Enhanced MALDI-TOF MS Spectra of Serum Peptides Using Ultrafiltration

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Abstract

Proteomic analysis of complex samples, such as serum or plasma is frequently influenced by the presence of high protein concentrations that hinder peptide detection. These proteins suppress the ionization of native peptides during MALDI-TOF MS analysis. As a result, sample complexity reduction to lower the level of abundant proteins is rapidly becoming an essential first step of many proteomics analysis schemes. Several pre-fractionation strategies using chromatographic absorbents have been employed to remove abundant proteins such as albumin. As an alternative to adsorption chromatography, we have treated mammalian (human, murine, & bovine) serum or plasma samples with ultrafiltration (UF) membranes to produce relatively protein free filtrates. Experiments were performed to select the best molecular weight cutoff (MWCO) centrifugal device, establish the recoveries of spiked known peptides, and optimize the protocol. The UF filtrate was then acidified with 1% TFA and treated with reverse phase C18 and/or ion exchange ZipTip for de-salting and concentration. The results demonstrated that the 10K MWCO membrane gave the optimal results based on significantly improved detection of serum peptides in the 800-4000m/z range. The sample complexity reduction technology described provides a convenient and rapid method for the enhancement of native low molecular weight peptides in biological fluids such as serum or plasma.

Introduction

Peptides and other low molecular weight molecules have been associated with many pathological states such as cancers, AIDS, diabetes, cardiovascular and neurological diseases (1-3). Many of the potential research & diagnostic applications of these "biomarkers" have not been fully realized because of their difficulty in analysis & detection. Many of these significant factors are often measured by radio-immunoassay protocols for R&D or diagnostic applications. Analyzing low molecular weight biomarkers (peptides) in serum and plasma has been notoriously difficult due to the vast number of contaminating salts, proteins, and lipids present. These are extremely problematic for both multidimensional liquid chromatography (MDLC) and mass spectroscopy techniques. High concentrations of proteins, lipids and salts suppress the ionization of native peptides during MALDI-TOF MS analysis. As a result, sample complexity reduction to lower the level of abundant proteins is rapidly becoming an essential first step of many proteomics analysis schemes. Ultrafiltration has been investigated and found to be unsuitable for the removal of abundant proteins like albumin for subsequent protein analysis (4). Several other pre-fractionation strategies using chromatographic absorbents have been more successfully employed to remove abundant proteins such as albumin prior to MDLC or electrophoresis. However, the low molecular weight filtrate fractions are typically difficult to analyze and are frequently lost in these other approaches. As an alternative to adsorption chromatography, we have investigated ultrafiltration (UF) techniques to produce relatively protein free filtrates and enhance the MALDI-TOF detection of serum peptides and other low molecular weight molecules.

References

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3. Petricoin III, E., Ardekani, A.M., Hitt, B.A., Levine, P.J., Fusaro, V.A., Steinberg, S.M., Mills, G.B., Simone, C., Fishman, D.A., Kohn, E.C. and Liotta, L.A. Use of proteomic patterns in serum to identify ovarian cancer. The Lancet 2002 359:572-577.

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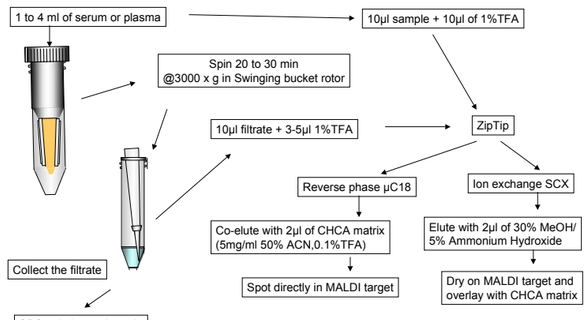
Materials and Methods

Amicon® Ultra-4 centrifugal filter devices (10 K MWCO, cat #UFC801008), µC18 ZipTip (cat # ZTC18M096) and SCX ZipTip (Cat # STSCXS008) were purchased from Millipore Corporation (Bedford MA). Bovine, Mouse, and Human serum, as well as Brilliant blue G-colloidal Coomasie™, were purchased from Sigma Co.(St. Louis MO). The rest of the human plasma samples were taken from healthy male and female volunteer donors. Ficoll was added to blood samples and centrifuged at 500xg. Ficoll diluted plasma was separated and keep frozen at -20°C.

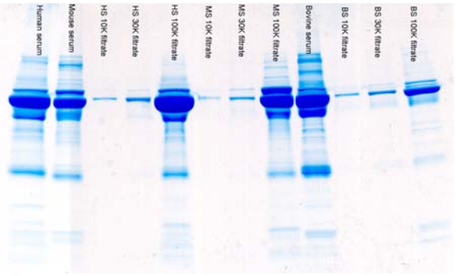
Trifluoroacetic acid (TFA), methanol, ammonium hydroxide and acetonitrile (ACN) were purchased from Fisher Co. (Pittsburg PA.). Alpha-cyano-4hydroxy cinnamic acid (CHCA) matrix was from Applied Biosystems (Foster City CA).10-20% tris-glycine SDS gels were purchased from Invitrogen Co. (Carlsbad CA).

Samples were analyzed in a linear Voyager-DE™ BioSpectrometry Workstation, Applied Biosystems (Framingham, MA), or in a reflectron Autoflex® Bruker Daltonics® (Billerica, MA.) MALDI-TOF Instruments.

Detailed Protocol Schematic

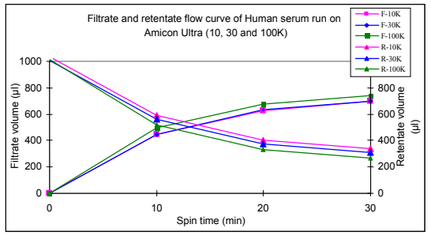


SDS-PAGE Gels of Original And Ultrafiltrate Serum Samples



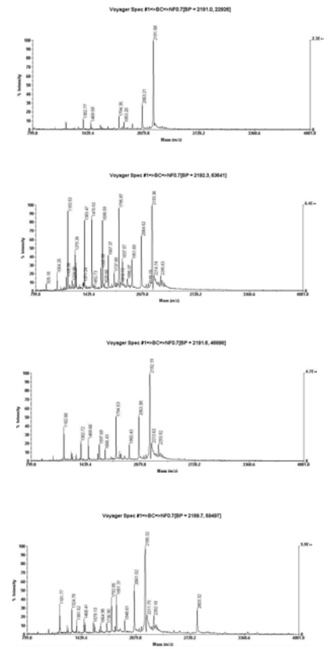
Human, Bovine and Mouse sera were passed through Amicon Ultra devices using three different MWCO (10, 30 and 100K) membranes. It is clear that the 10K MWCO membrane produced a filtrate with the lowest protein concentration for all three sera (~99% rejection of the starting total protein concentration). All filtrate samples were further processed as above for MALDI evaluations.

Human Serum Flow Curve



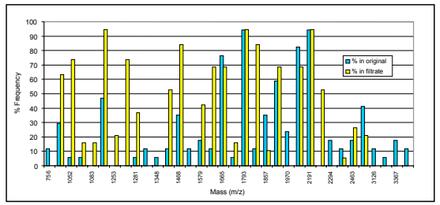
Samples were spun at room temperature, in a swinging bucket rotor at 3000 x g for the times indicated for the 3 different molecular weight cut off devices. Both the Filtrate (F) and retentate (R) volumes are shown.

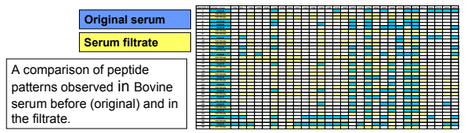
MALDI Spectra of Original Bovine Sera and Filtrate Samples Obtained from Different MWCO Devices



All samples were acidified with 1% TFA, then purified with µC18ZipTip, prior to MALDI analysis on the Voyager-DE BioSpectrometry workstation. The 10 K membrane was selected for all future work.

Frequencies of Peptides Observed in Bovine Serum Before and After Ultrafiltration



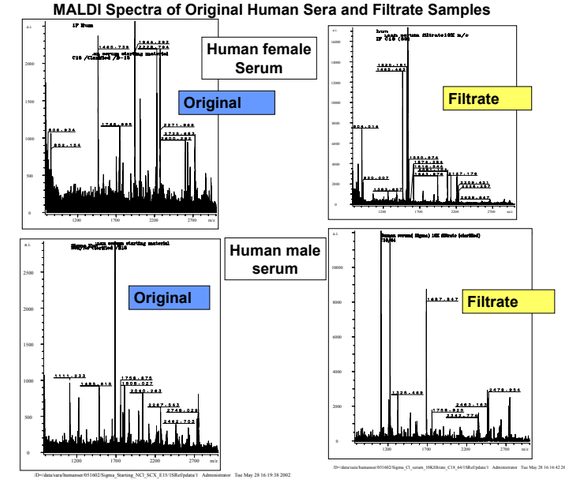


A comparison of peptide patterns observed in Bovine serum before (original) and in the filtrate.

Percent frequency of the five most common peaks observed in the adult bovine serum reported in this poster and the correspondent peptides reported in the literature on 10% fetal bovine serum (Basso, et. al., 2002, ref# 1).

10% Fetal bovine serum (1)		Adult Bovine serum	
Peptide (MW)	Frequency(%)	Peptide (MW)	Frequency(%)
1667	13.3	1668	76.5
1795	11.8	1793	94.7
1952	9.6	1949	68.4
2065	49.6	2062	82.3
2193	100	2191	94.7

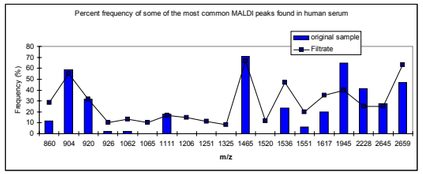
MALDI Spectra of Original Human Sera and Filtrate Samples



Human serum samples were acidified with 1%TFA and purified with µC18 ZipTip prior to analysis on a Autoflex® Bruker Daltonics ® reflectron mode. Higher signal and lower backgrounds were consistently observed in the spectra of the filtrate samples compared to the original sera.

The 10 Most Common Peptides Observed in Human Sera and Plasma

m/z	Frequency (%
903.82	58.82
919.82	31.37
1111.05	17.65
1465.21	70.59
1536.28	23.53
1551.20	5.88
1944.50	64.71
2227.89	41.18
2645.08	27.45
2659.46	47.06



More than 100 samples from 3 different males and 3 females were analyzed on the Autoflex MALDI. The 10 most common peaks appear in the original sample as well as in the filtrate with about the same frequency, however, other less abundant peaks were only present on the filtrates of the human samples.

Summary and Conclusions

•A significantly increased number of low molecular weight molecules (800-4000 m/z) were detected in the filtrate compared to the starting serum or plasma sample. The actual identity of these observed low molecular weight molecules is still under investigation in addition to the significance, if any, of the more complex peptide patterns observed.

• A higher signal intensity and lower background were observed in the MALDI spectra obtained on the filtrate samples, compared with the starting material.

•The 10K dalton MWCO ultrafiltration devices were determined to be optimal to prepare low molecular weight fractions from serum or plasma for MALDI analysis

•All samples required ZipTip processing to concentrate and de-salt the samples prior to analysis.

•The 5 most abundant peptides observed in bovine serum agreed with other literature reports. The results clearly demonstrates the increased observed frequencies after ultrafiltration for a number of peptide masses (1269, 1468, 1834 and 2211) while others were observed equally in both samples (such as 1793, 2191 and 1665).

•The sample complexity reduction technology described provides a convenient and rapid method for the enhancement and detection of native low molecular weight peptides in biological fluids such as serum or plasma.