Purification of Peptides from Serum and Cell Lysates for Mass Spectrometry

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Overview

Complex biological samples, such as serum, plasma, urine or cell lysates frequently present a major challenge for peptide analysis because of high salt, lipid and protein content. Sample complexity reduction is an essential first step of any peptide analysis.
In this paper, we show that ultrafiltration in combination with solid phase extraction (SPE) using C18 resin can be a convenient and efficient tool for serum peptide sample preparation. The method provides more peptides for mass spectrometry analysis than performing acetonitrile precipitation only.

Introduction

A biomarker can be defined as a molecule that indicates an alteration in physiology. Biomarkers play an essential role in the drug discovery and development process. They 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. Biomarkers can also provide valuable diagnostic and prognostic information that can facilitate personalized medicine. Peptide and protein patterns have been linked to ovarian cancer, breast cancer, prostate cancer and astrocytoma [1-5].

Most diagnostic tests are based on blood or urine analysis [5]. Serum is a key source of putative protein biomarkers, and by its nature, can elucidate organ-confined events. Use of mass spectroscopy coupled with Bioinformatics has demonstrated the capability of distinguishing serum protein pattern signatures of ovarian cancer in patients with early-and late-stage disease [6].
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 that make it difficult to detect and analyze peptides by mass spectrometry. Multiple protocols have been developed to extract and enrich peptides from tissues and body fluids. Methods such as extraction with 0.1% trifluoroacetic acid (TFA), or 50% acetonitrile to selectively precipitate large proteins while enhancing the solubility of smaller proteins and peptides, or by batch reversed phase chromatography using C18 resin.

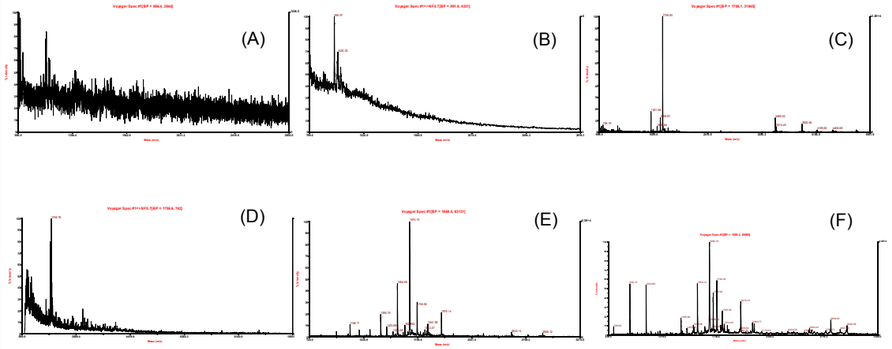


Figure 1. MALDI TOF spectra of (A) rat serum un-diluted, (B) serum peptides in 50% acetonitrile supernatant after precipitation, (C) serum peptides in 50% acetonitrile supernatant processed with ZipTip$_{C18}$ pipette tip, (D) rat serum processed with ZipTip$_{C18}$ pipette tip, (E) serum ultrafiltrate (10 KDa MWCO) processed with ZipTip$_{C18}$ pipette tip, (F) serum ultrafiltrate with 20% acetonitrile processed with ZipTip$_{C18}$ pipette tip

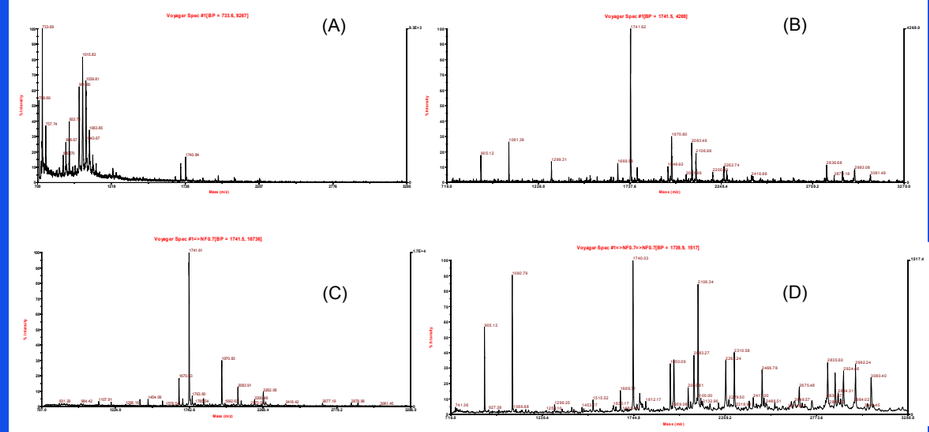
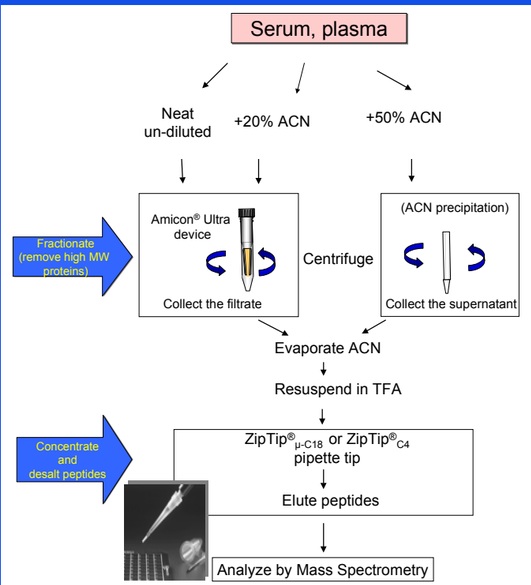


Figure 2. MALDI TOF spectra of rat plasma peptides after acetonitrile precipitation (A), plasma with 20% ACN after ultrafiltration through 10kDa (B), 30kDa (C) and 50kDa (D) cut-off membrane, all followed by concentration/desalting by reversed phase chromatography on ZipTip$_{C18}$ pipette tips.



Method

Rat serum and plasma samples were obtained from Zivic Inc. laboratories. Human serum samples were obtained from healthy donors. 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 10kDa, 30kDa and 50kDa centrifugal devices, and ZipTip pipette tips were from Millipore Corporation, (Billerica, MA). The samples were analyzed on Voyager-DE™ Workstation (Applied Biosystems) in linear mode. PSD analysis of serum peptides was done on AXIMA ™ -CFR Plus MALDI-TOF mass spectrometer (Shimadzu-Kratos).

Two milliliters of un-diluted serum or plasma, as well as samples diluted to 20% ACN, were filtered on Amicon Ultra-4 10kDa, 30kDa and 50kDa MWCO ultrafiltration devices 30 min at 3000 x g. Ten microliters of the filtrate were acidified with 5 µl of 1% TFA, desalted with ZipTip$_{µC18}$ or ZipTip$_{C4}$ pipette tips. Samples containing ACN were evaporated from 500µl to 20-30µl. Then samples were acidified with 2%TFA prior to desalting with ZipTip$_{µ\text{-}C18}$ 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). For acetonitrile precipitation, serum and plasma samples were diluted 1:1 with 100% ACN. Samples were centrifuged for 15 min at 12,000 rpm. Supernatant fluid was evaporated to a final volume of 20-30µl, acidified and desalted with ZipTip$_{µ\text{-}C18}$ pipette tip. Co-elution was performed directly onto the MALDI target as previously described.

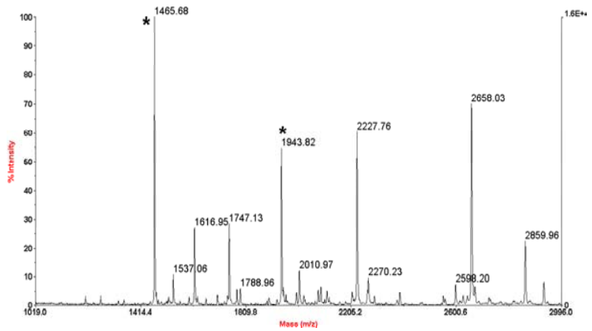


Figure 3. MALDI TOF spectrum of human serum peptides prepared by ultrafiltration and solid phase extraction. More than 50 peptides were observed in a single MALDI TOF spectrum from human serum. The strong signal and low background allowed the easy identification of peptides by MALDI TOF MS with PSD ionization. Two of the identified peptides are labelled: *m/z* 1465 – Fibrinopeptide A, with first and last amino acids truncated (DSGEGDFLAEGGGVR); *m/z* 1943 - Fragment of kininogen L, high MW (amino acids 63-79 NLGHGHKHERDQGHGHQ). Both peptides are normally present in human serum.

Conclusions

- Ultrafiltration is a convenient, fast and effective way of reducing complexity of serum, plasma or other biological samples prior to peptide analysis.
- Solid phase extraction combined with ultrafiltration resulted in substantial enhancement of detection of peptides in biological fluids.
- More serum peptides, with a higher signal intensity, were detected in the serum ultrafiltrate compared to the serum supernatant after 50% acetonitrile precipitation.

References

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