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REVIEW

Physiological Roles of Mammalian Sulfate Transporters NaS1 and Sat1

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Abstract This review summarizes the physiological roles of the renal sulfate transporters NaS1 (Slc13a1) and Sat1 (Slc26a1). NaS1 and Sat1 encode renal anion transporters that mediate proximal tubular sulfate reabsorption and thereby regulate blood sulfate levels. Targeted disruption of murine NaS1 and Sat1 leads to hyposulfatemia and hypersulfaturia. Sat1 null mice also exhibit hyperoxalemia, hyperoxaluria and calcium oxalate urolithiasis. Dysregulation of *NaS1* and *Sat1* leads to hypersulfaturia, hyposulfatemia and liver damage. Loss of Sat1 leads additionally to hyperoxaluria with hyperoxalemia, nephrocalcinosis and calcium oxalate urolithiasis. These data indicate that the renal anion transporters NaS1 and Sat1 are essential for sulfate and oxalate homeostasis, respectively.

Keywords Sulfate transport · Oxalate transport · Renal reabsorption · Anion exchange · NaS1 · Sat1 · SLC13A1 · SLC26A1

Abbreviations

NaS1	Sodium sulfate cotransporter-1
Sat1	Sulfate anion transporter-1
BBM	Brush-border membrane
BLM	Basolateral membrane
DIDS	4,4′-Diisothiocyanato-2,2′-disulfonate
SITS	4-Acetamido-4′-isothiocyanato-2,2′-disulfonate

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Introduction

Sulfate is among the most important macronutrients in the body. It is the fourth most abundant anion in human plasma ($\approx$300 μM) and is a hydrophilic anion that cannot passively cross the lipid bilayer of cell membranes. Therefore, all cells require plasma membrane sulfate transporters to ensure an optimal supply of sulfate in the body. The most widely studied organ in terms of sulfate transport is the mammalian kidney. Early studies demonstrated that sulfate was freely filtered and extensively reabsorbed by the kidneys (Goudsmit et al. 1939). Stop-flow experiments proposed that the kidney proximal tubule is the major site of active sulfate reabsorption (Hierholzer et al. 1960). The majority of filtered sulfate is reabsorbed, with fractional excretion only being approx. 10% (Goudsmit et al. 1939). In mammalian renal proximal tubular cells, sulfate transport systems exist on the luminal brush-border membrane (BBM) (Lucke et al. 1979) and the contraluminal basolateral membrane (BLM) (David and Ullrich 1992). The principal pathway for secondary active sulfate uptake across the BBM is sodium-coupled sulfate transport (Na^+–SO_4^{2-} cotransport), driven by the luminal membrane Na^+ gradient (Bastlein and Burckhardt 1986; Lucke et al. 1981; Schneider et al. 1984; Turner 1984). The exit of sulfate across the BLM occurs via an SO_4^{2-}/anion exchanger (Brazy and Dennis 1981; Kuo and Aronson 1988; Low et al. 1984; Pritchard and Renfro 1983), which completes the process of transcellular sulfate reabsorption in the proximal tubule. It is this pathway that regulates blood sulfate levels.

Renal Sulfate Transport Systems

Early transport studies using radiotracer ^{35}S-sulfate in renal proximal tubular BBM vesicles characterized a

Na^+-dependent sulfate transporter (Schneider et al. 1984; Turner 1984; Ullrich et al. 1980), which was inhibited by thiosulfate, molybdate, chromate and selenate (Ullrich et al. 1980). Renal proximal tubular BLM vesicles characterized a sulfate anion exchanger, that transports sulfate out of the cell (into the systemic circulation) and exchanges sulfate with thiosulfate, hydroxyl ions, bicarbonate, oxalate and a variety of organic ions and was inhibited by stilbene derivatives DIDS (4,4′-diisothiocyanato-2,2′-disulfonate) and SITS (4-acetamido-4′-isothiocyanato-2,2′-disulfonate) (Bastlein and Burckhardt 1986; Brazy and Dennis 1981; Hagenbuch et al. 1985; Low et al. 1984; Pritchard and Renfro 1983).

Cloning and Functional Characterization of Renal Sulfate Transporters

By expression cloning in *Xenopus* oocytes (Markovich 2008), two functionally and structurally distinct sulfate transporters were isolated and characterized: (1) the Na^+-Sulfate (NaS1; SLC13A1) cotransporter (Markovich et al. 1993), and (2) the sulfate anion transporter (Sat1, SLC26A1) (Bissig et al. 1994) (Fig. 1). For reviews on sulfate transport, see (Dawson and Markovich 2005; Lee et al. 2005; Markovich 2001; Markovich and Aronson 2007; Markovich and Murer 2004; Murer et al. 1994).

Na^+–Sulfate Cotransporter NaS1 (SLC13A1)

NaS1 encodes an electrogenic pH-insensitive Na^+-dependent SO_4^{2-} transporter (Na^+–SO_4^{2-} cotransporter/symporter) (Markovich et al. 1993), with substrate preferences for the anions sulfate (K_m = 93 μM), thiosulfate (K_m = 84 μM), selenate (K_m = 580 μM) and the cation Na^+ (K_{mNa^+} = 16−24 mM) (Busch et al. 1994). Significant *cis*-inhibition for NaS1-induced sulfate transport was observed with thiosulfate, selenate, tungstate and molybdate and with succinate and citrate (Lee et al. 2000a). The NaS1 protein is localized to the BBM (Regeer et al. 2007) of renal proximal tubular cells (Lotscher et al. 1996; Markovich et al. 1999a; Regeer et al. 2007) and encodes a protein of 595 amino acids (≈66 kDa) with 13 putative transmembrane domains (Beck and Markovich 2000; Lee et al. 2000a). The human *NaS1* gene (*SLC13A1*) consists of 15 exons (spanning >83 kb) localized on human chromosome 7q31–7q32 (Lee et al. 2000a).

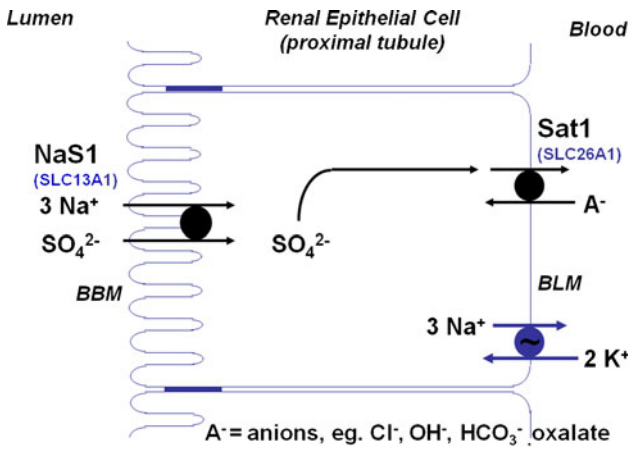

Fig. 1 Sulfate transporters in renal proximal tubule. *BBM* brush-border membrane, *BLM* basolateral membrane; NaS1 (SLC13A1): sodium sulfate cotransporter-1; Sat1 (SLC26A1): sulfate anion transporter-1

NaS1 mRNA and protein levels are down-regulated in the renal cortex by a variety of conditions, including: high sulfate diet (Markovich et al. 1998), hypothyroidism (Sagawa et al. 1999b), vitamin D depletion (Fernandes et al. 1997), glucocorticoids (Lee et al. 2000b), hypokalemia (Markovich et al. 1999a), metabolic acidosis (Puttaparthi et al. 1999) and non-steroidal anti-inflammatory drugs (Sagawa et al. 1998a), and upregulated by low sulfate diet (Sagawa et al. 1998b), thyroid hormone (Sagawa et al. 1999b), vitamin D supplementation (Fernandes et al. 1997), growth hormone (Sagawa et al. 1999a), chronic renal failure (Fernandes et al. 2001) and during post-natal growth (Markovich et al. 1999b). *NaS1* promoter studies showed up-regulation by vitamin D, thyroid hormone and the xenobiotic 3-methycholanthrene (Dawson and Markovich 2002; Lee et al. 2005; Lee and Markovich 2004) and down-regulated by glucocorticoids (Beck and Markovich 2000).

Generation of *Nas1* null ($Nas1^{-/-}$) mice revealed numerous physiological roles of *NaS1* in vivo. When compared to wild-type littermates, $Nas1^{-/-}$ mice exhibit: hyposulfatemia and hypersulfaturia (Dawson et al. 2003), reduced body weights (Dawson et al. 2003), reduced fertility (Dawson et al. 2003), reduced circulating steroid levels and increased urinary glucocorticoid excretion (Dawson et al. 2008), altered hepatic lipid and cholesterol metabolism (Dawson et al. 2006), enhanced tumor growth (Dawson et al. 2010a), reduced intestinal mucin sulfonation and impaired intestinal barrier function (Dawson et al. 2009), spontaneous clonic seizures (Dawson et al. 2003), impaired memory and olfactory performance (Dawson et al. 2005), decreased object-induced anxiety and locomotor activity (Dawson et al. 2004), hyperserotonemia and reduced brain serotonin levels (Lee et al. 2007), increased sensitivity to acetaminophen hepatotoxicity, increased liver damage and decreased hepatic glutathione (Lee et al. 2006). These data indicate that NaS1 is essential for maintaining sulfate homeostasis and is involved in wide range of physiological functions.

Sulfate Anion Transporter Sat1 (SLC26A1)

Sat1 encodes a protein of 703 amino acids (≈75.4 kDa) with 12 transmembrane domains (Dawson and Markovich 2005; Markovich 2001). Functional characterization in *Xenopus* oocytes revealed Sat1 to encode a Na^+-independent sulfate transporter, with a $K_m = 136$ μM for sulfate, that was strongly inhibited by oxalate, molybdate, selenate, tungstate, thiosulfate, phenol red, probenecid and DIDS ($IC_{50\ DIDS} = 28$ μM) (Bissig et al. 1994; Markovich et al. 1994). Sat1 expression in *Xenopus* oocytes leads to a ~20-fold induction in Na^+-independent sulfate transport, a ~sixfold induction in oxalate and chloride transport when compared to controls (Lee et al. 2003; Markovich 2006).

The *Sat1* gene (*SLC26A1*) is localized to human chromosome 4p16.3 (Regeer et al. 2003). Northern blot analysis detects a single mRNA (3.8 kb) transcript for *Sat1* very strongly in rat kidney and liver, with weaker signals in skeletal muscle and brain (Bissig et al. 1994; Markovich et al. 1999b). The *Sat1* gene is up-regulated by thyroid hormone (Beck and Markovich 2000) and AP-1 (Lee et al. 2003; 2005). Using Sat1 polyclonal antibodies, Sat1 protein was localized to the BLM (Regeer and Markovich 2004) of kidney proximal tubules by immunocytochemistry (Karniski et al. 1998).

Generation of *Sat1* null ($Sat1^{-/-}$) mice revealed the physiological roles of *Sat1* in vivo. $Sat1^{-/-}$ mice exhibit hyperoxaluria with hyperoxalemia, nephrocalcinosis and calcium oxalate stones in their renal tubules and bladder, as well as hypersulfaturia, hyposulfatemia and enhanced liver toxicity (Dawson et al. 2010b). These data suggest Sat1 regulates both oxalate and sulfate homeostasis and is critical to the development of calcium oxalate urolithiasis and hepatotoxicity.

Conclusions

There has been great progress in the functional characterization of sulfate transporters in the kidney and elucidation of their physiological roles in the body. The apical membrane Na^+-sulfate cotransporter NaS1 plays a major role in mediating proximal tubular sulfate reabsorption, as shown by the striking hyposulfatemia and hypersulfaturia in Nas1 null mice. Sat1 null mice have recently revealed the important role of Sat1 in the homeostasis of both sulfate and oxalate. Future research on these proteins (and their murine models), will not only elucidate their roles in human disorders (Dawson and Markovich 2005) with perturbed homeostasis of sulfate (sulfate wasting disorders) and oxalate (urolithiasis), but may allow the development of novel therapeutics for human kidney stones and drug-induced hepatotoxicity.

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