Enhanced Recovery of Trypsin Digested Proteins Using Dispersive Pipette Extraction for Downstream Proteomic Analysis

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INTRODUCTION

For clinical proteomics, targeted peptide extraction and desalting efficiencies are essential to obtaining high quality mass spectra data. In this study, we report a novel peptide purification method using IMCStips™, a disposable pipette extraction tip with sorbent loosely contained inside a pipette tip. Using bovine serum albumin as a model protein and four other human serum proteins, trypsin-digested samples were readily desalted using IMCStips™ with reverse phase resin. The purification improved signal intensities, signal-to-noise ratios and sequence coverage in comparison to other commercially available tips. Since this method can be effortlessly adapted to an automated online format, we envision it to be applied in the high throughput clinical proteomics application.

MATERIALS/METHODS

Four purified human protein samples (albumin, prealbumin, alpha-1-acid glycoprotein, transferrin) were purchased from Athens Research and Technology (Athens, GA). Bovine serum albumin (BSA) was purchased from Fisher Scientific. Acclaim™ 120, C18, 5 µm (4.6 × 100 mm) from Thermo Scientific was used for peptide separation on UPLC with a diode array detector (Vanquish™, Thermo Scientific). All solvents were mass spectrometry grade purchased from Fisher Scientific. Sequencing grade trypsin was purchased from Sigma Aldrich. IMCStips were provided by the vendor. Ziptips® (Millipore) and Pierce C18 tips were purchased from Fisher Scientific.

Initial workflow validation studies and product comparison studies were conducted using 0.01 mg/mL BSA in 10 mM PBS (phosphate buffered saline) and 0.1% TFA (trifluoroacetic acid)

Ziptip® purification- followed the vendor's suggested method using 10 µL of 0.01 mg/mL BSA and eluted with 5 µL 50% acetonitrile/0.1% TFA;

Pierce® C18 tip purification- followed the vendor's suggested method using 100 µL of 0.01 mg/mL BSA and eluted with 50 µL 50% acetonitrile/0.1% TFA;

IMCStips purification using following protocol:

1. Condition the tip with 100 µL acetonitrile, repeat once;
2. Equilibrate by aspirating 100 µL water/0.1% TFA, repeat once;
3. Aspirate 100 µL of sample, dispense and aspirate sample for 3 cycles;
4. Rinse the tip by aspirating 100 µL water/0.1% TFA and discarding solvent, repeat once;
5. Elute the sample with 50 µL 50% acetonitrile/0.1% TFA, aspirate and dispense 3 cycles

MALDI-TOF MS Method

Samples for MALDI-TOF MS were prepared by spotting 1 µL of the eluted solution on the steel target, then drying the sample with N_2. An additional 0.6 µL of the CHCA matrix (20 mg/mL in 70% acetonitrile and 0.1% TFA) was spotted on the dry sample, and then dried by blowing nitrogen gas. MS data was obtained on Bruker Daltonics Ultraflex TOF/TOF through positive ion mass spectra in linear mode. A 337 nm nitrogen laser was set 70% power at a shot rate of 40 Hz. Each spectrum has a summation of 1000 laser shots with a mass range of 680–4000 Da. MS peaks were analyzed by mMass software, peak picking filters was set S/N > 10, absolutely intensity threshold > 10 and relative intensity threshold > 0.2 %. And sequence match search was set max miss cleavage site is 1, mass match tolerance: ± 0.5 Da.

RESULTS

Comparison of Sequence Coverage Provided by Three Different Commercial Products

Sample	1	2	3	Average
IMCStips	27%	27%	21%	25.0%
Pierce C 18	20%	20%	24%	21.3%
Ziptip®	18%	22%	20%	20.0%

Table 1. Three different products were used to desalt trypsin-digested BSA from 10 mM phosphate buffered solution. Sequence coverage is based on the sequence match algorithm using mMass with peak filter set at S/N > 10 with a maximum missed cleavage site at 1 and mass match tolerance of 0.5 Da

Figure 1

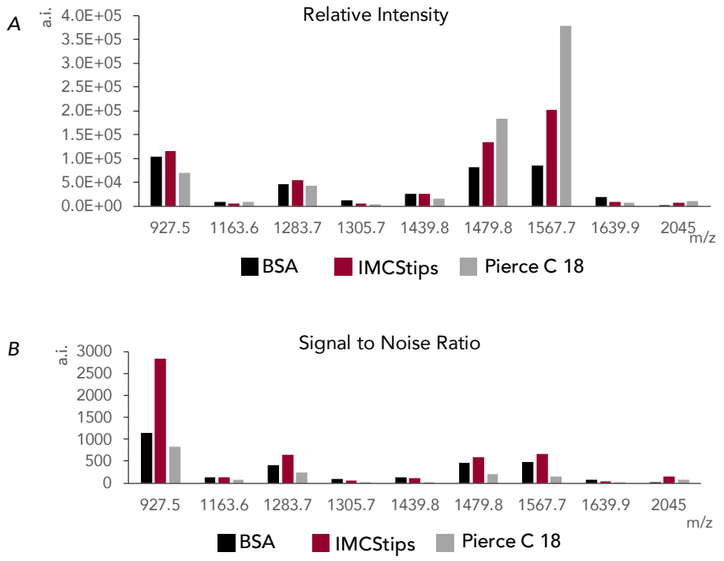
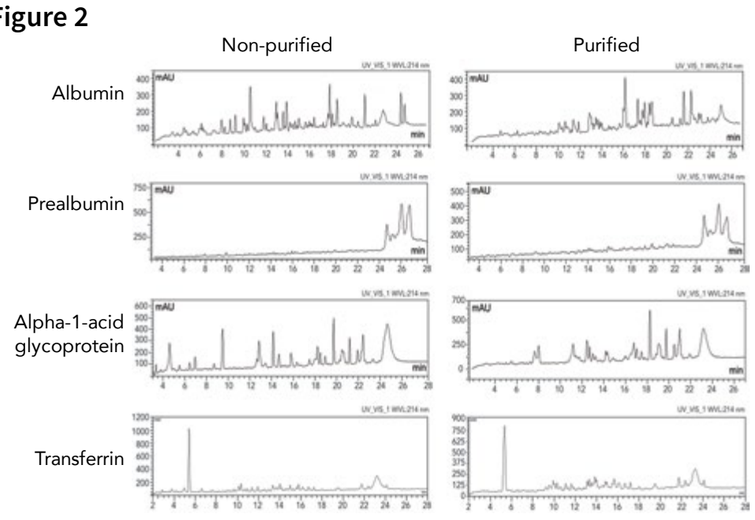


Figure 1. *Trypsin-digested bovine serum albumin (BSA) samples (0.01 mg/mL) were analyzed by MALDI-TOF MS (Bruker Daltonics). BSA denotes neat, non-extracted trypsin digested BSA which was used as a control sample. (A) Signal intensities are higher for the larger peptide peaks using Pierce C-18 and higher for the lower molecular weights using IMCStips. (B) Signal to noise ratios were calculated by dividing the maximum signal intensity by the average baseline signal intensity. Among 34 matched peptide peaks, only 9 peaks were presented due to space constraints.*

Figure 2



Peptide Percent Recovery

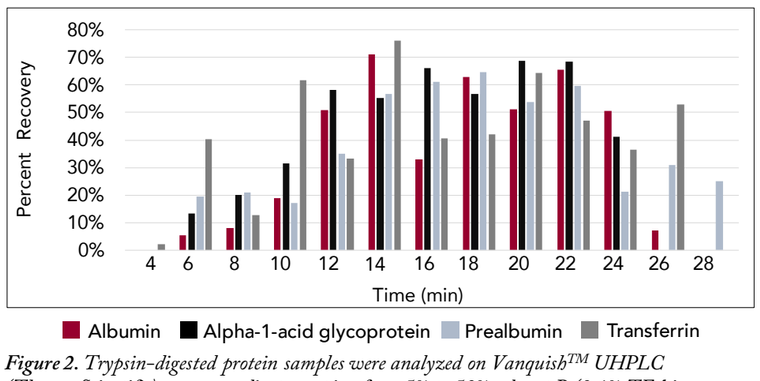


Figure 2. *Trypsin-digested protein samples were analyzed on Vanquish™ UHPLC (Thermo Scientific) over a gradient ramping from 5% to 50% solvent B (0.1% TFA in acetonitrile) over 30 minutes on a C-18 column (Acclaim 120, 5 µm, 4.6 x 100 mm). 20 µL of the sample was injected, and the flow rate was 1.2 mL/min with the column temperature held at 60°C. Percent recovery was calculated based on peak area of the extracted peptides over the non-purified peptide peak areas.*

Figure 3

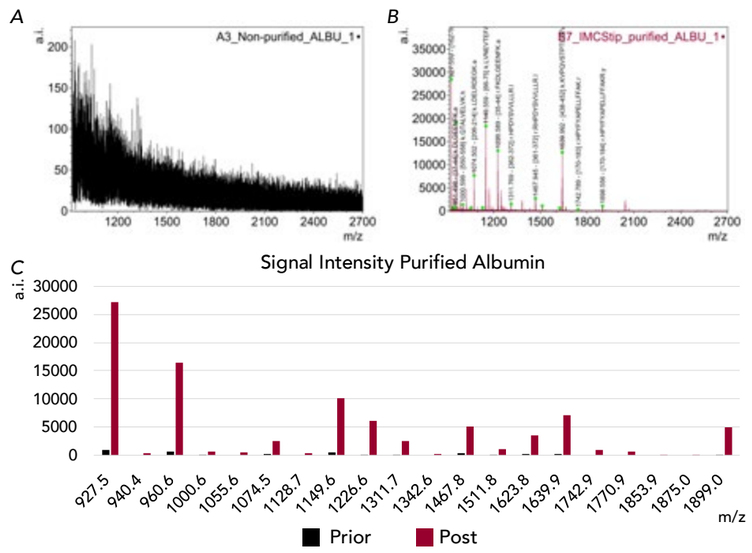


Figure 3. *Human serum albumin samples digested with trypsin and analyzed by MALDI-TOF MS. (A) Prior to desalting and removing the buffer; (B) post enrichment and desalting with IMCStips™ significantly improves signal intensities (C).*

Figure 4

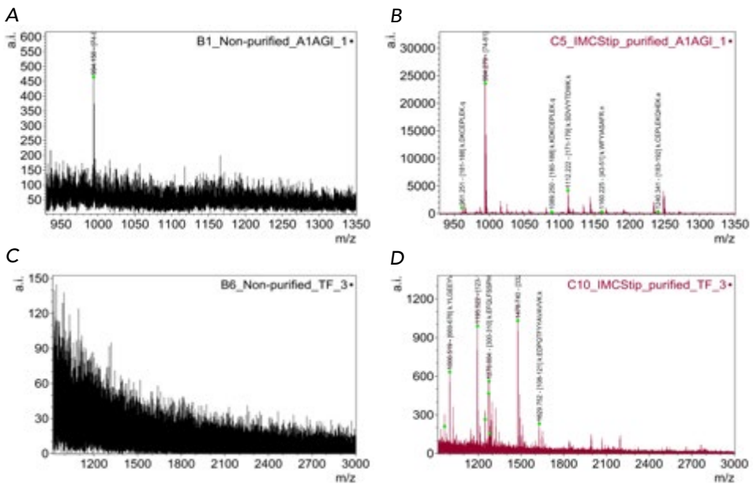


Figure 4. *MALDI-TOF MS spectra of human serum protein samples (A) alpha-1-acid glycoprotein prior to desalting and (B) after desalting; (C) human transferring denatured using ProteaseMax™ surfactant (Promega) prior to desalting and (D) after desalting. Overall signal intensities are significantly improved after desalting process.*

CONCLUSION

IMCStips™ achieves similar or greater recoveries compared to the other commercial products for desalting enzyme digested peptides for mass spectrometry applications with less repetitive aspiration steps. The products are adapted for use on automated platforms such as the Hamilton system to facilitate a high throughput workflow. Future variants of this product could incorporate other resins ranging in affinity, hydrophobicity, and ionic strengths to further facilitate the isolation and analysis of proteins.

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