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Topical Review

Molecular Mechanisms in Renal and Intestinal Sulfate (Re)Absorption

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Introduction

Inorganic sulfate is an essential cofactor for sulfate conjugation reactions that are responsible not only for the detoxification of many endogenous and exogenous compounds, but also for the biosynthesis of biologically active compounds. Xenobiotics including steroids, anti-inflammatory agents, analgesics, adrenergic stimulants and blockers undergo biotransformation via sulfate conjugation (Mulder, 1981). Sulfate conjugation is essential for the biological activity of many endogenous compounds; the degree of sulfation determines the biological activity of heparin, heparan sulfate, dermatan sulfate, gastrin and cholecystokinin (Mulder, 1981; Ofosu et al., 1987). Inorganic sulfate is also necessary for biosynthesis of numerous structural components of membranes and tissues, such as glycosaminoglycans or cerebroside sulfate (Humphries, Silbert & Silbert, 1986). In tissues, sulfated glycosaminoglycans occur as covalent complexes with a core protein in the form of proteoglycans, and cell differentiation appears to be guided by a tissue-specific composition of sulfated proteoglycans (Dietrich et al., 1977). The importance of proteoglycan sulfation has been clearly recognized after the identification of three chondrodysplasias (achondrogenesis type 1B, atelosteogenesis type 2 and diastrophic dysplasia) (Superti-Furga et al., 1996). These diseases result from a deficient sulfate transport and are characterized by deficient intracellular pools of sulfate in chondrocytes, leading to the production of undersulfated proteoglycans; the clinical features of these chondrodysplasias include dwarfism, spinal deformation and joint abnormalities (Hastbacka et al., 1994).

Inorganic sulfate homeostasis is largely maintained by reabsorption in the renal proximal tubule. Sodium-dependent sulfate cotransport in the brush border membrane (BBM) is of primary importance in the regulation of plasma inorganic sulfate concentrations. Sulfate is actively absorbed in the ileum; however the role of intestinal absorption in sulfate homeostasis is largely unknown. The recent identification of a number of sulfate transporter genes has allowed the investigation of the molecular mechanisms of the renal and intestinal transport of inorganic sulfate. In this review, the intestinal and renal sulfate transporters and the regulation of sulfate homeostasis will be discussed. Previous reviews in this area include the following: Murer, Markovich & Biber, 1994; Murer et al., 1986; Murer, 1988.

Renal Transport of Inorganic Sulfate

Inorganic sulfate is eliminated from the body mainly by urinary excretion; biliary excretion is of little importance (Walser, Seldin & Grollman, 1953). The urinary recovery of a tracer dose of sodium ^{35}S-sulfate in healthy men is approximately 86% in a 24-hr urine sample (Bauer, 1976) and 95% within 5 days (Ryan et al., 1956), predominantly as unconjugated (free) sulfate (Walser et al., 1953; Lundquist et al., 1980). The predominant process in the kidney appears to be absorptive, rather than secretory, since clearance ratios of sulfate to creatinine or inulin do not indicate secretion (Becker et al., 1960; Ber-

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glund, 1960). This capacity-limited reabsorption is of primary importance in the regulation of plasma inorganic sulfate concentrations. The renal reabsorption of inorganic sulfate in humans and animals (dogs, rats, rabbits, chicken) is approximately 10–35% of the GFR under normal physiological conditions and increases to a rate approximately equal to GFR when serum sulfate concentrations are increased (Becker et al., 1960; Berglund, 1960). The site of renal reabsorption of inorganic sulfate has been localized to the proximal tubules (Hierholzer et al., 1960; Quamme, 1981; Lötscher et al., 1996).

The luminal or brush border membrane (BBM) transport of sulfate is sodium-dependent, as reported in studies utilizing BBM vesicles and intact tubules isolated from rats and rabbits (Lücke, Stange & Murer, 1979; Ullrich et al., 1980; Schneider, Durham & Sacktor, 1984). The apparent K_m for sulfate is between 0.3 and 0.6 mM (close to physiological plasma concentrations) and that for sodium is between 25 and 50 mM (Lücke et al., 1979; Schneider et al., 1984; Sagawa et al., 1999*b*). This step has been reported to be electroneutral in numerous studies and kinetic analysis suggests a stoichiometry of 2:1 (Na^+:SO_4^{2-}) (Lücke et al., 1979; Schneider et al., 1984; Turner, 1984). However, electrophysiological analysis of the sodium/sulfate transport expressed in *Xenopus laevis* oocytes injected with NaSi-1 cRNA suggests an electrogenic cotransport of Na^+ and sulfate with a stoichiometry of 3:1 (Busch et al., 1994). The sodium-sulfate cotransporter is separate and distinct from the proximal tubular sodium-dependent glucose, phosphate, amino acid and monocarboxylic acid cotransporters (Tenenhouse, Lee & Harvey, 1991).

Sulfate exits from the proximal tubule cell across the contraluminal or basolateral membrane (BLM) via an anion-exchange mechanism for which bicarbonate is the most effective counterion although hydroxyl or sulfate ions can also function as counterions (Pritchard & Renfro, 1983; Löw et al., 1984). Sulfate/bicarbonate exchange is unaltered by the membrane potential, suggesting that sulfate BLM transport is electroneutral (Löw, Friedrich & Buckhardt, 1984). This anion-exchange mechanism has been identified at the BBM (Pritchard, 1987); however, this represents only a minor portion of sulfate transport in BBM (Darling et al., 1994). Sulfate BLM transport is strongly inhibited by thiosulfate, selenate, molybdate, oxalate and stilbene derivatives (Ullrich, Rumrich & Kloss, 1984). BLM sulfate/oxalate exchange may play an important physiological role in the secretion of oxalate (Brändle, Bernt & Hautmann, 1998). The sulfate transport system in BLM is also shared by a number of sulfate esters, sulfonates, aminosulfonates, disulfonates, di- and tricarboxylates, sulfocarboxylates and salicylates (Ullrich, Rumrich & Kloss, 1985*a,b,c;* Darling et al., 1994). The BLM transport of sulfate and thiosulfate can be inhibited by high concentrations of p-aminohippurate (PAH), probenecid, carinamide and salicylate; however, PAH and salicylate transport is not inhibited by sulfate or thiosulfate (Ullrich et al., 1980; Darling et al., 1994). Ullrich et al. (1994) concluded that the substrate components necessary for interaction with the contraluminal sulfate transporter are: "one negative ionic charge, several nearby electronegative groups (O, OH, or anilinonitrogen), and a possibly flat (nonbulky) structure of the interacting molecule."

Intestinal Transport of Inorganic Sulfate

Inorganic sulfate can be absorbed from foods in the diet or from drinking water. Foods high in sulfate include commercial breads, dried fruits and vegetables, nuts, fermented beverages and brassica vegetables (Florin et al., 1991). Sulfate is present in drinking water, with concentrations in some rural regions being greater than 250 mg/L (Chien, Robertson & Gerrard, 1968). It is estimated that dietary sulfate accounts for about 42% of available sulfate in humans; the rest is formed from the oxidation of sulfhydryl-containing amino acids (Florin et al., 1991). Maximum absorption of inorganic sulfate by the intestine is limited. As a result, sulfate salts are effective osmotic cathartics when ingested in sufficient amounts. In humans, a tracer dose of sulfate is well-absorbed: 80% of an oral dose is recovered in the urine over 24 hr compared with 86% of an i.v. dose (Bauer, 1976). Higher doses of sulfate demonstrate a decreased bioavailability with a dose of 0.75 mmoles/kg sodium sulfate administered in 4 hourly divided doses having an average bioavailability of 43.5% (Cocchetto & Levy, 1981) and a similar dose of magnesium sulfate having an average bioavailability of 30.2% (Morris & Levy, 1983). Inorganic sulfate is actively absorbed in the distal ileum of rats, rabbits and hamsters (Anast et al., 1965). Transport involves sodium-dependent influx across the brush border membrane, and efflux across the basolateral membrane by sulfate/chloride exchange. The properties of sodium/sulfate cotransport in the ileum are similar to those described for the kidney cortex. Studies using rabbit ileal BBM vesicles have demonstrated that sulfate uptake is saturable with a K_m of 0.52 mM, requires the presence of luminal sodium, and leads to intracellular accumulation of sulfate above the electrochemical equilibrium (Ahearn & Murer, 1984). Thiosulfate and other oxyanions are also substrates for the sodium/sulfate transporter, but uptake is not inhibited by the anion exchange inhibitor DIDS or by phosphate (Lücke et al., 1979; Smith, Orellana & Field, 1981). There is also evidence for a sulfate/hydroxyl ion exchange system in BBM (Schron et al., 1985): the importance of this process is unknown.

In rat and rabbit ileal BLM vesicles, sulfate/chloride exchange has been characterized (Smith et al., 1981;

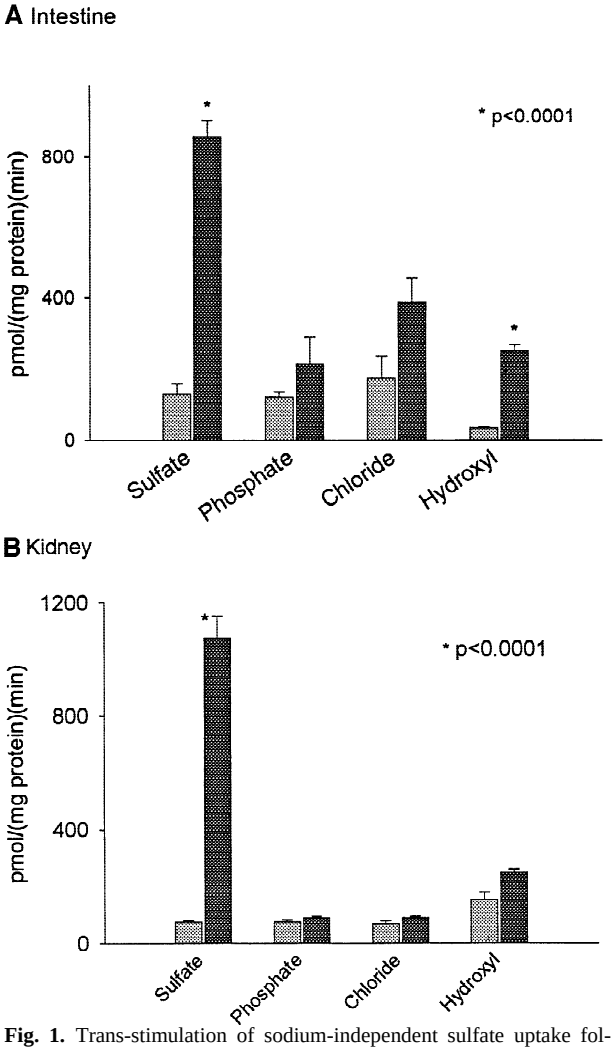

Fig. 1. Trans-stimulation of sodium-independent sulfate uptake following preincubation with sulfate, phosphate, chloride or hydroxyl ion (represented by dark bars) in (*A*) rat jejunal and (*B*) rat proximal tubular basolateral membrane vesicles. The uptake is given as mean ± SD of 5 determinations. Control data (no preincubation) are given by the light-grey bars. Adapted from Hagenbuch et al., 1985.

Hagenbuch et al., 1985). Preloading vesicles with sulfate or chloride or an outwardly directed hydroxyl ion gradient resulted in stimulation of tracer sulfate uptake (Fig. 1); an outwardly directed bicarbonate gradient had no effect. These results suggest that intestinal BLM transport occurs via anion exchange involving chloride or hydroxyl, but not bicarbonate. This contrasts with the findings in renal BLM membrane vesicles in which an outwardly directed bicarbonate gradient stimulates sulfate transport (Hagenbuch, Stange & Murer, 1985). Therefore, it is unlikely that this anion exchanger is the same sulfate anion exchanger (sat-1) present in the kidney and liver.

Ruppin et al. (1980) reported colonic sulfate absorption in humans. Florin et al. (1991) in their studies with normal subjects and subjects with ileostomies, reported a maximum net intestinal absorption of 5 mmol/day sulfate at dietary intakes of 7 mmol/day or above in humans, with colonic sulfate absorption accounting for 10 mmol/day with an intake of 16.6 mmol/day. The importance of colonic absorption of sulfate is largely unstudied. The sulfate transporter DRA is present in high amounts in the colon, but its role in sulfate absorption is unknown (Silberg et al., 1995).

Characterization of Renal and Intestinal Sulfate Transporters

A summary of the characteristics of the sulfate transport proteins is provided in the Table.

Sodium/Sulfate Cotransporter (NaSi-1)

cDNAs responsible for sodium/sulfate renal and ileal transport in the rat (NaSi-1) have been identified (Markovich et al., 1993). Expression cloning in *Xenopus laevis* oocytes was used to identify the cDNA related to sodium-dependent cotransport in rat kidney. The NaSi-1 cDNA contains 2239 base pairs (bp), including a poly(a) tail and encodes for a protein of 595 amino acids with an expected molecular weight of 66 kDa. Northern blot analysis shows signals of 2.3 and 2.9 Kb in rat kidney cortex, kidney medulla, upper small intestine (duodenum and jejunum) and lower small intestine (ileum) (Markovich et al., 1993). No hybridization signals were detected from RNAs in proximal colon, lung, liver, brain, heart, and skeletal muscle in rats (Markovich et al., 1993). Cross-species hybridization was detected in kidney cortex RNAs of mouse, rabbit, and pig (Markovich et al., 1993). Comparison of the deduced amino acid sequence revealed little similarity to other sodium-dependent transporters, except for the sodium/dicarboxylate cotransporter (Pajor et al., 1998). Hydrophobicity analysis suggested at least eight membrane-spanning domains (Markovich et al., 1993). The substrate recognition site of NaSi-1 is in the carboxy-terminal portion of the protein (Pajor et al., 1998).

A cDNA from rat small intestine (ileal NaSi-1) has been isolated by homology screening with renal NaSi-1 cDNA (Norbis et al., 1994). Ileal NaSi-1 cDNA contains 2722 bp (500 bp more than the renal NaSi-1 cDNA), but encodes a protein of 595 amino acids, identical to the renal NaSi-1 protein. Ileal NaSi-1 cDNA differs from renal NaSi-1 only in the length of the 3′ untranslated region. The predicted amino acid sequences of the two cloned NaSi-1 transporters (renal and ileal) are 100% identical. Concentration- and sodium-dependent sulfate transport is observed in oocytes injected with 1 ng NaSi-1 cRNA (Norbis et al., 1994) (Fig. 2). mRNA-induced transport was inhibited by thiosulfate, but not by

Table. Sulfate transport proteins present in kidney and intestinal tissue

Sulfate transporter[1]	NaSi-1	sat-1	DTDST	DRA
Functional description	Sodium/sulfate symporter	Sulfate/bicarbonate antiporter	Sulfate/chloride antiporter	Sulfate(chloride)/hydroxyl antiporter
Chromosomal location	Chromosome 6 (mouse)	not known	5q32–q33.1 (human)	7q22.31.1 (human)
Cloning species	Rat, mouse	Rat	Human, rat, mouse	Human, rat, mouse
Physiological location	Kidney Intestine	Liver Kidney Muscle (low) Brain (low)	Intestine Cartilage	Colon Intestine Cecum
Protein: Amino acids	595	703	739	764
Molecular size (kDa)	66	75.4	82	84.5
Amino acid similarity to rat sat-1	44.6% (rat)	100% (rat)	48% (human)	59% (human)
Transport Characteristics:				
Sodium-dependence	Yes	No	No	No
K_m values (mM):				
Tissue	0.3–0.6 (Kidney, intestine)	0.2–0.5 (kidney)	0.03 (fibroblasts)	ND
cRNA-injected oocytes	0.28	0.14	ND	ND
Other anions transported	Thiosulfate Selenate	Bicarbonate Thiosulfate Oxalate Succinate	Thiosulfate Oxalate	Chloride Oxalate
Cis inhibitors[2]				
Selenate	+	+	−	−
Thiosulfate	+	+	+	+
DIDS	−	+	+	+
Oxalate	−	+	+	+
Chloride	−	−	+	+
Bicarbonate	−	+	+	−
Regulation	Diet Thyroid hormone Growth hormone Age Pregnancy Glucocorticoids Metabolic acidosis Vitamin D Hypokalemia	None known	Bone morphogenetic protein-2	Adenoma and adenocarcinomas Intestinal inflammation
Relationship with human disease	None known	None known	Diastrophic dysplasia Achondrogenesis IB Atelosteogenesis II	Congenital chloride Diarrhea

[1] References are provided in the text ND = not determined.
[2] (+) significant inhibition at 1 mM or lower; (−) little inhibition at 1 mM or lower.

phosphate or DIDS, consistent with previous observations in rat and rabbit ileal BBM vesicles (Lücke et al., 1979; Smith et al., 1981).

Using a polyclonal antibody of NaSi-1 amino acid sequence raised against a synthetic C-terminal peptide of 12 amino acids, Custer, Murer and Biber (1994) demonstrated that this antibody recognized a protein with a molecular mass of 130 kDa in purified rat kidney BBM. However, the open reading frame of the isolated NaSi-1-related cDNA encodes for a protein of about 66 kDa (Markovich et al., 1993). The observed 130 kDa protein therefore may represent a dimeric form of the NaSi-1-related protein. Analysis of NaSi-1 expression in microdissected nephron segment of rat kidney showed that

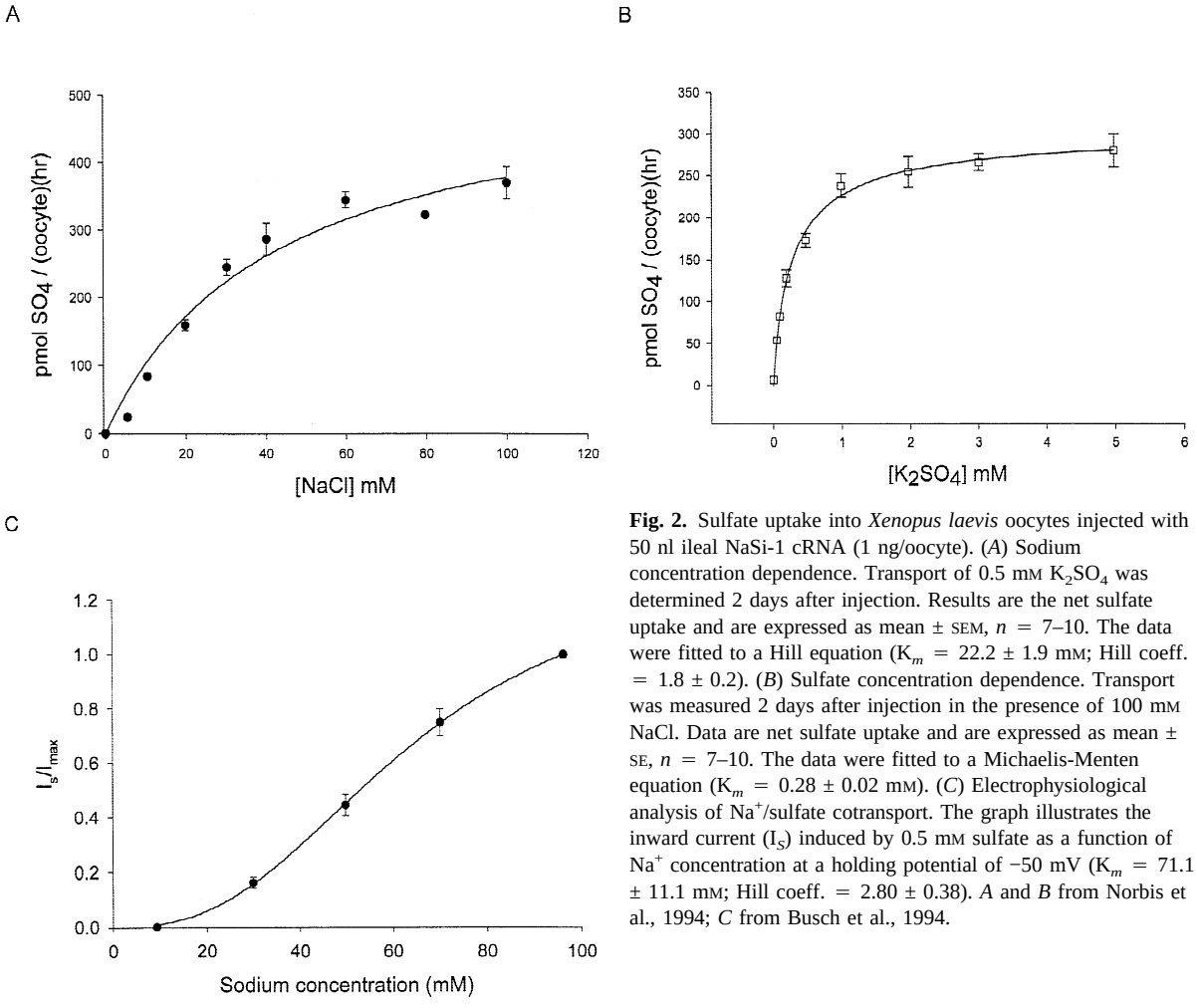

Fig. 2. Sulfate uptake into *Xenopus laevis* oocytes injected with 50 nl ileal NaSi-1 cRNA (1 ng/oocyte). (*A*) Sodium concentration dependence. Transport of 0.5 mM K_2SO_4 was determined 2 days after injection. Results are the net sulfate uptake and are expressed as mean ± SEM, $n = 7–10$. The data were fitted to a Hill equation ($K_m = 22.2 \pm 1.9$ mM; Hill coeff. $= 1.8 \pm 0.2$). (*B*) Sulfate concentration dependence. Transport was measured 2 days after injection in the presence of 100 mM NaCl. Data are net sulfate uptake and are expressed as mean ± SE, $n = 7–10$. The data were fitted to a Michaelis-Menten equation ($K_m = 0.28 \pm 0.02$ mM). (*C*) Electrophysiological analysis of Na^+/sulfate cotransport. The graph illustrates the inward current (I_S) induced by 0.5 mM sulfate as a function of Na^+ concentration at a holding potential of −50 mV ($K_m = 71.1 \pm 11.1$ mM; Hill coeff. $= 2.80 \pm 0.38$). *A* and *B* from Norbis et al., 1994; *C* from Busch et al., 1994.

NaSi-1 mRNA is expressed in proximal tubules and collecting ducts (Custer et al., 1994). However, a more recent immunohistochemistry study using rat kidney cortex slices showed that NaSi-1 protein (68 kDa) is present at the apical membrane of proximal tubules, and no immunoreactivity was detected in the other nephron segments (Lötscher et al., 1996).

Recently, the mouse NaSi-1 cDNA, gene (*Nas1*) and promoter region have been characterized (Beck & Markovich, 2000). The *Nas1* gene contains 75 kb pairs and maps to mouse chromosome 6. Transcription initiation occurs from a site 29 bp downstream to a TATA boxlike sequence. The promoter region contains a number of potential *cis*-acting elements recognized by well characterized transcription factors that may be important in the regulation of the *Nas1* gene. The kinetic characteristics of expressed activity of the mouse NaSi-1 protein in *Xenopus laevis* oocytes and the tissue distribution of the mouse NaSi-1 mRNA are very similar to the rat NaSi-1.

An additional sodium/sulfate cotransporter (SUT-1) present in human high endothelial venules (HEV) and the placenta, but not kidney or intestine, has been recently described (Girard et al., 1999). HEV endothelial cells incorporate large amounts of sulfate into the lymphocyte homing receptor L-selectin. SUT-1 exhibits 40–50% amino acid identity with the rat and mouse NaSi-1. The human SUT-1 gene was mapped to chromosome 7q33.

Sulfate Anion Transporter (Sat-1)

A cDNA encoding the renal sulfate anion transporter (sat-1), which is related to hepatic canalicular membrane sodium-independent sulfate transport, has been identified (Markovich et al., 1994). The cDNA encoding the canalicular sulfate anion transporter of rat liver (sat-1) has been cloned using a *Xenopus laevis* oocyte expression system (Bissig et al., 1994). The cloned cDNA sulfate transporter (sat-1) is a sulfate/bicarbonate exchanger (Fig. 3) which exhibits saturation kinetics with a K_m of 0.26 mM; sulfate transport is inhibited by DIDS (IC_{50}

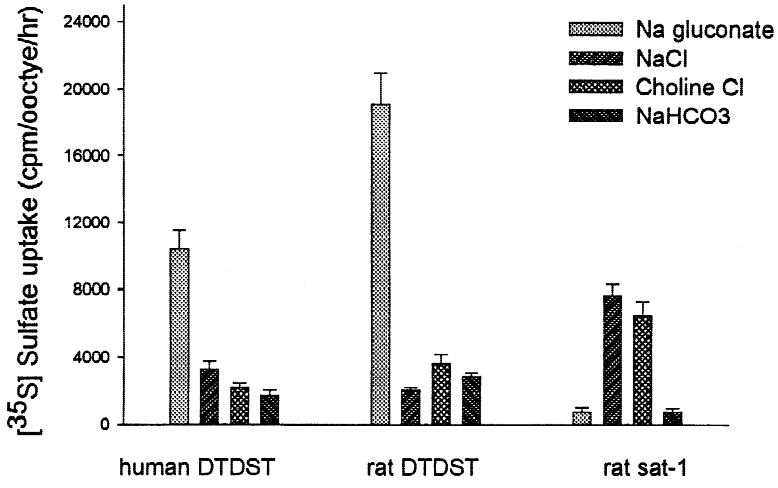

Fig. 3. Effects of extracellular ions on sulfate uptake of *Xenopus laevis* oocytes treated with human or rat DTDST cRNA or rat sat-1 cRNA. ^{35}S-sulfate uptake was measured for 1 hr in the presence of various extracellular ions (100 mM) at 20°. Values represent the means ± SE for 9–10 oocytes. From Satoh et al., 1998.

value of 0.02 mM) and oxalate (IC_{50} value of 31 mM). These characteristics are similar to the sulfate anion-exchanger evaluated in rat liver canalicular membrane vesicles. Sat-1 cDNA has a total length of 3726 bp with an open reading frame of 2109 bp. The coding region predicts a protein of 703 amino acids with a calculated molecular mass of 75.4 kDa. Computer analysis of the amino acid sequence suggests 3 potential glycosylation sites, and hydrophobicity analysis, 12 putative membrane-spanning domains. Sequence comparison between NaSi-1 and sat-1 proteins resulted in 44.6% similarity and 18.5% identity (Bissig et al., 1994). Similar sulfate anion transporters are also present in the kidney, muscle and brain, but not in the duodenum, ileum or proximal or distal colon of the rat, as determined from Northern hybridization experiments. A recent study has characterized sat-1 expression and activity in rat brain (Lee et al., 1999*a*). Northern blot analysis of sat-1 cDNA against rat kidney cortex RNA showed very strong levels of hybridization (Markovich et al., 1994). Karniski et al. (1998) have identified rat renal sat-1 from a renal cortex cDNA library using rat liver sat-1 as a screening probe. Western blot and immunohistochemistry analysis using sat-1 monoclonal antibodies showed the sat-1 protein is localized in the basolateral membrane, but not the apical membrane, of the proximal tubule (Karniski et al., 1998). Thus, it was concluded that sulfate anion exchange transporters at the apical and basolateral membranes of the proximal tubule might involve two different anion exchangers (Karniski et al., 1998).

Down Regulated in Adenoma (DRA)

An additional sulfate transport protein expressed in intestinal tissue is the gene product of Down Regulated in Adenoma (DRA). This is a membrane glycoprotein with high sequence homology with sat-1 (59% similar, 32% identical) and with the diastrophic dysplasia sulfate transporter (DTDST) (60% similar, 33% identical) (Hastbacka et al., 1994). The protein product of the DRA gene is expressed in intestinal columnar epithelial cells, particularly in the brush border cells of duodenum, ileum, and colon and cecum (Byeon et al., 1996), although its expression is much higher in the colon (Silberg et al., 1995). Interestingly, the pattern of DRA expression with regards to subcellular localization, cell-type specificity and portion of the gastrointestinal tract is very similar to the multidrug resistance transporter protein, MDR1. DRA protein is a membrane glycoprotein with 10–14 predicted transmembrane domains (Byeon et al., 1996). DRA was initially cloned as a tumor-suppressor gene based on its reduced expression in colon adenomas and carcinomas; it is not known whether loss of DRA transport function is involved in the development of the malignant phenotype (Silberg et al., 1995). Recent studies have indicated that DRA is the gene responsible for the recessively inherited disease, congenital chloride diarrhea (CLD), which is characterized by a defect in the intestinal absorption of chloride in the distal ileum and colon (Hoglund, 1996). The evaluation of CLD patients in worldwide epidemiological studies has revealed mutations in the DRA gene (Kere, Lohi & Hoglund, 1999). Functional studies in *Xenopus laevis* oocytes injected with wild-type DRA cRNA have demonstrated sodium-independent sulfate transport (Fig. 4), which can be inhibited by the anion transport inhibitor DIDS. Oxalate and chloride are also transported, and at high chloride concentrations, sulfate transport is inhibited and is similar to that in oocytes injected with water (Silberg et al., 1995; Moseley et al., 1999). Mutations in the DRA gene characterized in patients with CLD result in decreased chloride and sulfate uptake (Moseley et al., 1999). It appears that DRA represents a Cl^-, SO_4^{-2}/OH^- exchanger. Its role in the intestinal absorption of sulfate is not known; however, DRA may be important for sulfate absorption in the colon.

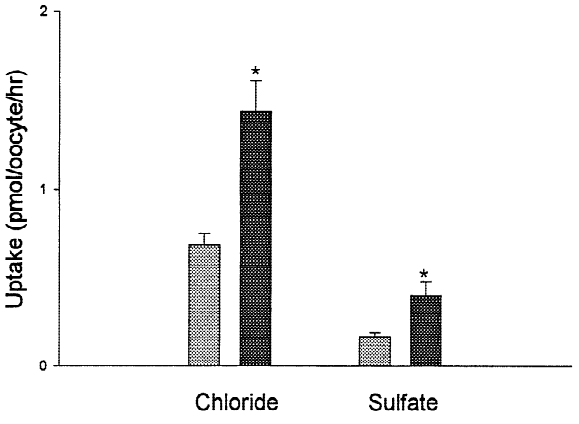

Fig. 4. Sulfate and chloride uptake into *Xenopus laevis* oocytes treated with either DEPC-treated water or 500 pg WT DRA cRNA. Four days after injection, 1-hr uptake of 1 mM ^{36}Cl or 1 mM ^{35}S was determined at 25°. Uptake values represent the means ± SE of 25–54 determinations from 3 separate oocyte preparations. Water-treated: grey bars; DRA-treated: dark bars. *$P < 0.005$ compared with water. Adapted from Moseley et al., 1999.

Diastrophic Dysplasia Sulfate Transporter (DTDST)

The diastrophic dysplasia sulfate transporter (DTDST) is a sulfate/chloride anion exchanger which is expressed in many tissues, but with highest expression in the small intestine and cartilage (Hastbacka et al., 1994; Satoh et al., 1998). The DTDST gene encodes a membrane transporter with 12 membrane-spanning domains, which demonstrates high amino acid similarity to the 5′ end of the coding region of sat-1 (Hastbacka et al., 1994). Mutations in DTDST result in a family of recessively inherited osteochondroplasias including diastrophic dystrophy, achondrogenesis type 1B and atelosteogenesis type II (Superti-Furga et al., 1996) due to the undersulfation of proteoglycans in chondrocytes (Satoh et al., 1998). Injection of rat and human DTDST cRNA into *Xenopus laevis* oocytes results in sodium-independent sulfate transport which is markedly inhibited by extracellular chloride and bicarbonate (Fig. 3), as well as by thiosulfate, oxalate and the anion exchange inhibitor DIDS. While sat-1 has been demonstrated to be a sulfate/bicarbonate exchanger, DTDST-mediated sulfate transport is not stimulated by intracellular bicarbonate (Satoh et al., 1998). The physiological role of DTDST expressed in the intestine remains to be elucidated, but it may represent the sulfate/chloride exchanger that has been characterized in ileal BLM vesicle preparations.

Regulation of Renal and Intestinal Sulfate Transport

Extensive efforts have been made in the last several years to understand the cellular mechanisms involved in the regulation of inorganic sulfate homeostasis. Recent work has demonstrated that the renal reabsorption of inorganic sulfate is regulated via the sodium/sulfate cotransporter which is located in the brush border membrane of epithelial cells in the proximal tubule. Renal sodium/sulfate cotransport is increased in infants and young children (Cole, Shafai & Scriver, 1982; Lee, Balasubramanian & Morris, 1999*b*), in pregnant women (Cole et al., 1985; Lee, Balasubramanian & Morris, 1999*b*), following sulfate restriction (Benincosa, Sagawa & Morris, 1995; Markovich et al., 1998; Sagawa et al., 1998*b*), and following treatment with growth hormone (Gershberg & Casch, 1956; Sagawa et al., 1999*a*) or thyroid hormone (Tenenhouse et al., 1991). Decreased sodium/sulfate cotransport has been observed following treatment with glucocorticoids (Sagawa et al., 2000), nonsteroidal antiinflammatory agents (Sagawa et al., 1998*a;* Benincosa et al., 1999b), under conditions of dietary excess of methionine (a sulfate precursor) (Sagawa et al., 1998*b*), with vitamin D (Fernandes et al., 1997) or potassium deficiency (Markovich et al., 1999), metabolic acidosis (Puttaparthi et al., 1999) and hypothyroidism (Sagawa et al., 1999*b*). The mechanism associated with these changes involves regulation of the steady-state levels of NaSi-1 protein, although altered membrane composition and fluidity may also contribute to the altered affinity or capacity of the NaSi-1 transporter (Lee et al., 1999*c*).

Little is known regarding the regulation of intestinal sulfate absorption. Smith and coworkers reported a modest increase in sodium-dependent sulfate influx in rabbit ileum by theophylline and heat-stable enterotoxin which stimulate the production of cGMP (Smith et al., 1981). In everted gut sac preparations, hypophysectomy reduced sulfate transport while growth hormone treatment restored transport (Anast et al., 1965). DRA protein expression is selectively decreased in mild inflammation and in patients with ulcerative colitis, a process which may be regulated at the level of gene transcription by proinflammatory cytokines (Yang et al., 1998). DTDST gene expression in osteoblasts is induced by bone morphogenetic protein-2 (BMP-2), a member of the transforming growth factor-β superfamily (Kobayashi et al., 1997). Further research in the areas of sulfate transport in the intestine and other tissues via NaSi-1, sat-1, DRA and DTDST is needed in order to fully understand the regulation of sulfate concentrations in blood and tissues.

Summary

Sulfate transporters have been conserved across eukaryotic organisms ranging from filamentous fungi, yeast and plants to mammals and humans. This conservation among species and the ubiquitous distribution in mam-

malian tissues, suggest an essential role for sulfate transporters in the maintenance of intracellular sulfate concentrations. The cloning of the cDNA for the mammalian sodium/sulfate cotransporter and sulfate/anion exchangers (sat-1, DRA and DTDST) have resulted in investigations characterizing sulfate membrane transport and its regulation. Sodium/sulfate cotransport in the kidneys is essential for the maintenance of plasma sulfate concentrations within the physiological range, and is highly regulated by its external environment. Although little is currently known concerning intestinal sodium/sulfate cotransport, it is likely that it may be regulated in a similar manner. The role of sulfate/anion transporters such as DRA and DTDST in the absorption of sulfate in the intestine and colon, and their importance in disease states including colon adenomas and carcinomas, ulcerative colitis and congenital chloride diarrhea, is an area of current investigation.

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