Tamm–Horsfall protein or uromodulin: new ideas about an old molecule

Olivier Devuyst, Karin Dahan and Yves Pirson

Divisions of Nephrology and Genetics, Université catholique de Louvain Medical School, B-1200 Brussels, Belgium

The discovery and re-discovery of Tamm–Horsfall protein/uromodulin

More than 50 years ago, Tamm and Horsfall isolated a mucoprotein from the human urine, and showed that the protein was able to interact and inhibit viral haemagglutination [1,2]. Of interest, the protein was found to be heavily glycosylated, containing up to 30% of its mass in carbohydrates [3]. It was then discovered that the Tamm–Horsfall protein (THP), as it was readily named, was the most abundant protein in normal human urine, with a migration pattern at ~90 kDa in SDS–PAGE [4]. In 1985, Muchmore and Decker [5] identified a 85 kDa glycoprotein in the urine of pregnant women. The protein was named uromodulin, due to its potent immunosuppressive activity reflecting its ability to inhibit antigen-induced T-cell proliferation and monocyte cytotoxicity in vitro [5]. Besides the molecular mass and the abundance in urine, the characterization of uromodulin revealed a number of resemblances with THP, including a ~30% carbohydrate content, a tendency to form aggregates and a significant number of intrachain disulfide bridges [5]. Based on sequence analysis, Pennica et al. [6] later confirmed that uromodulin was indeed THP. For the sake of clarity, we will use the term uromodulin to discuss the THP/ uromodulin protein in this review.

Uromodulin: biochemical properties and distribution

Uromodulin is a glycosylphosphatidylinositol (GPI) anchor-linked protein characterized by a remarkable structure and unique properties [reviewed in 7]. The mature protein contains 616 amino acids, including 48 cysteine residues potentially involved in 24 disulfide bridges, which are important for its conformation. Eight potential sites of N-glycosylation are also present, explaining the high carbohydrate content of uromodulin [3,5]. The predicted structure of uromodulin contains three epidermal growth factor (EGF) domains that contain a calcium-binding consensus sequence and mediate protein–protein interactions, as well as a zona pellucida-like domain. The C-terminus of the protein includes a stretch of hydrophobic amino acids that acts as a signal for the attachment of a preformed GPI anchor within the endoplasmic reticulum (ER). Following this addition, the membrane-bound protein is transported to the Golgi complex, where glycans are fully processed, then delivered to the luminal cell surface, and finally released into the urine by proteolytic cleavage.

Initial hybridization studies showed that uromodulin mRNA is only expressed in the kidney [6]. In this organ, uromodulin is distributed to the cells lining the thick ascending limb (TAL) of Henle’s loop, with an extension to the early distal convoluted tubule (DCT) in some species [7]. Evidence obtained at the light and electron microscope levels showed that uromodulin is sorted to the apical cell membrane of the TAL epithelial cells (Figure 1), probably resulting from the addition of the GPI anchor and/or N-linked glycans.
Uromodulin is excreted in the urine at a rate of \( \frac{1}{24} \) 50 mg/day, but this amount can be influenced by many factors, including urine volume, diet and exercise [10]. The biochemical characteristics of the protein can be assessed by analysis of the urine [11], for instance by examining the influence of deglycosylation or reducing conditions on the migration pattern in SDS–PAGE (Figure 1). It must be noted that a single immunostaining study has detected uromodulin in the ependymal cells and astrocytic processes of rat brain [12].

**Role and pathophysiology of uromodulin**

As recently reviewed [7], the biological role of uromodulin remains enigmatic. The protein tends to form large aggregates in solution and, via its glycans, interferes with lymphocytes and competes with uroplakin receptors for the adhesion of type 1 fimbriated *Escherichia coli*. Based on these properties, it has been postulated that uromodulin modulates cell adhesion and signal transduction by interacting with cytokines and, more specifically, inhibits the aggregation of calcium oxalate crystals and provides a defence against urinary tract infection. Also, the selective distribution of uromodulin in the TAL led to suggestions that it could play a role in the ion transport processes (and the lack of water permeability) that take place in that nephron segment. Furthermore, uromodulin is the major constituent of hyaline casts, and is also involved in the matrix of other types of casts, including those formed by IgG light chains.

The recent association of mutations in the uromodulin gene with a peculiar type of chronic interstitial nephritis has shed new light on the pathophysiological
The role played by this protein in the kidney. Familial juvenile hyperuricemic nephropathy (FJHN; OMIM no. 162000) is an autosomal dominant disorder characterized by hyperuricaemia due to reduced urinary excretion of urate, and the development of chronic interstitial nephritis leading to progressive renal failure [13]. The phenotype of FJHN resembles autosomal-dominant medullary cystic kidney disease (MCKD2; OMIM no. 603860), a rare form of chronic interstitial nephritis developing during adulthood and characterized by late and non-constant development of corticomedullary cysts. Mapping studies identified a locus for FJHN on chromosome 16p11.2, i.e. within <5 cM from the previously localized MCKD2 locus, which raised the hypothesis that the two disorders were actually two facets of the same disease [13]. This hypothesis was confirmed by Hart et al. [14], who first reported mutations of the UMOD gene (that encodes uromodulin) in FJHN as well as MCKD2 kindreds. The causative link between mutations in UMOD and FJHN/MCKD2 was confirmed by several independent groups [11,15–18]. To date, 37 distinct mutations have been reported in association with FJHN/MCKD2, with a clustering in exons 4 (32/37) and 5 (5/37). Most of the mutations are single amino acid changes (33/37), modifying cysteine (23/33) or charged residues (10/33). The exon 4 of UMOD contains a strongly conserved, cysteine-rich sequence as well as three Ca\(^{2+}\)-binding EGF domains (cbEGF). Missense mutations of UMOD affecting cysteine residues are likely to alter disulfide bridges and disrupt the correct folding of the protein. Similarly, mutations affecting the calcium-binding affinity of the EGF domains will likely lead to structural destabilization or increased sensitivity to proteolytic degradation [11,15].

Pathogenic mutations of UMOD impair uromodulin trafficking

A detailed analysis of the expression and distribution pattern of uromodulin demonstrated that mutations in UMOD lead to a gross accumulation of uromodulin in TAL cells (Figure 1), together with a drop in the urinary excretion of wild-type protein [11]. Both findings were specifically related to UMOD mutations because they were not observed in FJHN patients without mutations in this gene. Taken together, these observations led us to suggest that mutations in UMOD may critically affect the function and expression of uromodulin, resulting in abnormal accumulation of the mutated protein within tubular cells [11]. This conclusion was supported by Rampoldi et al. [17], who further analysed the trafficking defect of uromodulin in cultured cells. These experiments clearly established that UMOD mutations affecting cysteine residues cause a delayed export of mutant uromodulin to the plasma membrane, with a longer retention time in the ER probably reflecting an abnormal folding of the protein. The essential role of cysteine residues in the folding process is also supported by the slower maturation of uromodulin observed in reducing conditions (Figure 1). Of note, mutations that disrupt conserved cysteine residues within EGF-like domains of fibrillin-1, the protein involved in Marfan syndrome, are also associated with delayed intracellular processing and/or secretion of the mutant protein [19].

In concordance with the abnormal processing in the kidney, a significant decrease in the urinary excretion of uromodulin was reported in patients harbouring mutations of UMOD, irrespective of age, gender or degree of renal failure [11,17,20]. Biochemical and mass spectrometry analyses have shown that patients with UMOD mutations only excrete wild-type uromodulin in the urine [11]. The fact that the latter represents apparently <50% of the expected excretion may suggest a dominant-negative effect, in which the mutated protein may interfere with the normal trafficking of the wild-type copy in the TAL [11,17,20].

Uromodulin knock-out mouse models

The physiological role of uromodulin has been recently clarified by examination of two knock-out (KO) mouse models that have been generated by independent laboratories. Mo et al. [21] first generated a uromodulin-null mouse by deleting the first four exons and a 650 bp proximal promoter region of Umod, which resulted in a complete ablation of the gene. The deletion of Umod had no major effect on embryonic development and histology of the kidney. However, uromodulin KO mice were predisposed to bladder infections following inoculation of type 1-fimbriated E.coli possessing a FimH adhesin [21]. Similar results have been obtained in a second KO mouse obtained by targeted disruption of exon 2 of Umod, also resulting in a lack of uromodulin expression in kidney and urine and an increased bacterial sensitivity of the urothelium [22]. Taken together, these studies demonstrated that uromodulin plays a role in host-defence against E.coli adhesion to the urothelium in vivo. This protective effect is probably mediated by the high mannose glycosylation of uromodulin, which binds type 1-fimbriated E.coli in vitro [23]. Thus, the abundant uromodulin amounts excreted in normal urine could act as a competitive inhibitor for the FimH adhesin of type 1-fimbriated E.coli, thereby preventing them from binding the uroplakin receptors located on the surface of the urothelium.

The KO mice were also used to demonstrate the role of uromodulin in preventing urinary stones and nephrocalcinosis [24]. A sizeable fraction (~15%) of adult uromodulin KO mice showed spontaneous formation of calcium crystals located in the collecting ducts of the medulla, whereas such crystals were never found in wild-type littermates. The development of calcium oxalate crystals was triggered by exposure
Of mouse and man: unsolved issues and future perspectives in uromodulin research

The increased susceptibility to urinary tract infection (UTI) and renal stone formation observed in uromodulin KO mice is unlikely to be extrapolated to the patients harbouring UMOD mutations. Actually, an increased prevalence of UTI or kidney stones has been reported in none of the patient series with UMOD-associated FJHN/MCKD2. This lack of evidence is not surprising since, in contrast to KO mice lacking uromodulin expression, all patients harbouring UMOD mutations that have been investigated thus far show a grossly abnormal expression of uromodulin in the kidney [11,17]. Moreover, KO mice have no uromodulin excretion in the urine [21], whereas some wild-type protein is detected in the urine of patients with UMOD mutations [11,17,20]. This small amount of bioactive, residual protein may be sufficient to prevent UTI or renal stone formation. In that respect, a detailed phenotypic analysis of two patients harbouring a homozygous UMOD mutation, who have apparently no detectable uromodulin in the urine, could be of interest [27].

A second issue raised by the mouse models is the link between mutations in UMOD and structural abnormalities in the kidney. As noted above, the uromodulin KO mice lack any structural abnormality in the kidney, whereas patients with UMOD mutations have an interstitial nephritis with focal tubular atrophy, occasional lymphocytic infiltration, thickening and splitting of tubular basement membranes, and cortico-medullary cysts [11,13,14]. We have shown that uromodulin accumulates in a subset of dilated tubule profiles derived from the TAL [11]. This intracellular accumulation of mutated uromodulin may actually trigger an inflammatory response and a pro-fibrotic cascade leading to tubulo-interstitial fibrosis. The link with (non-constant) cystic changes is unclear: one hypothesis is that they could arise from tubule swelling due to luminal obstruction [17].

A third issue concerns the putative link between uromodulin and ion transport in the TAL, emphasized by the high prevalence of an urinary concentrating defect in patients with UMOD mutations [28]. The TAL reabsorbs ~25% of filtered NaCl and plays an essential role in the generation of the medullary interstitial hyperosmolality. It has been suggested that uromodulin could participate in the water impermeability of the cells lining the TAL [7]. Accordingly, a failure of this role would interfere with NaCl reabsorption, decrease the interstitial osmolality and impair the urine concentrating ability. The above mechanism could also explain another cardinal manifestation of FJHN/MCKD2, i.e. hyperuricaemia. The transport mechanisms of urate are localized in the PT, whereas no experimental evidence supports urate permeability in more distal segments of the nephron. Furthermore, there is no expression of normal or mutated uromodulin in cells lining the PT [11]. The hyperuricaemia associated with UMOD mutations could thus reflect an increase in proximal tubular reabsorption of urate secondary to volume contraction caused by a deficient transport in the TAL [11,14,17]. The correlation between urine osmolality and serum urate level supports this hypothesis [28]. Furthermore, recent investigations have shown that components of the juxtaglomerular apparatus and ion transporters in the kidney are both altered in uromodulin KO mice, in parallel with impaired urinary concentrating ability [29]. These changes, which include a reduction in juxtaglomerular COX-2 and renin expression, and a significant upregulation of the major transporters of the TAL (including NKCC, ROMK, CIC-Kb and barttin) and DCT (NCC), are compatible with an adaptive mechanism in response to altered reabsorptive activity in the TAL [29]. Other important questions, such as the specific role played by uromodulin during gestation, remain to be solved.

Conclusion

Recent evidence, obtained both in man and mouse, highlights the multi-faceted aspects of uromodulin and supports a significant biological role for this abundant constituent of normal urine. In addition to its role in urine, uromodulin could also play a functional role in the TAL, thereby influencing NaCl reabsorption and urinary concentrating ability. Disease resulting from mutations of the uromodulin gene (UMOD) has to be added to the expanding family of trafficking disorders in the kidney tubule [30]. Developing a knock-in mouse model, by directed mutagenesis affecting a conserved cysteine residue within the corresponding exon 4 in man, will be essential in deciphering the pathophysiology of uromodulin-associated diseases.

Acknowledgements. The authors work mentioned in this review has been supported by grants from the FNRS and FRSM, the Communaute Franc¸ aise de Belgique, the Fondation Alphonse et Jean Forton and the ARC 00/05-260. The authors apologize to the many colleagues whose work could not be mentioned because of space constraints.

Conflict of interest statement. None declared.
References


