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 (containing gel separator) were centrifuged for 15 mins at 1,100xg. P100 tubes were centrifuge for 15 min at 1,500xg allowing the mechanical separator to isolate the plasma from blood cells.
- Plasma was aliquoted at various time intervals.
- The peptidome was 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

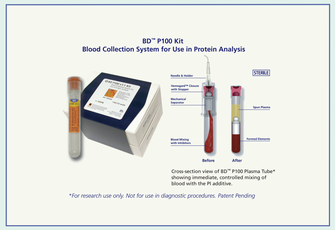
The search for proteomic biomarkers from human plasma holds both incredible clinical potential as well as significant challenges.[1] The dynamic range of concentration, known to exceed ten orders of magnitude, is the primary limitations in plasma proteomic analyses, typically limited to 10^4–10^6 with current instrumentation. Beyond the well-known dynamic range issues, plasma proteome analysis is further complicated by 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-stable and intact samples.[2] 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 Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS). MS results indicate that "new" peptides were being generated ex vivo 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-UV for a quantitative assessment of the sample followed by direct MALDI and LC-MALDI for an in-depth look at the peptidome. Methodical probing of plasma peptides enables deeper understanding of preanalytical variables associated with sample collection and handling. It further elucidates the beneficial aspect of *in vitro* protease inhibitors and their role in establishing plasma proteome sample acquisition and handling standards.

**For research use only. Not for use in diagnostic procedures.*

Experimental



Cross-section view of BD™ P100 Plasma Tube* showing immediate, controlled mixing of blood with the PI additive.

**For research use only. Not for use in diagnostic procedures. Patent Pending*

Draw Blood into P100 and PPT Tubes

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Plasma Separated from Cells

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Plasma Time-Course Incubation

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Peptides Extracted with 3kDa MWCO filter

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Cleanup for MALDI

↓

MALDI or LC or LC-MALDI-MS

The above method was evaluated to ensure a high level of reproducibility. Thus, 3 MWCO filters and 2 ziptips were pooled together to reduce inherent process variability. Also, it was found that PAC384 targets (Bruker) gave the most consistent and reproducible MALDI spectrums and thus were used for the LC-MALDI data collection. Finally, it should be noted that we have data (not shown) indicating that each LC, MALDI, and LC-MALDI experiment can be reproduced with a low level of variation.

LC-UV was first performed on three different subjects, in P100 and PPT blood collection tubes, at different time intervals of room temperature incubation of the plasma after blood draw. Figure 1 displays good quantitative UV chromatograms of the peptidomes and how they change over time.

Next, direct MALDI of the fractions was conducted, enabling a relative comparison of peptides. Figure 2. Displays the MALDI spectra for time intervals 0 and 240 min for each tube.

Finally 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.

Results

Figure 1.
Monitoring peptide variations in P100 and EDTA plasmas over time with LC-UV

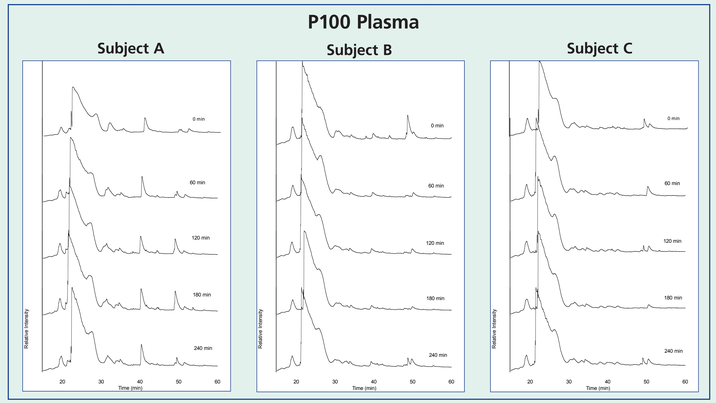


Figure 2.
Monitoring peptide variations with Direct MALDI.

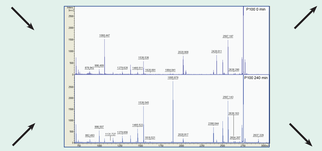
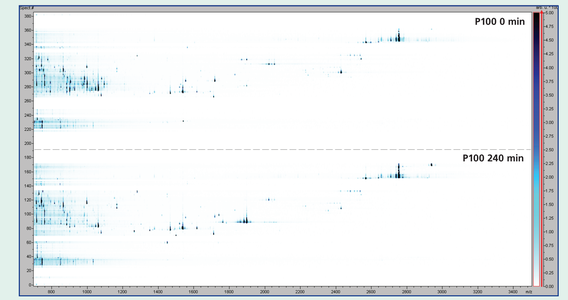


Figure 3.
Monitoring peptide variations using LC-MALDI-MS.



- Figure 1. Displays LC-UV traces for peptidomes extracted from 3 different subjects. UV traces enable a broad quantitative comparison of how the peptidome changes overtime in P100 *vs.* PPT tubes.
  - > LC-UV 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 a 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 virtual 2D plots clearly vary more over 240 min in EDTA *vs.* P100 tubes.
  - > Also, note more peptides detected in EDTA at both time 0 and 240 min, suggesting an increase due to peptides being created *ex-vivo*, at very early time, and then general instability of peptides over longer times

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 anomalous 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.** [2]
- **Protease inhibitors present in the BD™ P100 Tube increase both protein and endogenous peptide stability starting at the time of blood collection.**

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References:

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2. Yi J, Kim C, Gelfand CA. Inhibition of Intrinsic Proteolytic Activities Moderates Preanalytical Variability and Instability of Human Plasma. *Journal of Proteome Research.* 2007 (inpress).
3. Campbell,DJ, Kinins in Humans. *Am J Physiol Regulatory Integrative Comp Physiol.* 2000; 278: 897-904.