The physiological roles of sulfate transporters

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Sulfate (SO_4^{2-}) is an essential anion involved in many cellular processes. Cells obtain sulfate primarily *via* sulfate transporters that mediate the movement of sulfate across their plasma membranes. Renal sulfate transporters (NaS1 and Sat1) are responsible for regulating serum sulfate levels and sulfate homeostasis. Despite their important roles in sulfate homeostasis, very little is known about the structural identities and sorting mechanisms that control NaS1 and Sat1 expression in cells. NaS1 encodes a Na^+-sulfate cotransporter expressed in renal and intestinal epithelial cells. It contains 595 amino acids with 13 putative transmembrane domains. In the renal proximal tubule, NaS1 is localized to the apical (brush-border) membrane, for which the sorting mechanisms have not been determined. The Sat-1 transporter mediates sulfate/bicarbonate/oxalate anion exchange *in vivo* at the basolateral membrane of the kidney proximal tubule. The aims of this study were to biochemically characterize the NaS1 and Sat1 proteins and identify the sorting mechanisms responsible for their membrane trafficking. For structural characterization of NaS1, a C-terminally hexahistidyl tagged NaS1 construct (NaS1-His) was expressed and metabolically labeled in *Xenopus* oocytes. SDS-PAGE revealed NaS1-His as two bands (50 kD and 60 kD). Treatment with endoglycosidase H led to a small (1–2 KD) shift in the 50 kD (but not the 60kD) band, suggesting complex glycosylation and the presence of a single glycosylation motif. Mutagenesis of a putative N-glycosylation site (N591S) produced a single band (50 kD) that was not shifted by endoglycosidase H, suggestive of a true glycosylation site. Transient transfection of EGFP/NaS1 in renal epithelial cells (OK, LLC-PK1 and MDCK) demonstrated apical membrane expression, which was not affected by tunicamycin. Transfection of the EGFP/NaSi-1 N591S glycosylation mutant still led to apical expression, suggesting that apical sorting was independent of the glycosylation of this site. Treatment with cholesterol depleting compounds (lovastatin and methyl-β-cyclodextrin) was also unable to disrupt apical sorting, suggesting that NaS1 apical trafficking may be independent of membrane lipid rafts. NaS1-His proteins when analyzed by BN-PAGE appeared as a single complex. Dissociation revealed one additional band, indicating a dimeric structure of the complex. Our data demonstrate that NaS1 most likely forms a dimeric protein which is glycosylated at N591, whose sorting to the apical membrane of renal epithelial cells may be independent of lipid rafts and glycosylation. Since Sat1 is not endogenously expressed in any renal cells line, here we transfected Sat1 into two renal cell lines (MDCK and LLC-PK1 cells), which sorted Sat1 exclusively, to the basolateral membrane, as observed *in vivo*. To identify possible sorting determinants, truncations of the Sat1 cytoplasmic C-terminus were generated, fused to enhanced green fluorescence protein (EGFP) or the human IL2R α-chain (Tac) protein and both fusion constructs were transiently transfected into MDCK cells. Confocal microscopy revealed the removal of the last three residues on the Sat1 C-terminus, a putative PDZ domain, had no effect on the basolateral sorting in MDCK cells, nor an effect on sulfate transport in *Xenopus* oocytes. Removal of the last 30 residues led to an intracellular expression for the GFP fusion protein and an apical expression for the Tac fusion protein, suggesting a possible sorting motif lies between the last 3 and 30 residues of the Sat1 C-terminus. Elimination of a dileucine motif at position 677/678 resulted in the loss of basolateral sorting, suggesting this motif is required for Sat1 targeting to the basolateral membrane. This post-translational mechanism may be important for the regulation of sulfate reabsorption by Sat1 in the kidney proximal tubule. Recently, single nucleotide polymorphisms (SNPs) have been identified in the coding regions of human NaS1 and Sat1 genes. These SNPs and their effects on protein sorting may have relevance to the regulation of serum sulfate levels in humans.

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