The sweet side of urea transporters

Rebecca P. Hughey
Department of Medicine, Renal-Electrolyte Division and Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

UREA IS PRODUCED AS AN END product of protein catabolism in the liver and is ultimately excreted by the kidney. However, this unique small molecule of only 60 daltons is also used by the kidney to control water balance by a complex process that involves both secretion and reabsorption of urea throughout the entire renal tubule (11). Disturbance of these processes can cause a toxic buildup of urea in the blood termed uremia. Clearly, urea transporters (UT) are key players in this important process, and understanding UT activity, synthesis, turnover, and membrane trafficking has been a focus of researchers since the first gene was cloned in 1993 (17).

The UT-A subfamily represents the renal tubule transporters, and the 12-transmembrane UT-A1 protein is the largest and best studied of this group (12). The transport-competent UT-A1 includes two pores and two NH2-linked glycans, and, interestingly, a splice variant UT-A3 representing only the NH2-terminal half of UT-A1 is independently expressed with one pore, one N-glycan, and robust transport activity (1, 4, 8). UT-A1 and UT-A3 are both expressed in the inner medullary collecting duct (IMCD) (7). Although UT-A1 is expressed in both the inner medulla (IM) tip and base, UT-A3 is found only in the IM tip, consistent with a unique function for the two variants within the renal tubule. However, each variant also exhibits alternatively localized and processed forms within the cell, consistent with fine tuning of the transporter activity by specific cell types (7). The article by Su et al. (13) in an issue of the American Journal of Physiology-Renal Physiology is providing new significant clues to our understanding of UT expression and regulation by dissecting the basis and potential consequences of the UT alternative processing. Su et al. report that UT-A3 with a unique modification of the N-glycan is localized to a specific plasma membrane domain within cells that correlates with increased activity and remarkable stability.

Immunoblot analysis of rat IM extracts reveals two distinct forms of both UT-A1 (97 and 117 kDa) and UT-A3 (44 and 67 kDa). Previous studies revealed that both forms of UT-A1 and UT-A3 are resistant to treatment with endoglycosidase H (Endo H), an enzyme that can remove immature Asn (N)-linked oligosaccharides from glycoproteins (3, 14). Acquisition of Endo H resistance is a hallmark feature of glycoproteins that transit the Golgi complex where N-glycans are first trimmed and then usually remodeled by addition of sugars such as GlcNAc, galactose, fucose, and sialic acid. The extent of remodelling can also be assessed by simple pull-down assays with plant lectins that exhibit established specificities for binding glycoconjugate structures. Chen et al. (5) previously used this approach to show that the 117-kDa form of UT-A1 from IM was modified with poly-N-acetyllactosamine extensions (repeats of Galβ1,4GlcNAc), whereas the more abundant 97-kDa form lacks this modification, which likely accounts for the mobility differences of the two forms on SDS gels (5). More importantly, the 117-kDa form of UT-A1 is localized primarily to a unique membrane subdomain (termed a lipid raft) defined by its appearance exclusively in a low-density fraction of a sucrose flotation gradient after extraction with the detergent Brij 96V. Using a similar approach, Su et al. (13) now show that the 67-kDa form of UT-A3 is also modified with poly-N-acetyllactosamine extensions when expressed in HEK293 cells while the 44 kDa form is not and that the 67-kDa form partitions exclusively into these low-density lipid rafts. Most interestingly, the 67-kDa form of UT-A3 is absolutely stable in HEK293 cells over 18 h, whereas the 44-kDa form (and the 97-kDa form of UT-A1) has a more typical half-life of ~10 h.

The correlation of UT modification with poly-N-acetyllactosamine extensions and its lipid raft association is intriguing. It is possible that the N-glycan modification with poly-N-acetyllactosamine extensions targets UT to lipid rafts or that association of UTs with lipid rafts results in modification of the UT N-glycans with poly-N-acetyllactosamine extensions. Both could be true. Chen et al. (5) already showed that the 97-kDa form of UT-A1 is present in lipid rafts within an hour of its synthesis in Madin-Darby canine kidney cells, whereas a mutant lacking consensus sites for N-linked glycosylation is not found in lipid rafts. These data could simply reflect reduced exit of mutant UT-A1 from the endoplasmic reticulum, which is often N-glycan-dependent for glycoproteins, or it could reflect a role for N-glycans in surface expression of UTs, since N-glycans on some transmembrane proteins act as apical targeting signals in polarized epithelial cells (10, 15). Both roles for N-glycans could affect UT membrane trafficking and subsequent association with lipid rafts (16). On the other hand, both the 97-kDa UT-A1 and the 44-kDa UT-A3, which both lack poly-N-acetyllactosamine extensions, are present in both the low-density lipid raft fractions and the high-density fractions of the sucrose gradient, consistent with modification of UT N-glycans once they are present in the lipid rafts. Because some apically expressed proteins are targeted by signals within their transmembrane domains through association with lipid rafts (defined by cold detergent insolubility and low density) (10, 15), it becomes important to determine the mechanism for UT association with Brij 96V rafts.

Finally, it is not yet clear why the UT-A3 with poly-N-acetyllactosamine extensions has such a protracted stability or enhanced activity compared with UT-A1. The enhanced enzymatic activity of sucrase-isomaltase localized in lipid rafts (defined by its cold detergent solubility in Triton X-100) is dependent on terminal processing of both its N-linked and O-linked glycans and is attributed to cluster formation and cooperative interactions (16). One factor that could be directly associated with the terminal processing of glycans (and clustering) is an association with galectins. Galectins are small
lectin-like proteins that bind β-galactose-containing glycoconjugates and have been implicated to play a role in apical targeting of glycoproteins through cross-linking of either N-linked or O-linked glycans, or even glycolipids (2, 6, 9). Formation of multivalent lattices or microdomains through galectin cross-linking of cell surface receptors or solute transporters is a well-established phenomenon that reduces glycoprotein trafficking into endocytic compartments and thereby decreases the potential for degradation (2). In short, UT modification with poly-N-acetyllactosamine extensions provides a prime target for binding of galectins and formation of seemingly stable complexes on the cell surface. Cell-specific regulation of glycosyltransferases that form these extensions could provide an additional level of regulation of UT expression and activity throughout the kidney tubule.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

Author contributions: R.P.H. drafted manuscript; R.P.H. edited and revised manuscript; R.P.H. approved final version of manuscript.

REFERENCES