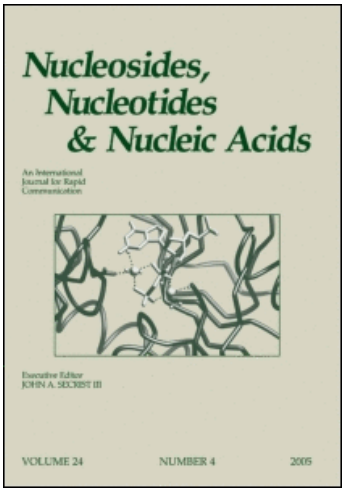

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Detection of Prothrombin and Osteopontin in a Renal Stone Found in a Hyperuricemic Patient Using 2D-PAGE and LC-MS Analysis

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ABSTRACT

The liquid chromatography-mass spectrometry (LC-MS) following on from the two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technique was applied for the analysis of proteins in a renal stone found in a hyperuricemic patient. This technique was sensitive enough to detect small quantities of proteins even in a renal stone.

Key Words: Renal stone; Analysis of protein; 2D-PAGE and LC-MS; Prothrombin; Osteopontin.

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INTRODUCTION

Urinary stones are known to be often found in patients with hyperuricemia and/or hypouricemia. For preventing recurrence of calculi, it is considered important to carefully analyze each individual pathological calculus in every patient. Recently, LC-MS following 2D-PAGE has been applied in the analysis of various proteins.[1–3] We employed this method to analyze the matrix in a calculus. Nanoflow LC-MS equipped with a nano ESI interface, and database searching on an ion trap mass spectrometer were used in this study.

MATERIALS AND METHODS

Materials: A renal stone (size: 7–9 mm) from a male patient (64-years-old) with hyperuriceamia was examined. *IR analysis*: For the determination of inorganic components in renal stones, infrared (IR) spectroscopy is generally used. IR analysis was undertaken with a KBr tablet. *Extraction*: The stone was extracted with EDTA and guanidine hydrochloride, following dialysis, concentration followed the method noted by Yamate et al.[4] *2D-PAGE*: Proteins were analyzed by isoelectric focusing polyacrylamide gel electrophoresis (IEF-PAGE) and sodium-dodesyl sulfated polyacrylamide gel electrophoresis (SDS-PAGE).[1] *In gel digestion*: Protein spots were excised from the gel and digested with trypsin according to published procedure.[5] *LC-MS/MS*: A nano LC-MS/MS equipped with a nano ESI interface and an ion trap were used. With an ion trapping detection system, the mass fragmentation from the selected ions was

- >gi|135807|sp|P00734|THRB_HUMAN PROTHROMBIN PRECURSOR (COAGULATION FACTOR II) gi|625232|pir||TBHU thrombin (EC 3.4.21.5) precursor - human gi|339641 (M17262) prothrombin [Homo sapiens] [MASS=70037]
  MAHVRGLQLP GCLALAALCS LVHSQHVFLA PQQARSLLQR VRRANTFLEE VRKGNLEREC
  VEETCSYEEA FEALESSTAT DVFWAK**YTAC ETART**PRDKL AACLEGNCAE GLGTNYRGHV
  NITR**SGIECQ LWR**SRYPHKP EINSTTHPGA DLQENFCRNP DSSTTGPWCY TTDPTVRRQE
  CSIPVCGQDQ VTVAMTPRSE GSSVNLSPPL EQCVPDRGQQ YQGRLAVTTH GLPCLAWASA
  QAKALSKHQD FNSAVQLVEN FCRNPDGDEE GVWCYVAGKP GDFGYCDLNY CEEAVEEETG
  DGLDEDSDRA IEGR**TATSEY QTFFNPRTFG SGEADCGLRP LFEK** KSLEDK TERELLESYI
  DGRIVEGSDA EIGMSPWQVM LFRK**SPQELL CGASLISDRW** VLTAAHCLLY PPWDKNFTEN
  DLLVRIGKHS RTRYERNIEK ISMLEKIYIH PRYNWRENLD RDIALMKLKK PVAFSDYIHP
  VCLPDRETAA SLLQAGYKGR VTGWGNLKET WTANVGKGQP SVLQVVNLPI VERPVCKDST
  RIRITDNMFC AGYKPDEGKR GDACEGDSGG PFVMKSPFNN RWYQMGIVSW GEGCDRDGK**Y**
  GFYTHVFRLK KWIQKVIDQF GE
  >average mass = **70018**
- position sequence (**NCBI BLAST** link)
- 87- 94 YTACETAR
- 600- 608 YGFYTHVFR
- 125- 133 SGIECQLWR
- 328- 344 TFGSGEADCGLRPLFEK
- 315- 327 TATSEYQTFFNPR
- 385- 399 SPQELLCGASLISDR
- **Protein Coverage:** 71/622 = **11.4%** by amino acid count, 8064/70018 = **11.5%** by mass

Figure 1. Prothrombin in the a renal stone (spot7).

easily collected. The MS/MS spectra were submitted to a database search, SEQUEST,[6] and the containing proteins determined.

RESULTS

Absorption around 3200 and 1620 cm^{-1} were found in IR spectrum. They were derived from hydroxy and carbonyl group, respectively. IR analysis showed the renal stone was mainly composed of calcium oxalate. In 2D-PAGE, several proteins were detected in the acid rich area (pI 3–4). They were excised from the gel and digested with trypsin and then applied to LC-MS. From LC-MS analysis of proteins in the spot with pI 3 and molecular weight of 72 kDa, prothrombin was determined. Six peptides, whose positions were 87–94, 125–133, 315–327, 328–344, 385–399, and 600–608 were detected, respectively (Fig. 1). 71 amino acids in 622 of prothrombin were determined with LC-MS analysis (protein coverage rate: 11.4%). Osteopontin was also determined to be contained as an acidic protein.

DISCUSSION

The method of the nanoflow LC-MS/MS equipped with a nano ESI interface and an ion trap, following 2D-PAGE, was so sensitive that even a small amount of protein (approximately 300 fmol) in just a part of the renal calculus could be determined. As prothrombin and osteopontin were detected in this calculus, it is suggested that calcium binding proteins play an important role in the growing process of the calcium oxalate stones. In order to investigate the cause of pathological stone recurrence, analysis of each stone in detail is thought to be helpful.

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