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Molecular properties of the SLC13 family of dicarboxylate and sulfate transporters

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Abstract

The SLC13 gene family consists of five members in humans, with corresponding orthologs from different vertebrate species. All five genes code for sodium-coupled transporters that are found on the plasma membrane. Two of the transporters, NaS1 and NaS2, carry substrates such as sulfate, selenate and thiosulfate. The other members of the family (NaDC1, NaDC3, and NaCT) are transporters for di- and tri-carboxylates including succinate, citrate and α-ketoglutarate. The SLC13 transporters from vertebrates are electrogenic and they produce inward currents in the presence of sodium and substrate. Substrate-independent leak currents have also been described. Structure–function studies have identified the carboxy terminal half of these proteins as the most important for determining function. Transmembrane helices 9 and 10 may form part of the substrate permeation pathway and participate in conformational changes during the transport cycle. This review also discusses new members of the SLC13 superfamily that exhibit both sodium-dependent and sodium-independent transport mechanisms. The *Indy* protein from *Drosophila*, involved in determining lifespan, and the plant vacuolar malate transporter are both sodium-independent dicarboxylate transporters, possibly acting as exchangers. The purpose of this review is to provide an update on new advances in this gene family, particularly on structure–function studies and new members of the family.

Keywords

Succinate; Citrate; Dicarboxylates; Sulfate; Sodium

Introduction

The SLC13 gene family consists of five members in humans, all of which are sodium-coupled transporters for anions such as sulfate or dicarboxylates. SLC13 transporters with related sequences and function are found in other vertebrates, ranging from fish to mammals. These transporters are secondary active transporters that couple multiple sodium ions to the movement of an anion substrate. The SLC13 family transporters are typically found on plasma membranes in mammalian cells. However, the newer members of the family that have been identified in insects and plants do not necessarily couple sodium and can be found in intracellular organelles.

This review is intended to provide an update on developments in the field since the publication of previous reviews on the members of the SLC13 family [44,45,47,53,54]. The most recent review of the field, by Markovich and Murer [47] focused primarily on cloning of mammalian members of the family, their physiological function and regulation. The present review adds

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new information published in the last year, as well as more details on structure–function studies, electrophysiological properties and non-vertebrate members of the family.

SLC13 gene family: vertebrates

Humans contain five genes coding for SLC13 family members, all of which are approximately 40%–50% identical in protein sequence. The genes have been named in the order in which they were identified (Table 1). Although some attempts have been made to organize the protein names [47], the issue has not been completely resolved. The suggested use of NaC for the di- and tri-carboxylate transporters [47] is somewhat problematic since the name is already in use for the NaC family of voltage-gated ion channels that includes ENaC. The gene nomenclature also needs revision. The current SLC system is limited because it does not provide a mechanism for including family members from distantly related species, including bacteria and plants, nor does it allow the naming of subfamilies. A more comprehensive nomenclature based on evolution and independent of substrate should be developed, similar to the one proposed for the organic anion transporting polypeptide family, OATP [24]. In order to allow the naming of subfamilies of OATPs, the gene family name was changed from SLC26 to SLCO. A similar change to the SLC13 family nomenclature would allow the inclusion of the newer family members from non-vertebrate species.

SLC13A1: NaS1

The first member of the SLC13 family to be identified was the sodium-coupled inorganic sulfate transporter, originally named NaSi-1 but more recently renamed NaS1 [47]. The human gene name is SLC13A1 (Table 1). NaS1 carries a range of divalent oxyanion substrates such as sulfate (SO_4^{2-}), selenate (SeO_4^{2-}), and thiosulfate ($S_2O_3^{2-}$) [11,40]. The transport is sodium dependent, with at least three sodium ions coupled to the transport of each substrate. The K_m for sulfate in the human NaS1 is around 0.4 mM, and the half-saturation constant for sodium (K_{Na}) is about 30 mM [41]. The K_m for sulfate in rat NaS1 is about 0.7 mM (radiotracer uptakes) and 0.1 mM (substrate-dependent inward currents) [11,46]. Initially, the NaS1 transporter was assumed to be electroneutral because the Hill coefficient in non-voltage clamped cells was only around 1.4. However, the transporter produces substrate-dependent inward currents and the Hill coefficient measured under voltage clamped conditions is 2.8 [11].

The NaS1 protein is found on the apical membrane in the renal proximal tubule. In mouse and rat, the protein or message is found in both kidney and small intestine, but in humans the transporter may be restricted to the kidney [5,40,43]. Antibodies against human NaS1 recognize a glycosylated protein in the kidney membrane vesicles [42] of both pig and rat. Most of the recent studies on NaS1 have focused on the production and characterization of the Nas1 knockout mouse [14,16,17]. Mice with a disruption in the Nas1 gene exhibit increased renal excretion of sulfate and a general decrease in growth. These mice also exhibit behavioral abnormalities and memory disturbances.

SLC13A2: NaDC1

The SLC13A2 gene codes for the low affinity Na^+/dicarboxylate transporter, NaDC1 (Table 1). NaDC1 is found on apical membranes of the cells of the renal proximal tubule and small intestine where it functions to absorb citric acid cycle intermediates from the renal filtrate or the intestinal lumen (see reviews [53,54]). The preferred substrates of NaDC1 are dicarboxylates such as succinate. Substrates such as citrate that exist mostly as tricarboxylates at physiological pH, are transported in protonated form as $citrate^{2-}$. The K_m for the test substrate succinate varies between 0.3 and 1 mM, depending on the species. Transport is sodium dependent, with three sodium ions coupled to the transport of a divalent anion substrate. This

transporter also interacts with lithium, which at low concentrations can compete with sodium to inhibit transport [54].

NaDC1 has a broad substrate specificity, in general the preferred substrates are four carbon dicarboxylates in trans configuration. One measure of substrate specificity that is rarely reported for the SLC13 family is the transport efficiency, estimated from the ratio of V_{max}/K_m (for radiotracer uptakes) or $I_{max}/K_{0.5}$ (for two-electrode voltage clamp measurements). Typically, K_m values for substrates are compared but those do not always accurately reflect transport efficiency. For example, in the case of hNaDC1, the $K_{0.5}$ for succinate is lower than that for fumarate, which might suggest that the preferred substrate is succinate [68]. However, the I_{max} value for fumarate is much larger than that for succinate, making the efficiency or $I_{max}/K_{0.5}$ ratio greater for fumarate than succinate [68]. The transport efficiency for citrate is usually underestimated since the K_m for citrate is given as total citrate even though the true substrate, citrate^{2-}, is only about 7% of the total at pH 7.5. For example, the $K_{0.5}$ of total citrate is 6.8 mM in hNaDC1, but the citrate^{2-} $K_{0.5}$ is approximately 0.5 mM at pH 7.5. Since the I_{max} value represents inward current, carried by sodium, this reflects the movement of the true substrate, citrate^{2-}. The $I_{max}/K_{0.5}$ ratio for citrate in hNaDC-1 is 1134 nA/mM, followed by fumarate at 365 and succinate at 166 [68]. Therefore, when the $I_{max}/K_{0.5}$ ratios are considered, the efficiency of transporting citrate by NaDC1 is much greater than fumarate or succinate. Because of differences in protein expression between different transporters, the relative efficiencies for different substrates can be compared but not the actual $I_{max}/K_{0.5}$ values unless there is independent evidence that the protein concentrations are the same. In addition to the transport efficiency for a given substrate, the plasma concentrations of substrates are also important determinants of the physiological function of the transporters. In humans, plasma concentrations of substrates such as succinate, α-ketoglutarate and citrate are approximately 5, 25 and 100 μM, respectively [27,39].

There is relatively little information about the in vivo regulation of NaDC1. Most of the research with the dicarboxylate transporters has focused on functional characterization and structure–function studies. There is evidence that NaDC1 in the kidney is regulated by metabolic acidosis [3]. The trafficking of NaDC1 is also affected by kinases, including SGK-1, that are involved in regulating transporters involved in acid–base balance [6]. The recent development of a knockout mouse model may help clarify the physiological functions of NaDC1. Mice with disruptions in the Slc13a2 gene survive to adulthood with no obvious phenotypic aberrations and exhibit increased urinary excretion of citrate (C.D. Smith, personal communication).

SLC13A3: NaDC3

The high affinity Na^+/dicarboxylate transporter, NaDC3, is encoded by the SLC13A3 gene (Table 1). The human NaDC3 is 43% identical in protein sequence to hNaDC1 [47]. NaDC3 has a broader tissue distribution than NaDC1 and is found on basolateral membranes in renal proximal tubule cells [28], as well as liver, brain and placenta [12,37,56]. The K_m for succinate in NaDC3 is between 10 μM and 140 μM, depending on the species [56,66]. The substrate specificities of NaDC1 and NaDC3 overlap somewhat but NaDC3 appears to handle a larger range of structures, particularly longer chain or branched dicarboxylates, compared with NaDC1. For example, an important physiological substrate of NaDC3 is α-ketoglutarate [12], whereas NaDC1 has a very low transport capacity for this substrate [68]. NaDC3 handles substrates with substitutions on the third carbon, such as 2,3-dimethylsuccinate or the heavy metal chelators, *meso*-2,3-dimercaptosuccinate and dimercaptopropane-1-sulfonate [7]. In the brain, NaDC3 mediates the transport of the modified amino acid, *N*-acetylaspartate, with a relatively high affinity (K_m 60–250 μM) [21]. NaDC3 may also interact with some monovalent organic anions, such as benzylpenicillin [8]. The transport of substrates by NaDC3 is sodium-

dependent, probably involving three Na^+ ions with each substrate, and transport is electrogenic. Similar to NaDC1, the transport by NaDC3 is inhibited by millimolar concentrations of lithium [12,66]. There is relatively little information on the structure or in vivo regulation of NaDC3, although there has been one study showing that treatment with protein kinase C activators produces endocytosis of the transporter [25]. There is no knockout mouse model yet.

SLC13A4: NaS2

NaS2, originally named SUT-1, is a sodium-dependent sulfate transporter found predominantly in human placenta, high endothelial venules and testis [22,48]. In rats the message is also found in brain and liver [15]. The sequence of hNaS2 is 49% identical to that of hNaS1 [47]. The functional properties of NaS2 are very similar to those of NaS1: the K_m for sulfate in NaS2 is approximately 0.4 mM and the K_{Na} is around 20 mM [48]. In rat NaS2, the K_m for sulfate is 1.3 mM [15]. Although it has not been examined in detail, the range of substrates handled by NaS2 also appears to be very similar to the substrate specificity of NaS1. To date there is relatively little information on NaS2, and it is not known whether there are any functional differences between NaS2 and NaS1.

SLC13A5: NaCT

The most recent addition to the human SLC13 family is the Na^+-citrate transporter, NaCT. This transporter was first identified in brain, but NaCT message is also found in liver and testis [32,33]. The sequence of the human NaCT is 54% identical to that of hNaDC1 and 47% identical to hNaDC3 [47]. A splice variant of NaCT, missing 43 amino acids due to the loss of exon 2, has been reported in human brain, but the function and tissue distribution of this variant is unknown (Genbank AJ489980). In preliminary studies, we have detected a similar splice variant in mouse brain by RT-PCR (unpublished observations).

NaCT is a sodium-dependent transporter for citrate and dicarboxylates. In the rat and human transporters, the K_m for total citrate is lower than for dicarboxylates such as succinate. In mouse NaCT, the K_m is the same for citrate and succinate, around 38 μM [31]. However, the citrate V_{max} is larger than the succinate V_{max}, thus the transport efficiency with citrate is approximately twice that of succinate [31]. There are large species differences in citrate affinity, for comparison the K_m for citrate in human NaCT is around 0.6 mM [32]. There is some evidence that NaCT transports citrate as a trivalent anion coupled with four sodium ions. NaCT has a pH optimum of seven for citrate transport [31], compared with the pH profiles of NaDC1 and NaDC3, which show the highest transport of citrate at acidic pH, in accordance with the highest concentration of protonated citrate [62]. In sodium-activation experiments, the Hill coefficient of NaCT is greater than three, suggesting that more than three sodium ions are involved in activating transport [33]. In the rat NaCT, the charge to substrate ratio was determined to be 1:1 for citrate and 2:1 for succinate, again suggesting that four sodium ions may be coupled to the transport of trivalent citrate or divalent succinate [31]. NaCT exhibits species differences in interaction with lithium [34]. The rat NaCT is inhibited by lithium, similar to other dicarboxylate transporters, but the human NaCT is stimulated by lithium. The addition of lithium appears to increase the efficiency of transporting citrate by the human NaCT, mediated by a combination of increased V_{max} and decreased K_m for citrate [34].

SLC13 family: electrical properties

The members of the SLC13 family found in vertebrates are electrogenic transporters that couple multiple Na^+ ions to the movement of a divalent or trivalent anion substrate. The electrical properties of many of the members of the SLC13 family have been examined by measuring whole-cell currents using the two-electrode voltage clamp technique. At least three different types of currents have been associated with the expression of SLC13 transporters.

Substrate dependent currents

In all members of the family studied to date, the addition of substrate in the presence of sodium induces inward currents that are voltage-dependent. Although most of the electrophysiological studies have been done with the dicarboxylate transporters, there is one published report of inward currents in the rat NaS1 with sulfate, thiosulfate and selenate [11]. We have also observed inward currents of about −50 to −100 nA with sulfate and thiosulfate in oocytes expressing the human NaS1 (Yao and Pajor, unpublished observations). Most of the kinetic characterization has been done on the dicarboxylate transporters. In all of the transporters examined to date, the $K_{0.5}$ for substrate appears to be relatively insensitive to changes in membrane voltage [9,10,13,57]. The I_{max}, however, becomes larger with more negative membrane voltage [9,10,13,57]. The charge to uptake ratio was measured for NaDC1 and shown to be 1:1, supporting the idea that there is a net movement of one positive charge for each transport cycle, consistent with coupled transport of three sodium ions with each divalent anion substrate [13]. Measurement of substrate-induced inward currents has proven useful for examining substrate specificity and identification of potential inhibitors [8,9]. For example, a recent study with hNaDC3 identified folate as a potential inhibitor since it does not induce substrate-dependent currents, but it can inhibit succinate-dependent currents [9].

Cation-dependent leak currents

Several of the Na^+/dicarboxylate cotransporters exhibit leak currents of cations in the absence of substrate, which may represent an uncoupled movement of cations through the transporter. In the rat and human NaDC1, the sodium leak current has hyperbolic kinetics, suggesting a single sodium binding site, with a $K_{0.5}$ of 185 mM and inhibition by phloretin [13,68]. Recently, a different type of cation current associated with NaDC3 has been characterized in the presence of flufenamate [8]. Flufenamate is a non-steroidal anti-inflammatory drug that inhibits transport by NaDC1 and NaDC3 [8,62]. The inhibition is dependent on sodium but also appears to activate a K^+ conductance, with a reversal potential of −60 mV [8]. It is not known yet whether the K^+ conductance is mediated by NaDC3 or through a protein associated with the transporter [8]. It has been suggested that the mechanism of flufenamate inhibition may be similar to that of palytoxin, which inhibits the Na^+/K^+-ATPase by converting it into a non-selective cation channel [1].

Anion currents

The NaDC3 transporter from *Xenopus laevis* exhibits a substrate-independent current that may be mediated by anions, such as chloride [50]. Oocytes expressing xNaDC3 have what appear to be leak currents in the presence of sodium or lithium. However, when the concentrations of sodium or lithium are varied, the reversal potential remains constant at about −20 mV suggesting that the currents are not mediated by the cations. Replacement of chloride with other anions such as gluconate reduces the current amplitude and shifts the reversal potential. The results are consistent with an anion current that is activated by cations, and mediated by xNaDC3 either directly or through an interaction of another protein with xNaDC3. These anion currents have not been reported for any other member of the family.

Structure–function studies

Earlier studies, summarized in previous reviews, have shown that carboxy terminal half of the SLC13 transport proteins is required for substrate recognition [47,54]. For example, chimeras made between NaS1 and NaDC1 have the substrate specificity of the donor of transmembrane helices (TM) 7–11. Furthermore, residues involved in binding substrates and cations, either directly or indirectly, have also been identified in TM 7–11 [54].

N-glycosylation sites

All of the vertebrate members of the SLC13 family contain one or two N-glycosylation sites at the C-terminal tail [47,54]. In rabbit NaDC1, N-glycosylation affects protein sorting or targeting since the activity and protein expression are both reduced in mutants lacking the N-glycosylation site at position 578 [61]. In contrast, N-glycosylation appears to be required for function in hNaS1 [42]. NaS1 contains a single N-glycosylation site at amino acid 591, also in the C-terminal tail. Mutagenesis of Asn-591 in NaS1 to alanine or tyrosine reduces the V_{max} without affecting the K_m for sulfate, but the protein abundance on the plasma membrane is increased compared with wild-type [42]. Therefore, glycosylation appears to affect the rate of substrate turnover in NaS1, although the mechanism is not known.

Cysteine substitutions and other mutagenesis experiments

The substituted-cysteine accessibility method (SCAM) has been used extensively to identify functionally important residues in transport proteins [36]. In this method, individual amino acids are mutated to cysteine, which can then be chemically modified by reagents, such as the methanethiosulfonates (MTS). Particularly, [2-(trimethylammonium)ethyl]-methanethiosulfonate or MTSET has proved to be very useful for testing topology models since it is membrane impermeant and it reacts rapidly with exposed thiol groups from cysteine residues located on the outside of the cell or accessible to the outside through a water-filled pore in the protein. The method is also useful for identifying residues that are accessible to the MTS reagents in different conformational states of the protein.

Amino acids in TM 5 appear to be involved in transport of substrate and cations. All of the Na^+/sulfate transporters contain serines at positions 260 and 288 (hNaS1 numbering), and the dicarboxylate transporters contain alanine or threonine at these positions [41]. The S260A mutant of hNaS1 had a decreased affinity for both sulfate and sodium [35]. The S288A mutant appeared to affect only cation affinity and specificity, with an increased sodium affinity and decreased cation selectivity. The two serines and surrounding amino acids were mutated to cysteine and the MTSET sensitivity of the mutants was examined. Four amino acids around Ser-260 in TM5 (Fig. 1) were sensitive to MTSET and the accessibility appeared to change in the presence and absence of sodium. There was also substrate protection of MTSET binding. Therefore, TM 5 appears to be involved in conformational changes that occur during the transport cycle.

Amino acids at the extracellular ends of TM 7 and 8 of NaDC1 were also mutated to cysteine and tested for sensitivity to MTS reagents [67]. Only three substituted cysteines in TM 7 and 8 were sensitive to chemical modification by MTS reagents (Fig. 1). In TM 7, the R349C mutant was sensitive to MTSEA or (2-amino-ethyl)methanethiosulfonate, but was not affected by chemical modification with MTSET. Arg-349 is a conserved residue that is important for substrate binding in NaDC1, and it appears that the charge and size of substitution at this position is important for function. The modification of a cysteine residue by MTSEA resembles the side chain of arginine in volume and charge. The R349C mutant had reduced activity which was restored by chemical modification of the cysteine with MTSEA. The amino acid at position 349 appears to be most accessible to the outside of the cell in the conformational state seen in the presence of sodium. Two residues in TM8, Ser-372 and Asp-373, were also found to be sensitive to MTS reagents. The D373C mutant had an increased affinity for succinate, and was sensitive to inhibition by MTSET in both sodium and choline. However, the addition of substrate prevented chemical modification by MTSET.

A more extensive cysteine scan was done of TM 9 and 10 and the connecting extracellular loop [55,59] (Fig. 1). TM 9 was chosen because it contains Glu-475, which determines affinity for both sodium and succinate, and the endogenous Cys-476, which mediates inhibition by the

cysteine-selective reagent *p*CMBS (*p*-chloromercuri-benzene sulfonate) [23,58]. Although most of the cysteine-substituted mutants exhibited succinate transport activity, only four residues at the outer end of TM9 were sensitive to inhibition by MTSET (Fig. 1). All four residues showed differences in sensitivity to MTSET in different conformational states, and there was substrate protection of MTSET labeling. The cysteine scan was continued through the extracellular loop between TM 9 and 10, extracellular loop 5 (EL5), as well as the TM 10 (amino acids 483–528). This part of the protein was previously shown to mediate affinity for citrate and sodium in NaDC1 [35]. In a preliminary study, Phe-500 in TM 10 of NaCT also affects affinity for citrate and lithium [31]. In the cysteine scan of NaDC1, we found that all of the residues sensitive to MTSET are located in the extracellular loop and not in the TM [59]. The cysteine sensitive residues in extracellular loop 5 between TM 9 and 10 are conformationally sensitive, with the highest accessibility in the presence of sodium and protection of inhibition in the presence of substrate. Interestingly, the substrate protection was also observed in the cold, suggesting that the mechanism of substrate protection probably involves steric hindrance of MTSET binding rather than a large scale conformational change. Taken together, the results indicate that at least ten amino acids in TM 9–10 may participate in the conformational changes seen during the transport cycle of NaDC1 [55,59].

The current secondary structure models of members of the SLC13 family are based on hydropathy analysis and contain between 11 and 14 predicted transmembrane helices. These models are largely untested. There is experimental evidence supporting an odd number of TM, either 11 or 13, because the *N*- and *C*-termini are on opposite sides of the membrane [69]. So far, the results of SCAM are consistent with the proposed topology of 11 transmembrane helices for NaDC1 (shown in Fig. 1), but 13 TMs could also be possible. The secondary structure model proposed for NaS1 has 13 TMs [47]. The NaS1 model accommodates the extracellular location of the MTS-sensitive residues from TM 5, 7 and 8 (Fig. 1). However, the position of helices 10–12 of NaS1 is inconsistent with the experimental results since the MTS-sensitive residues would be placed on the inside of the cell rather than the outside. Given that the structures of bacterial transporters are used for homology modeling of mammalian proteins, it is unlikely that the mammalian sulfate and dicarboxylate transporters will differ by that much in their overall structures.

SLC13 gene family: other species

Several new members of the SLC13 family have been identified in insects, nematode worms, plants and bacteria. These transporters exhibit a greater variety of cellular distribution and transport mechanism compared with the vertebrate transporters.

Insects

The Indy gene (I'm not dead yet) from *Drosophila melanogaster* was identified in a genetic screen of flies with lifespan extensions [63]. P-element insertions into the gene reduced transcription and resulted in decreased protein expression [38]. Interestingly, the increased lifespan is seen only in heterogyzotes, the homozygotes have a slight decrease in lifespan, indicating that the amount of knockdown is related to lifespan extension. The *Indy* protein is a sodium-independent transporter for dicarboxylates with a relatively high affinity for succinate (K_m 40 μM) [30,38]. The *Indy* protein is expressed in metabolically important organs such as the plasma membrane of oenocyte and fat body as well as the basolateral membrane of midgut [33]. At present, there is no known mammalian protein with a similar function. NaDC1 is found in the small intestine, analogous to the fly midgut, although it is located on the apical membrane. However, since two transporters in series are required to mediate transepithelial transport of dicarboxylates in the gastrointestinal tract, a reduction in either the basolateral or apical transporter could produce a similar effect. The current hypothesis to explain lifespan extension in flies containing mutations in the *Indy* gene is that the reduced activity of *Indy* protein

produces an effect similar to caloric restriction [63]. The correlation between lifespan extension and a partial reduction of *Indy* activity resembles the effects of caloric restriction since a moderate restriction is beneficial but starvation is detrimental [63].

Caenorhabditis elegans

Three members of the SLC13 superfamily have been identified in *C. elegans*: gene F31F6.6 (protein ceNaDC1, later renamed ceNAC-1), gene R107.1 (protein ceNAC-2) and gene K08E5.2 (protein ceNaDC2, later renamed ceNAC-3) (Table 2, Refs. [19,20] and http://www.wormbase.org). The three proteins are sodium-coupled dicarboxylate transporters that resemble the mammalian transporters somewhat in substrate specificity. The sequences of these proteins are about 35% identical to the mammalian SLC13 transporters. Interestingly, the genes for these transporters appear to be expressed only in the intestinal tract. NAC-2 and NAC-3, but not NAC-1, may contribute to lifespan determination. Worms fed bacteria that produce NAC-2 and NAC-3 RNAi exhibit lifespan extensions of about 15–20% [19,20]. The amount of protein or mRNA in these treated worms was not quantitated, so it is not clear whether the treatment was successful in knocking down NAC-1, or how much reduction in NAC-2 and NAC-3 had occurred. The study also showed that the NAC-2 RNAi treatment decreased both body size and the amount of fat droplets in the worms [20].

Plants

SLC13 family members have also been identified in plants (Table 2). The only functionally characterized transporter from plants is called AttDT, the tonoplast dicarboxylate transporter from *Arabidopsis thaliana* [18]. This transporter is the first member of the SLC13 family that is located on an intracellular organelle, the vacuole. AttDT is not sodium dependent, and the transport mechanism has not been determined. AttDT appears to transport malate and fumarate, but a separate transporter or channel mediates transport of citrate [29]. Plants with a disruption in the gene coding for AttDT have decreased malate and fumarate concentrations in their vacuole but normal or higher citrate concentrations [29].

Bacteria

Genome sequencing projects have identified many SLC13 homologs in microorganisms but, to date, only one of these transporters has been characterized functionally. The SdcS (Sodium dicarboxylate symporter) from *Staphylococcus aureus* is approximately 35% identical in amino acid sequence to hNaDC1 [26]. SdcS is a sodium-dependent transporter for four carbon dicarboxylates, such as succinate, fumarate and malate with K_m values between 7 μM and 15 μM. One interesting characteristic of SdcS is the inhibition of transport at high sodium concentrations. Dicarboxylate transport activity is highest at approximately 5 mM sodium, and almost complete inhibition is seen by 100 mM sodium. A similar effect is seen with lithium, except that the optimal cation concentration is shifted to about 50 mM. This transporter should prove useful for future structural studies of the SLC13 transporters.

Conclusion

The SLC13 family is relatively small, comprising only five genes in mammals, and with only two categories of substrates, either sulfate or di-/tricarboxylates. Considerable advances have been made in this area in recent years, although some unanswered questions remain including: what is the structure of the proteins, how do the transporters work, how are they regulated, and what are their physiological functions? The addition of newer members of the family, such as *Indy*, has helped to identify potential functions, including the possible involvement of dicarboxylate transporters in metabolic regulation and lifespan determination. The development of knockout mouse models should also help to identify physiological roles of

these transporters. Finally, the identification of homologs from bacteria should allow the over expression and purification of the proteins for structural analysis.

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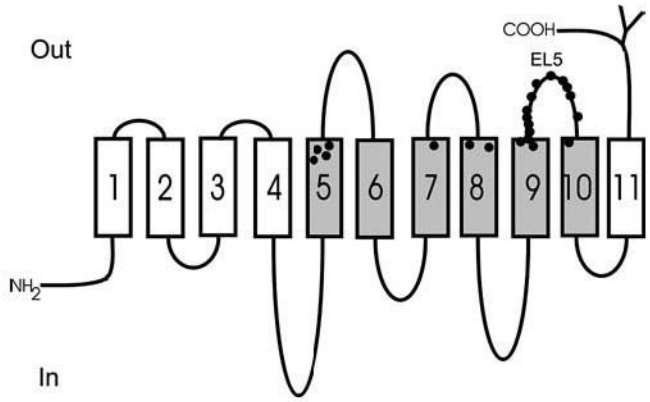

Fig. 1.

Secondary structure model of NaDC1 summarizing the results of cysteine scanning mutagenesis experiments. The rectangles represent the 11 transmembrane helices (TM). The protein has an intracellular N-terminus and an extracellular C-terminus containing the N-glycosylation site (shown by a Y) at Asn-578. Amino acids in TM 5–10 (shaded rectangles) and extracellular loop 5 (EL5) have been mutated to cysteines. The filled circles indicate the positions of substituted cysteines that are accessible to the methanethiosulfonate reagents, MTSET or MTSEA, at different conformational states of the protein. The results for TM 5 and 6 are based on studies with hNaS1 [41]. The results for the rest of the protein are from experiments done with rbNaDC1 [55,59,67]

Table 1

Characteristics of human members of SLC13 family and vertebrate orthologs. All are sodium dependent transporters found on plasma membranes. Only functionally characterized transporters are included

Human gene	Protein names	Tissue distribution	Substrates	Orthologs	References
SLC13A1	NaS1 (NaSi-1)	Kidney (human, rat), intestine (rat)	Sulfate, low affinity, K_m sulfate 0.4 mM	Human, rat, mouse, eel	[5,40,46,49]
SLC13A2	NaDC1 (NaDC-1, NaC1)	Kidney (apical membrane), small intestine	Dicarboxylates, low affinity, K_m succinate 0.8 mM	Human, rabbit, opossum, rat, mouse, *Xenopus* [1]	[2,4,13,51,52,60,64]
SLC13A3	NaDC3 (SDCT2, NaC3)	Kidney (basolateral membrane), placenta, liver, brain	Dicarboxylates, high affinity, K_m succinate 20 μM	Human, rat, mouse, *Xenopus*, flounder	[12,37,56,65,66]
SLC13A4	NaS2 (SUT-1)	Placenta, endothelial venules, testis, heart, liver	Sulfate, low affinity, K_m sulfate 0.4 mM	Human	[22,48]
SLC13A5	NaCT (NaC2)	Liver, brain, testis	Citrate (K_m 0.6 mM), dicarboxylates	Human, rat, mouse	[31–33]

[1] The *Xenopus laevis* NaDC-2 has been renamed xNaDC1 because of sequence similarity to other NaDC1 orthologs.

Table 2

New members of SLC13 superfamily. Only functionally characterized transporters are included

Species	Protein/gene name	Percent identity with hNaDC1	Substrates	Na^+-dependent?	References
Drosophila melanogaster	*Indy*	34%	Succinate (K_m 40 μM), citrate, dicarboxylates	No, electroneutral	[30,38,63]
Caenorhabditis elegans	ceNAC-1 (ceNaDC1), F31F6.6	38	Succinate (K_m 0.7 mM), citrate, dicarboxylates	Yes	[19]
Caenorhabditis elegans	ceNAC-2, B0285.6	39	Succinate (K_m 88 μM), citrate (K_m 76 μM), dicarboxylates	Yes	[20]
Caenorhabditis elegans	ceNAC-3 (ceNaDC2), K08E5.2	35	Succinate (K_m 60 μM), dicarboxylates	Yes	[19]
Arabidopsis thaliana	AttDT	34	Malate, fumarate	No	[18]
Staphylococcus aureus	SdcS	35	Succinate (K_m 7 μM), malate (K_m 8 μM), fumarate (K_m 15 μM)	Yes, Na^+ and Li^+, inhibited by high [cation]	[26]