

Enhanced detection of low-abundance human plasma proteins using tandem IgY14-SuperMix immunoaffinity depletion and LC-SRM-MS

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Overview

- IgY14-SuperMix on-line immunoaffinity depletion process was coupled with LC-SRM-MS to analyze human female plasma spiked with five non-human proteins and human PSA at 7 concentration ranges in addition to three endogenous proteins.
- A side-by-side comparison of the limit of detection (LOD) for IgY14 only and IgY14-SuperMix tandem depletion was performed.
- Analytes processed with IgY14-SuperMix tandem depletion provided considerable enhancement in sensitivity for detecting low-abundance spiked-in proteins in human female plasma.

Introduction

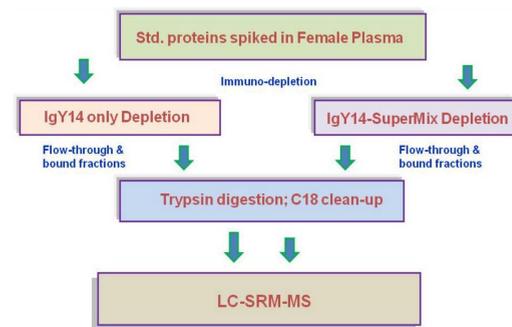
With improved capability for high throughput candidate quantification and verification, selected reaction monitoring mass spectrometry (SRM-MS) coupled with liquid chromatography (LC) is playing an increasingly important role in quantitative proteomics and biomarker verification.

Even with the competitive advantage in sensitivity and quantification accuracy, current LC-SRM-MS technologies are still challenged to detect and quantify low-abundance proteins in blood plasma/serum^[1], which are often considered an attractive source of potential biomarkers. The major difficulty in characterizing blood-based biomarkers is the overall complexity and high dynamic range of protein concentrations in human plasma/serum.

To address these challenges, we incorporated an on-line tandem IgY14-SuperMix immunoaffinity depletion technique with an LC-SRM-MS workflow for enhanced detection of low-abundance proteins in human plasma.

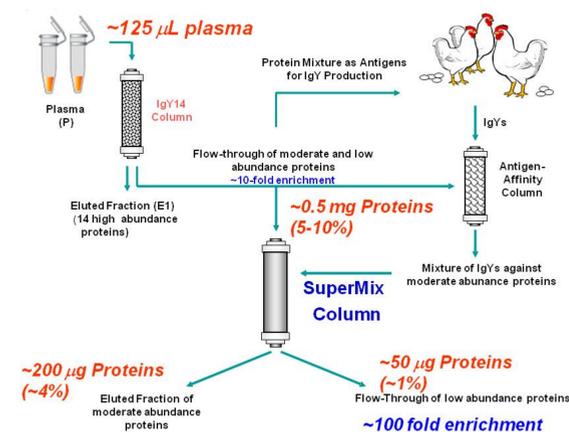
Methods

Experimental overview



- First, the loading capacity for the depletion system was optimized.
- Five non-human proteins and PSA were spiked into human female plasma at different conc. ranges (0, 0.1, 0.3, 1, 5, 25, 100, and 500 ng/mL).
- Each sample was depleted individually through either IgY14 (LC10, 10 mL bed volume) only or IgY14 (LC10)-SuperMix (LC5) tandem columns (Sigma, MA) for comparison.
- Applying the method, both the flow-through and bound fractions were collected and then digested with trypsin using an urea-based protocol.
- Heavy-labeled (¹³C, ¹⁵N) synthetic peptides were added as internal standards prior to LC-SRM-MS analysis. (LC: nanoAcquity UPLC™ from Waters; Column: 1.7 μm BEH 130 C18, 75 μm x 250 mm, 45-min LC run time; MS: TSQ Vantage™ from Thermo Scientific)
- Two process and two technical replicates were analyzed for each data point.
- 'Skyline' data analysis software was used to measure the limit of detection (LOD) of the seven spiked proteins, as well as to detect 3 targeted endogenous low-abundance proteins.

Tandem IgY14-SuperMix immunoaffinity separation strategy^[2]



Results

Evaluation of the loading capacity for immunodepletion columns

- Three different amounts of plasma samples (see below) in triplicate were injected into the IgY14-SuperMix depletion system to assess the loading capacity.
- Trypsin digestion with TFE treatment followed by LC-MS/MS (Thermo LTQ mass spectrometer) analysis.
- Loading volume of 125 μL was used for the remainder of the experiments.

	240 μL	120 μL	60 μL
Flow-through recovery	3.4%	1.0%	0.6%
# proteins among a list of low-abundance proteins with literature-reported plasma concentrations	3	14	15
Total protein identifications*	137	209	169

* By spectral counting analysis with at least two spectral counts

Selection of peptides, transitions, and Optimization of SRM-MS analysis

- Two heavy synthetic peptides per protein (only one peptide from CRP) were analyzed by LC-SRM-MS, and three transitions were selected for each peptide in the SRM-MS analysis.
- Scheduled SRM-MS was employed with five segments.
- Optimized collision energies were used for each transition. For data analysis purposes, only the best transition from each peptide was considered.

Proteins	Peptides	Precursor (m/z)	Product (m/z)
Bovine carbonic anhydrase (CAH2_BOVIN)	DFPIANGER	509.7513 ⁺⁺	756.3999 [*] , 546.2631 [*] , 378.7036 ⁺⁺
	DGPLTGTYSR	490.2458 ⁺⁺	597.2991 [*] , 496.2514 [*] , 404.2216 ⁺⁺
Bovine beta-lactoglobulin (LACB_BOVIN)	VYVEELKPTPEGDLLEILLQK	771.7578 ⁺⁺⁺	1026.067 ⁺⁺ , 976.533 ⁺⁺ , 627.8506 ⁺⁺
	VLVLDTDYK	597.3424 ⁺⁺	981.5251 [*] , 882.4567 [*] , 491.2662 ⁺⁺
Bovine cytochrome c (CYC_BOVIN)	TGPNLHGLFGR	584.8147 ⁺⁺	686.3733 [*] , 549.3144 [*] , 505.7802 ⁺⁺
	EDLIAYLK	482.7711 ⁺⁺	494.2973 [*] , 423.2602 [*] , 260.1969 [*]
<i>E. coli</i> beta galactosidase (BGAL_ECOLI)	LWSAEIPNLYR	681.3642 ⁺⁺	1062.558 [*] , 904.4887 [*] , 662.362 [*]
	VDEQPPFAVPK	671.3379 ⁺⁺	755.445 [*] , 511.3239 [*] , 244.1656 [*]
Chicken ovalbumin (OVAL_CHICK)	GGLEPINFQTAADQAR	844.4236 ⁺⁺	1121.533 [*] , 732.3635 [*] , 666.3388 ⁺⁺
	AFKDEDQAMPFR	519.2452 ⁺⁺⁺	621.3177 [*] , 550.2806 [*] , 419.2401 [*]
Prostate specific antigen (KLK3_HUMAN)	IVGGWECEK	539.2553 ⁺⁺	964.4193 [*] , 865.3509 [*] , 436.186 [*]
	LSEPAELTDAVK	636.8377 ⁺⁺	943.5095 [*] , 533.293 [*] , 472.2584 ⁺⁺
C-reactive protein (CRP_HUMAN)	GYSIFSATK	568.7848 ⁺⁺	716.3614 [*] , 916.4775 [*] , 829.4454 [*]
Myelin basic protein (MBP_HUMAN)	HGFLPP	363.7059 ⁺⁺	589.3457 [*] , 532.3242 [*] , 385.2558 [*]
	TQDENPVVHFFK	487.5771 ⁺⁺⁺	677.377 [*] , 616.3089 ⁺⁺ , 578.3085 [*]
Troponin T (TNNT2_HUMAN)	YEINVLR	453.7558 ⁺⁺	743.441 [*] , 614.3984 [*] , 501.3144 [*]
	VLAIDLHLEDQLR	512.6107 ⁺⁺⁺	887.4581 [*] , 774.3741 [*] , 662.3362 ⁺⁺

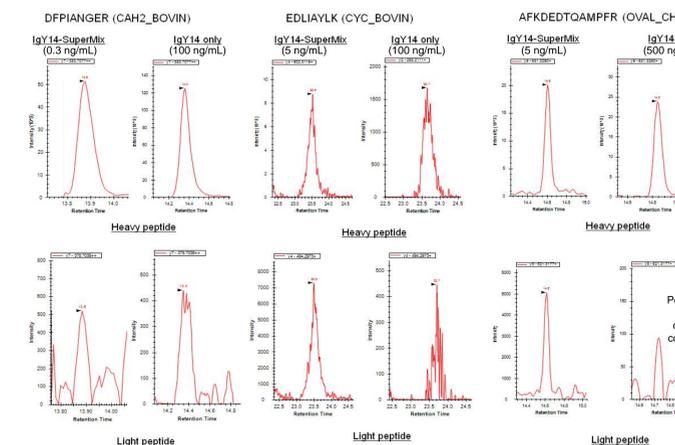
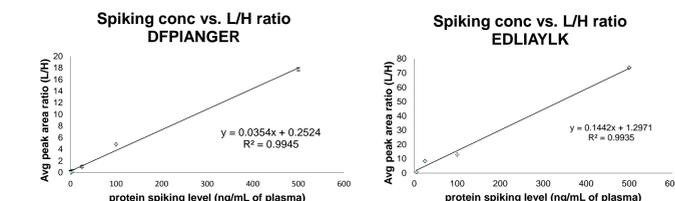
Limit of detection (LOD)

Proteins	Peptides	LOD (IgY14-SuperMix depletion)	%CV at LOD level	LOD (IgY14 only depletion)	%CV at LOD level
Bovine carbonic anhydrase	DFPIANGER	0.3 ng/mL	25.55	100 ng/mL	8.08
	DGPLTGTYSR	5 ng/mL	28.7	500 ng/mL	-
Bovine beta-lactoglobulin	VYVEELKPTPEGDLLEILLQK	5 ng/mL	12.32	500 ng/mL	-
	VLVLDTDYK	5 ng/mL	15.46	500 ng/mL	20.24
Bovine cytochrome c	TGPNLHGLFGR	500 ng/mL	6.8	500 ng/mL	11.18
	EDLIAYLK	5 ng/mL	4.77	100 ng/mL	49.06
<i>E. coli</i> beta galactosidase	LWSAEIPNLYR	500 ng/mL	42.45	> 500 ng/mL #	-
	VDEQPPFAVPK	1 ng/mL	6.66	500 ng/mL	37.46
Chicken ovalbumin	GGLEPINFQTAADQAR	5 ng/mL	26.48	> 500 ng/mL	-
	AFKDEDQAMPFR	5 ng/mL	5.22	> 500 ng/mL	-
Prostate specific antigen	IVGGWECEK	5 ng/mL	24.71	5 ng/mL	52.94
	LSEPAELTDAVK	1 ng/mL	21.79	1 ng/mL	3.53
C-reactive protein ^a	GYSIFSATK	detected [*]	32.35	detected [*]	38.16
		(intensity: ~ 9000)		(intensity: ~ 350)	
Myelin basic protein ^b	HGFLPP	not detected		not detected	
	TQDENPVVHFFK	not detected		not detected	
Troponin T ^c	YEINVLR	not detected		not detected	
	VLAIDLHLEDQLR	not detected		not detected	

^a Comparatively higher signal intensity; [#] interferences from matrix - %CV could not be determined
^a: ~1400 ng/mL; ^b: ~0.65 ng/mL; ^c: ~0.05 ng/mL; (normal amount in plasma from literatures)

Selected response curve and spectra

- Transitions m/z 509.7513⁺⁺ > 378.7036⁺⁺ from the peptide DFPIANGER (CAH2_BOVIN) and m/z 482.7711⁺⁺ > 494.2973⁺ from the peptide EDLIAYLK (CYC_BOVIN) are shown in the following graph.
- Selected spectra (both heavy and light) are at their respective LOD levels.



Conclusions

- IgY14-SuperMix tandem depletion coupled with scheduled SRM-MS provided ~1-5 ng/mL detection limit with ~ 10-100 times sensitivity enhancement (without fractionation) in detecting low-abundance spiked proteins in human plasma compared to IgY14 only depletion.
- IgY14-SuperMix tandem depletion not only afforded improvements in LOD values, but also in the reproducibility for the majority of peptides in this study compared to IgY14 only depletion
- CRP was detected in both depletion processes, however, the signal intensity was comparatively higher for the IgY14-SuperMix tandem depleted samples.
- Tandem depletion flow-through was found to provide only free PSA whereas the IgY14 only depleted flow-through contained both free and bound PSA (with ACT).

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