Toward Proteomics in Uroscopy: Urinary Protein Profiles after Radiocontrast Medium Administration

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Abstract. Previous attempts to use urinary protein profiles for diagnostic purposes have been rather disappointing with respect to their clinical validity, in part because of the insufficient reproducibility, sensitivity, and rapidity of available techniques. Therefore, a newly developed, high-throughput technique, namely surface-enhanced laser desorption/ionization (SELDI) ProteinChip array-time of flight mass spectrometry, was studied, to assess its applicability for protein profiling of urine and to exemplify its use for a group of patients receiving radiocontrast medium. Assessment of the accuracy, sensitivity, and reproducibility of SELDI in test urinary protein profiling was performed. Renal function was studied in 20 male Sprague-Dawley rats before and after intravenous administration of either 1.25 g/kg ioxilan (n = 10) or hypertonic saline solution (n = 10) as a control. Urine samples from 25 patients undergoing cardiac catheterization were obtained before, immediately after, and 6 to 12 h after the procedure. Administration of ioxilan to rats resulted in changes in the abundance of proteins of 9.9, 18.7, 21.0, and 66.3 kDa. For patients, even in uncomplicated cases of radiocontrast medium infusion during cardiac catheterization, perturbations in the protein composition occurred but returned to baseline values after 6 to 12 h. Proteins with molecular masses of 9.75, 11.75, 23.5, and 66.4 kDa changed in abundance. For patients with impaired renal function, these changes were not reversible within 6 to 12 h. As a proof of principle, one of the peaks, i.e., that at 11.75 kDa, was identified as β2-microglobulin. SELDI is a promising tool for the detection, identification, and characterization of trace amounts of proteins in urine. Even for patients without renal complications, proteins with a broad range of molecular masses either appear in or disappear from the urine. Some of these might represent markers of impending nephropathy.

Urine has long been known as a rich source of diagnostic information, because of its physical properties and chemical composition. Previous attempts to use urinary protein profiles for diagnostic purposes resulted in some remarkably successful applications, such as pregnancy tests based on human chorionic gonadotropin excretion (1); in most cases, however, these profiles have been rather disappointing in their predictive value (2,3). Exhaustive studies launched in the 1970s used two-dimensional gel electrophoresis to reveal hundreds of proteins in normal human urine, depending on the resolution of individual techniques, but the reproducibility and sensitivity of this method were inadequate, and it remained too complicated and time-consuming for most practical purposes (4). Therefore, the initial enthusiasm regarding the search for diagnostic markers in the urine has subsided considerably, because of the lack of a rapid, high-throughput technique for analysis of native biologic samples that would allow unbiased screening of the entire repertoire of urinary proteins.

The recent development of surface-enhanced laser desorption/ionization (SELDI)-time of flight (TOF) technology, based on improved methods for the chemical preparation of absorptive surfaces and their use for solid-state mass spectrometry (5), allows high-throughput protein analysis of crude biologic samples. Key components of this technology, in addition to the mass spectrometer, are ProteinChip arrays that contain 2-mm-diameter absorptive target spots. Each of these targets represents either a chemical (e.g., ionic, hydrophobic, or hydrophilic) or immunosorbptive (antibody) surface designed to capture proteins of interest. After removal of unbound proteins and interfering substances, the molecular masses of the proteins retained on the ProteinChip can be determined by TOF analysis. The potential advantages of SELDI ProteinChip technology include rapidity and reproducibility in the screening of protein expression profiles known as “phenomic fingerprints.” In general, this technology can be used to provide phenomic fingerprints of complex protein mixtures; however, there are no published data on the use of this technique in studies of normal and pathologic urinary protein profiles. This work summarizes our investigation designed to test the utility (accuracy, reproducibility, and sensitivity) of this technique in studies of urinary protein composi-
tion and to exemplify its use with a study of the changes in urine composition associated with radiocontrast medium administration. This latter condition was selected for two main reasons, i.e., the large number of patients who undergo cardiac catheterization and the poor predictability of its renal complications.

The incidence of acute renal failure triggered by the intravenous administration of radiocontrast media for diagnostic purpose ranges from 1.2 to 100%, depending on the number of risk factors (6–8). Despite the routine precautionary measures taken for each patient, radiocontrast medium-induced nephropathy remains a serious risk for procedures (such as cardiac catheterization) performed in already-compromised hosts. This condition is especially dangerous because overt signs of such complications are detectable 24 to 48 h after the procedure, when patients have been discharged or have been scheduled for or even undergone cardiac surgery. Early knowledge of impending renal complications could significantly reduce the morbidity associated with this diagnostic procedure. The existing markers of developing radiocontrast medium-induced nephropathy, based on measurements of enzymuria, are non-specific, and their detection remains technically cumbersome (2). SELDI-TOF-generated data on urinary protein compositions after radiocontrast medium administration are summarized in this report.

Materials and Methods

**SELDI-TOF Protein Analysis**

After deposition of 1 μl of sample directly onto a spot of a hydrophobically coated aluminum ProteinChip array (Ciphergen Biosystems, Palo Alto, CA), hydrophobic proteins were captured and retained. Nonbound proteins and other contaminants were washed from the ProteinChip array with deionized distilled water, an energy-absorbing molecule (EAM1; Ciphergen) was applied in solvent containing 50% acetonitrile and 0.5% trifluoroacetic acid, and the sample was allowed to dry. After insertion of the ProteinChip array into the ProteinChip reader, a laser beam was focused on the sample in vacuo. This caused the proteins absorbed to the matrix to become ionized and, simultaneously, to be desorbed from the ProteinChip array surface. The ionized proteins were detected and their molecular masses were determined using TOF analysis. TOF mass spectra were collected with a ProteinChip System (PBS I and PBS II series; Ciphergen), using Ciphergen Peaks (versions 1.5 and 2.0) software. Spectra were collected in the positive-ion mode. Real-time signal averages of 100 laser shots were used to generate each spectrum.

The ProteinChip system was calibrated with bovine superoxide dismutase and bovine β-lactoglobulin A (both from Sigma Chemical Co., St. Louis, MO). Identification of α-defensin-2 [human neutrophil peptide-2 (HNP-2)] in urine samples, on the basis of mass, was performed using the SWISS-PROT protein database, allowing 0.1% error in observed mass. To confirm the identity, the mass of synthetic human HNP-2 (Sigma) was compared with findings for the urine sample. To assess the sensitivity of the ProteinChip system, purified HNP-2 was diluted from 10 fmol to 0.1 fmol and the peak amplitudes were determined. Peaks with amplitudes at least 3 times greater than the average background noise level were considered meaningful. The reproducibility was tested by depositing different aliquots of the same urine sample on six different spots of the ProteinChip array.

**Identification of β2-Microglobulin**

To confirm the identity of β2-microglobulin (β2M), the masses of purified β2M from human urine (Calbiochem, San Diego, CA) and immunoprecipitated β2M from the samples were compared with findings for the original urine samples. For immunoprecipitation of β2M, urine samples were incubated for 2 h at 4°C, with agitation, either with anti-β2M antibodies (Sigma) or with irrelevant polyclonal goat anti-mouse antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) as a negative control. After incubation for 1 h at 4°C, with agitation, with protein A/G beads (Protein A/G PLUS agarose; Santa Cruz Biotechnologies, Santa Cruz, CA) in a 1:1 suspension in phosphate-buffered saline, the samples were centrifuged at 10,000 × g for 5 min at 4°C. The beads were washed twice with washing buffer I (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM ethylenediaminetetraacetate, 1% Triton X-100, 1% Nonidet P-40, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 10 μg/ml trypsin inhibitor, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate) and once with washing buffer II (10 mM Tris-HCl, pH 7.4, 1 mM ethylenediaminetetraacetate). After final centrifugation, the pellets were reconstituted in 20 μl of water.

To dissociate antigen-antibody complexes from the protein A/G beads, the samples containing the beads were vortex-mixed for 2 min at 37°C and then cooled on ice. After addition of elution buffer (ImunoPure Gentle Ag/Ab Elution Buffer; Pierce, Rockford, IL) to each of the samples and vortex-mixing for 15 min at room temperature, the samples were centrifuged at 16,000 × g for 10 min. Guanidine (8 M in 0.25% trifluoroacetic acid) was added to the pellet, and the mixture was vortex-mixed for 15 min at room temperature and centrifuged at 16,000 × g for 2 min. Solid-phase extraction was performed using ZipTips (Millipore Corp., Bedford, MA), according to the instructions provided by the manufacturer, except that proteins were eluted with 2,2,2-trifluoroethanol (Aldrich, Milwaukee, WI).

**Animal Studies**

All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (9). Twenty male Sprague-Dawley rats (270 to 290 g) were maintained on standard rat chow, with water available ad libitum and with an automatically controlled 12-h light/dark cycle, in the accredited Department of Laboratory Animal Research at the State University of New York at Stony Brook. The animals were placed in individual metabolic cages, and baseline 24-h urine samples were collected after a 1-d adaptation period. The rats were divided into four experimental groups of five animals each. In animals in groups 1 and 3, hypertonic saline solution (690 mOsm) intravenously and were used as control animals. The rats in groups 2 and 4 received intravenous injections of 1 ml (1.25 g/kg body wt) of the nonionic, low-osmolar (695 mOsm), radiocontrast agent ioxilan (Oxilan-350; Cook Imaging Corp., Bloomington, IN), which was first described in 1988 (10). One-half of the rats (groups 3 and 4) were pretreated with NG-nitro-L-arginine methyl ester (L-NAME) (50 mg/L in water) to mimic the endothelial dysfunction observed in patients with cardiovascular disease (modified from references 11 and 12). Twenty-four-hour urine samples were collected for 2 d consecutively. Serum and urine creatinine levels were measured by colorimetric assay (Beckman Synchron CX and kit 443340); blood urea nitrogen (BUN) levels were measured using the enzymatic conductivity rate method (Beckman Synchron CX and kit 443350).
**Clinical Studies**

Twenty-five patients admitted to the University Hospital of the State University of New York at Stony Brook for diagnostic or therapeutic cardiac catheterization were recruited to participate in the study. Patients who had undergone any invasive procedure 2 wk before admission and those with severe nephrotic syndrome, active lupus nephritis, genitourinary tract infection, or liver failure were excluded from the study. Three freshly voided urine samples were studied; the first was obtained before cardiac catheterization, the second immediately after it, and the third approximately 6 to 12 h after the procedure. In the course of routine follow-up monitoring, serum creatinine and BUN levels were measured 48 h after the procedure. The patients were divided into two groups; one group consisted of patients with normal renal function, as indicated by plasma creatinine levels ($n=20$), and the other group consisted of patients with deteriorations in renal function ($n=5$). Ioxilan was administered as a radiocontrast agent to all patients studied. The study was approved by the Committee on Research Involving Human Subjects of the State University of New York at Stony Brook. All patients signed an informed consent form for participation.

**Sample Preparation**

Urine samples were immediately centrifuged at $3000 \times g$, the sediments were analyzed microscopically, and the supernatants were stored frozen at $-80^\circ C$ until further analysis. Supernatants were slowly thawed on ice and either filtered at $7500 \times g$ for 20 min at $4^\circ C$ through a Microcon centrifugal filter device with a 50-kD cut-off (Millipore) or analyzed directly. The retentates were filtered three times and each time reconstituted with deionized water.

**Statistical Analyses**

Results are reported as mean $\pm$ SEM. Comparisons between control samples and each of the treatment groups were made using $t$ tests. Analyses of differences in multiple groups were performed using one-way ANOVA. A $P$ value of $<0.05$ was considered to indicate a statistically significant difference.

**Results**

**Characterization of SELDI ProteinChip Technology**

The calibration was performed using two protein standards, as indicated in Figure 1. The molecular masses of the detected peaks closely resembled their predicted masses, i.e., a peak with an apparent molecular mass of 15,591.4 D (bovine superoxide dismutase; calculated mass, 15,591 D) and a peak with a mass of 18,364.6 D (bovine $\beta$-lactoglobulin A; calculated mass, 18,363 D). Additionally, there were smaller peaks at 7796 D (15,591.4+$2H$) and 9182 D (18,364.6+$2H$), which represented doubly charged molecular standards. The accuracy of calibration was well within 0.01% of the molecular mass.

Figure 2 displays protein peaks in the range between 3000 and 4000 D. In Figure 2 (upper), the test urine sample demonstrates three unknown peaks, with apparent molecular masses of 3370, 3441, and 3484 D. Using the SWISS-PROT protein database, peaks with these masses were tentatively identified as HNP-2 (calculated masses, 3371.0 D), HNP-1 (calculated mass, 3442.1 D), and HNP-3 (calculated mass, 3486.2 D). Human $\alpha$-defensins-1 to -3 (also termed HNP-1 to -3, respectively) are a group of small cationic peptides (average mass, 3.3 to 3.5 kD) that are derived from neutrophils and exhibit broad-spectrum antimicrobial activity (13,14). They are almost identical in sequence. HNP-1 and -3 are 30 amino acids long and differ only at the first amino acid, which is alanine in HNP-1 and aspartate in HNP-3. HNP-2 is only 29 amino acids in length (15). Application of purified HNP-2 peptide to the target, followed by SELDI-TOF analysis, revealed a peak at 3369.6 D ($<0.05\%$ difference from the mass measured in the complex biologic sample), thus confirming the presence of HNP-2 in the test urine sample.

The sensitivity of SELDI-TOF is illustrated in Figure 3; sensitivity was assessed by titration of HNP-2 from 10 fmol down to 0.1 fmol. A level of 0.1 fmol of HNP-2 yielded a peak at 3370 D that was significantly greater than the background noise, and this level was defined as the detection limit in this setting. The test urine sample used to determine the reproducibility demonstrated, among other proteins, the triple peak that

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![Figure 1](image1.png)

**Figure 1.** Representative surface-enhanced laser desorption/ionization (SELDI)-time of flight (TOF) mass spectrum, showing calibration of the instrument with the molecular mass standards bovine superoxide dismutase (15,591 D) and bovine $\beta$-lactoglobulin A (18,363 D). The smaller peaks at 7796 D (15,591.4+$2H$) and 9182 D (18,364.6+$2H$) represent doubly charged molecular standards.

![Figure 2](image2.png)

**Figure 2.** Identification of $\alpha$-defensin-2 [human neutrophil peptide-2 (HNP-2)] in a test urine sample.
is characteristic of α-defensins (Figure 4). The peak representing HNP-2 demonstrated an average mass of 3372.2 Da (SD, ±0.1; calculated mass, 3371.0 Da). The protein profiles of the test urine sample preabsorbed on six target spots of the ProteinChip array were nearly identical. Collectively, these data demonstrate that SELDI-TOF is a highly sensitive, reproducible, accurate technique that allows the detection of minute protein fractions in biologic samples as complex as urine. These findings permitted the use of SELDI-TOF to study urinary protein composition of experimental animals and patients receiving radiocontrast medium.

Effects of Radiocontrast Medium in Experimental Animals

The four groups of rats received the nonionic radiocontrast medium ioxilan or vehicle. The rate of urine output was unaffected by this treatment (Figure 5C). There was a marginal decrease in creatinine clearance in animals that received either ioxilan or L-NAME alone, from 3.63 ml/h to 2.79 and 2.90 ml/min, respectively. In contrast, animals that received both ioxilan and L-NAME exhibited a significant reduction in creatinine clearance to 2.38 ml/min ($P < 0.05$, compared with control), as summarized in Figure 5B.

The protein profiles of the urine samples from control rats that were studied using SELDI-TOF indicated that the most prominent peaks occurred at 9.9, 18.7, 21.0, 33.3, and 66.3 kDa (Figure 5A). For animals pretreated with L-NAME, there were only minor changes in the 21.0-, 33.3-, and 66.3-kDa proteins. For rats given infusions of ioxilan, amounts of a protein with a molecular mass of 21 kDa were decreased. In contrast to these relatively minor changes, a dramatic reduction or disappearance of all five protein peaks was observed in the urine of rats treated with both ioxilan and L-NAME, which was the group characterized by reduced creatinine clearance.

Effects of Radiocontrast Medium in Patients

Plasma creatinine and BUN levels in patients with normal kidney function exhibited no significant differences before and after cardiac catheterization (creatinine, 0.9 ± 0.06 versus 0.8 ± 0.05 mg/dl, $P = 0.455$; BUN, 16.3 ± 1.3 versus 15.6 ± 1.28 mg/dl, $P = 0.404$). In patients with impaired renal function, plasma creatinine and BUN levels were elevated before the catheterization and plasma creatinine levels exhibited a trend toward further increase after the procedure (creatinine, 1.9 ±
0.24 versus 2.2 ± 0.37 mg/dl, P = 0.152; BUN, 41.4 ± 13.0 versus 40.2 ± 13.7 mg/dl, P = 0.235). Urinalyses and sediment analyses revealed no major interval changes in the urine samples after cardiac catheterization, compared with the initial samples.

The screening of patients with unimpaired renal function and without clinically detectable proteinuria demonstrated several prominent peaks detected by SELDI-TOF analysis, which represented proteins with molecular masses of 4.87, 9.75, 11.75, 23.5, 33.3, and 66.4 kD (Figure 6). Urine samples obtained

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**Figure 5.** (A) SELDI-TOF protein profiles of urine samples from rats, before and after administration of radiocontrast medium. L-NAME, N\(^\text{G}\)-nitro-L-arginine methyl ester. (B) Creatinine clearance (Crea Cl.) of experimental animals (*, P = 0.044, compared with control animals). (C) Urine output of experimental animals.
after the administration of radiocontrast medium, i.e., immediately after the completion of cardiac catheterization, displayed changes in the abundance of normally detectable proteins; increases in proteins with molecular masses of 4.87, 9.75, and 11.75 kD were accompanied by the almost complete disappearance of a peak at 66.4 kD. Six to 12 h after the procedure, there was a strong trend toward the recovery of the precatheterization urinary protein pattern in patients with normal renal function who did not develop renal complications (as judged by plasma creatinine levels).

A strikingly different pattern of urinary proteins was observed for five patients with mild chronic renal insufficiency (Figure 7). Proteins with apparent molecular masses of 9.75, 11.75, 23.5, 33.3, and 66.4 kD, as well as several higher-molecular mass species of 79.3, 90.3, 133, and 199.2 kD (not shown), were detected using SELDI-TOF analysis. After cardiac catheterization and infusion of radiocontrast medium, urine samples exhibited the appearance of low-molecular mass peaks at 4.87 and 7.8 kD, increases in peaks at 9.75, 11.75, and 23.5 kD, and suppression of peaks at 33.3, 66.4, and 199.2 kD, which was not reversible within the first 6 to 12 h after the procedure. The 9.75-, 11.75-, and 23.5-kD peaks were increased even further after 6 to 12 h, as opposed to the patterns observed for patients with normal renal function, which tended to recover the baseline protein profile (Figure 6).

Because the 66.6-kD protein peak represented albumin and exhibited dynamic changes indicative of its perfusion-dependent excretory changes, we next used the amplitude of this peak as a common denominator for studies of the relative excretion of other proteins. The calculated ratio of the amplitude of the 9.75-kD or 11.75-kD peak to that of the 66.6-kD protein peak revealed marked amplification of differences for each of these peaks separately (Figure 8). There was a 10-fold increase in both ratios for patients with normal renal function immediately after cardiac catheterization, with a return to baseline values 6 to 12 h after the procedure. In contrast, for patients with impaired renal function, the 9.75 kD/66.6 kD ratio, which was initially <0.1, increased 60- and 70-fold immediately and 6 to 12 h after catheterization, respectively. The 11.75 kD/66.6 kD ratio also increased approximately 15-fold immediately after the procedure and remained elevated 6 to 12 h after the administration of contrast medium.

The 11.75-kD protein in the urine exhibited the same mass and pattern as purified β2M from human urine (Figure 9A). Furthermore, the same peak could be observed in the immunoprecipitate but not the immunodepleted supernatant of samples incubated with specific anti-β2M antibodies (Figure 9B). In contrast, urine samples incubated with irrelevant goat anti-rabbit antibodies as a negative control demonstrated no peak in the immunoprecipitate but an 11.744-kD peak in the supernatant (Figure 9C). The 11.75-kD peak thus can be safely identified as β2M.

Discussion

This study was intended to evaluate the applicability of the recently developed high-throughput technique of SELDI-TOF mass spectrometry for detection of the protein repertoire in urine. The data presented demonstrate subfemtomolar sensitivity, measurement accuracy such that actual results differed from predicted molecular masses by <0.01%, and high reproducibility of results obtained with the SELDI-TOF technique (Figures 3 and 4). These findings proved the applicability of
SELDI-TOF for studies of urinary protein profiles and justified attempts to clinically define protein components of urine from patients and experimental animals receiving radiocontrast medium.

The radiocontrast medium used in this study, ioxilan, is a modern, nonionic, low-osmolar agent known to be stable, water-soluble, and well tolerated (10), with reportedly minimal effects on systemic and renal hemodynamics (16–18). Urine profiles, including albumin, glucose, lactate dehydrogenase, N-acetyl-β-D-glucosaminidase, and γ-glutamyltransferase measurements, obtained for rabbits after administration of ioxilan demonstrated no significant changes (19). In experimental rats, however, increases in albumin, lactate dehydrogenase, and γ-glutamyltransferase excretion were reported (20). In view of the reported minimal nephrotoxicity of ioxilan, the changes in urinary protein composition observed for both patients and experimental animals attest to the high sensitivity of SELDI-TOF in detecting trace amounts of proteins, which are usually undetectable by other techniques. However, dramatic changes in urinary protein composition were detected mainly in patients and experimental animals with pre-existing renal dysfunction. In the animal model that mimics the endothelial dysfunction observed in patients with cardiovascular disease (inhibition of endothelial nitric oxide synthase with L-NAME), there was a significant reduction in creatinine clearance after the injection of ioxilan, compared with control findings. This might reflect the limited nephrotoxicity of radiocontrast agents themselves, and it is consistent with previously reported observations (11,12). Concurrent with these functional abnormalities, SELDI-TOF analysis revealed a different urinary protein pattern; all protein components observed in normal urine were decreased or nearly absent (Figure 5A). These findings probably reflect the cumulative effects of contrast agent-induced nephrotoxicity and vascular dysfunction, leading to reductions in GFR and protein excretion. Even more dramatic changes were observed in human patients with renal dysfunction (see above).

Using the SELDI-TOF technique, it has become possible to detect previously unrecognized proteins in urine obtained from patients with otherwise normal kidney function and no history of renal diseases. Specifically, we demonstrated that, in uncomplicated cases of radiocontrast medium infusion during cardiac catheterization, proteins with a broad range of molecular masses either appear or disappear from the urine, with the tendency to recover after 6 to 12 h (Figure 6). These might represent a previously unrecognized response of the kidney to the administration of radiocontrast medium. The increase in low-molecular mass proteins (9.75 and 11.75 kD) and the decrease in higher-molecular mass proteins (66.6-kD albumin) after the administration of radiocontrast medium can be attributed to combined glomerular and tubular dysfunction. Although it was expected that the repertoire and abundance of proteins in the urine of patients with known renal diseases would be more pronounced, SELDI-TOF analysis revealed a distinctive protein pattern and a lack of reversibility for changes in several protein components that appeared in the urine after cardiac catheterization. Surprisingly, there was no clear-cut correspondence of the urinary protein profiles in the experimental animals and the patients receiving ioxilan. However, possible differences in the molecular masses of proteins, attributable to different post-translational modifications or species-specific sequence variations, may be responsible for the observed lack of similarities between experimental animals and human subjects.

Figure 7. SELDI-TOF protein profiles of urine samples from patients with impaired renal function, before and after administration of radiocontrast medium.
The analysis of SELDI-TOF protein peaks revealed substantial differences in the dynamics of protein patterns in the urine of patients with or without pre-existing renal insufficiency, as summarized in Figure 8. The latter group demonstrated a lack of reversibility in the depressed excretion of 66.6-kD albumin and increased excretion of proteins with molecular masses of 9.75 and 11.75 kD. The fact that these peptides exhibited changes in the opposite direction, compared with changes in albumin, argues against the possibility that changes in GFR are entirely responsible for the observed protein pattern dynamics. Although this could be true for albumin excretion, increased levels of proteins with molecular masses of 9.75 and 11.75 kD would be inconsistent with a causal role for renal hemodynamics. The observed reciprocal changes in proteins with molecular masses of 9.75 or 11.75 and 66.6 kD could be further exploited as a potentially useful index, i.e., 9.75 kD/66.6 kD or 11.75 kD/66.6 kD ratio. As demonstrated in Figure 8, these ratios are dramatically increased immediately and 6 to 12 h after cardiac catheterization in patients with impaired renal function, as opposed to patients with no renal complications. In future studies, we shall assess the utility of these indices in predicting impending contrast agent-induced nephropathy.

There is an urgent need for such early markers, because the
frequency of renal complications resulting from radiocontrast medium administration remains high and therapeutic interventions to treat such complications are quite limited. Our search of protein databases identified several plausible candidates that were revealed using SELDI-TOF analysis. Species of 9.75 kD are most probably heparin-binding epidermal growth factor-like growth factor (9.729 kD; species of 4.87 kD are the same molecules but doubly charged). Proteins of 11.75 kD most likely represent β2M (11.731 kD), as shown in Figure 9. The peak in the urine samples from patients with impaired renal function demonstrates the same mass and pattern as purified β2M from human urine (Figure 9A). Furthermore, the same peak can be observed in the immunoprecipitate but not the immunodepleted supernatant of samples incubated with specific anti-β2M antibodies (Figure 9B), as opposed to urine samples incubated with irrelevant goat anti-rabbit antibodies (Figure 9C). This is evidence that the protein represented by the peak with a molecular mass of 11.74 kD bound specifically to anti-β2M antibodies and can thus be identified as β2M. The candidate marker with an apparent molecular mass of 23.5 kD is most likely cathepsin O (23.460 kD). There is convincing evidence that the 66.4-kD (and doubly charged 33.3-kD) peaks observed in urine from rats and patients represent albumin (66.480 kD), especially from experiments in which the samples were mixed with an albumin standard (data not shown), although there is not yet an explanation for why albumin excretion decreases after the administration of radiocontrast medium. There are data to support heparin-binding epidermal growth factor-like growth factor playing an important role in the pathophysiologic processes of acute renal injury (21–23), making it a potentially meaningful marker. Different investigators have used β2M as a marker of nephrotoxicity (24,25), with controversial results (26). However, there are no published data on the role of cathepsins as markers of any nephropathy. Definitive proof can be obtained through the isolation and microsequencing of these proteins. At that stage, it will be possible to perform a complete analysis of samples from patients receiving radiocontrast medium, in the search for candidate markers that are predictive of impending radiocontrast agent-induced nephropathy.

The proteins detected in the urine fall into several categories (4), as follows: (1) proteins that are also present in plasma and appear in the urine either intact or as fragments (>31 proteins) (27,28); (2) proteins that are produced by the kidney (approximately 30 different enzymes) or lower urinary tract (12 derived from the bladder, ureters, urethra, and/or prostate gland); (3) proteins that leak into the circulation, and eventually into the urine, from other organs, i.e., liver-, testicle-, or skeletal muscle/myocardium-specific proteins; (4) hormones or other signaling molecules (in the case of human chorionic gonadotropin, diagnosis of pregnancy has been made simple) (1); and (5) tumor-associated, bacterial, or viral products. These theoretical considerations should underscore the applicability of SELDI-TOF uroscopy (in conjunction with the analysis of serum) to a broader range of clinical problems.

In conclusion, the data presented here demonstrate the applicability of SELDI-TOF for the detection of unique proteins in the urine and the potential of this technique for the identification of marker proteins to aid in the prediction of impending renal complications of radiocontrast agent administration during procedures such as cardiac catheterization. As a proof of principle, we have identified one of the peaks in the SELDI protein profiles, i.e., that at 11.75 kD, as β2M. Application of the SELDI-TOF technique to studies of urinary protein composition has the potential to revolutionize this field, making mass spectrometric uroscopy an important predictive and diagnostic tool.

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