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Research Article

Glomerulus proteome analysis with two-dimensional gel electrophoresis and mass spectrometry

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Abstract. A proteome analysis of mammalian glomeruli was performed in mouse using two-dimensional-gel electrophoresis with separate Coomassie and silver staining and subsequent mass spectrometric identifications. Altogether, 414 protein spots were identified, revealing 232 different proteins representing a wide spectrum of activities, including enzymes (27%), cell-signalling proteins (22%), structural proteins (12%), protein folding and metabolism (13%), cell-growth-related proteins (6%), and replication, transcription and translation (4%). Only 53 of the proteins were detected in another proteome study, showing the value of analyses with different methodologies. However, 50 of the proteins were also identified in a proteome analysis of endothelial cells and 42 in one of glomerular mesangial cells, revealing distinct similarities between these tissues, but also unique differences. Finally, 80 of the proteins were not identified in a separate transcriptome analysis, while 10 of the present proteins were then indentifiable with genes implicated in glomerulus development and function, allowing direct correlation with expression data.

Keywords. Kidney glomerulus, two-dimensional gel electrophoresis, MALDI-MS, LC-MS/MS, marker protein, endothelial cell.

Proteome analysis constitutes a central tool providing information on proteins expressed in different cells, tissues or organs in normal and pathological states. It can contribute to a better understanding of the molecular basis of normal physiology by revealing broad expression profiles of interconnected processes. Comparison of such profiles at different stages of disease can shed new light on the molecular nature of diseases and provide novel biomarkers for disease processes [1].

Diuresis in the kidney is the main route for excretion of soluble waste products from the body. The filtration of plasma occurs in the renal glomeruli, which are specialized filtration units that ensure the retention of plasma macromolecules of the size of albumin or larger. The glomeruli constitute a tuft of capillaries in the Bowman's capsule. A human kidney contains about one million and a mouse kidney about 100,000 glomeruli. The capillary wall has three separate layers: fenestrated endothelial cells, the glomerular basement membrane (GBM) and the epithelial podocytes with the cell bodies floating in the urinary space. The glomerular tuft has only three types of cell: capillary endothelial cells, podocytes and mesangial cells. Several components of the GBM and slit diaphragm are known [2], but the mechanisms by which glomerular filtration is regulated are still poorly

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understood, and glomerular proteome studies are rare [3, 4].

The glomeruli are affected in both genetic and acquired progressive kidney diseases, characterized in turn by proteinuria, nephrotic syndrome, and eventually renal failure that need treatment with dialysis or kidney transplantation. About two-thirds of end-stage renal diseases originate in the glomeruli. These diseases are traumatic to patients and costly to society but the molecular mechanisms are often not known. It is important to generate more information about the glomerular proteome in humans and other mammals to better understand the normal physiology and the pathological processes leading to renal disease [5]. In the present study, we have identified glomerulus-specific proteins in murine glomeruli using two-dimensional (2-D) gel electrophoresis and mass spectrometry subsequent to two different staining methods intended to increase identifications and sensitivity. Many novel protein identifications were obtained, allowing comparisons with different studies of glomeruli [3, 4], endothelial [6] and mesangial [7] tissues, and with cDNA analyses of transcriptomes [8, 9].

Materials and methods

Materials. Kidney glomeruli were isolated from C57BL/6 female mice (approved by the local committee for ethics in animal research). Deoxyribonuclease I (DNAse I) and Hanks′ balanced salt solution (HBSS) were from Invitrogen (Carlsbad, CA), tosyl-activated Dynabeads M-450 and a magnetic particle concentrator from Dynal (Oslo, Norway), cell strainers from BD Falcon (Franklin Lakes, NJ), Immobiline pH gradient (IPG) strips, bis-acrylamide and carrier ampholytes from Bio-Rad Laboratories (Hercules, CA), thiourea, DTT, nonylphenyl ethoxylate (NP-40), Triton X-100 and CHAPS from Sigma Aldrich (St. Louis, MO), urea and EDTA from Amersham Biosciences (Uppsala, Sweden), and protease inhibitor cocktail tablets from Roche (Mannheim, Germany). α-Cyano hydroxy-cinnamic acid was from Bruker Daltonics. C-18 reverse-phase ZipTips were purchased from Millipore (Bedford, MA).

Isolation of glomeruli. Mouse glomeruli were isolated from about 50 C57BL/6 mice using the method of Takemoto et al. [9]. Dynabeads were inactivated according to the manufacturer's instructions. The magnetic beads were washed for 5 min at 4 °C with ice-cold 1 × PBS, pH 7.4, 0.1 % w/v BSA, 0.02 % w/v sodium azide, and then incubated for 24 h at 20 °C with 0.2 M Tris, pH 8.5, 0.1 % w/v BSA. The mice were anesthetized by intraperitoneal injection of avertin (2,2,2 tribromoethanol and tertiary amyl alcohol; Sigma Aldrich) at a dosage of 0.5 mg/g and perfused through the left ventricle with HBSS until the color of the kidneys was pale, and subsequently with 40 ml HBSS containing 8×10^7 Dynabeads in 40 ml of HBSS. The kidneys were removed, minced and digested with 100 U/ml DNase I in HBSS for 30 min at 4 °C. The tissue was then pressed through a 100-μm cell strainer using a pestle, the strainer was washed with 8 ml HBSS (with 100 U/ml DNase I), and the filtrate was centrifuged for 10 min at 5000 g at 4 °C. The supernatant was discarded and the pellet was resuspended in 1.5 ml HBSS (with 100 U/ml DNase I). The suspension was filtrated through a 100-μm cell strainer and washed with 6 ml HBSS (with 100 U/ml DNase I). The filtrate was then transferred to 2-ml tubes and the glomeruli-containing magnetic Dynabeads were collected with a magnetic particle concentrator. The glomeruli were counted, controlled for purity (Fig. 1) and stored at –80 °C.

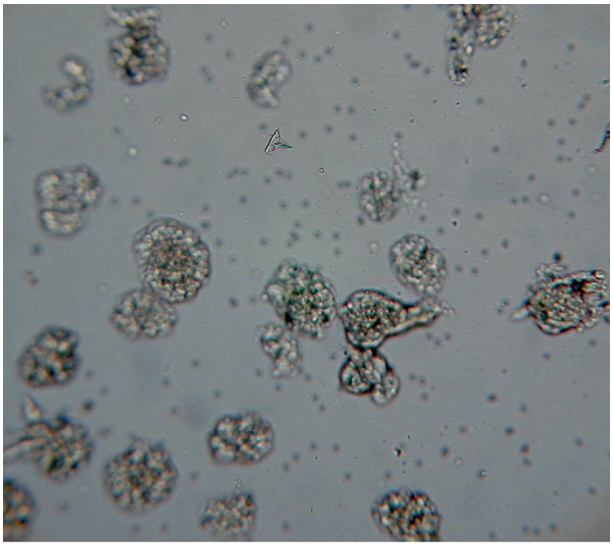

Figure 1. Histological purity control of the isolated glomeruli, showing normal-shaped glomeruli without surrounding kidney tissue. Free magnetic beads were removed from the sample before the 2-D gel electrophoresis and do not affect the results.

Sample preparation and 2-D electrophoresis. Proteins of the isolated glomeruli were extracted [10] to a suspension in MilliQ water (Millipore) twice the glomerular wet weight (WW). The suspension was freeze-thawed four times in liquid nitrogen to disrupt the cells, after which 0.1 × WW, 10 % SDS/33 % mercaptoethanol and 0.33 × WW, DNase I/RNase A (0.15 mg/ml/0.072 mg/ml in 2.4 mM Tris-base, 47.6 mM Tris-HCl, 5 mM $MgCl_2$) was added. The samples were incubated at 4 °C for 10 min, frozen in liquid

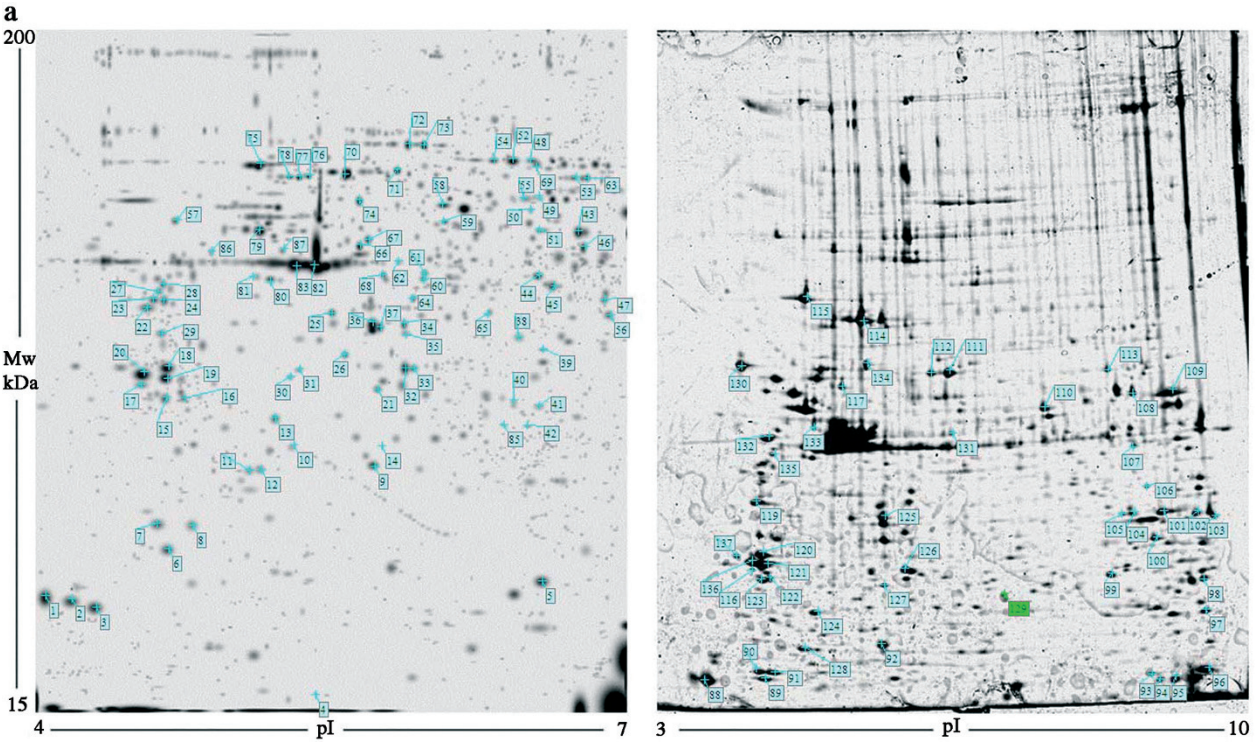

Figure 2. 2-D gel electrophoretic patterns of mouse glomerular proteins. Coomassie staining of an extract (~500 µg) showing 625 protein spots on a pI 4–7 gel and 342 spots on a pI 3–10 gel (*a*) and silver staining of an extract (~75 µg) showing 922 protein spots on a pI 4–7 gel (*b*). Together, the annotated 414 excised spots were identified with peptide mass fingerprinting and sequence analyses as given in Table 1 using Voyager MALDI DE-PRO (*a*) and a MALDI Q-Tof Ultima instrument (*b*).

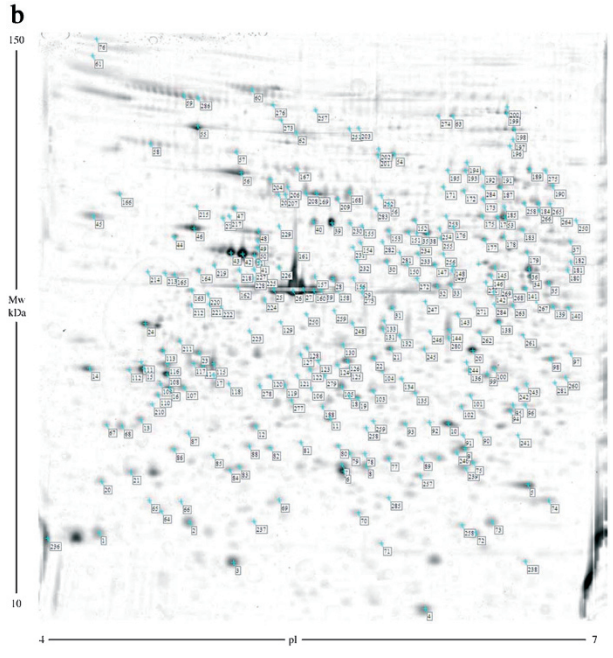

Figure 2. (continued).

nitrogen and then solubilized in 6 × WW lysis buffer [9 M urea, 65 mM DTT, 0.5 % NP-40, 2 % CHAPS, 2 % carrier ampholytes, 35 mM Tris-base, protease inhibitor complete mini cocktail tablet (Roche, Basel, Switzerland)] in a shaker for 4 h at 1300 rpm and 20 °C. After centrifugation at 13 000 g for 15 min, supernatants were collected and protein concentrations were measured with the Bradford protein assay (BioRad) after which the samples were stored at –80 °C until use.

Isoelectric focusing was performed on 17-cm Ready-Strip IPG strips (Bio-Rad), pI gradients 4–7 and 3–10. The IPG strips were actively rehydrated at 20 °C for 12 h with 300 µl of 7 M urea, 2 M thiourea, 4 % w/v CHAPS, 0.5 % Triton X-100 and 0.5 % IPG buffer containing the sample. Samples of 75 µg protein were loaded for the silver-stained gels, and 500 µg for the Coomassie brilliant blue (CBB)-stained gels. The focusing was carried out in an isoelectric focusing cell (Bio-Rad) with total voltage hours (Vh) of 51 000 for the pI 4–7 strips and 35 000 Vh for the pI 3–10 strips.

After isoelectric focusing, the strips were equilibrated at 20 °C for 15 min with 10 ml of equilibration buffer A (6 M urea, 20 % glycerol, 10 % SDS, 50 mM Tris-HCl pH 8.8, 65 mM DTT) and 15 min in 10 ml of buffer B (6 M urea, 20 % glycerol, 10 % SDS, 50 mM Tris-HCl pH 8.8, 135 mM IAA, and a trace of bromophenol blue).

Table 1. Identified glomerular proteins.

			Acc. no.			Peptides				Mowse	
CBB	**Ag**	**Name**	**SwissProt**	**pI**	**Mw**	**Searched**	**M**	**C (%)**	**Expect**	**Score**	**MS/MS**
	15	14–3-3 protein epsilon	P62259	4.63	29 326	24	13	47	6.3E-11	143	
15,123	16	14–3-3 protein zeta/delta	P63101	4.73	27 925	31	16	55	6.1E-13	164	
16,122	107	14–3-3 protein beta/alpha	Q9CQV8	4.77	28 052	19	7	40	0.00042	75	
	109	14–3-3 protein theta	P68254	4.69	28 046	28	12	28	0.00022	80	
	269	26S proteasome non-ATPase regulatory subunit 11	Q8BG32	6.09	47 561	27	8	19	0.0016	69	
	198	2-oxoglutarate dehydrogenase E1 component	Q60597	6.51	117 298	37	28	33	8.0E-25	282	
	272[1]	2-oxoisovalerate dehydrogenase alpha subu	P50136	6.05	45 674	47	8	28	0.028	56	
	134	3’(2’),5’-bisphosphate nucleotidase 1	Q9Z0S1	5.54	33 517	41	8	31	0.0056	64	
	99	3-hydroxyisobutyrate dehydrogenase	Q99L13	8.37	35 816	30	11	20	2.7E-5	87	
	163	40S ribosomal protein SA	P14206	4.74	32 681	20	7	21	0.022	58	
	206[1]	5’-nucleotidase precursor	Q61503	6.18	64 280	52	13	31	1.6E-5	89	YPFIVTADDGR
74	39, 40, 230	60 kDa heat shock protein	P63038	5.91	61 088	22	16	40	1.0E-15	191	
	133	60S acidic ribosomal protein P0	P14869	5.91	34 366	36	8	45	3.0E-5	86	
75, 115	56	78 kDa glucose-regulated protein precursor	P20029	5.07	72 492	25	22	37	2.7E-7	307	
	188	Acetyl-coenzyme A synthetase 2-like	Q99NB1	6.51	75 317	49	10	16	0.023	57	
39	25	Actin, cytoplasmic 1[2]	P60710	5.29	42 052	23	12	41	1.2E-9	130	
	122	Actin, cytoplasmic 2	P63260	5.31	42 108	23	7	23	0.016	56	
	284[1]	Actin-like protein 2	P61161	6.3	45 017	30	8	21	0.008	62	
	30	Actin-like protein 3	Q99JY9	5.61	47 783	27	20	45	1.0E-20	241	
	260	Actin-related protein 2/3 complex subunit 2	Q9CVB6	6.3	34 450	20	5	27	0.023	58	
	70	Actin-related protein 2/3 complex subunit 5	Q9CPW4	5.47	16 204	20	6	55	0.00071	72	
	141[3]	Acyl-coenzyme A thioesterase 2	Q9QYR9	6.14	44 934	19	11	32	3.2E-8	116	
	268	Adenosylhomocysteinase	P50247	6.08	48 039	31	13	29	2.5E-9	127	
	80	Adenylate kinase isoenzyme 1	Q9R0Y5	5.67	21 640	59	6	46	0.29	46	YGYTHLSTGDLLR
43, 110		Aldehyde dehydrogenase	P47738	7.53	57 017	19	13	31	4.2E-12	121	
	178	Aldehyde dehydrogenase family 7 member A1	Q9DBF1	5.99	55 935	40	10	26	4.5E-5	84	

Table 1 *(Continued)*

CBB	Ag	Name	Acc. no. SwissProt	pI	Mw	Peptides Searched	M	C (%)	Expect	Mowse Score	MS/MS
	36[3], 145[3]	Aldehyde dehydrogenase	P47738	6.05	54 375	25	12	37	1.0E-10	141	
	258	ADP-ribosylation factor 3	P61205	7.03	20 514	32	7	46	0.0015	69	
46	34,146	Alpha-enolase	P17182	6.36	47 322	59	13	23	1.1E-5	90	
	58,62,206	Alpha-actinin-4	P57780	5.25	105 368	53	31	36	9.9E-19	221	
	127	Alpha-soluble NSF attachment protein	Q9DB05	5.3	33 624	51	16	43	8.1E-11	142	
51, 105		Annexin A1	P10107	7.15	38 864	18	13	45	0.88	184	
101, 104		Annexin A2	P07356	7.53	38 806	20	13	36	5.3E-9	151	
37		Annexin A3	O35639	5.33	36 389	14	9	28	5.5E-9	125	
26	123	Annexin A4	P97429	5.43	36 121	10	8	30	1.7E-8	120	
41,129	23	Annexin A5 (Annexin V)	P48036	4.83	35 787	29	12	32	6.1E-8	114	
109		ATP synthase subunit alpha	Q03265	9.22	59 830	12	8	18	5.5E-7	96	
79	41	ATP synthase beta chain[2]	P56480	5.19	56 265	38	20	45	1.3E-14	180	
9,92	6	ATP synthase D chain	Q9DCX2	5.52	18 664	30	9	63	2.5E-6	97	
	270	Beta-centractin	Q8R5C5	5.98	42 369	22	8	28	1.4E-5	89	
	210	Calbindin	P12658	4.71	30 072	52	7	28	0.42	45	SFVDQYGQR
	85	Calcium-binding protein p22	P61022	4.97	22 287	15	8	56	5.0E-9	124	
28		Caldesmon	gi21704156[4]	6.97	60 531	15	15	25	1.1E-14	191	
1,88	236	Calmodulin (CaM)	P62204	4.09	16 696	14	4	31	0.06	53	
	166	Calnexin precursor	P35564	4.5	67 635	62	11	16	0.0095	61	
130	45	Calreticulin precursor	P14211	4.33	48 136	42	11	22	5.1E-5	84	
34	120	Chloride intracellular channel protein 1	Q9Z1Q5	5.09	27 207	15	9	39	0.00064	73	
40	101	Chloride intracellular channel protein 3	Q9D7P7	5.98	27 171	38	8	47	5.1E-5	84	APLDHELAQEPHLR
	106	Chloride intracellular channel protein 4	Q9QYB1	5.44	28 939	40	9	45	1.0E-4	84	
	104, 125	Chloride intracellular channel protein 5	Q8BXK9	5.64	28 440	26	10	56	6.3E-9	123	
	87	Chromobox protein homolog 1	P83917	4.85	21 519	34	6	39	0.0062	63	
96		Cofilin-1	P18760	8.26	18 645	13	6	49	1.5E-6	87	
	60[3]	Collagen alpha-1(VI)	Q04857	5.15	106 387	13	8	10	0.0002	78	VAVVQYSGQGQQQPGR
99		Col4a2 protein	gi113819968[4]	7.95	38 092	8	4	12	0.53	54	

Table 1 *(Continued)*

CBB	Ag	Name	Acc. no. SwissProt	pI	Mw	Peptides Searched	M	C (%)	Expect	Mowse Score	MS/MS
	14	Complement component 1	O35658	4.77	31 348	47	6	31	6.9	41	EVSFQATGDSEWR
	156[1]	Creatine kinase B-type	Q04447	5.4	42 971	43	10	41	1.0E-5	91	
47		Chain A, crystal structure of nidogen laminin	gi34810643[4]	7.26	30 168	14	8	32	2.2E-5	98	
	2	Cytochrome b5	P56395	4.96	15 100	34	5	48	0.029	57	
	13	Cytochrome c oxidase subunit 2	P00405	4.6	26 130	29	7	27	0.059	53	
	243	Cytochrome c1	Q9D0M3	9.24	35 533	30	9	36	0.00038	75	
	231	Cytosolic nonspecific dipeptidase	Q9D1A2	5.43	53 190	54	13	40	2.5E-7	107	
	100	Delta3,5-delta2,4-dienoyl-CoA isomerase	O35459	7.6	36 437	14	8	47	6.4E-6	93	YCTQDAFFQIK
	261	Delta-aminolevulinic acid dehydratase	P10518	6.32	36 456	27	9	23	6.3E-5	83	
	221	Desmin	P31001	5.37	42 229	31	6	17	2.3	38	FASEANGYQDNIAR
135	161, 222	Desmin	P31001	5.21	53 391	19	10	34	2.0E-8	118	FASEANGYQDNIAR
95		Destrin	Q9R0P5	8.2	18 721	14	5	34	0.43	58	
	52	Dlat protein	Q8R339	8.81	68 473	52	20	37	6.8E-11	151	
	37[3],182	Dihydrolipoyl dehydrogenase	O08749	6.43	50 183	35	11	28	1.6E-5	89	
	147[3], 150[3]	Dihydrolipoyllysine-residue succinyltransferase	Q9D2G2	5.98	41 470	20	10	22	0.0059	63	
49,55	176,235,254	Dihydropyrimidinase-related protein 2	O08553	5.95	62 531	51	18	43	5.0E-10	134	
	284[1]	DnaJ homolog subfamily B member 11 precursor	Q99KV1	5.92	40 815	30	12	31	1.3E-7	110	
	148	D-lactate dehydrogenase	Q7TNG8	6.15	52 499	25	9	26	0.00056	74	
	250	EH-domain containg protein 1	Q9WVK4	6.35	60 622	26	8	17	0.014	60	
53,63	175	EH-domain-containing protein 3[1]	Q9QXY6	6.03	60 888	54	16	33	2.8E-6	96	
	265	EH-domain-containing protein 4 (mPAST2)	Q9EQP2	6.33	61 670	20	13	26	1.3E-10	140	LIEAVDMNLTNK, EGADEEEWVVAK
4		Enhancer of rudimentary homolog	P84089	5.63	12 422	6	5	45	7.9E-5	82	
	135	Endoplasmic reticulum protein ERp29	P57759	5.9	28 862	50	9	37	0.049	67	
	55	Endoplasmin precursor	P08113	4.74	92 703	27	16	18	0.002	112	
	237	Eukaryotic translation initiation factor 5A-1	P63242	5.08	16 918	52	7	42	0.023	57	
	95	ETHE1 protein	Q9DCM0	6.78	28 234	29	7	37	0.002	76	
48,52,54	183	Ezrin[1]	P26040	5.83	69 347	26	15	23	1.6E-8	119	IGFPWSEIR
	128	F-actin capping protein alpha-1 subunit	P47753	5.34	33 090	20	8	40	8.1E-6	92	

Table 1 *(Continued)*

CBB	Ag	Name	Acc. no. SwissProt	pI	Mw	Peptides Searched	M	C (%)	Expect	Mowse Score	MS/MS
25, 32	22	F-actin capping protein alpha-2 subunit	P47754	5.58	32 987	15	12	65	7.9E-12	211	
126	103	F-actin capping protein beta subunit	P47757	5.47	31 480	22	6	19	0.0099	61	
	285	Ferritin light chain 1	P29391	5.66	20 715	56	7	56	0.0027	67	
	246,262	Fructose-1,6-bisphosphatase 1	Q9QXD6	6.18	37 157	25	12	46	3.2E-10	136	
72,73	201	Gelsolin precursor[1]	P13020	5.83	86 287	46	17	31	4.0E-9	125	
98		Glutathione S-transferase Mu 1	P10649	8.13	25 936	6	6	28	8.4E-7	106	
108		Glutamate dehydrogenase 1	P26443	8.05	61 640	9	6	12	0.017	70	
	61	Glutamyl aminopeptidase	P16406	5.28	108 401	19	13	16	5.1E-10	134	
	239	Glutathione peroxidase 1	P11352	6.74	22 553	22	8	46	1.6E-7	109	
	7[3],8[3], 77[3]	Glutathione peroxidase 3 (chain)	P46412	6.38	22 883	49	8	43	0.01	62	
	172	Glycerol-3-phosphate dehydrogenase	Q64521	6.17	81 362	51	20	30	1.3E-10	140	
31		Glyoxalase domain-containing protein 4	Q9CPV4	5.26	33 581	12	7	28	1.7E-7	109	
	92	Growth factor receptor-bound protein 2	Q60631	5.89	25 336	13	5	23	0.015	59	
	143	GTPase, IMAP family member 4	Q99JY3	5.94	38 362	34	16	40	6.8E-8	121	
	129	Guanine nucleotide-binding protein G(i), alpha-2 subun	P08752	5.28	40 884	15	8	27	2.0E-5	88	
	249	Guanine nucleotide-binding protein G(q) subunit alpha	P21279	5.58	41 742	36	10	36	0.043	88	
	247	Guanine nucleotide-binding protein alpha-11 subunit	P21278	5.7	42 189	23	6	25	0.056	54	
29,36,125		Guanine nucleotide-binding protein beta 1	P62874	5.6	38 020	17	10	26	0.095	98	
	130	Guanine nucleotide-binding protein beta subunit 2	P62880	5.6	38 048	18	7	39	0.0026	70	
35		Guanine nucleotide-binding protein beta 4	P29387	5.74	37 965	13	6	17	0.0006	74	
42		Heat shock 27 kDa	P14602	6.12	23 057	11	6	29	7.4E-6	92	
	273	Heat shock 70 kDa protein 4	Q61316	5.15	94 133	34	15	20	3.2E-7	106	
70,114	169, 208	Heat shock cognate 71 kDa protein	P63017	5.37	71 055	28	18	30	3.1E-13	166	
	171	Heat shock protein 75 kDa	Q9CQN1	6.25	80 501	54	15	28	8.5E-5	82	
	57	Heat shock protein HSP 90-beta	P11499	4.97	83 484	39	12	19	0.00087	221	
103		Heterogeneous nuclear ribonucleoproteins A2/B1	O88569	8.67	36 028	7	4	14	6.7E-9	53	
59		Heterogeneous nuclear ribonucleoprotein K	P61979	5.39	51 230	17	10	23	1.3E-8	120	
	90, 241	High mobility group protein B1	P63158	5.62	24 918	31	9	39	0.00042	75	

Table 1 *(Continued)*

CBB	Ag	Name	Acc. no. SwissProt	pI	Mw	Peptides Searched	M	C (%)	Expect	Mowse Score	MS/MS
	238	Histidine triad nucleotide-binding protein 1	P70349	6.39	13 751	16	4	66	0.0038	65	
33	44	Histone-binding protein RBBP4	Q60972	4.79	47 779	15	8	19	0.00012	94	YMPQNPCIIATK
	121	Hypothetical protein, 2700085E05Rik	Q9CY26	5.28	33 630	41	9	42	7.8E-5	90	
	257	Integrin alpha-3 heavy chain	Q62470	5.9	93 165	34	15	20	0.00014	106	
	59,286[3]	Integrin alpha-3 precursor	Q62470	5.9	113 454	21	16	15	8.1E-13	162	
5	73	Ionized calcium-binding adapter molecule 2	Q9EQX4	6.63	17 070	6	5	37	0.99	78	ANESSPKPAGPPPER
58	248	Isocitrate dehydrogenase [NAD] subunit alpha	Q9D6R2	6.27	40 069	28	8	25	0.043	117	
	131	Isocitrate dehydrogenase, Splice isoform 1	Q9D6R2	6.27	40 069	22	11	31	1.2E-8	121	
	132	Isocitrate dehydrogenase, Splice isoform 2	Q9D6R2	5.7	31 898	42	13	37	1.7E-7	117	
	139[3]	Isovaleryl-CoA dehydrogenase	Q9JHI5	6.29	42 971	41	12	35	3.2E-5	86	
	136	Lactamase, beta 2	Q99KR3	5.89	33 019	24	6	37	0.0078	70	
10	88	Lactoylglutathione lyase	Q9CPU0	5.25	20 836	40	7	32	0.007	63	
76,77,78	205, 207	Lamin-B1	P14733	5.11	66 842	52	29	42	1.0E-20	241	
	186, 264	Lamin-A/C	P48678	6.37	74 238	21	17	31	3.1E-16	197	
	173	Leukotriene A-4 hydrolase	P24527	5.98	69 473	41	14	30	6.3E-8	113	
	21	L-lactate dehydrogenase B chain	P16125	5.7	36 703	40	15	48	5.0E-11	144	
	203 ,251	Major vault protein (MVP)	Q9EQK5	5.43	96 047	64	12	21	0.034	56	
	67, 68	Membrane-associated progesterone receptor	O55022	4.57	21 664	28	5	26	0.045	55	FYGPEGPYGFAGR
	244	Metaxin-1	P47802	5.82	35 886	48	8	28	0.0045	64	
	275	Mitochondrial inner membrane protein (Mitofilin).	Q8CAQ8	6.18	84 247	45	14	22	6.5E-6	93	
	190	Moesin	P26041	6.24	67 708	47	21	29	1.6E-8	119	
2,3	1	Myosin light polypeptide 6	Q60605	4.56	16 788	17	10	60	0.0024	115	
6	64, 66	Myosin regulatory light chain 2[1]	Q9CQ19	4.8	19 767	37	14	63	4.0E-7	105	
51,68	97, 98	Na(+)/H(+) exchange regulatory cofactor NHE-RF2	Q9JHL1	7.23	37 712	30	10	32	1.6E-7	109	LIEVNGQNVEGLR
	82	NADH-ubiquinone oxidoreductase 23 kDa subunit	Q8K3J1	5.89	24 479	21	10	40	4.0E-8	115	GLGMTLSYLFR
	11	NADH-ubiquinone oxidoreductase 24 kDa subunit	Q9D6J6	7	27 640	35	7	35	0.017	59	
	19	NADH-ubiquinone oxidoreductase 30 kDa subunit	Q9DCT2	6.4	30 360	50	16	48	8.0E-15	182	
	33	NADH-ubiquinone oxidoreductase 49 kDa subunit	Q91WD5	6.52	52 991	26	12	28	9.9E-7	111	

Table 1 *(Continued)*

CBB	Ag	Name	Acc. no. SwissProt	pI	Mw	Peptides Searched	M	C (%)	Expect	Mowse Score	MS/MS
	167	NADH-ubiquinone oxidoreductase 75 kDa subunit	Q91VD9	5.51	80724	19	7	12	0.031	57	
	137[3]	NADH dehydrogenase 1 alpha subcomplex subunit 10	Q99LC3	5.96	36943	57	13	39	2.2E-5	96	
	69	NADH dehydrogenase 1 beta subcomplex subunit 8	Q9D6J5	6.15	21919	15	5	28	0.015	59	QYPYNNLYLER
	76[3]	Nephrin (Chain)	Q9QZS7	5.34	132733	47	8	11	2.7	38	LSCQSYNSVSAETQER
	196, 197	Nidogen-1 precursor	P10493	5.25	139387	50	20	20	1.5E-9	130	
	242	Nit protein 2	Q9JHW2	6.44	30825	17	6	30	0.0026	75	
93		Nucleoside diphosphate kinase B	Q01768	6.97	17466	7	4	31	0.0038	66	
	215 ,216	Nucleobindin-1 precursor	Q02819	4.99	53376	28	12	35	6.3E-10	133	
	267	Nucleoside diphosphate-linked moiety X motif 19	P11930	6.22	40799	22	7	20	0.00033	76	
	32	Ornithine aminotransferase	P29758	6.19	48723	25	18	49	1.0E-20	241	
	126	Pdhb protein	Q99LW9	5.63	35156	26	9	38	0.00048	82	
94		Peptidyl-prolyl cis-trans isomerase	P17742	7.88	18000	10	5	25	0.00015	81	
97		Peroxiredoxin-1	P35700	8.26	22390	11	5	20	2.1E-7	55	
11,128	84	Peroxiredoxin-2	Q61171	5.2	21936	18	5	26	0.092	65	
85	102	Peroxiredoxin-4	O08807	6.67	31261	24	7	29	9.3E-5	81	QITLNDLPVGR
	94	Peroxiredoxin-6	O08709	6.01	24794	50	5	35	0.01	61	VVFIFGPDKK
12	83	Phosphatidylethanolamine-binding protein	P70296	5.19	20857	29	7	68	5.5E-6	94	
21,127	18	Prohibitin	P67778	5.57	29859	17	5	25	0.015	59	
	187,189,284	Propionyl-CoA carboxylase alpha chain	Q91ZA3	6.83	80498	51	27	43	1.3E-19	230	
	181	Propionyl-CoA carboxylase beta chain	Q99MN9	7.18	58984	50	10	30	0.00047	74	FANPFPAAVR
	110	Proteasome subunit alpha type 5	Q9Z2U1	4.74	26565	21	6	30	0.016	59	
	96	Proteasome subunit alpha type 6	Q9QUM9	6.34	27811	27	7	49	4.0E-8	115	
	75	Protein DJ-1	Q99LX0	6.32	20236	16	6	37	0.056	54	
	46	Protein disulfide-isomerase	P09103	4.79	57507	45	16	34	7.8E-10	132	
	151	Protein disulfide-isomerase associated 3	Q99LF6	5.88	56678	34	8	19	0.037	64	
57,111,112	38,235[1]	Protein disulfide-isomerase A3	P27773	5.98	57042	43	12	28	0.00026	77	
	204	Protein disulfide-isomerase A4 precursor	P08003	5.13	72271	51	7	15	4.0	36	VSGYPTLK
	50	Protein disulfide-isomerase A6 precursor	Q922R8	5	48469	19	11	31	6.4E-11	143	

Table 1 *(Continued)*

CBB	Ag	Name	Acc. no. SwissProt	pI	Mw	Peptides Searched	M	C (%)	Expect	Mowse Score	MS/MS
	71	Protein mago nashi homolog	P61327	5.74	17210	38	5	35	0.032	56	
	112	PSD-95 alpha	Q6XVF4	4.95	28241	55	7	27	0.27	106	
	272[1]	Protein NDRG1	Q62433	5.69	43437	47	15	33	0.0013	70	LNITPNSGATGNNAGPK
	199, 200	Pyruvate carboxylase	Q05920	6.25	130344	26	22	25	5.0E-20	234	
107		Pyruvate dehydrogenase E1 component a subunit	P35486	8.49	43888	6	4	10	0.054	52	
	217	Rab GDP dissociation inhibitor alpha	P50396	4.96	51059	49	11	31	0.00029	76	
	149	Rab GDP dissociation inhibitor beta	Q61598	5.93	51018	54	14	42	3.9E-7	106	
69	191, 192	Radixin (ESP10)	P26043	5.85	68672	39	20	30	5.0E-12	154	
	78	Ras-related protein Rab-1A	P62821	5.93	22760	22	4	27	0.53	45	LQLWDTAGQER
	240	Ras-related protein Rab-11A	P62492	6.12	24492	21	7	37	1.4E-7	93	
	74	Ras-related protein Rap-1b precursor	Q99JI6	5.65	21040	28	6	26	0.0075	63	INVNEIFYDLVR
	81	Ras-related protein Rab-1B	Q9D1G1	5.55	22344	26	9	53	5.1E-7	104	
14	79	Ras-related protein Rab-11B	P46638	5.64	24589	23	10	46	1.8E-9	131	
	89	Ras-related protein R-Ras2	P62071	5.74	23613	26	6	33	0.0066	63	
	17	Renin-1 precursor	P06281	6.71	44714	29	6	17	0.05	54	EEVFSVYYNR
	115	Renin-1 precursor	P06281	5.74	36012	16	3	9	37	26	EEVFSVYYNR
	144	Renin-1	P06281	5.82	44343	24	6	13	0.11	59	
13,124	12	Rho GDP-dissociation inhibitor 1	Q99PT1	5.12	23450	9	7	33	4.2E-5	85	
	91	Rho-related GTP-binding protein RhoC	Q62159	6.2	22334	57	7	42	0.012	60	
	214	Ribonuclease/angiogenin inhibitor 1	Q91VI7	4.69	49816	25	8	26	0.00026	85	
	179	RuvB-like 1	P60122	6.02	50524	61	10	31	0.0056	64	
	232	RuvB-like 2	Q9WTM5	5.49	51121	53	10	24	0.0022	68	
	233, 256	Selenium-binding protein 2	Q63836	5.78	53165	53	12	33	9.3E-6	91	
	259	Sepiapterin reductase	Q64105	5.58	28208	38	8	34	0.025	57	
44		Septin 2	P42208	6.1	41727	10	7	23	0.00012	95	
	180	Septin-11	Q8C1B7	6.38	49088	27	9	32	4.6E-6	94	
	47	Serine/threonine-protein phosphatase 2A	Q76MZ3	5	65948	38	18	34	1.6E-11	149	
65	280	Serine/threonine-protein phosphatase PP1-alpha	P62137	5.94	38257	38	13	46	6.4E-10	133	

Table 1 *(Continued)*

CBB	Ag	Name	Acc. no. SwissProt	pI	Mw	Peptides Searched	M	C (%)	Expect	Mowse Score	MS/MS
59	245	Serine/threonine-protein phosphatase PP1-beta	P62141	5.84	37 961	34	9	24	2.5E-5	88	
	252	Serum albumin precursor	P07724	5.75	70 700	39	15	31	1.3E-9	130	
	140[3]	Short-chain specific acyl-CoA dehydrogenase	Q07417	7.12	42 231	26	15	51	5.5E-6	180	
	271	Short/branched chain specific acyl-CoA dehydrogenase	Q9DBL1	8	48 300	29	9	29	2.0E-5	88	YYASEVAGLTTSK
	263	Short-chain specific acyl-CoA dehydrogenase	Q07417	8.96	45 203	31	9	29	0.00013	80	LYEIGAGTSAVR, YYASVIGLTTSK
	229	Splicing factor 3A subunit 3	Q9D554	5.23	59 147	46	12	29	2.9E-6	96	
	276	Spectrin alpha chain, brain	P16546	5.17	98 017	49	25	33	5.4E-16	203	
71,113	168	Stress-70 protein, mitochondrial precursor	P38647	5.91	73 768	43	16	31	6.2E-8	113	
	184[3]	Succinate dehydrogenase	Q8K2B3	6.32	68 032	25	8	16	0.0031	66	
	29	Succinyl-CoA ligase beta-chain	Q9Z2I8	5.75	44 028	27	14	30	3.1E-10	137	
	153	Sulfite oxidase	Q8R086	5.69	54 413	30	6	23	0.063	53	
	72	Superoxide dismutase	P08228	6.03	15 973	21	7	43	1.3E-6	100	
	255	T-complex protein 1 subunit alpha A	P11984	5.76	60 930	30	10	21	5.5E-5	84	
50	177	T-complex protein 1 subunit beta	P80314	5.98	57 652	41	12	32	3.1E-6	96	
74	152	T-complex protein 1 subunit epsilon	P80316	5.72	60 042	24	11	29	4.6E-6	94	
	266	T-complex protein 1 subunit gamma	P80318	6.28	61 162	29	10	19	0.00032	76	
	65	Thioredoxin	P10639	4.8	11 879	15	4	32	0.017	60	
	225	Thioredoxin domain-containing protein 4	Q9D1Q6	5.09	47 222	50	10	33	0.00016	79	
	226	Thioredoxin domain-containing protein 5	Q91W90	5.51	47 070	27	7	21	0.0071	62	FFDVGSNK
	93	Thioredoxin-dependent peroxide reductase	P20108	5.73	28 127	15	4	21	0.25	47	HLSVNDLPVGR
	10	Thioredoxin-dependent peroxide reductase	P20108	7.15	28 337	23	6	28	0.0014	70	
	118	Toll-interacting protein	Q9QZ06	5.04	30 345	15	3	16	10	32	GPVYIGELPQDFLR
	281, 283	Plastin-3 (T-plastin)	Q99K51	5.54	70 835	50	16	30	0.034	111	
45		Transcriptional activator protein Pur-alpha	P42669	6.07	34 976	10	5	14	0.011	61	
	9	Transforming protein RhoA	Q9QUI0	5.83	22 110	33	14	56	4.0E-10	64	
	86	Translationally-controlled tumor protein	P63028	4.76	19 564	16	6	37	0.0041	65	DLISHDELFSDIYK
80, 81		Tropomodulin-3	Q9JHJ0	5.02	39 592	8	7	20	7.7E-5	111	
19	113, 281	Tropomyosin 1 alpha chain[2]	P58771	4.69	32 718	46	9	33	0.0051	64	
18,136	116	Tropomyosin alpha-3 chain	P21107	4.75	29 100	30	10	22	3.2E-7	106	

Table 1 *(Continued)*

CBB	Ag	Name	Acc. no. SwissProt	pI	Mw	Peptides Searched	M	C (%)	Expect	Mowse Score	MS/MS
84	111	Tropomyosin alpha-4 chain[2]	Q6IRU2	4.65	28 433	17	5	17	0.042	55	
22	24, 108	Tropomyosin beta chain	P58774	4.63	32 995	34	9	30	0.00072	72	KLVILEGELER
64	35[3]	Tu translation elongation factor	Q8BFR5	6.2	44 971	50	18	51	3.9E-12	156	
	48	Tubulin alpha-6 chain	P68373	4.96	50 562	19	7	19	0.0024	55	
	223	Tubulin beta 2	Q9DCR1	4.78	34 146	46	16	42	1.7E-7	117	FPGQLNADLR
67, 131		Tubulin beta-5 chain	P99024	4.78	50 095	10	8	15	8.6E-5	113	
	234	Tubulointerstitial nephritis antigen-like	Q99JR5	6.31	53 998	33	6	18	1.2	40	VSDNCYPFSGR
	4	Ubiquitin	P62991	6.56	8560	19	8	43	0.044	55	TLSDYNIQ
66	28,156[1],157	Ubiquinol-cytochrome-c reductase, core protein I	Q9CZ13	5.75	53 420	26	13	37	5.0E-10	134	
	209	Vacuolar ATP synthase catalytic subunit A	P50516	5.62	68 567	31	12	22	4.7E-6	94	
	154, 282	Vacuolar ATP synthase subunit B, brain isoform	P62814	5.57	56 857	56	22	51	1.3E-16	200	
24	164	Vimentin[2]	P20152	5.06	53 581	36	24	53	6.3E-20	233	
	63, 274	Vinculin	Q64727	5.77	117 083	26	18	20	2.0E-11	148	
	258	WD-repeat protein 1 (Actin-interacting protein 1)	O88342	6.12	66 918	29	12	25	2.5E-8	117	

Spot numbers refer to the gel spots in Figure 2 and accession numbers to the SwissProt and NCBI databases. In the columns marked Peptides, 'searched', indicates the number of determined peptide masses searched against the databases; M, the number of matched peptides, and C (in %), the covered sequence of the protein identified. The columns Expect and Mowse score give values from the search engine (Mascot) and indicate the expected chance occurrence against the database (with E meaning 10 raised to the power shown) and the significance of the match, respectively. The MS/MS column shows the sequence obtained from MS/MS analyses performed when the mass scores were considered insufficient.
Underlining in the first two columns (staining method) indicates the spot (in the case of several) that gives the best peptide coverage, with the values as given.
[1] Mixture of two proteins.
[2] Additional identifications were obtained for actin, cytoplasmic, in spots 60, 61, 62, 82 (CBB) and 26, 27, 31, 105, 114, 117, 124, 142, 158, 159, 160, 162, 277 (Ag), ATP synthase beta chain in spots 42, 43, 51, 119, 218, 228, 278, 279 (Ag), EH-domain-containing protein 3 in spots 53, 174, 185 (Ag), ezrin in spots 193, 194, 195, 253 (Ag), gelsolin in spots 54, 170, 201, 202, myosin regulatory light chain 2 in spots 7, 8, 89, 90, 91, 118, 119, 120 (CBB), tropomyosin 1 alpha chain in spots 23, 84, 116, 121 (CBB), tropomyosin alpha-4 chain in spots 116, 117, 137 (CBB), vimentin in spots 27, 30, 86, 87, 132, 133 (CBB) and 46, 165, 211, 212, 213, 219, 220, 224, 227 (Ag).
[3] Calculated pI/Mw.
[4] NCBI database.

The second dimension was performed on 8–16% gradient SDS/PAGE gels, run at 5 W per gel for 1 h and thereafter at 17 W per gel in an Ettan Dalt Six instrument (Amersham Biosciences). Silver staining was according to the PlusOne silver stain protocol (Amersham Biosciences). Coomassie blue gels were fixed in 30% ethanol, 2% phosphoric acid for 12 h, and then stained with 0.01% colloidal CBB in 10% ethanol, 2% phosphoric acid, 5% aluminium sulfate. Molecular masses were determined by comparisons with positions of standard protein markers (Invitrogen).

All gels were scanned with the S710 scanner from Biorad. 2-D gel image analysis was performed using PDQuest 2-D image analysis software (BioRad). Spot detection wizard was used to detect the protein spots.

In-gel digestion. Protein spots were excised manually from both the CBB-stained and the silver-stained gels. For mass spectrometrical analysis of the silver-stained spots, correlating spots from three gels were pooled (in a few cases with weak spots, further pooling was attempted, and single identifications were obtained after pooling of spots from six gels). Gel pieces were cut into small pieces and digested in a MassPrep Station (Micromass/Waters, Manchester, UK) with a protocol based on an in-gel digestion method described elsewhere [11]. Gel pieces were destained twice with 100 µl 50 mM ammonium bicarbonate/50% v/v acetonitrile at 40 °C for 10 min each. Proteins were reduced with 10 mM DTT in 100 mM ammonium bicarbonate for 30 min, dehydrated in acetonitrile and alkylated with 55 mM iodoacetamide in 100 mM ammonium bicarbonate for 20 min. Each protein spot was digested with 300 ng trypsin (Promega, Madison, WI) in 50 mM ammonium bicarbonate and incubation for 4.5 h at 40 °C. Peptides were first extracted with 30 µl 5% formic acid/2% acetonitrile, followed by an extraction with 25 µl 2.5% formic acid/50% acetonitrile. Acetonitrile was evaporated at atmospheric pressure before desalting and mass spectrometry.

Sample preparation before mass spectrometry. Prior to mass spectrometry, the Coomassie-stained samples were desalted with an automated CD-based Gyrolab MALDI SP1 Workstation (Gyros AB, Uppsala, Sweden) as described elsewhere [12] or in a conventional manner manually with C_{18} ZipTips, while the silver-stained samples were desalted manually with an improved method reducing matrix adduct ions [13]. For desalting with the Gyrolab Workstation, the CD reverse-phase column was conditioned with 50% acetonitrile, 1 µl of peptide sample solution was applied, washed with 200 nl 0.1% TFA, and eluted with 200 nl 50% acetonitrile/0.1% TFA containing 1 mg/ml α-cyano-4-hydroxycinnamic acid matrix onto a MALDI target area. For the conventional manual desalting, C18 ZipTips were first activated with 70% acetonitrile/0.1% TFA and equilibrated with 0.1% TFA. Peptide samples were then bound to the resin by pipetting 10 µl peptide solution 30 times. The resin was washed with 3 × 10 µl 0.1% TFA, and peptides were eluted with 5 µl 75% acetonitrile/0.1% TFA. One microliter of eluted sample was mixed 1:1 directly on the target plate with saturated matrix in 50% MeOH/0.1% TFA. For reduced matrix adduct formation, samples from the ZipTip column were mixed 1:1 with a matrix solution also containing ammonium monobasic phosphate [13] (5 mg α-cyano-4-hydroxycinnamic acid in 500 µl 50% aqueous MeOH and 500 µl 50 mM $NH_4H_2PO_4$) and spotted directly onto the target plate.

Mass spectrometry. Mass fingerprinting of tryptic digests was carried out with a MALDI-Tof Voyager DE-PRO workstation (Applied Biosystems) for the CBB-stained gels and a MALDI Q-Tof Ultima (Waters Inc) for the silver-stained gels. Both instruments were operated in the single reflector mode and with positive ion data. In MALDI Voyager, each spectrum was calibrated either internally against known trypsin fragments or externally with a standard calibrant mixture (Applied Biosystems). The Voyager software created a peak list which was searched against protein databases. The MALDI Ultima instrument was calibrated with a polyethylene glycol standard between 80–2500 m/z. MassLynx Version 3.4 was used to create a peak list. MaxEnt3 [min. mol. mass 800 Da and max. mol. mass 3000 Da, max number of charges: 1, peak width (Da): 0.1] was applied for each list before submitting into the Mascot search engine.

The MALDI Q-Tof Ultima was also used for CID fragmentation to confirm the sequence of peptides of interest. A second spot on the target plate for the sample was then used. The precursor for the peptide of interest was selected and the collision energy was altered between 60–90 eV. The data were acquired for 10 min with a scan rate of 5 s. Amino acid sequences of relevant peptide samples were also determined using a Micromass LC-API Q-Tof Ultima tandem mass spectrometer with an orthogonal sampling ES-interface (z-spray; Micromass). Those samples were desalted using an Atlantis dC18 5-µm nanoease Trap column (Waters) with 5% acetonitrile/0.1% formic acid for 3 min and separated on an Atlantis dC18 column (3 µm, 100 Å, 75 µm ID × 15 cm) with the solvent system 5% acetonitrile/0.1% formic acid (solvent 1) and 95% acetonitrile/0.1% formic acid (solvent 2) in a gradient (5–

80 % solvent 2 for 42 min at 200 nl/min). For the electrospray, a PicoTip EMITTER (SilicaTip; New Objective, Woburn, Mass.) was used. The sample aerosol was desolvated in a stream of nitrogen. Argon was used as the collision gas at 5.5×10^{-5} mbar. Data-dependent acquisition (DDA) was used at 300–1600 m/z in ms mode and 50–1600 in ms/ms mode with a scan rate of 1 s and automatic switching from ms to ms/ms mode on multiply charged ions. The data analyses used PLGS 2.2.3 (ProteinLynx Global Server 2.2.3; Waters) software to generate a pkl-file for protein data searches.

The data were searced using the Mascot search engine by Matrix Science [Mascot Server (www.matrixcience.com), Windows version 2.1] with the following parameters: Database, SwissProt or NCBInr; Taxonomy, Mus musculus; Enzyme, trypsin; Fixed Modification: Carbamidomethyl (C); Variable Modifications, Oxidation (M); Peptide tol, ± 0.10 Da with up to 1 missed cleavage; Mass Value, MH+. In the MALDI experiments, four or more matched peptides, an expected value ≤ 0.05, and a Mowse score ≥ 53 were required for direct identification. MS/MS experiments were performed to confirm identifications with insufficient values, scores, or pI and mass agreements, and a sequence of at least eight residues was then required for consideration as a match.

Results

Kidney glomeruli were isolated from approximately 50 mice. After the isolation, the purity and number of glomeruli were checked under a light microscope (Fig. 1). The results revealed that the number of glomeruli obtained from one adult mouse was approximately 20 000, and as seen from the figure, the glomeruli were highly pure and devoid of Bowman's capsule. The total amount of protein extracted from the glomeruli collected from 50 mice was approximately 5 mg.

Total glomerular, denatured, water-soluble protein extracts were subjected to 2-D gel electrophoresis as described before. In the pI 4–7 and 3–10 CBB-stained gels 625 and 342 and in the pI 4–7 silver-stained gel 922 spots were detectable. From the CBB-stained gel with pI 4–7 and pI 3–10, 192 and 96 protein spots, respectively, and 480 spots from the silver-stained gel were excised for further mass spectrometrical analysis. Each excised spot was analyzed individually first by MALDI-TOF MS for identification according to the tryptic peptide masses, using a minimum of four matching peptides, a coverage of 12 % of the protein, minimal scores and maximal expected values as given (see Materials and methods, and Table 1) for approval of the identity assignment. In this manner, 414 protein spots were identified (Table 1). Relevant protein spot identifications were further checked by amino acid sequence analysis upon LC-MS/MS as also shown in Table 1.

The proteins identified were found to belong to at least 12 different groups according to the functional categories of the SwissProt database (www.ebi.ac.uk/uniprot/database/download.html). However, classification of proteins is not simple and many of the proteins fall into several categories. Nevertheless, 27 % are common cellular metabolic enzymes, 22 % take part in cell signalling, 12 % are structural proteins and 13 % are protein-folding and protein metabolic enzymes, while 5 % of the identified proteins did not have an annotated function. Most of the identifications concern housekeeping proteins and are therefore ubiquitous, while many are cytoskeletal structural proteins (actin, F-actin, vimentin, α-tubulin, β-tubulin) in the podocytes, consistent with the report of Yoshida et al. [4]. However, we were able to identify α-actinin-4 and nephrin, which play a role in the glomerular filtration barrier. Actinin-4 is not itself located in the glomerular slit but is an actin filament cross-linker and therefore has a significant role in the maintenance of podocyte integrity. Mutation in the *ACTN4* gene has been shown to cause familial focal segmental glomerulosclerosis [14]. Previous 2-D gel electrophoresis studies of the kidney glomeruli have not been able to identify any of the kidney podocyte slit diaphragm proteins. This is probably due to their low abundancy and hydrophobicity. We could identify nephrin, which is a large 134.7-kDa transmembrane protein located in the glomerular slit diaphragm, has been proposed to anchor the slit diaphragm to the actin cytoskeleton and has a role in the Finnish-type congenital nephritic syndrome [15].

Discussion

This study represents a proteome analysis of isolated mammalian glomeruli. The glomeruli were prepared from 50 C57BL/6 female mice and contributed a total of 5 mg of extracted proteins. This material was enough for optimization of the sample preparation method and for the analysis of 768 protein spots from two Coomassie- and three silver-stained gels. The whole protein extract was analyzed in direct succession without pre-fractionation. In particular, the many identifications after silver staining, derived from pooling of spots from more gels than the Coomassie staining and using a method for reducing matrix adduct ions, are noticeable and contribute to the large number of identifications.

Table 2. Comparative analysis of the present identifications and those in other proteome expression studies.

Protein	SwissProt	Reference			cDNA library
		3,4	6	7	
14-3-3 protein beta/alpha	Q9CQV8				2
14-3-3 protein epsilon	P62259				3
14-3-3 protein theta	P68254				1
14-3-3 protein zeta/delta	P63101				6
26S proteasome non-ATPase regulatory subunit 11	Q8BG32				1
2-oxoglutarate dehydrogenase E1 component	Q60597				2
2-oxoisovalerate dehydrogenase alpha subu	P50136				2
3'(2'),5'-bisphosphate nucleotidase 1	Q9Z0S1				1
40S ribosomal protein SA	P14206				15
5'-nucleotidase precursor	Q61503				1
60 kDa heat shock protein	P63038	x	x	x	1
60S acidic ribosomal protein P0	P14869		x		
78 kDa glucose-regulated protein precursor	P20029	x	x	x	5
Actin, cytoplasmic 1	P60710	x	x	x	49
Actin, cytoplasmic 2	P63260				13
Actin-like protein 2	P61161				2
Actin-like protein 3	Q99JY9				3
Actin-related protein 2/3 complex subunit 2	Q9CVB6				3
Actin-related protein 2/3 complex subunit 5	Q9CPW4	x			5
Adenosylhomocysteinase	P50247				1
Adenylate kinase isoenzyme 1	Q9R0Y5				2
Aldehyde dehydrogenase	P47738	x	x		1
Alpha-actinin-4	P57780	x		x	13
Alpha-enolase	P17182		x	x	
Alpha-soluble NSF attachment protein	Q9DB05				2
Annexin A1	P10107	x	x	x	
Annexin A2	P07356	x		x	4
Annexin A3	O35639		x	x	6
Annexin A4	P97429		x	x	2
Annexin A5 (Annexin V)	P48036	x	x		3
ATP synthase beta chain	P56480	x	x		10
ATP synthase D chain	Q9DCX2	x	x	x	2
ATP synthase subunit alpha	Q03265	x			6
Beta-centractin	Q8R5C5				4
Calcium-binding protein p22	P61022	x			4
Calmodulin (CaM)	P62204				9
Calnexin precursor	P35564				4
Calreticulin precursor	P14211	x			4
Chloride intracellular channel protein 1	Q9Z1Q5	x	x		
Chloride intracellular channel protein 3	Q9D7P7				1
Chloride intracellular channel protein 4	Q9QYB1			x	2
Chloride intracellular channel protein 5	Q8BXK9				13
Chromobox protein homolog 1	P83917				1
Cofilin-1	P18760	x		x	4
Collagen alpha-1(VI)	Q04857	x			1

Table 2 *(Continued)*

Protein	SwissProt	Reference			cDNA library
		3,4	6	7	
Complement component 1	O35658				1
Creatine kinase B-type	Q04447				4
Cytochrome b5	P56395	x			
Cytochrome c1	Q9D0M3				2
Cytosolic nonspecific dipeptidase	Q9D1A2				2
Delta3,5-delta2,4-dienoyl-CoA isomerase	O35459				1
Delta-aminolevulinic acid dehydratase	P10518				4
Desmin	P31001			x	1
Destrin	Q9R0P5			x	3
Dihydrolipoyllysine-residue succinyltransferase	Q9D2G2				1
Dihydropyrimidinase-related protein 2	O08553	x	x		3
Dlat protein	Q8R339				1
DnaJ homolog subfamily B member 11 precursor	Q99KV1		x		1
EH-domain containg protein 1	Q9WVK4				3
EH-domain-containing protein 3	Q9QXY6	x			13
EH-domain-containing protein 4 (mPAST2)	Q9EQP2				2
Endoplasmic reticulum protein ERp29	P57759		x		2
Endoplasmin precursor	P08113	x	x	x	5
ETHE1 protein	Q9DCM0				1
Eukaryotic translation initiation factor 5A-1	P63242		x		1
Ezrin	P26040	x			3
F-actin capping protein alpha-1 subunit	P47753		x		
F-actin capping protein alpha-2 subunit	P47754	x			1
F-actin capping protein beta subunit	P47757				3
Ferritin light chain 1	P29391			x	
Glutamate dehydrogenase 1	P26443	x			3
Glutamyl aminopeptidase	P16406				17
Glutathione peroxidase 3 (chain)	P46412				7
Glutathione S-transferase Mu 1	P10649				1
Glycerol-3-phosphate dehydrogenase	Q64521				2
Growth factor receptor-bound protein 2	Q60631				1
GTPase, IMAP family member 4	Q99JY3				6
Guanine nucleotide-binding protein beta 1	P62874		x	x	3
Guanine nucleotide-binding protein beta 4	P29387	x			2
Guanine nucleotide-binding protein beta subunit 2	P62880		x		6
Guanine nucleotide-binding protein G(i), alpha-2 subunit	P08752		x		6
Guanine nucleotide-binding protein G(q) subunit alpha	P21279				2
Heat shock 27 kDa	P14602	x	x	x	
Heat shock 70 kDa protein 4	Q61316		x		1
Heat shock cognate 71 kDa protein	P63017		x		20
Heat shock protein 75 kDa	Q9CQN1				2
Heat shock protein HSP 90-beta	P11499				14
Heterogeneous nuclear ribonucleoprotein K	P61979		x		
Heterogeneous nuclear ribonucleoproteins A2/B1	O88569	x			2
High mobility group protein B1	P63158				4

Table 2 *(Continued)*

Protein	SwissProt	Reference			cDNA library
		3,4	6	7	
Histone-binding protein RBBP4	Q60972				2
Hypothetical protein, 2700085E05Rik	Q9CY26				1
Integrin alpha-3 heavy chain	Q62470				1
Ionized calcium-binding adapter molecule 2	Q9EQX4				2
Isocitrate dehydrogenase [NAD] subunit alpha	Q9D6R2			x	
Lactoylglutathione lyase	Q9CPU0				2
Lamin-B1	P14733		x		
Leukotriene A-4 hydrolase	P24527				2
L-lactate dehydrogenase B chain	P16125	x	x		2
Mitochondrial inner membrane protein (Mitofilin).	Q8CAQ8		x	x	1
Moesin	P26041	x	x		
Myosin light polypeptide 6	Q60605	x			
Na(+)/H(+) exchange regulatory cofactor NHE-RF2	Q9JHL1				4
NADH dehydrogenase 1 alpha subcomplex subunit 10	Q99LC3				2
NADH-ubiquinone oxidoreductase 24 kDa subunit	Q9D6J6				1
NADH-ubiquinone oxidoreductase 30 kDa subunit	Q9DCT2	x			1
NADH-ubiquinone oxidoreductase 49 kDa subunit	Q91WD5				1
NADH-ubiquinone oxidoreductase 75 kDa subunit	Q91VD9		x		2
Nephrin (Chain)	Q9QZS7				11
Nidogen-1 precursor	P10493				5
Nucleobindin-1 precursor	Q02819				6
Nucleoside diphosphate kinase B	Q01768			x	
Ornithine aminotransferase	P29758				3
Pdhb protein	Q99LW9				2
Peptidyl-prolyl cis-trans isomerase	P17742	x		x	
Peroxiredoxin-1	P35700	x			
Peroxiredoxin-2	Q61171	x	x	x	5
Peroxiredoxin-4	O08807		x		1
Peroxiredoxin-6	O08709	x	x	x	
Phosphatidylethanolamine-binding protein	P70296			x	2
Plastin-3 (T-plastin)	Q99K51				1
Prohibitin	P67778	x	x		1
Propionyl-CoA carboxylase alpha chain	Q91ZA3				1
Propionyl-CoA carboxylase beta chain	Q99MN9				1
Proteasome subunit alpha type 6	Q9QUM9			x	4
Protein disulfide-isomerase	P09103	x	x	x	5
Protein disulfide-isomerase A3	P27773	x	x	x	5
Protein disulfide-isomerase A4 precursor	P08003		x		
Protein disulfide-isomerase A6 precursor	Q922R8	x		x	2
Protein DJ-1	Q99LX0				4
Protein mago nashi homolog	P61327				1
Protein NDRG1	Q62433				6
Pyruvate dehydrogenase E1 component a subunit	P35486				4
Rab GDP dissociation inhibitor alpha	P50396			x	2
Radixin (ESP10)	P26043				7

Table 2 *(Continued)*

Protein	SwissProt	Reference			cDNA library
		3,4	6	7	
Ras-related protein Rab-11A	P62492		x		4
Ras-related protein Rab-1A	P62821				2
Ras-related protein Rap-1b precursor	Q99JI6	x			7
Rho GDP-dissociation inhibitor 1	Q99PT1		x	x	3
Ribonuclease/angiogenin inhibitor 1	Q91VI7			x	2
RuvB-like 1	P60122	x			2
Septin 2	P42208	x	x		1
Septin-11	Q8C1B7				2
Serine/threonine-protein phosphatase 2A	Q76MZ3				2
Serine/threonine-protein phosphatase PP1-beta	P62141				2
Short/branched chain specific acyl-CoA dehydrogenase	Q9DBL1				1
Short-chain specific acyl-CoA dehydrogenase	Q07417				1
Spectrin alpha chain, brain	P16546	x			5
Splicing factor 3A subunit 3	Q9D554				1
Stress-70 protein, mitochondrial precursor	P38647		x	x	3
Succinate dehydrogenase	Q8K2B3				3
Succinyl-CoA ligase beta-chain	Q9Z2I8				1
Superoxide dismutase	P08228	x	x	x	
T-complex protein 1 subunit beta	P80314	x			
T-complex protein 1 subunit epsilon	P80316		x		7
Thioredoxin domain-containing protein 4	Q9D1Q6				2
Thioredoxin domain-containing protein 5	Q91W90		x		2
Thioredoxin-dependent peroxide reductase	P20108	x	x		2
Toll-interacting protein	Q9QZ06				1
Transforming protein RhoA	Q9QUI0				3
Translationally-controlled tumor protein	P63028		x	x	15
Tropomodulin-3	Q9JHJ0	x			2
Tropomyosin 1 alpha chain	P58771			x	3
Tropomyosin alpha-3 chain	P21107	x			1
Tropomyosin alpha-4 chain	Q6IRU2	x	x	x	2
Tropomyosin beta chain	P58774	x		x	1
Tu translation elongation factor	Q8BFR5	x		x	
Tubulin alpha-6 chain	P68373	x			1
Tubulin beta 2	Q9DCR1				5
Tubulin beta-5 chain	P99024				5
Tubulointerstitial nephritis antigen-like	Q99JR5				2
Ubiquinol-cytochrome-c reductase, core protein I	Q9CZ13		x		7
Vimentin	P20152	x	x	x	11
Vinculin	Q64727			x	
	Sum:	53	50	41	152

Left column, those of the presently identified proteins in the mouse glomerular proteome that were also detected in at least one of the other studies; second column, SwissProt accession numbers; subsequent three columns: x mark identifications also in a human glomerular proteome study [3, 4], a human endothelial proteome analysis [6] and a human mesangial proteome analysis [7]. The last column shows the number of transcripts found in a mouse cDNA library [8].

Of the excised spots, 414 could be identified which represents 232 different proteins. Unexpectedly, only 53 of these were detected in another glomerular proteome study [4]. This low fit could be due to many factors, including the presently extended identifications from silver staining (now 31 % of the identifications uniquely found with that staining) emphasizing comparatively low-abundant forms, the use here of several identification methods and the quality of our tissue preparations (Fig. 1), but shows, nevertheless, the value of repeated proteome analyses using different methodologies. However, almost the same number of the present proteins (50 forms) were also identified in a similar proteome analysis of endothelial cells [6], revealing distinct similarities between these tissues, as expected. Similarly, somewhat fewer of the present proteins (42 forms) were identified in a study of mesangial proteins [7]. The coinciding identifications are shown in Table 2. These values are compatible with the fact that the proteome studies detect many of the housekeeping proteins shared by similar tissues.

It is also of interest to compare the results of these direct proteome studies with those of separate transcriptome analyses. Thus, an independent glomerular-specific cDNA library analysis [8] identified many of these proteins as expected, but not all. In fact, 80 of the present identifications were not reported in the cDNA analysis (Table 2), showing that multiple approaches are valuable to complement each other in final interpretations of functional relationships. Notably, only 10 proteins were identified in all of the five separate analyses performed (Table 2). They are well-established in many cells and constitute the 60-kDa heat shock protein, the 78-kDa glucose-regulated protein, cytoplasmic actin, an ATP synthase, endoplasmin, a peroxiredoxin, two protein disulfide isomerases, tropomysosin and vimentin. Combined, the results show that the separate analyses complement each other in giving several types of data on normal glomerular protein patterns (including these found only in this study; 62 enzymes, 28 structural proteins, 30 heat shock proteins, 9 elongation and initiating factors, and 12 proteins of unknown function), now accessible to analysis of changes in renal disease states.

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