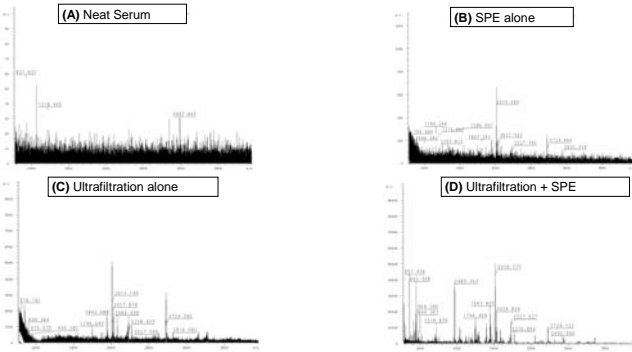


Figure 1: MALDI TOF Spectra of Human Serum Before and After Ultrafiltration and Solid Phase Extraction (SPE). Comparison of a MALDI spectra of human serum spotted neat on a MALDI target (A), after desalting with ZipTip_{μC18} pipette tip(B), following ultrafiltration (C), and finally after ultrafiltration, desalting and concentration with ZipTip_{μC18} pipette tip(D).

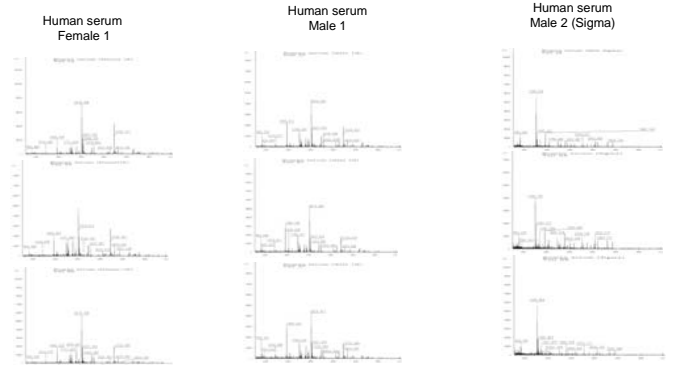
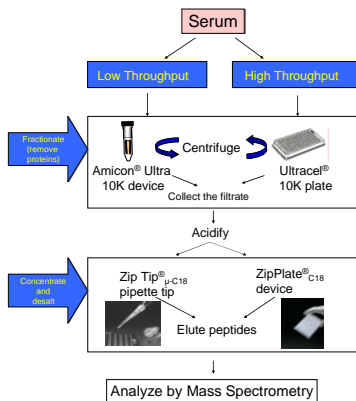


Figure 2: High Throughput Serum Peptide Analysis. Reproducibility of peptide MALDI spectra of 4 different human serum samples. Serum samples were filtered, acidified and desalted and concentrated with ZipPlate_{μC18} device. MALDI spectra demonstrate good well-to-well reproducibility within the same sample but differences in the peptide profile between different individuals.

Experimental Procedure



Methods

Human serum samples were obtained from healthy individuals. Trifluoroacetic acid (TFA), Methanol, and Acetonitrile (ACN) were purchased from Fisher Co. (Pittsburgh PA), Alpha-cyano-4-hydroxy cinnamic acid (CHCA) matrix was obtained from Applied Biosystems, (Foster City CA). Amicon Ultra 10Kcentrifugal devices, Zip Tip pipette tips, ZipPlate_{μC18} micro-SPE plate and Ultracel 10K ultrafiltration plate were from Millipore Corporation, (Billerica MA). The samples were analyzed on Voyager-DE™ Workstation (Applied Biosystems) in linear mode, or Autoflex® MALDI TOF mass spectrometer (Bruker Daltonics) in reflector mode. PSD analysis of serum peptides was done on AXIMA™ -CFR Plus MALDI-TOF mass spectrometer (Shimadzu-Kioto).

Low throughput method:

One milliliter of human serum was filtered on Amicon® Ultra-4 10,000 MWCO centrifugal device. The ultrafiltration devices were centrifuged in a swinging bucket rotor for 15 to 30 min at 3000 x g. Ten microliters of the filtrate was acidified with 5 μl of 1% TFA, desalted and cleaned with ZipTip_{μC18} pipette tips. Co-elution was performed directly onto the MALDI target with 2 μl of Alpha-cyano-4-hydroxy cinnamic acid matrix (5 mg/ml in 50% acetonitrile, 0.1% TFA).

High throughput method:

300μl of serum (undiluted or diluted 1:1 with 10mM Tris) was added to each well of a Ultracel 10K plate. Samples were centrifuged in a swinging bucket rotor at 2,500 x g or filtered by vacuum at 18"Hg for about 45 to 50 min. The filtrates collected were transferred to a 96 well ZipPlate device for desalting and concentration. Previous to the addition of the serum, 5μl of 100% ACN were added to each ZipPlate well, and after 3min at RT, 50μl of 0.5% TFA was added followed by 50μl of serum filtrate collected from Ultracel 10K. Samples were mixed up and down with a pipette and bound to the C18 at low vacuum (5 to 7"Hg). The samples were washed four times with 200μl of 0.1% TFA using full vacuum. Peptides were eluted by centrifugation with 4μl of 50%ACN, 0.1%TFA and collected in a polypropylene™ bottom collection plate. 2μl were spotted on the MALDI target and overlaid with 1μl of Alpha-cyano-4-hydroxy cinnamic acid matrix (5mg/ml in 50%ACN, 0.1%TFA).

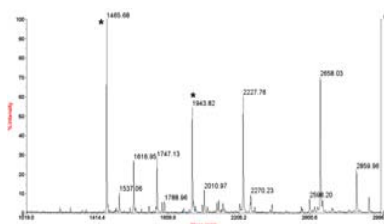


Figure 3. MALDI TOF Spectrum of Human Serum Peptides Prepared by Ultrafiltration and Solid Phase Extraction. More than 50 peptides were observed in a single MALDI TOF spectrum from human serum. The strong signal and low background allowed the easy identification of peptides by MALDI TOF MS with PSD ionization. Two of the identified peptides are labelled: *m/z* 1465 – Fibrinopeptide A, with first and last amino acids truncated (DSGEFDLAEGGVNR); *m/z* 1943 – Fragment of kininogen L, high MW (amino acids 63-79 NLGHCYKHERDQGHGHQ). Both peptides are normally present in human serum.

Summary

- Ultrafiltration is convenient, fast and reproducible way of preparing serum or other complex biological fluids for peptide analysis.
- An increased number of low molecular weight peptides, with a higher signal intensity, were detected in the serum ultrafiltrate compared to the starting serum sample.
- Solid phase extraction combined with ultrafiltration has demonstrated the dramatic enhancement of detection of low molecular weight peptides in biological fluids by mass spectrometry.
- Serum peptide purification was shown to be efficient in 96-well format with high well to well reproducibility.