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Differential expression of proteins in renal cortex and medulla: A proteomic approach[1]

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Differential expression of proteins in renal cortex and medulla: A proteomic approach.

Background. Western blotting has previously been used to identify changes in protein expression in renal tissue. However, only a few proteins can be studied in each experiment by Western blot. We have used proteomic tools to construct protein maps of rat kidney cortex and medulla.

Methods. Expression of proteins was determined by silver stain after two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Protein spots were excised and digested with trypsin. Peptide masses were identified by MALDI-TOF mass spectrometry. The Mascot search engine was used to analyze the peptide masses and identify the proteins.

Results. Seventy-two proteins were identified (54 unique proteins) out of approximately 1000 spots visualized on each gel. Most of the spots were expressed both in cortex and medulla. Of the identified proteins, three were expressed only in medulla and one only in cortex. Nine proteins were expressed in both regions but to a greater extent in cortex and three proteins were expressed more in medulla. Differential expression was confirmed for three proteins by Western blot.

Conclusions. A large group of proteins and their relative expression levels from cortical and medullary portions of rat kidneys were found. Sixteen proteins are differentially expressed. Proteomics can be used to identify differential expression of proteins in the kidney on a large scale. Proteomics should be useful to detect changes in renal protein expression in response to a large range of physiological and pathophysiological stimuli.

Renal cortex and medulla serve different functions. Structures in the cortex filter fluid and electrolytes from proteins and formed elements, reabsorb glucose, amino acids, water and electrolytes, and produce hormones that modulate blood pressure, hematopoiesis and calcium homeostasis. The medulla is largely responsible for concentrating urine and as a consequence medullary cells must survive in an environment where the osmolality is much higher than the rest of the body. Since specific functions of cortex and medulla are mediated by proteins, it would be expected that protein expression would differ between cortex and medulla. Techniques such as Western blotting are able to identify differences in protein expression, but these techniques are limited to identification of a single protein at a time and are only available for proteins for which antibodies exist. Recently, methods have become available that allow comparison of expression of many proteins simultaneously. In 1975 O'Farrell developed a technique for resolution of proteins using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) [1]. Proteins are separated using isoelectric focusing in the first dimension and polyacrylamide gel electrophoresis in the second dimension, resulting in a unique spot for each protein that differs in molecular size and/or pI. Using this technique O'Farrell was able to resolve 1100 different components from *Escherichia coli*. More recently, technical modifications including large format gels, complex ampholyte sets, use of ready-made gel solutions and improvements in staining have resulted in resolution of as many as 10,000 polypeptide spots [2]. The analysis of separated proteins by mass spectrometry has permitted analysis of proteins on a "genomic" scale [3]. Analysis of proteins on a genomic scale has acquired the name "proteomics." The most common means of proteomic analysis uses high-resolution 2D-PAGE, peptide mass fingerprinting and bioinformatics to identify proteins in a high-throughput fashion. Peptide mass fingerprinting is done by excising specific protein spots from the gel, digesting them with trypsin and measuring the sizes of the peptide fragments by mass spectrometry. The group of peptide sizes obtained from a specific protein is then compared to the predicted fragment sizes that would be

[1] **See Editorial by Bonventre, p. 1470.**

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obtained by cleavage of all proteins in a large database. A match between actual peptide fragments obtained from digestion of the protein spot and the expected fragments obtained from a specific database allows a prediction that the proteins are the same [4]. Databases of proteins expressed in specific tissues have been established and published. Fifty protein spots have been identified from rat liver [5]. Many of these proteins were identified from several different spots. For instance six spots were identified as peroxisomal bifunctional enzyme, so the number of unique proteins identified is considerably smaller. Proteomic analysis has been used to identify eighteen proteins that were previously not shown to be present in that species [6]. Relatively large databases of protein spots have been identified from yeast (401 spots representing 279 different proteins) [7]. Protein databases for cerebrospinal fluid, heart, kidney and multiple cell lines are available on the Internet at the Expert protein analysis system (ExPASy) site *www.expasy.ch* that is maintained by the Swiss Institute of Bioinformatics [8]. Limited databases of proteins expressed in renal tissue have been compiled [9]. Once proteins are identified in a specific tissue, a map can be generated that links individual spots with the protein identification of that spot. As these maps are developed, they can be used to compare changes in protein expression within that tissue for multiple interventions. We have prepared protein from rat renal cortex and medulla and analyzed a number of proteins by two-dimensional gel electrophoresis, excision and tryptic digestion of the spots and peptide mass fingerprinting using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. A map of renal protein expression in the cortex and medulla was generated. Once the protein spots were identified, we determined protein quantity based on spot intensity in a series of gels from cortex and medulla. Relative expression of all proteins identified was then calculated for cortex and medulla, and statistically significant differences between kidney regions were determined.

METHODS

Rat kidney protein preparation

All studies using rats have been approved by the University of Louisville Institutional Animal Care and Use Committee. Sprague-Dawley rats (150 to 200 g) were fed standard rat chow obtained from PMI Nutrition International (Richmond, IN, USA). Rats were sacrificed using a CO_2 chamber. The kidney was dissected and the capsule removed. The cortex was separated from the medulla and cut into several pieces and washed in ice-cold saline. The pieces of renal tissue were frozen in liquid nitrogen and ground to a powder using a chilled mortar and pestle. Tissue was resuspended in a buffer containing 50 mmol/L Tris, 0.3% sodium dodecyl sulfate (SDS) and 200 mmol/L dithiothreitol (DTT), incubated at 100°C for five minutes and transferred to ice. One tenth volume of a buffer containing 500 mmol/L Tris, 50 mmol/L $MgCl_2$, 1 mg/mL DNAse I and 0.25 mg/mL RNAse A was added and incubated for an additional 10 minutes. The 12,000 × *g* supernatant was obtained, trichloroacetic acid (TCA) added to 10% and the 12,000 × *g* pellet obtained. After several washes with acetone, the pellet was suspended in a sample buffer containing 40 mmol/L Tris, 7.92 mol/L urea, 0.06% SDS, 1.76% ampholytes, 120 mmol/L DTT and 3.2% Triton X-100.

Two-dimensional gel electrophoresis

Proteins obtained from rat kidney cortex (75 μg/sample) were separated by two-dimensional gel electrophoresis using a two-dimensional electrophoresis system (Genomic Solutions, Ann Arbor, MI, USA). Proteins were separated by isoelectric focusing over a pH range of 3 to 10 using precast first dimension gels for 17.5 hours at 110 μA/gel. The first dimension gel was then loaded on a large format (22 × 22 cm) second-dimension slab gel to separate proteins by molecular weight. After electrophoresis, proteins were silver stained using a modified Morrissey stain without glutaraldehyde according to the manufacturer's specifications. For identification of proteins using peptide mass fingerprinting, gels were stained with European Molecular Biology Laboratory (EMBL) silver stain [10] or Brilliant Blue G-colloidal concentrate (Sigma Chemical Co. St. Louis, MO, USA) according to the manufacturer's specifications, except that gels were fixed for one hour in a solution of 7% glacial acetic acid in 40% methanol.

In-gel tryptic digestion

Samples were prepared using a modification of the technique described by Jensen et al [11]. The stained gel slabs were washed with dI water (18 mega Ohm) four to five times. Spots on the gel were excised with a clean scalpel into one mm cubes. The gel pieces were transferred to clean 0.5 mL microfuge tubes. Ammonium bicarbonate (0.1 mol/L) was added to the gel pieces to twice the volume of the gel and incubated at room temperature for 15 minutes. Acetonitrile was added to the gel pieces and incubated at room temperature for 15 minutes. The solvent was removed and the gel pieces were dried using a vacuum centrifuge. The gel pieces were rehydrated with 20 μL of 20 mmol/L DTT in 0.1 mol/L NH_4HCO_3 and incubated at 56°C for 45 minutes to reduce the protein. The tubes were chilled at room temperature and the DTT solution was removed and replaced with 20 μL of 55 mmol/L iodoacetamide in 0.1 mol/L NH_4HCO_3 and incubated at room temperature in the dark for 30 minutes. The iodoacetamide was removed and replaced with 0.2 mL of 50 mmol/L NH_4HCO_3 and incubated at room temperature for 15 minutes. Acetoni-

trile (0.2 mL) was added and the sample was incubated at room temperature for 15 minutes. The solvent was removed and the gel pieces were dried with a vacuum centrifuge. The gel pieces were rehydrated with 5 μL of 20 ng/μL modified trypsin (Promega, Madison, WI, USA) in 50 mmol/L NH_4HCO_3. After the gel pieces were rehydrated, 50 mmol/L NH_4HCO_3 was added to cover the gel pieces and they were incubated overnight at 37°C.

Sample preparation for matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry

A nitrocellulose solution was made by dissolving a nitrocellulose membrane in 1:1 acetone/isopropanol solvent. Alpha-cyano-4-hydroxycinnamic acid (α-CN) was washed with 50 μL of acetone and acetone phase was discarded. The α-CN was dissolved in acetone to a concentration of 10 mg/mL, and the nitrocellulose and α-CN solutions were mixed to 1:4 ratio; 1 μL of this mixture was deposited onto the 96-well MALDI target plate. The sample was prepared for addition to the plate by adding 2 μL of sample to 2 μL of a solution of acetone washed α-CN dissolved in 0.1% trifluoroacetic acid and added to a 1:1 H_2O/acetonitrile to a final concentration of 10 mg/mL α-CN. One microliter of the sample mixture was loaded onto each thin film. After the sample mixture was dried, 1.5 μL of 2% formic acid in dI water was added to each spot. The formic solution was removed by gentle blotting. This washing step was performed twice. The samples were then dried at room temperature. Fragment size was determined by MALDI-TOF mass spectrometry.

MALDI-TOF mass spectrometry

Mass spectral data were obtained using a Micromass Tof-Spec 2E instrument equipped with a 337 nm N_2 laser at 20-35% power in the positive ion reflectron mode. Spectral data were obtained by averaging 10 spectra each of which was the composite of 10 laser firings. The mass axis was calibrated using known peaks from tryptic autolysis.

Analysis of peptide sequences

Protein identification from tryptic fragment sizes was made using the Mascot search engine (*www.matrixscience.com*) based on the entire NCBI protein database using the assumption that peptides are monoisotopic, oxidized at methionine residues and carboxamidomethylated at cysteine residues. Up to one missed trypsin cleavage was allowed, although most matches did not contain any missed cleavages. A mass accuracy tolerance of a maximum of 100 ppm was used for matching tryptic peptides. Probability based Mowse score defined as $-10*\log(P)$, where P was the probability that the observed match was a random event were determined [12]. Scores greater than 70 were considered significant ($P < 0.05$). All protein identifications were in the expected size range based on position in the gel.

Identification of proteins with changes in quantity

Silver stained gels were scanned using a high-resolution 12-bit camera. Scanned gel files were analyzed using Bioimage 2-D gel software. A composite gel was formed from five cortical and five medullary gels. Bioimage software was initially used to match the same spots together from each tissue according to the manufacturer's documentation. This software uses easily identified protein spots or "anchors" to serve as a starting point for spot matching and then matches additional spots based on pattern recognition. In addition, the accuracy of the protein spot matching was determined manually for each spot on each gel. Protein intensity was compared for each protein identified from cortex and medulla. The integrated intensity of each spot was determined from each of 10 gels obtained from 10 different rats. Mean and SEM was calculated and a two-tailed, non-paired *t* test was done on each to determine differences.

Western blot

Proteins were prepared from four rat kidneys using the same protocol and buffers as those described for 2-D gel electrophoresis. Western blotting was done as previously described using polyclonal antibodies against GRP-78 (1:2000; Accurate Chemical, Westbury, NY, USA) and retinol binding protein (1:1000; Cortex Biochem, San Leandro, CA, USA) or a monoclonal antibody against fatty acid binding Protein (1:1000; Chemicon, Temecula, CA, USA).

RESULTS

Two-dimensional gel electrophoresis was performed on proteins extracted from cortical and medullary tissue derived from Sprague-Dawley rat kidney. Digital images of stained spots were obtained with a CCD camera and individual images were matched together to form composite images of renal cortex and medulla. Each spot on the composite represented the appropriate spot within each individual gel. The initial matching process was done by the Bioimage software program. Accuracy of the matches was confirmed by manually comparing each gel image to the composite and to other individual gels. Composites of silver stained gels were generated from both regions, and 1095 spots were visualized by spot picking software in the cortex and 885 in the medulla (Fig. 1). Protein spots were picked, digested with trypsin and identified by peptide mass fingerprinting. A typical mass spectrum from the protein calbindin is shown in Figure 2. The peptide masses determined by mass spectrometry were used to identify the protein spot. In many cases additional peptides were seen after digestion that

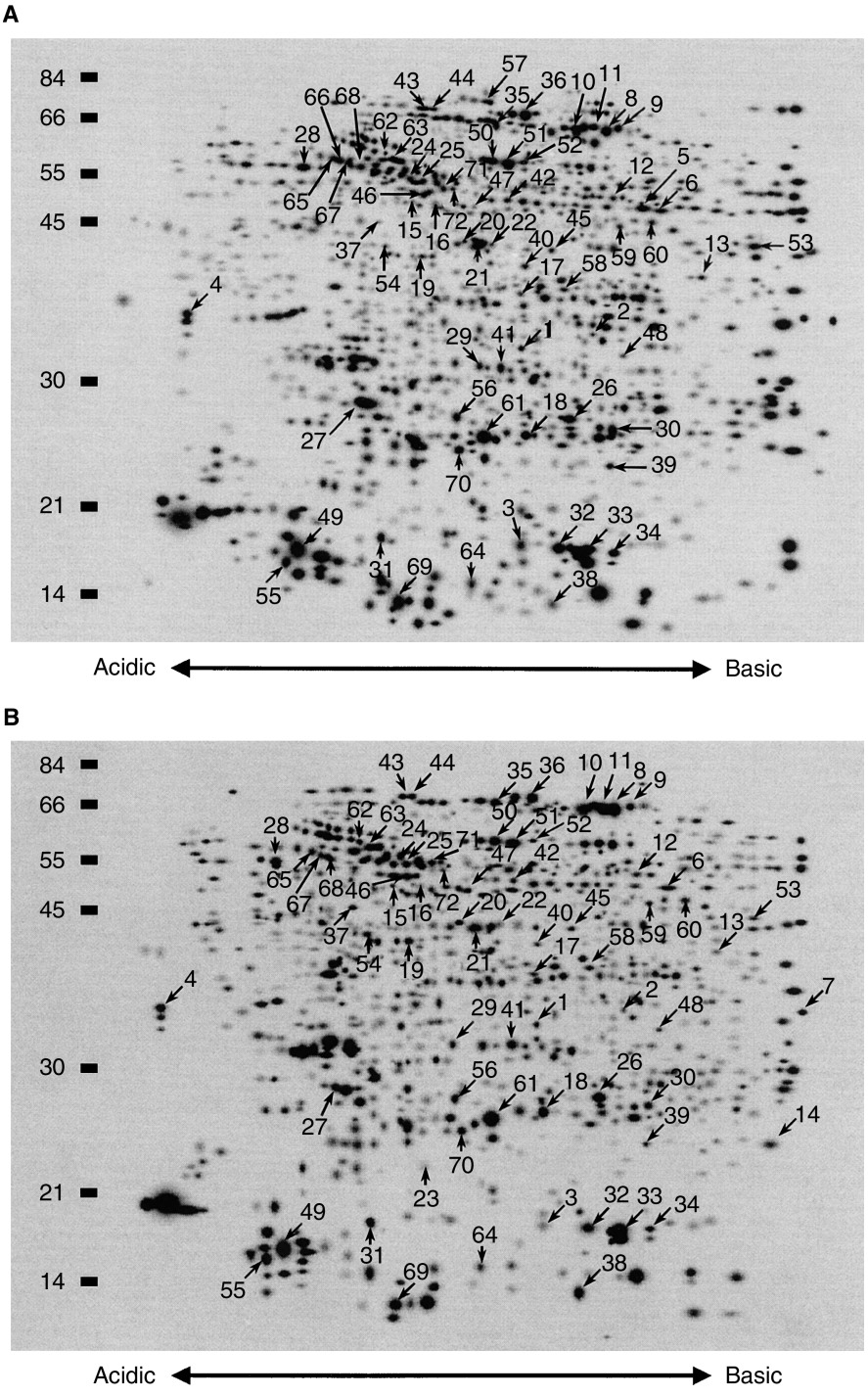

Fig. 1. Composite images of two-dimensional gels from renal cortex (*A*) and medulla (*B*). A total of 1095 spots were visualized in the cortex and 885 in the medulla by spot picking software. Numbers represent proteins that were identified by tryptic digestion, MALDI-TOF mass spectrometry and peptide mass fingerprinting. The numbers correspond to the peptides listed in Table 1.

did not correspond to the identified protein. These peptides likely represented other proteins present in the same spot, but we were not able to identify these other proteins with confidence. From the 180 spots picked, 72 spots representing 54 unique proteins were identified (Table 1 and Fig. 1). Predicted pI and molecular size were determined for each identified protein and compared to the pI and molecular size predicted from the location of the spot on the gel. Predicted and actual pI and size were nearly identical for all spots. Several horizontal rows of spots were seen on the gels. Within these rows analysis of multiple spots identified the same protein. These rows of proteins spots, which typically had similar molecular sizes but slightly different isoelectric points, probably were due to posttranslational modifications which cause changes in the protein charge but little

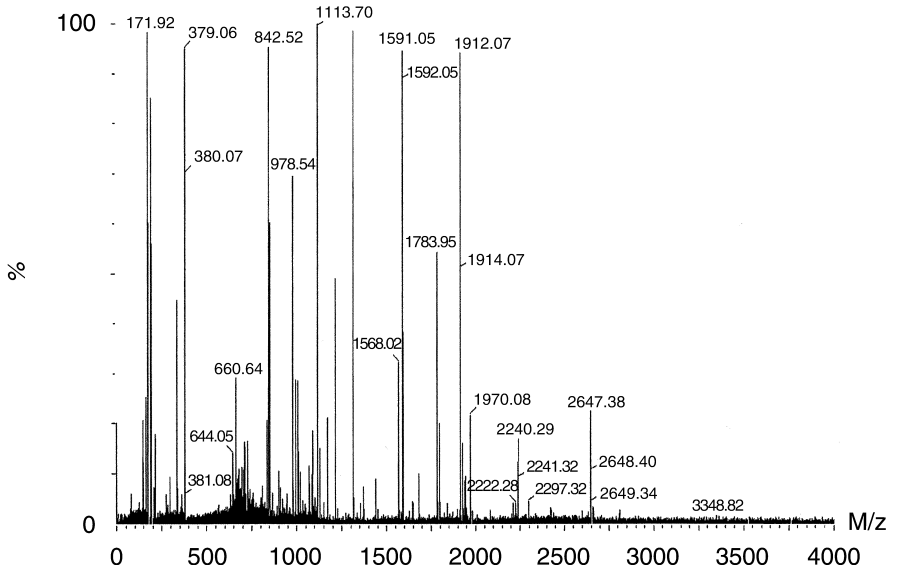

Fig. 2. Typical spectrum obtained from peptide mass fingerprinting of a gel spot. After separation of renal cortical tissue by two-dimensional gel electrophoresis, the gel spot was excised and digested with trypsin. Peptides obtained from the digestion were analyzed by MALDI-TOF mass spectrometry. The abscissa represents the mass/charge ratio of ions detected in the MALDI-TOF experiment. Peaks are almost always for the protonated molecular ion [M + H^+]. The ordinate represents the relative intensity of the averaged ion current in arbitrary units.

change in the protein mass as seen on the gel. The protein identities from all spots were entered in the composite gel database so that changes in protein expression could be determined for each protein spot. Integrated protein intensity was determined for each identified spot in ten gels each from cortex and medulla. Mean intensity of each spot was calculated by silver stain intensity and compared between cortex and medulla. Protein concentration within each region was calculated by silver stain intensity and relative intensities between cortex and medulla were compared. The average coefficient of variation for multiple replicates of a single spot was relatively large (65%). Table 2 lists the proteins that were differentially expressed. Only proteins identified by peptide mass fingerprinting were analyzed for differential expression. Alpha enolase was expressed only in cortex while aflatoxin B1 aldehyde reductase, alpha B crystallin and BH3 interacting death domain were expressed only in medulla. Nine proteins were expressed to a greater extent in cortex than medulla. They were 3-mercaptopyruvate sulfurtransferase, alpha 2u globulin, aldehyde dehydrogenase, contraception associated protein 1, heat shock 60 kD protein, isocitrate dehydrogenase, NADH-ubiquinone oxidoreductase 24 kD subunit precursor, ornithine aminotransferase and retinol binding protein. Among the proteins identified only albumin, glucose-regulated protein precursor (78 kD) and fatty acid binding protein were found to have a statistically significant increase in expression in the medulla. Four adjacent spots were identified as albumin and only one of them differed between cortex and medulla, probably representing a difference in posttranslational modification between cortex and medulla.

In order to confirm the differences in expression found by the proteomics approach, we performed Western blots on several proteins found to be differentially expressed. Expression as determined by densitometry was found to be qualitatively similar to that determined using two-dimensional gels. Figure 3 shows the expression of alpha-B crystallin, GRP78 and fatty acid binding protein by Western blot.

DISCUSSION

We have created a map of renal protein expression consisting of a 2-dimensional representation of renal protein expression based on pI and molecular size. This map permits consistent identification of a protein following multiple interventions since the protein location can be reproducibly identified on a two-dimensional gel. We have visualized about 1000 protein spots and identified the protein by peptide mass fingerprinting from 72. Using this technique to look at protein expression will allow protein expression to be determined for multiple proteins simultaneously instead of the small number possible with Western blotting. This technique is useful in hypothesis testing as well as for generating new hypotheses and screening for proteins involved in a specific physiological pathway.

While two-dimensional gels provide high resolution separation, they have a number of shortcomings. It is difficult to identify proteins of certain types. In particular, proteins of low abundance, membrane proteins, proteins at the extremes of molecular size and basic proteins have been difficult to identify using classical techniques. Fortunately, refinements in technique have improved the ability to identify some of these difficult proteins by two-dimensional gel analysis. Our current study found

Table 1. Differentially expressed proteins

Spot no.	Proteins	GenInfo identifier	Accession no.
1	3-hydroxyantranilate 3,4-dioxygenase	gi|9910256	NP 064461
2	3-mercaptopyruvate sulfurtransferase	gi|3122930	P 97532
3	Alpha 2u globulin	gi|204261	AAA 41198
4	Acidic nuclear phosphoprotein 32	gi|730318	P 39687
5	Alpha-enolase	gi|12667141	AAK01319
6	Alpha-enolase	gi|12667141	AAK01319
7	Aflatoxin B1 aldehyde reductase	gi|728819	P 38918
8	Albumin	gi|113580	P 02770
9	Albumin	gi|113580	P 02770
10	Albumin	gi|113580	P 02770
11	Albumin	gi|113580	P 02770
12	Aldehyde dehydrogenase	gi|14785467	XP007526
13	Aldehyde reductase 1	gi|6978491	NP 036630
14	Alpha B crystallin	gi|13162243	CAC 33095
15	Alpha-1-antitrypsin precursor	gi|203063	AAA 40788
16	Alpha-1-antitrypsin precursor	gi|203063	AAA 40788
17	Alpha-1-macroglobulin	gi|202857	AAA 40723
18	Apolipoprotein A-I	gi|2145147	AAB58430
19	Apolipoprotein A-IV	gi|71798	LPRTA4
20	Actin, beta	gi|224305	224305
21	Actin, beta	gi|224305	224305
22	Actin, beta	gi|224305	224305
23	BH3 interacting domain death agonist	gi|6680782	NP 031570
24	Tubulin, beta	gi|92930	A25113
25	Tubulin, beta	gi|92930	A25113
26	Ca-independent phospholipase A2	gi|5902791	O35244
27	Calbindin-d28K	gi|2119348	I 54042
28	Calreticulin	gi|117505	P 18418
29	Chloride intracellular channel protein 1	gi|6685328	Q9Z1Q5
30	Contraception associated protein 1	gi|7429594	JE 0344
31	Cytochrome b5	gi|554539	AAA 72420
32	Dismutase	gi|818029	CAA 29121
33	Superoxide dismutase	gi|203658	AAA 40996
34	Dismutase	gi|818029	CAA 29121
35	Heat shock 70 kD protein 10 (HSC71)	gi|5729877	NP 006588
36	DNAK type molecular chaperone	gi|2119726	I 56581
37	Vimentin	gi|1353212	P 48670
38	Fatty acid binding protein	gi|119804	P 07483
39	Ferritin light chain	gi|2119695	I 54774
40	Fructose-1,6-bisphosphatase	gi|119740	P 19112
41	Glutamate cysteine ligase	gi|8393446	NP 059001
42	Glutathione synthetase	gi|1170038	P 46413
43	Glucose-regulated protein precursor (78 kD)	gi|4033392	Q 90593
44	Glucose-regulated protein precursor (78 kD)	gi|4033392	Q 90593
45	GTP-specific succinyl-CoA synthetase beta subunit	gi|3766203	AAC 64399
46	H^+ transporting ATP synthase	gi|92350	A 28701
47	Heterogeneous nuclear ribonucleoprotein F	gi|4826760	NP 004957
48	High mobility group protein 1	gi|123367	P 10103
49	Histone H3.2	gi|70755	HSXL 32
50	Heat shock 60 kD protein	gi|1334284	CAA 37654
51	Heat shock 60 kD protein	gi|1334284	CAA 37654
52	Heat shock 60 kD protein	gi|1334284	CAA 37654
53	Isocitrate dehydrogenase	gi|1170478	P41562
54	Laminin-binding protein	gi|34234	CAA 43469
55	Myosin, light chain, smooth muscle	gi|12737351	XP 012180
56	NADH-ubiquinone oxidoreductase 24 kD subunit precursor	gi|128867	P 19234
57	NADH-ubiquinone oxidoreductase 75 kD subunit precursor	gi|128825	P 15690
58	N^G, NG-dimethylarginine dimethylaminohydrolase 1	gi|6831527	O 08557
59	Ornithine aminotransferase	gi|129019	P 04182
60	Ornithine aminotransferase	gi|129019	P 04182
61	Phosphotidylethanolamine binding protein	gi|8393910	NP 058932
62	Protein disulfide isomerase	gi|6981324	NP 037130
63	Protein disulfide isomerase	gi|6981324	NP 037130
64	Retinol binding protein	gi|809309	809309
65	Serine protease inhibitor 2	gi|57233	CAA 34407
66	Serine protease inhibitor 2	gi|57233	CAA 34407
67	Serine protease inhibitor 2	gi|57233	CAA 34407
68	Serine protease inhibitor 2	gi|57233	CAA 34407
69	Thioredoxin	gi|135776	P 11232
70	Thioredoxin peroxidase 1	gi|8394432	NP 058865
71	Tubulin, alpha chain	gi|422542	S 33517
72	Tubulin, alpha chain	gi|422542	S 33517

Table 2. Differentially expressed proteins

Spot no.	Proteins	Cortex			Medulla			*P* value
		Mean	SEM	*N*	Mean	SEM	*N*	
2	3-Mercaptopyruvate sulfurtransferase	1.18	0.18	10	0.59	0.13	10	0.016
3	Alpha 2u globulin	9.85	3.38	8	1.98	0.43	9	0.033
5	Alpha enolase	3.84	1.08	10	0.00	0.00	0	0.004
7	Aflatoxin B1 aldehyde reductase	0.00	0.00	10	1.79	0.30	8	0.000
10	Albumin	5.35	1.14	10	7.27	0.87	10	0.046
12	Aldehyde dehydrogenase	2.94	0.53	10	1.28	0.35	10	0.040
14	Alpha B crystallin	0.00	0.00	10	1.30	0.46	7	0.002
23	BH3 interacting domain death agonist	0.00	0.00	10	0.44	0.12	10	0.004
30	Contraception associated protein 1	5.38	0.57	10	3.06	0.42	10	0.005
38	Fatty acid binding protein	10.62	1.56	9	14.43	2.95	6	0.047
44	Glucose-regulated protein precursor (78 kD)	1.15	0.29	8	2.15	0.43	8	0.040
52	Heat shock 60 kD protein	2.39	0.43	8	1.20	0.22	10	0.042
53	Isocitrate dehydrogenase	4.79	0.91	7	2.56	0.36	9	0.045
56	NADH-ubiquinone oxidoreductase 24 kD subunit precursor	3.77	0.46	10	2.20	0.44	10	0.035
60	Omithine aminotransferase	2.01	0.30	8	1.05	0.21	9	0.019
64	Retinol binding protein	3.65	0.46	10	1.87	0.38	10	0.016

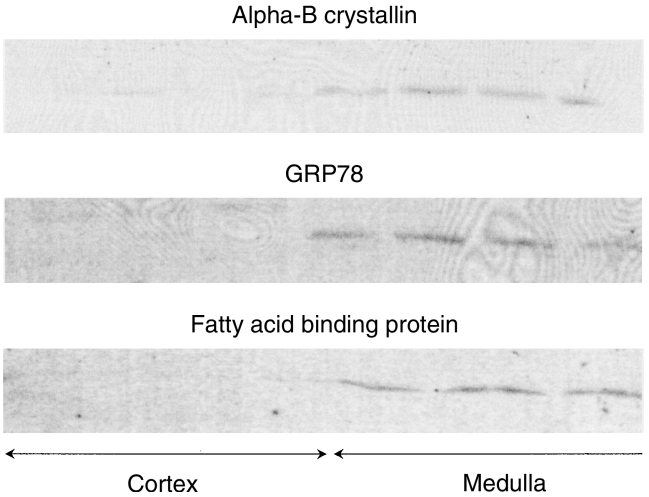

Fig. 3. Western blot of proteins determined to be differentially expressed by two-dimensional gel analysis. Alpha-B crystallin ($N = 4$), GRP78 ($N = 4$) and fatty acid binding protein ($N = 3$) were all determined to be differentially expressed between cortex and medulla in a manner similar to that seen by two-dimensional gel.

a positive identifications from 72 out of 180 spots analyzed or 40%. Our data demonstrate that some of the spots likely contained multiple proteins since unmatched peptides remained after identification of a first protein. We were not able to identify a second protein with statistical confidence from any spot in this study, however.

Using the two-dimensional gel technique we have identified a number of renal proteins. Many of the proteins are expected to be present in renal tissue. For instance alpha 2u globulin binds pheromones in rat urine and allows for their release during drying [13]. Calbindin is a vitamin D-dependent calcium binding protein that is found primarily in the distal nephron and the intestine [14]. A number of proteins that are widely distributed were identified including cytoskeletal and mitochondrial proteins. Cytoskeletal proteins like actin, myosin and tubulin were present in relatively high abundance. Several mitochondrial proteins were found like the H^+ transporting adenosine 5′-triphosphate (ATP) synthase [15], isocitrate dehydrogenase [16] and heat shock protein 60 kD (HSP 60) [17]. Some of the proteins are somewhat unexpected to find in the kidney. Alpha B-crystallin is abundant in lens and skeletal muscle but has also been identified in kidney [18]. 3-Mercaptopyruvate sulfurtransferase is an enzyme responsible for transfer of a sulfur ion to thiols or cyanide. It has been identified in the liver but not previously seen in the kidney [19]. While the preparation of the tissue should have removed most blood proteins, we cannot exclude the possibility that some of the proteins present were derived from residual blood in the tissue.

Cytosolic proteins from rat kidney and medulla have previously been compared using proteomics by Witzman and colleagues [9]. They resolved 727 protein spots in cortex and 716 in medulla (compared to 1095 and 885, respectively, in the current study). One hundred and twenty-seven of these protein spots were found to differ in abundance between cortex and medulla, and 30 were found to be unique to one region or the other. They used mass spectrometry, Western blotting and comparison of homologous spot positions from other databases to identify 20 of these spots representing 14 different proteins and variants. Eight of these spots representing seven different proteins were identified by mass spectrometry. We have expanded on these findings to use whole kidney homogenates rather than cytosolic fractions and to identify a much larger group of expressed proteins. Seventy-two spots representing 54 different proteins were identified. In addition, all of the identifications in the current study were done with peptide mass

fingerprinting using MALDI-TOF mass spectrometry, a technique that is more amenable to high-throughput operation. Matching of large numbers of gels with many proteins can be time consuming and error prone. Recent improvements in software promise to make this task much easier and more accurate, but great care must be taken to ensure accurate spot matching.

Some differences between our findings and those of Witzman and colleagues [9] exist for the relative expression of cortical and medullary proteins. They found that actin was expressed to a greater extent in the medulla. We identified three actin spots, all of which had a numerically greater expression in the medulla but none reached statistical significance. The previous study had identified aflatoxin B1 aldehyde reductase expressed in both cortex and medulla but to a greater extent in medulla, while our study found it only in medulla. These differences can probably be accounted for at least in part by differences in technique. The primary difference was that the previous study had looked only at cytosolic proteins while we used whole cell homogenates.

A targeted proteomic approach has been used to quantify expression of specific transport proteins in Na^+-/H^+ exchanger and thiazide-sensitive Na^+Cl^- cotransporter knockout mice [20]. This technique uses antibodies against known proteins to determine abundance of proteins. It is more sensitive than looking at protein expression using silver stain but is limited to detection of known proteins for which antibodies exist.

Proteomics was used here to identify a large component of renal proteins and compare expression of these proteins between cortex and medulla. This technique provides a mechanism to characterize changes in large numbers of proteins simultaneously. Once the proteins are identified, this technique can be used to monitor changes in protein expression in response to any intervention. Two-dimensional gel electrophoresis can be done by investigators using identical protocols to identify protein spots that change their expression. These spots can then be compared to known maps of proteins such as the ones in our study to determine the identity of those spots. Further work will need to be done to fully characterize the renal proteome. Separation using another characteristic of proteins such as solubility will add a third dimension of separation, which will greatly increase both the usefulness and the number of proteins that can be identified. Eventually a nephron specific or cell type specific map will be necessary to gain the full value of proteomics for the renal community. We anticipate that this technique will be widely and effectively used in renal research to characterize physiological processes and disease.

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