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Diagnosis of albuminuria by tryptic digestion and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry

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ABSTRACT

Background: Matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry has been successfully used to detect trace albumin in urine for the diagnosis of albuminuria. However, only the monomeric form of albumin was detected with this approach.
Methods: Trypsin was used to digest urinary albumin and its related compounds in urine to produce characteristic peptides. The digest solution was subsequently analyzed by MALDI-TOF MS to obtain peptide ion signals which were used as diagnostic biomarkers for albuminuria.
Results: The analytical protocol was optimized for efficient digestion and high-performance MALDI-TOF MS analysis. The limit of detection (LOD) of albumin in urine was about 5×10^{-7} M.
Conclusions: Trypsin digestion combined with MALDI-TOF MS analysis is an efficient and simple approach for rapidly diagnosing albuminuria.

1. Introduction

Excessive albumin excretion in urine indicates a problem with the glomerular barrier and is recognized as a risk factor for the progression of renal and cardiovascular diseases [1–3]. Normal adults excrete <30 mg of albumin in their daily urine. If the quantity of excreted albumin is in the range of 30–300 mg/day, the individual has a condition known as microalbuminuria; if the quantity is >300 mg/day, the condition is known as macroalbuminuria. Screening for albuminuria allows early stage diagnosis of renal function impairment, thereby allowing preventive or therapeutic treatments to begin earlier.

Several techniques are currently used to measure the total amount of urinary proteins, and many more are used to measure urinary albumin concentrations, including turbidimetric assays, protein dye-binding assays, urine dipstick testing, immunochemical albuminassays, and methods based on size-exclusion high-performance liquid chromatography (HPLC) [4–14]. Turbidimetric assays (using trichloroacetic acid, sulfosalicylic acid, or benzethonium chloride) and dye-binding assays (biuret assays or coomassie brilliant blue assays) are mainly used to determine total urine protein [4–10]. Urine dipstick testing, which can be found in most outpatient settings, mainly detects albumin. A typical urinary dipstick contains tetrabromophenol blue (TBB) as the indicator [11]; when it comes into contact with excreted urinary albumin, an alkaline color reaction is set off, turning the color of the pad from yellow to green or blue for a positive protein result (>10 mg/dl). Because the urine dipstick indirectly detects proteins through color changes resulting from chemical reactions, this method is readily influenced by pH or the presence of many chemical and biochemical compounds in urine samples, potentially leading to false-positive or false-negative results [12]. Immunochemical albumin assays are applied specifically for the quantitative analysis of albumin in urine [13]; although this approach has been widely used, some controversy exists regarding whether immunoassays detect all forms (e.g., intact, modified, fragmented) of albumin in urine [14–19]. Size-exclusion HPLC is an alternative method for quantitative analysis of albumin in urine; for most specimens, the measured values are higher than those provided by immunoassays [20]. Nevertheless, data obtained using this method might be questionable if it detects other urinary protein molecules that have approximately the same size as albumin [21].

Even though several analytical methods exist, there is clinical interest for the development of approaches for accurately measuring urine albumin as an early indicator of kidney disease [22,23]. Such methods should enable the sensitive, automatic, rapid, and reliable detection of trace albumin in urine. Matrix-assisted laser desorption

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ionization/time-of-flight mass spectrometry (MALDI-TOFMS) is an extremely sensitive technique that permits the detection of large biomolecules such as protein at sub-femtomole abundances [24–26]. In addition, the experimental procedures required for a typical MALDI-TOF MS analysis are very simple; the reagents for sample preparation are inexpensive and, more importantly, the instrumentation is capable of automation so that it can be used to screen large numbers of samples. As a result, MALDI-TOF MS is a potentially useful tool for routine clinical diagnoses [27–33].

In previous studies, we found that MALDI-TOFMS is an alternative approach for the detection of urine albumin with a greater sensitivity than that of dipstick testing, and also for the rapid diagnosis of albuminuria [24,25]. Diagnostic success was determined through the observation of multiply charged albumin ions on MALDI mass spectra. Trace amounts of urine were directly analyzed without the need for tedious sample pretreatment [24]. We also demonstrated that MALDI-TOF analysis can overcome problems associated with interferences from high pH, salts, blood, drugs, detergents, proteins, and pigments in urine samples. In addition, since the intensity of individual albumin ions with different charged states changes with concentration, this said relationship has been successfully used to quantify albumin in urine [25].

Unfortunately, such an approach only examines the dominant monomeric and intact albumin molecules. Several different types of albumin, including bound and clustered forms which contribute to total urinary albumin excretions, are difficult to detect with MALDI-TOF due to their high mass ranges and low abundances, leading to potential underestimation of the total albumin in urine. Another problem

Table 1
Peptides generated from tryptic-digested urine/albumin samples that match with the "PeptideMass" of ALBU_HUMAN (P02768) from UniProtKB/Swiss-Prot.

Mass (observed)	Mass (expected)	Peptide sequence	Position	Number of missed cleavages
1226.98	1226.61	FKDLGEENFK	35–44	1
1149.95	1149.62	LVNEVTEFAK	66–75	0
876.77	876.50	LCTVATLR	98–105	0
658.27	658.32	QEPER	118–122	0
927.79	927.49	YLYEIAR	162–168	0
1083.90	1083.59	YLYEIARR	162–169	1
2055.50	2055.10	RHPYFYAPELLFFAKR	169–184	2
1899.40	1899.00	HPYFYAPELLFFAKR	170–184	1
1341.76	1341.76	AACLLPKLDELR	199–210	1
544.19	544.36	QRLK	220–223	1
508.21	508.25	FGER	230–233	0
1019.87	1019.58	AFKAWAVAR	234–242	1
673.21	673.38	AWAVAR	237–242	0
875.78	875.51	LSQRFPK	243–249	1
1624.20	1623.79	DVFLGMFLYEYAR	348–360	0
1468.28	1467.84	RHPDYSVVLLLR	361–372	1
1312.12	1311.74	HPDYSWLLLR	362–372	0
960.87	960.56	FQNALLVR	427–434	0
1640.40	1639.94	KVPQVSTPTLVEVSR	438–452	1
1511.84	1511.84	VPQVSTPTLVEVSR	439–452	0
674.27	674.35	TPVSDR	491–496	0
801.49	801.49	ERQIKK	544–549	2
1490.90	1490.91	QTALVELVKHKPK	550–562	1
509.20	509.32	H KPK	559–562	0

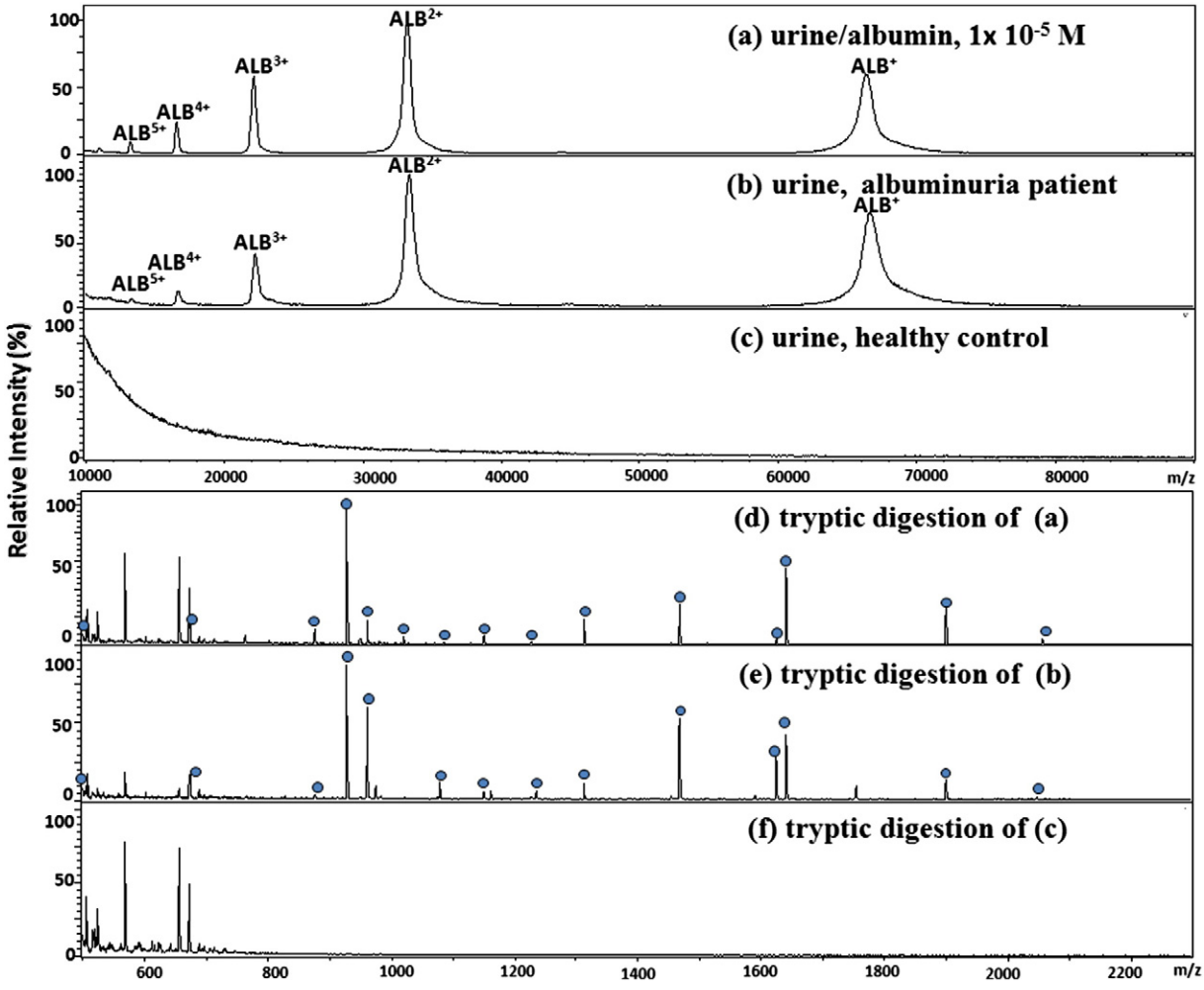

Fig. 1. Positive-ion MALDI mass spectra of (a) normal urine sample spiked with 1×10^{-5} mol/l albumin; (b) urine sample obtained from an albuminuria patient; (c) normal urine sample; (d) trypsin-digested sample of (a); (e) trypsin-digested sample of (b); and (f) trypsin-digested sample of (c). ALB: albumin. ●:Tryptic peptide ions of albumin.