Monitoring Peptidome Variance within Different Blood Collection to Further Understand Human Plasma Proteome Stability

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Overview

Purpose

Compare plasma peptidome stability provided by protease inhibitors present in BD P100™ plasma vs EDTA plasma.

Methods

• Collect blood in EDTA (PPT) and EDTA with on-board protease inhibitors (P100) plasma tubes.
• EDTA tubes (conventional gel separation) were centrifuged for 15 min at 1,000g. P100 tubes were centrifuged for 15 min at 1,500g allowing the mechanical separator to isolate the plasma from blood cells.
• Plasma was aliquoted at various time intervals.
• The peptides were recovered using three separate 3kDa MWCO filters.
• Peptides were cleaned with two Ziptips™ (Millipore) each and pooled for MALDI, LC, and LC-MALDI.

• Virtual 2-D plots were created from LC/MALDI data, enabling an in-depth comparison of EDTA and P100 plasma peptidomes.

Result

• Successful comparison of peptidomes from EDTA vs P100.
• Presence of protease inhibitors in the BD P100 tubes stabilizes both native proteins and endogenous peptides.

Introduction

The search for proteomic biomarkers from human plasma holds both immediate clinical potential as well as significant challenges: The dynamic range of concentration, known to exceed ten orders of magnitude, is the primary limitation in plasma proteome analyses, typically limited to 10^−8 with current instrumentation. Beyond the well-known dynamic range issues, plasma proteome analysis is further complicated by a preanalytical variability, in particular, during blood collection and early sample handling. These sample processing issues need to be evaluated.

Our previous experiments demonstrated that plasma, collected in evacuated blood tubes including protease inhibitors present at the moment of phlebotomy, yields more time-resolved and intact samples. Standard serum and anticoagulated plasma samples, in parallel studies, are measurably less stable. After centrifuging, separating plasma from blood cells, the samples were first incubated for different lengths of time, then passed through 3kDa molecular weight (MW) cutoff filters. The resulting peptides were analyzed by high-resolution Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS). MS results indicate that "new" peptides were being generated more rapidly in standard EDTA tubes, as compared to measurably increased stability using protease inhibitors (BD P100™ tubes).

In the previous studies, the entire peptide content from each sample was analyzed by direct MALDI-MS. In the current study, we use LC-LDI for a quantitative assessment of the sample followed by direct MALDI and MALDI-MS for an in-depth look at the peptide. Methodological proof of plasma peptidome enables deeper understanding of functional proteome associated with sample collection and handling. It further elucidates the beneficial aspect of ex vivo protease inhibitors and their role in establishing plasma proteome sample acquisition and handling standards.

Experimental

• Figure 1. Displays LC-LDI traces for peptide pools extracted from 3 different subjects. UV traces enable a broad quantitative comparison of how the peptidome changes overtime in P100 vs PPT tubes.
  > LC-LDI traces vary slightly more over time in EDTA vs P100 tubes.

• Figure 2. Displays direct MALDI analysis of Subject C at time 0 and 240 min for each tube. This enables the relative comparison of highly abundant peptides.
  > MALDI spectra vary more over time in EDTA vs P100 tubes.

• Figure 3. Displays LC-MALDI virtual 2D plot of Subject C at time 0 and 240 min for each tube. This enables a deeper look in to the plasma peptidome.
  > LC-MALDI-MS was performed on these same two time intervals for each tube. This method reduces ion suppression of direct MALDI enabling the detection of more peptides.

Conclusions

• The combination of LC-UV, Direct MALDI, and LC-MALDI-MS successfully displays different aspects of the plasma peptidome.
• The deeper data provided by LC-MALDI method shows even greater variations over time for EDTA plasma, compared to the increase stability of P100.
• LC-MALDI clearly displays the magnitude of peptides changing over time in the EDTA, also displaying the relative stability of peptides in the P100 tube.
• Observation of more abundant peptides in EDTA plasma at both 0 and 240 mins compared with P100 samples demonstrates greater protein stabilization in P100 as a result of the protease inhibitors.
• Data suggests that detection of more peptides in plasma is due to ex vivo protease activity, consistent with previous reports.
• Protease inhibitors present in the BD P100 Tube increase both protein and endogenous peptide stability starting at the time of blood collection.

References:


www.bd.com/proteomics

*The image contains a diagram illustrating the experimental setup and results. Please refer to the original source for detailed visual content.