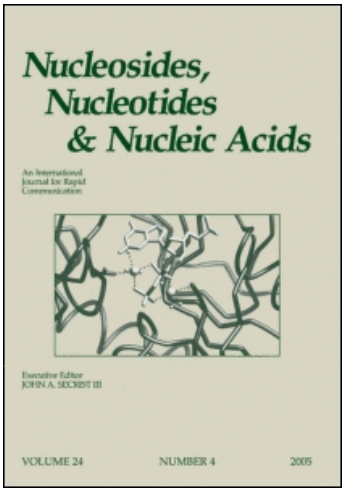

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DEVELOPMENT OF LC-MS METHOD FOR DETECTION OF MUTANT UROMODULIN PROTEIN

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□ *Mutations in the uromodulin gene cause the autosomal disorders familial juvenile hyperuricemic nephropathy (FJHN) and medullary cystic kidney disease type 2 (MCKD2). However, methods to detect the mutant form of the uromodulin protein have not been developed. In this study, we developed a liquid chromatography-mass spectrometry (LC-MS) method for detection of the mutated uromodulin peptide (C148W). Our method can distinguish the mutant peptide, GWHWE, from wildtype peptide, GWHC*E. Using MS/MS analysis with a selected reaction monitoring (SRM) mode, peptide-specific fragment ions (m/z 714 → 381, 471, 567, and 679 for GWHWE and m/z 688 → 381, 445, 541, and 653 for GWHC*E) were detected.*

Keywords FJHN/MCKD2; uromodulin; LC-MS; selected reaction monitoring

INTRODUCTION

Familial juvenile hyperuricemic nephropathy (FJHN) and medullary cystic kidney disease type 2 (MCKD2) are autosomal dominant genetic disorders characterized by hyperuricemia, gout, and chronic interstitial nephritis.[1] FJHN/MCKD2 are caused by mutations in the gene encoding uromodulin, the most abundant protein in urine. Although urinary uromodulin protein is severely reduced in patients with FJHN/MCKD2, it is not known if the uromodulin protein present in the urine is mutant, wildtype, or both. To investigate how mutant uromodulin causes disease, we developed an animal model by expressing mutant human uromodulin (C148W) in transgenic

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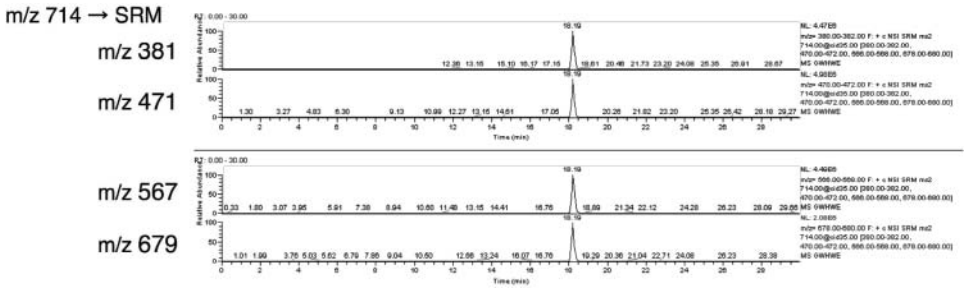

FIGURE 1 SRM chromatograms of GWHWE.

mice.[2] In this study, we developed a liquid chromatography-mass spectrometry (LC-MS) method for the detection of mutant and wildtype uromodulin proteins.

MATERIALS AND METHODS

To detect mutant and wildtype uromodulin protein with LC-MS, we synthesized the peptide, GWHWE, considered to be generated by *Staphylococcus aureus* V8 protease (V8 protease) digestion of mutant uromodulin (C148W). The wildtype peptide GWHCE was also synthesized, and the reduced cysteine residue was alkylated by iodoacetamide to generate the peptide GWHC*E. All three peptides, GWHWE, GWHCE, and GWHC*E, were purified by reversed phase HPLC. MS/MS analysis with selected reaction monitoring (SRM) method was used to detect each peptide individually. To confirm this method, wildtype uromodulin was separated by SDS-polyacrylamide gel electrophoresis and digested with V8 protease; the resulting peptide mixture was analyzed.

The peptide solution was analyzed by LC-MS/MS. The HPLC system used was the Magic 2002 from MICHROM Bioresources, Inc. (Auburn, CA, USA). The analytical conditions were as follows: column, Magic C18, 0.2

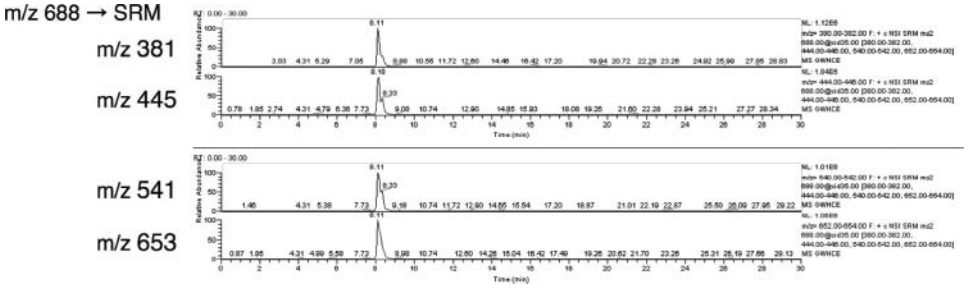

FIGURE 2 SRM chromatograms of GWHC*E.

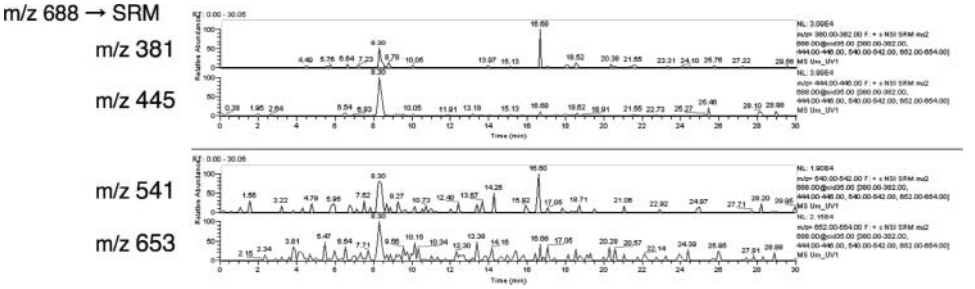

FIGURE 3 SRM chromatograms of digested wildtype uromodulin.

mm ID × 50 mm; solvent, 0.1 M formic acid with CH_3CN gradient (5% to 65% in 20 minutes); flow rate, 2 μl/min. The mass spectrometer used was LCQ_{DECA} (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an ion trap, and a nano ESI interface from MICHROM Bioresources. The electrospray voltage was 1.5 kV and the temperature of the heated capillary was 170°C.[3]

RESULTS AND DISCUSSION

Purified peptides GWHWE, GWHCE, and GWHC*E were identified by MS as $[GWHWE + H]^+$ (m/z 714.2), $[GWHCE+H]^+$ (m/z 631.2), and $[GWHC^*E + H]^+$ (m/z 688.1). Using MS/MS analysis with SRM mode, peptide-specific fragment ions, m/z 381, 471, 567, and 679 from GWHWE and m/z 381, 445, 541, and 653 from GWHC*E, were detected. GWHWE and GWHC*E were eluted at 18 and 8 minutes, respectively, and the corresponding fragment ions were detected also detected at 18 and 8 minutes (Figures 1 and 2). When wildtype uromodulin was analyzed by LC-MS/MS with SRM, GWHC*E and related fragment ions were detected (Figure 3). This method will be applied to examine the relative abundance of wildtype and mutant uromodulin protein in the urine of transgenic mice.

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