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A Depletion Strategy for Improved Detection of Human Proteins from Urine

Mark M. Kushnir,[1] Peter Mrozinski,[2] Alan L. Rockwood,[3] and David K. Crockett[1]

[1]*ARUP Institute for Clinical and Experimental Pathology®, Salt Lake City, UT;* [2]*Agilent Technologies, Santa Clara, CA;* [3]*Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT*

With rapidly growing interest in the urine proteome, methods for reducing sample complexity are becoming increasingly important. Depletion strategies for removal of high-abundance proteins from human urine have not been reported. A commercial kit designed for depletion of abundant proteins from plasma was evaluated for removing top proteins from urine of patients with proteinuria. The number of low-abundance proteins identified in urine after depletion increased nearly 2.5-fold.

KEY WORDS: affinity chromatography, protein depletion, proteinuria, proteomics, urine

INTRODUCTION

The detection and identification of trace amounts of proteins in complex samples is a major challenge in bio-markers discovery and validation. Depletion of proteins present in high concentrations is one common approach for improved detection of low-abundance proteins, which may serve as potential biomarkers.[1–3] The depletion strategy often employs antibody affinity chemistry using commercially available kits.[4,5]

Samples of interest for detection of protein biomarkers are typically serum or plasma. Thus, commercial kits for the depletion of abundant proteins are generally developed and optimized for plasma or serum samples. However, with the rapidly growing interest in the human urine proteome, methods for reducing the complexity of urine samples are needed.[6–8] To date, depletion strategies for removal of high-abundance proteins from human urine have not been reported.

Fractionation of samples has been recognized as one common approach for reducing complexity and making the samples amenable for instrumental analysis. One of the most efficient methods for reduction of sample complexity is affinity capture of abundant proteins followed by analysis of the remaining fraction.[1,4,9]

Because of the simplicity and noninvasiveness of collection, urine is an attractive sample type for many diagnostic tests. Furthermore, urine contains a relatively small number of proteins typically present at low concentrations, and thus a simpler matrix for detecting proteins as compared to serum or plasma. However, in some human diseases excess protein is found in the urine, as can occur in patients with compromised kidney function. As a result, many of the proteins normally present in blood will also be excreted into the urine. This condition, known as proteinuria, is often observed in acute inflammation, acute urinary tract infection, amyloidosis, diabetic nephropathy, kidney failure, multiple myeloma, nephrotic syndrome, and severe yeast infections. [10–14]

When searching for diagnostic markers of the above diseases and conditions, plasma proteins present at high concentrations in urine samples can hinder the ability to detect potential protein biomarkers present at low concentrations. Depletion of high-abundance plasma proteins from urine is one approach that would simplify sample complexity and improve the chances of finding potential biomarkers. In this study we report the use of a commercially manufactured protein depletion kit for the removal of the six most abundant human plasma proteins from urine samples.

EXPERIMENTAL PROCEDURES

To evaluate the commercial protein depletion kit, human serum samples were prepared and processed according to the manufacturer's instructions. Test samples of pooled human urine were then prepared. A protein-containing urine pool was collected from the urine of patients with proteinuria. For the negative control, pooled urine samples from healthy individuals were used. All samples were de-identified in accordance with a University of Utah Institutional Review Board approved protocol.

ADDRESS CORRESPONDENCE TO: David K. Crockett, ARUP Laboratories, 500 Chipeta Way, Salt Lake City, Utah 84132 (phone: 801-583-2787; fax: 801-584-5109; email: david.crockett@aruplab.com).
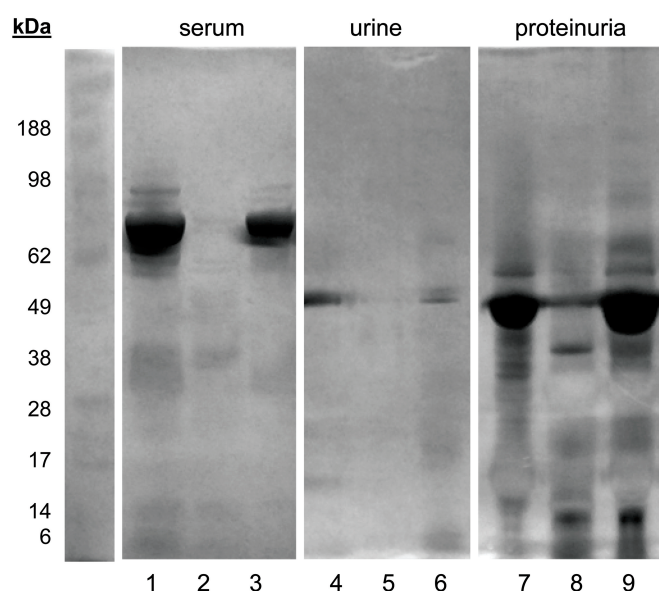
FIGURE 1

Depletion of high-abundance proteins from serum (lanes 1–3) and urine (lanes 4–9) samples. *Lane 1*: Serum control; *lane 2*: depletion column flow-through; *lane 3:* column elution of the bound serum proteins. *Lane 4:* normal urine control; *lane 5:* column flow-through; *lane 6:* column elution. *Lane 7:* proteinuria control; *lane 8:* flow-through fraction; *lane 9*: column elution of the bound protein fraction.

The multiple affinity removal (MARS) column for the depletion of 6 high-abundant proteins (Agilent Technologies, Santa Clara, CA, Part #5185-5984) contains affinity binders for the depletion of albumin, transferrin, haptoglobin, IgG, IgA, and alpha-1 antitrypsin. Samples were prepared by ultrafiltration (Amicon Ultra-4 filters, 5 kDa cutoff, Millipore, Billerica, MA), then processed with the recommended column run cycle consisting of loading the sample (serum or urine), collecting the flow-through (depleted fraction) proteins, washing, and eluting bound proteins.

The efficiency of the depletion of the proteins from urine was confirmed using SDS-PAGE. Where possible, equal loading of total protein onto the depletion column and for gel analysis was employed. After electrophoresis, the gel was silver stained (Invitrogen, Carlsbad, CA) and imaged.

Samples were processed by alkylating cysteine residues with iodoacetamide, then either in-gel or in-solution tryptic digest for 18 h at 37°C. The samples were then analyzed on the Agilent 6510 Q-TOF (Agilent Technologies, Santa Clara, CA) equipped with a ChipCube (G2240A) and an Agilent 1200 nano-HPLC system using a 30-min acetonitrile gradient (5%–40%) and C18 reversed-phase separation. Data acquisition (2 GHz extended dynamic range) was performed with MassHunter Q-TOF Acquisition software B.01.03 with an acquisition rate of 3 scans/s followed

by tandem mass spectrometry (MS/MS) scans of the three most intense ions using the acquisition rate of 2 scans/s. Exclusion was set for 12 s after two consecutive MS/MS scans of a precursor ion. The time-of-flight (TOF) analyzer was tuned to a resolution of 12,000 and calibrated prior to each experiment for a mass accuracy < 2 ppm.

The acquired MS/MS spectra were searched using Spectrum Mill MS Proteomics Workbench Rev A.03.03.075 (Agilent) against the SwissProt human database (v 13.2–53,541 entries). Mass tolerance was set to 20 ppm for precursor ions and 50 ppm for fragment ions. An enzyme-specific search (trypsin) was used with two missed cleavages allowed. Variable carbamidomethylation (Da 57.0214) was also included for peptide scoring during the database search. Search results were filtered to approximately 5% error using Spectrum Mill's autovalidation tool, with an individual peptide score threshold of 10 or summed score of 15.

RESULTS

High-abundance proteins were removed from human serum using the Agilent MARS column. The depletion was performed according to the method recommended by the manufacturer for serum samples. Figure 1 shows the silver-stained SDS gel of the control serum sample (lane 1), flow-through of the depletion column (lane 2), and the eluted fraction of serum proteins retained by the column (lane 3).

Performance of the depletion kit was also compared between serum and urine samples. The pool of urine samples collected from healthy individuals contained traces of proteins (lane 4), which were removed in the flow-through fraction (lane 5) and accounted for in the eluted fraction (lane 6). The gel analysis of proteinuria samples contained large amounts of proteins (lane 7), which were removed in the flow-through fraction (lane 8) and seen in the eluted fraction (lane 9).

To further evaluate the performance of depletion in urine, the total number of proteins identified by liquid chromatography (LC)-MS/MS analysis of urine samples was compared. While only 29 proteins were identified in urine from healthy individuals (Fig. 1, lane 4 and Table 1), some 60 proteins were identified in urine from patients with proteinuria (Fig. 1, lane 7 and Table 2). However, after depletion of high-abundance proteins, 142 proteins were identified (Fig. 1, lane 8 and Table 3). Table 4 summarizes these findings.

DISCUSSION

LC-MS/MS is a powerful technique available for proteomic studies. Depending on the type of separation and detection used, reliable detection range may span three to

TABLE 1

Human Proteins Identified in Urine of Healthy Individuals

Group No.	No. Spectra	No. Unique PEPs	% Coverage	Unique Score	Accession	Protein
1	20	10	20	147.9	P02768	Serum albumin precursor
2	18	7	65	117.5	P62988	Ubiquitin
3	4	3	2	41.8	P02452	Collagen alpha-1(I) chain precursor
4	3	2	8	38.7	P10645	Chromogranin-A precursor
5	3	3	12	29.9	P11684	Uteroglobin precursor
6	4	3	12	29.6	P01009	Alpha-1-antitrypsin precursor
7	3	2	4	28.5	P04080	Cystatin-B
8	13	4	33	27.2	P01834	Ig kappa chain C region
9	2	2	31	21.6	Q9UGM3	Deleted in malignant brain tumors 1 protein precursor
10	5	2	1	21.4	P01344	Insulin-like growth factor II precursor
11	2	2	4	21.2	P02814	Submaxillary gland androgen-regulated protein 3 homolog B
12	7	2	39	20.7	P68133	Actin, alpha skeletal muscle
13	2	3	2	20.4	P68363	Tubulin alpha-1B chain
14	2	2	3	20.3	P36578	60S ribosomal protein L4
15	2	2	4	19.7	P18135	Ig kappa chain V-III region HAH precursor
16	2	2	6	19.7	P02750	Leucine-rich alpha-2-glycoprotein precursor
17	2	2	2	19.0	P02760	AMBP protein precursor (contains alpha-1-microglobulin)
18	2	2	4	18.9	P00738	Haptoglobin precursor (contains haptoglobin alpha chain)
19	2	2	1	18.9	P01602	Ig kappa chain V-I region HK102 precursor
20	2	2	13	18.4	P10153	Nonsecretory ribonuclease precursor
21	2	2	9	18.2	P01842	Ig lambda chain C regions
22	2	2	27	17.0	P02753	Plasma retinol-binding protein precursor
23	2	2	4	16.8	P07602	Proactivator polypeptide precursor (contains Saposin-A)
24	2	2	2	16.8	P01703	Ig lambda chain V-I region NEWM
25	2	2	10	16.1	Q12907	Vesicular integral-membrane protein VIP36 precursor
26	4	2	2	15.3	P01857	Ig gamma-1 chain C region
27	3	2	3	15.1	P19971	Thymidine phosphorylase precursor
28	2	2	2	15.0	Q99459	Cell division cycle 5-like protein
29	3	2	2	15.0	P80748	Ig lambda chain V-III region LOI

T A B L E 2

Human Proteins Identified in Urine of Individuals with Proteinuria

Group No.	No. Spectra	No. Unique PEPs	% Coverage	Unique Score	Accession	Protein
1	42	12	17	218.0	P02768	Serum albumin precursor
2	28	6	9	107.8	P07911	Uromodulin precursor
3	11	5	1	98.0	P98160	Basement membrane-specific heparan sulfate proteoglycan core protein
4	11	4	4	75.4	P01133	Pro-epidermal growth factor precursor
5	7	3	7	58.9	P19961	Alpha-amylase 2B precursor
6	6	3	8	56.5	P10909	Clusterin precursor
7	6	3	5	56.3	Q6EMK4	Vasorin precursor
8	7	3	5	52.5	P01042	Kininogen-1 precursor
9	15	4	16	52.3	P05090	Apolipoprotein D precursor
10	4	2	3	40.3	P55290	Cadherin-13 precursor
11	6	2	5	37.6	P01876	Ig alpha-1 chain C region
12	7	2	9	37.0	P10451	Osteopontin precursor
13	6	2	3	36.1	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4 precursor
14	6	2	3	34.0	Q08380	Galectin-3-binding protein precursor
15	4	2	3	33.4	P01833	Polymeric-immunoglobulin receptor precursor
16	3	2	4	32.0	P68871	Hemoglobin subunit beta
17	2	2	9	27.2	P01009	Alpha-1-antitrypsin precursor
18	4	2	4	25.2	P10153	Nonsecretory ribonuclease precursor
19	2	2	18	25.1	P07288	Prostate-specific antigen precursor
20	3	3	8	24.9	P01834	Ig kappa chain C region
21	2	2	1	24.6	Q7Z5L0	Vitelline membrane outer layer protein 1 homolog precursor
22	2	2	2	24.4	P10253	Lysosomal alpha-glucosidase precursor
23	5	2	1	24.0	Q9HCU0	Endosialin precursor
24	4	3	5	23.7	P12109	Collagen alpha-1(VI) chain precursor
25	4	2	8	22.9	P05452	Tetranectin precursor
26	6	2	3	22.5	P41222	Prostaglandin-H2 D-isomerase precursor
27	2	2	4	21.2	P04004	Vitronectin precursor
28	2	2	4	20.7	Q6GTX8	Leukocyte-associated immunoglobulin-like receptor 1 precursor
29	2	2	2	20.6	P02774	Vitamin D-binding protein precursor
30	4	3	9	20.6	P01859	Ig gamma-2 chain C region
31	3	3	2	20.5	P06310	Ig kappa chain V-II region RPMI 6410 precursor
32	2	2	3	20.2	O94919	Endonuclease domain-containing 1 protein precursor
33	4	2	4	20.0	P15309	Prostatic acid phosphatase precursor
34	2	2	4	20.0	P06870	Kallikrein-1 precursor
35	2	2	15	19.9	O75594	Peptidoglycan recognition protein precursor
36	2	2	1	19.4	P01766	Ig heavy chain V-III region BRO
37	4	2	2	19.2	P04264	Keratin, type II cytoskeletal 1
38	2	2	7	19.2	Q12907	Vesicular integral-membrane protein VIP36 precursor
39	2	2	2	19.0	P01781	Ig heavy chain V-III region GAL
40	4	2	2	19.0	O00187	Mannan-binding lectin serine protease 2 precursor
41	3	3	1	18.8	P01871	Ig mu chain C region
42	2	2	5	18.8	P16070	CD44 antigen precursor
43	4	2	2	18.5	P25311	Zinc-alpha-2-glycoprotein precursor
44	2	2	1	18.4	P05155	Plasma protease C1 inhibitor precursor
45	2	2	2	17.4	P14543	Nidogen-1 precursor
46	6	2	1	17.0	Q9GZM5	Protein YIPF3
47	3	2	2	16.9	P35908	Keratin, type II cytoskeletal 2 epidermal
48	2	2	5	16.9	P08571	Monocyte differentiation antigen CD14 precursor
49	2	2	1	16.8	P18827	Syndecan-1 precursor
50	2	2	2	16.6	Q6UVK1	Chondroitin sulfate proteoglycan 4 precursor
51	3	3	1	16.6	P69905	Hemoglobin subunit alpha
52	6	2	1	16.3	P02760	AMBP protein precursor [Contains: Alpha-1-microglobulin]
53	4	2	1	16.1	P06396	Gelsolin precursor
54	2	2	1	15.1	Q6XZF7	Dynamin-binding protein
55	3	3	1	15.1	P02751	Fibronectin precursor
56	2	2	2	15.0	Q00796	Sorbitol dehydrogenase
57	2	2	3	15.0	P12830	Epithelial-cadherin precursor
58	3	2	9	15.0	P02750	Leucine-rich alpha-2-glycoprotein precursor
59	2	2	2	15.0	O75144	ICOS ligand precursor
60	6	2	1	15.0	Q8IZQ5	Selenoprotein H

T A B L E   3

Human Proteins Identified in Urine of Individuals with Proteinuria Using a Depletion Strategy

Group No.	No. Spectra	No. Unique PEPs	% Coverage	Unique Score	Accession	Protein
1	80	14	46	261.1	P25311	Zinc-alpha-2-glycoprotein precursor
2	33	10	11	161.7	P15144	Aminopeptidase N
3	80	8	69	152.5	P62988	Ubiquitin
4	66	6	27	117.2	P02763	Alpha-1-acid glycoprotein 1 precursor
5	47	6	30	114.1	P10451	Osteopontin precursor
6	92	7	21	107.4	P02760	AMBP protein precursor (contains alpha-1-microglobulin)
7	30	6	18	105.7	P80188	Neutrophil gelatinase-associated lipocalin precursor
8	17	6	43	103.3	P27487	Dipeptidyl peptidase 4
9	13	7	7	102.0	P10253	Lysosomal alpha-glucosidase precursor
10	23	6	10	100.8	P08571	Monocyte differentiation antigen CD14 precursor
11	21	5	22	96.7	P15586	N-acetylglucosamine-6-sulfatase precursor
12	42	5	9	89.4	P07602	Proactivator polypeptide precursor (contains saposin-A)
13	12	6	8	88.3	O43451	Maltase-glucoamylase, intestinal (includes maltase)
14	32	6	9	83.3	P07911	Uromodulin precursor
15	15	5	3	81.7	P07339	Cathepsin D precursor
16	13	5	8	81.3	P01011	Alpha-1-antichymotrypsin precursor
17	14	5	15	78.6	P04746	Pancreatic alpha-amylase precursor
18	9	4	15	77.4	Q13228	Selenium-binding protein 1
19	15	5	10	75.1	P01833	Polymeric-immunoglobulin receptor precursor
20	23	4	11	74.7	P02768	Serum albumin precursor
21	20	4	7	73.6	P17900	Ganglioside GM2 activator precursor
22	13	5	23	70.7	P00915	Carbonic anhydrase 1
23	9	5	26	68.9	Q92820	Gamma-glutamyl hydrolase precursor
24	7	3	21	63.5	P02452	Collagen alpha-1(I) chain precursor
25	7	4	2	61.2	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4 precursor
26	7	3	6	60.9	P22352	Glutathione peroxidase 3 precursor
27	10	3	19	59.1	P07686	Beta-hexosaminidase beta chain precursor
28	20	3	5	57.4	P05451	Lithostathine 1 alpha precursor
29	18	2	18	54.2	P01593	Ig kappa chain V-I region AG
30	10	3	30	53.3	Q9Y5Y7	Lymphatic vessel endothelial hyaluronic acid receptor 1 precursor
31	7	3	8	51.8	P09603	Macrophage colony-stimulating factor 1 precursor
32	12	3	7	51.3	O00584	Ribonuclease T2 precursor
33	6	3	10	51.2	P00751	Complement factor B precursor
34	13	3	6	50.9	P01620	Ig kappa chain V-III region SIE
35	25	3	38	50.7	P41222	Prostaglandin-H2 D-isomerase precursor
36	13	3	20	50.3	Q08380	Galectin-3-binding protein precursor
37	15	2	5	48.2	P01834	Ig kappa chain C region
38	8	3	34	47.9	P12830	Epithelial-cadherin precursor
39	12	3	5	45.9	P13473	Lysosome-associated membrane glycoprotein 2 precursor
40	9	3	7	44.8	P05155	Plasma protease C1 inhibitor precursor
41	7	2	5	44.0	P07478	Trypsin-2 precursor
42	6	2	7	43.1	Q13231	Chitotriosidase-1 precursor
43	8	2	5	43.0	Q9H299	SH3 domain-binding glutamic acid-rich-like protein 3
44	6	3	20	42.3	P06865	Beta-hexosaminidase alpha chain precursor
45	11	3	5	42.2	P02753	Plasma retinol-binding protein precursor
46	4	2	24	40.9	O94919	Endonuclease domain-containing 1 protein precursor
47	13	3	5	38.5	P55290	Cadherin-13 precursor
48	6	2	4	37.0	P61769	Beta-2-microglobulin precursor
49	5	2	16	36.7	P11279	Lysosome-associated membrane glycoprotein 1 precursor
50	12	2	4	36.5	P01625	Ig kappa chain V-IV region Len
51	9	2	22	36.1	P35754	Glutaredoxin-1
52	12	2	11	35.6	P19320	Vascular cell adhesion protein 1 precursor
53	7	3	2	35.4	P98160	Basement membrane-specific heparan sulfate proteoglycan core protein
54	4	3	1	35.2	P98164	Low-density lipoprotein receptor-related protein 2 precursor
55	7	2	1	35.1	P10153	Nonsecretory ribonuclease precursor
56	4	3	9	34.7	P02751	Fibronectin precursor
57	3	2	2	34.6	P02774	Vitamin D-binding protein precursor
58	7	3	6	34.1	P04080	Cystatin-B
59	23	3	24	33.9	P00441	Superoxide dismutase [Cu-Zn]

(continued)

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Human Proteins Identified in Urine of Individuals with Proteinuria Using a Depletion Strategy

Group No.	No. Spectra	No. Unique PEPs	% Coverage	Unique Score	Accession	Protein
60	8	2	12	33.5	P05090	Apolipoprotein D precursor
61	5	2	12	33.3	Q15828	Cystatin-M precursor
62	6	2	14	33.1	P29966	Myristoylated alanine-rich C-kinase substrate
63	4	2	15	32.0	P31025	Lipocalin-1 precursor
64	4	2	12	31.8	P78324	Tyrosine-protein phosphatase non-receptor type substrate 1 precursor
65	5	2	5	31.7	P06870	Kallikrein-1 precursor
66	9	2	8	31.2	O00468	Agrin precursor
67	4	2	1	29.5	Q07075	Glutamyl aminopeptidase
68	4	2	3	28.7	Q96S96	PEBP family protein precursor
69	4	2	6	28.5	P02765	Alpha-2-HS-glycoprotein precursor
70	5	2	4	28.2	P11684	Uteroglobin precursor
71	6	2	12	27.6	Q6GTX8	Leukocyte-associated immunoglobulin-like receptor 1 precursor
72	4	2	9	26.2	P02766	Transthyretin precursor
73	4	2	8	26.1	P01040	Cystatin-A
74	4	2	21	26.0	O75368	SH3 domain-binding glutamic acid-rich-like protein
75	6	2	20	25.2	P07360	Complement component C8 gamma chain precursor
76	3	2	7	24.2	P01008	Antithrombin-III precursor
77	4	2	4	24.2	Q01459	Di-N-acetylchitobiase precursor
78	6	2	3	24.1	Q13508	Ecto-ADP-ribosyltransferase 3 precursor
79	3	2	2	23.7	Q14019	Coactosin-like protein
80	6	2	16	23.5	P16070	CD44 antigen precursor
81	4	2	1	23.2	P06310	Ig kappa chain V-II region RPMI 6410 precursor
82	3	2	9	23.0	Q9UM22	Mammalian ependymin-related protein 1 precursor
83	3	2	8	22.8	Q14315	Filamin-C
84	2	2	1	22.8	P02735	Serum amyloid A protein precursor
85	11	2	29	22.3	Q02747	Guanylin precursor
86	4	2	16	21.8	P02671	Fibrinogen alpha chain precursor [Contains: Fibrinopeptide A]
87	6	2	2	21.6	Q9NP84	Tumor necrosis factor receptor superfamily member 12A precursor
88	4	2	7	21.6	Q9NQC3	Reticulon-4
89	6	2	1	21.6	P07148	Fatty acid-binding protein, liver
90	2	2	8	21.4	P53634	Dipeptidyl-peptidase 1 precursor
91	4	2	3	21.4	P02788	Lactotransferrin precursor
92	2	2	1	21.2	P10619	Lysosomal protective protein precursor
93	2	2	2	21.1	P08174	Complement decay-accelerating factor precursor
94	4	2	3	20.9	P01871	Ig mu chain C region
95	18	3	2	20.9	P01842	Ig lambda chain C regions
96	2	2	18	20.3	P37173	TGF-beta receptor type-2 precursor
97	2	2	2	20.1	Q03405	Urokinase plasminogen activator surface receptor precursor
98	4	2	3	19.9	P09668	Cathepsin H precursor
99	2	2	3	19.8	P02792	Ferritin light chain
100	2	2	8	19.8	P36957	Dihydrolipoyllysine-residue succinyltransferase, mitochondrial precursor
101	4	2	3	19.7	P01703	Ig lambda chain V-I region NEWM
102	5	2	10	19.6	P10599	Thioredoxin
103	7	2	12	19.0	P81605	Dermcidin precursor
104	4	2	10	19.0	P06396	Gelsolin precursor
105	2	2	1	19.0	P05413	Fatty acid-binding protein, heart
106	6	2	9	18.9	P01034	Cystatin-C precursor
107	2	2	7	18.7	P24855	Deoxyribonuclease-1 precursor
108	3	3	4	18.6	P01700	Ig lambda chain V-I region HA
109	4	2	11	18.3	A0AVF1	Tetratricopeptide repeat protein 26
110	5	2	1	18.1	P05060	Secretogranin-1 precursor
111	2	2	2	18.1	O75223	Uncharacterized protein C7orf24
112	2	2	5	18.1	Q09666	Neuroblast differentiation-associated protein AHNAK
113	6	2	1	17.8	Q9Y624	Junctional adhesion molecule A precursor
114	3	2	3	17.7	P08236	Beta-glucuronidase precursor
115	12	3	1	17.5	O43692	Peptidase inhibitor 15 precursor
116	2	2	3	17.3	P04207	Ig kappa chain V-III region CLL precursor

T A B L E   3   (continued)

Human Proteins Identified in Urine of Individuals with Proteinuria Using a Depletion Strategy

Group No.	No. Spectra	No. Unique PEPs	% Coverage	Unique Score	Accession	Protein
117	2	2	6	17.3	Q86Y38	Xylosyltransferase 1
118	2	2	1	17.3	Q13201	Multimerin-1 precursor
119	2	2	1	17.2	P15289	Arylsulfatase A precursor
120	2	2	3	17.1	Q16378	Proline-rich protein 4 precursor
121	2	2	11	17.1	P20142	Gastricsin precursor
122	3	2	2	17.0	P52758	Ribonuclease UK114
123	2	2	7	17.0	P04279	Semenogelin-1 precursor
124	11	3	3	16.5	Q9GZM5	Protein YIPF3
125	2	2	2	16.3	Q9Y4L1	Hypoxia up-regulated protein 1 precursor
126	2	2	1	16.2	P07711	Cathepsin L precursor
127	2	2	4	15.7	Q9Y279	V-set and immunoglobulin domain-containing protein 4 precursor
128	2	2	2	15.4	P01857	Ig gamma-1 chain C region
129	2	2	6	15.4	Q9BY77	Polymerase delta-interacting protein 3
130	3	3	3	15.3	P04433	Ig kappa chain V-III region VG precursor
131	2	2	7	15.2	Q9UGM3	Deleted in malignant brain tumors 1 protein precursor
132	2	2	1	15.1	P04430	Ig kappa chain V-I region BAN
133	2	2	8	15.1	O43653	Prostate stem cell antigen precursor
134	4	2	15	15.1	Q9UKL3	CASP8-associated protein 2
135	2	2	1	15.0	Q15149	Plectin-1
136	2	2	1	15.0	Q6UWV6	Ectonucleotide pyrophosphatase/phosphodiesterase family member 7 precursor
137	2	2	2	15.0	Q12830	Nucleosome remodeling factor subunit BPTF
138	2	2	1	15.0	P05543	Thyroxine-binding globulin precursor
139	2	2	2	15.0	P05067	Amyloid beta A4 protein precursor
140	2	2	1	15.0	P01598	Ig kappa chain V-I region EU
141	2	2	15	15.0	Q6EMK4	Vasorin precursor
142	2	2	2	15.0	P02689	Myelin P2 protein

four orders of magnitude of protein concentration. However, this is insufficient for detection of low-abundance proteins that may be present at concentrations up to 10 orders of magnitude lower than the most abundant proteins of the sample. Although the protein content in urine samples of patients with proteinuria is significantly lower compared to plasma, the samples are still very complex and detection of low-abundance proteins remains a difficult task.

The silver-stained SDS-PAGE (Fig. 1) demonstrated efficient depletion of major proteins from human urine. Performance of the affinity depletion MARS column for urine samples was comparable to the performance observed when the column was used for serum samples.

In addition, the protocol for removal of abundant proteins from urine required only slight modification, including the removal of low-molecular-weight components using ultrafiltration. Benefits of the commercial depletion kit included efficient removal of targeted proteins and the ability to reuse the column for multiple samples, thus decreasing processing cost per sample.

Importantly, the proteins targeted by the depletion kit (albumin, transferrin, haptoglobin, immunoglobulin G, immunoglobulin A, and alpha-1 antitrypsin) were noticeably absent or greatly reduced in the depleted data set, thus allowing many more moderate- or low-concentration proteins to be found. For example, serum albumin was identified with the highest score in both normal urine

T A B L E   4

Summary of Proteins Identified in Human Urine by LC-MS/MS

Sample Type	Depletion	Protein IDs	Fold
Healthy human urine	No	29	—
Proteinuria human urine	No	60	2.1
Proteinuria human urine	Yes	142	2.4

(10 peptides) and proteinuria sample (12 peptides), but was identified 20th on the list (only 4 peptides) in the proteinuria sample after depletion.

The human response to disease or infection often increases the number of proteins in the urine. For proteinuria samples, depletion of six high-abundance proteins allowed two-and-a-half times the number of proteins to be identified in urine from these patients. This study demonstrates that depletion is a useful strategy for reducing the overall complexity of the urine sample.

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