

Vitamin C

New Biochemical and Functional Insights

Edited by

Qi Chen, PhD

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First published 2020

978-1-138-33799-2 (hbk)

978-0-429-44202-5 (ebk)

Chapter 3

Vitamin C Alimentation via SLC Solute Carriers

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CHAPTER THREE

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INTRODUCTION

L-Ascorbic acid, also known as vitamin C, is an important cofactor for a great variety of metal ion-dependent enzymes, as well as an efficient antioxidant. All plants and most animals can synthesize vitamin C from glucose, except humans, guinea pigs, and certain birds, fishes, and nonhuman primates [1,2]. In humans, severe ascorbic acid deficiency (e.g., due to long voyages during the “Age of Sail”) results in scurvy, leading to impaired wound healing, anemia, fatigue, and ultimately death. To avoid ascorbic acid deficiency, humans rely on taking up ascorbic acid from the diet.

There are two ascorbic acid transporters belonging to the SLC23 family, SLC23A1/SVCT1 and SLC23A2/SVCT2. Both are Na⁺-coupled cotransporters [2]. SVCT1 is expressed in the intestines, the kidneys, the liver, the lungs, and skin [2,3]. It is the major uptake pathway for ascorbic acid in the intestine and, thus, is responsible for maintaining whole-body vitamin C levels [4]. SVCT2 is expressed in a variety of tissues such as the brain, lungs, liver, skin, spleen, muscles, and adrenal glands [3,5–7]. The uptake of ascorbic acid is regulated tightly, thereby limiting intestinal uptake, renal absorption, and delivery into target tissues. Ascorbic acid is an

essential cofactor of many enzymes, for example, as part of the synthesis of collagens [8]. Given the importance of vitamin C, it is not surprising that altered functions of the transporters of this essential vitamin are linked to different diseases. Thus, it is of fundamental importance to understand in detail the vitamin C uptake and dissemination pathways in our body.

INITIAL CLONING AND CHARACTERIZATION

The SLC23 family consists of four members. Of these, SVCT1 (SLC23A1) and SVCT2 (SLC23A2) are Na⁺-dependent vitamin C (ascorbic acid) transporters. The third member of the family, SVCT3 (SLC23A3), is an orphan transporter. A fourth member, SLC23A4 (SVCT4 or SNBT1), found in several organisms, exists in humans only as a pseudogene [2]. SVCT1, SVCT2, and SVCT3 sequences were previously identified as yolk sac permease-like proteins, YSPL3, YSPL2, and YSPL1, respectively [9,10], but were not functionally characterized. The Na⁺-coupled vitamin C transporter SVCT1 was first identified by functional expression of rat cDNA in *Xenopus* oocytes in our laboratory. The gene encoding SVCT2, SLC23A2, was identified by homologue screening of a rat brain cDNA library [6]. Several groups further cloned and characterized SVCT1 and SVCT2 from different species [2,6,11,12]. SVCT3 is an orphan transporter, since its transport substrates and physiological roles are still unknown [2].

FUNCTIONAL PROPERTIES OF THE SLC23 FAMILY

SVCT1 and SVCT2 facilitate the transport of ascorbic acid in a Na⁺-dependent manner across the cell membranes. Both transporters have a high affinity for L-ascorbic acid [4,13–15]. SVCT1 exhibits a $K_{0.5}$ of 20–100 μ M for L-ascorbic acid, depending on the species and the expression system. It displays a unique preference for L-ascorbic acid over the stereoisomer D-isoascorbic acid, as well as dehydroascorbic acid (DHA), various analogs, and intermediates of vitamin C metabolism [6]. The optimal pH for the electrogenic transport is around 7.5, while at pH 5.5, the transport rate is reduced by 50%–60% [2,6,14,15]. Both SVCT1 and SVCT2 cotransport ascorbic acid and Na⁺, most likely in a 1:2 stoichiometry, using the Na⁺ gradient as the driving force for the accumulation of ascorbic acid [3,15,16]. The binding order of the

substrates has been determined, with the first Na⁺ binding, then ascorbic acid, and finally the second Na⁺ [15,16]. SVCT2 function is not only Na⁺-dependent but is also affected by Ca²⁺ and Mg²⁺. Even in the presence of Na⁺, there is no transport in the absence of Mg²⁺/Ca²⁺. Without these ions, SVCT2 seems to be in an inactive state [2,16].

DHA is the oxidized form of vitamin C, which is not a substrate for SVCT1 or SVCT2. It is a substrate of SLC2A1/GLUT1, SLC2A3/GLUT3, and SLC2A4/GLUT4 [17]. DHA is toxic at high concentrations. When it enters into cells via GLUT transporters, it is reduced to ascorbic acid as part of the recycling process [18].

PHYLOGENETIC ASPECTS

The human SLC23 proteins belong to the nucleobase:cation symporter 2 (NCS2) family of membrane transporters, also called the nucleobase-ascorbate transporter (NAT) family. This family of proteins was originally described as a nucleobase:H⁺ symporter 2 family [19,20], containing transporters from Gram-negative and Gram-positive bacteria, fungi, plants, and animals, transporting various purines and pyrimidines, such as uracil, xanthine, or uric acid (Figure 3.1) [20,21]. Representative members of the family include the uracil transporter UraA [22] and the xanthine transporter YgfO/XanQ [23] from *Escherichia coli*, and UapA from *Aspergillus nidulans*/*Emericella nidulans* [24]. At around this time, rat SVCT1 and SVCT2 were identified by our group [6] and were subsequently included in the family as ascorbic acid transporters [21]. Interestingly, the only nucleobase transporter from the NCS2 family existing in mammals is SLC23A4 (SVCT4/SNBT1), which is active only in nonprimate mammals [25] and has been characterized to be a uric acid transporter [26].

STRUCTURE-FUNCTION RELATIONSHIP OF THE SLC23 FAMILY

Overall Fold and Transmembrane Architecture

Early transmembrane topology predictions based on hydropathy plots predicted 12 transmembrane helices for SLC23 proteins [6,9,14,32,33].

SLC23 transporters show an inverted repeat architecture with 7+7 transmembrane helices (TMHs) [34–36], due to which this fold family has been termed “7-TM Inverted Repeat” (7TMIR) fold [37]. One symmetric pair of the transmembrane

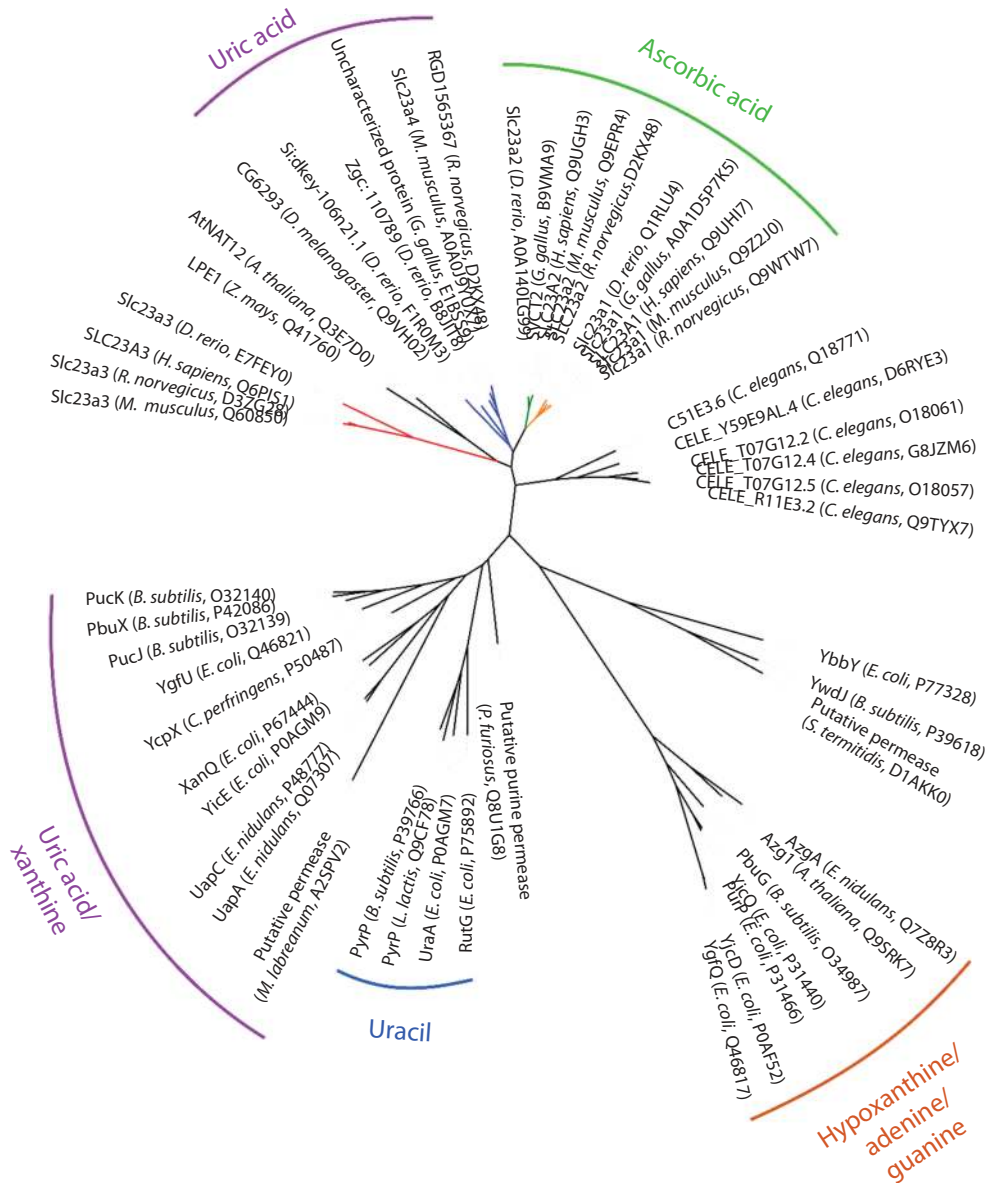


Figure 3.1. Phylogenetic tree of nucleobase:cation symporter 2 family transporters. Sequences were taken from family 2.A.40 of the Transporter Classification Database [27] and from in-house similarity searches within UniProt [28] sequences. SLC23A1-4 family branches are marked with orange, green, red, and blue, respectively. Predominant substrate groups are shown. The sequence alignment and the tree were created using ClustalO [29] and PhyML [30,31], respectively.

helices, TMH3 and TMH10, are shorter than usual and do not span the entire membrane. Instead, they are precluded by short β -strands that interact with each other to form a two-strand antiparallel β -sheet at the center of the protein, which is a hallmark element of the UraA/7TMIR fold. This structural feature is likely the reason why two TMHs were missed in the original topology predictions [38].

The UraA/7TMIR fold is represented in mammals by the SLC4/SLC23/SLC26 families, which share overall similar structural architectures [34,37,38].

Currently, there are three structures of NCS2-family transporters available at near-atomic resolution (Table 3.1), constituting a useful resource to predict the structural features of SLC23 transporters. In addition, structurally

TABLE 3.1

Currently available resolved three-dimensional structures for transporters of the 7-TM Inverted Repeat (7TMIR/UraA) fold

Protein	Protein Data		Cocrystallized		References
	Bank ID	Resolution [\AA]	Substrate	Conformation	
SLC23 Homologues					
UraA (<i>Escherichia coli</i>)	3QE7	2.781	Uracil	Inward-open	[34]
UraA (<i>E. coli</i>)	5XLS	2.5	Uracil	Occluded	[36]
UapA (<i>Emericella nidulans</i>)	5I6C	3.7	Xanthine	Inward-open	[35]
SLC4 Homologues					
AE1 (<i>Homo sapiens</i>)	4YZF	3.5	–	Outward-open	[39]
Bor1 (<i>Arabidopsis thaliana</i>)	5L25	4.11	–	Occluded	[40]
Bor1p (<i>Saccharomyces cerevisiae</i>)	5SV9	5.9	–	Inward-facing	[41]
SLC26 Homologues					
SLC26Dg (<i>Deinococcus geothermalis</i>)	5IOF	4.2	–	Inward-facing	[42]
SLC26Dg (<i>D. geothermalis</i>)	5DA0	3.2	–	Inward-facing	[42] (truncated construct)

similar homologues of SLC4 and SLC26 proteins can also be useful to understand the mechanism of transport (Table 3.1). Here we have used the substrate-bound occluded state of the UraA transporter (Protein Data Bank [PDB] ID: 5XLS) to generate a homology-based model of human SVCT1, SVCT2, and SVCT3 (see Figure 3.2a for hSVCT1; hSVCT2 and hSVCT3 models are not shown). Based on the structure of UraA and UapA, we also present a putative, refined transmembrane topology of SLC23 proteins (Figure 3.2c).

Interestingly, while members of the NCS1 family have been suggested to be structurally similar to APC transporters, such as LeuT (PDB ID: 2Q6H), this was initially not apparent for NAT/NCS2 family members [43]. As the structure of UraA was solved, it became clear that the overall structural fold of NCS2 and thus SLC23 transporters is distinct from APC transporters [34]. Despite the apparent structural dissimilarity, it was proposed that NCS2 transporters in fact belong to the APC superfamily, as they share a common evolutionary origin [44]. Later it was shown that the structural folds of LeuT and UraA indeed share common supersecondary structural elements [38].

Domain Architecture and Mechanism of Transport

The overall structure of the protein is often divided into the core domain comprising TMHs

1–4 and 8–11, and the gate domain that is formed by TMHs 5–7 and 12–14 [34]. While the core domain harbors most of the hydrophilic substrate-binding residues and a large number of buried hydrogen bonds, the gate domain as well as the interface between the two domains remain mostly hydrophobic [34].

Currently available structures of the 7TMIR fold show a variety of conformations (Table 3.1). Based on the superposition of these homologous structures, it has been suggested that SLC4/SLC23/SLC26 proteins function according to an elevator mechanism, as was also proposed for SLC1 transporters [35–37]. However, compared to a typical elevator model, the gate domain of 7TMIR transporters, unlike a typical scaffold domain, could undergo substantial local conformational changes. The overall mechanism is likely to be conserved among SLC23 family members [36].

Substrate-Binding Site

The structures of UraA and UapA have been crystallized in complex with uracil and xanthine, respectively (see Table 3.1) [34–36]. In all cases, the substrates have been unambiguously identified in the binding site, and residues likely to take part in substrate binding have been identified. In UraA, which is a H⁺-coupled symporter, residues E241, H245, and E290 cluster at the interface between the core and the gate domains

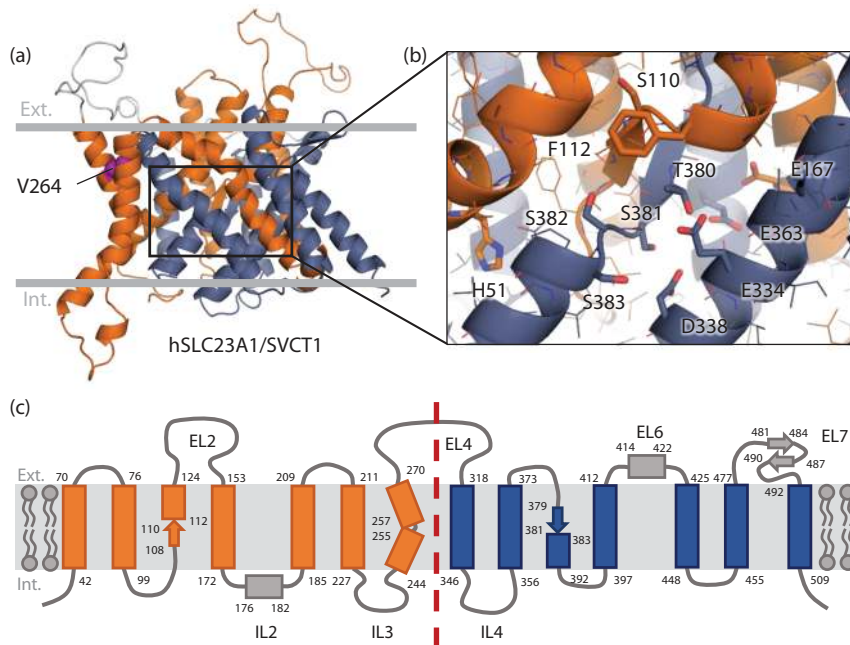


Figure 3.2. Structure and transmembrane topology of SLC23 transporters. (a) Homology-based model of human SLC23A1/SVCT1 based on the structure of the *Escherichia coli* UraA uracil:H⁺ symporter (Protein Data Bank [PDB] ID: 5XLS). Membrane orientation is based on the orientation of 5XLS from the Orientations of Proteins in Membranes (OPM) database [49]. The regions showing internal structural symmetry are colored orange and blue, respectively. The V264 residue corresponds to the location of the missense SNP rs33972313. The alignments for structure prediction were generated by PSI-COFFEE [50,51] and AlignMe [52] and modified manually. The structural model was generated using MODELLER 9.21 [53]. (b) Putative binding site of human SLC23A1/SVCT1 based on the UraA structure. Amino acid side chains that possibly take part in vitamin C and Na⁺ binding are highlighted in stick representation. (c) Predicted membrane topology based on the structure of UraA (PDB ID: 5XLS). The α -helices are shown as rectangles, β -strands as arrows, and bounding residues are numbered, based on similarity to the *E. coli* UraA structure and OPM predictions. Internally symmetric regions are colored orange and blue and separated by a red dashed line. (EL, extracellular loop; IL, intracellular loop.)

and have been implicated in proton binding [34]. Molecular dynamics simulations of the occluded UraA structure have shown that E241 needs to be deprotonated and H245 protonated to stabilize the uracil substrate in the binding site [36]. Based on these findings, the residue corresponding to H245 (D338 in hSVCT1 according to our alignment, see Figure 3.2b) in human SVCT1, SVCT2, and SNBT1 was suggested to bind the cotransported Na⁺ ion [34]. Interestingly, both hSVCT1 and hSVCT2 have been reported to be exclusively dependent on Na⁺ for transport and do not function with other relevant cations tested [33], suggesting that the functionally relevant cation binding sites in these proteins are specific for Na⁺.

The pH dependence of transport by hSVCT1 and hSVCT2 was early on linked with the presence of four conserved histidine residues [33]. A later study found that of these, only H51 in hSVCT1 (H109 in hSVCT2) is essential for transport activity but is

not responsible for the pH dependence of transport [45]. In line with this, the H109Q variant of hSVCT2 proved to be inactive, leading to the suggestion that H51 (hSVCT1) and H109 (hSVCT2) might be part of the substrate-binding site. In our model of hSVCT1, H51 is the only one of the four histidine residues that is buried in the protein; the other three are exposed to the solvent. Due to its orientation (Figure 3.2b), H51 is likely not in contact with the extracellular medium, which would explain why it does not affect the pH dependence of transport. Additionally, Varma et al. found that mutations of H51 affect the affinity of hSVCT1 toward ascorbic acid [45]. Based on our structural model, this is likely to be an indirect effect, as H51 is not predicted to be directly lining the substrate-binding site (Figure 3.2b). Interestingly, the analogous residue in the *E. coli* xanthine transporter YgfO/XanQ has also been reported to alter substrate selectivity [46]. Several histidine residues outside the putative

substrate-binding site in hSVCT2 have been shown to affect the Michaelis-constant of transport (K_m) of transport, while not affecting the cooperativity of cotransported Na^+ ions [47]. In the same report, H413 in hSVCT2 has been suggested to be responsible for the pH dependence of transport [47].

Dimerization

Both UraA and UapA, two homologues of SLC23 proteins with known structure, have been suggested to function as a dimer, with dimer formation essential for function [35,36]. Dimerization is mediated through the gate domains [36], and UapA was shown to be in a dynamic equilibrium between monomeric and dimeric states [36].

Interestingly, the positions identified by genetic screens for mutant variants of UapA that change substrate specificity have been mostly localized to either the interface between the core and gate domains or the linker between the two domains [35]. One of these residues, R481 [48], seems to lie closer to the substrate-binding site of the opposite UapA protomer in a homodimeric transporter complex. Molecular dynamics simulations also suggested that R481 could approach the central binding cavity and form cation- π interactions with the xanthine substrate, thereby modulating substrate specificity. This result also corroborates the idea that the functional unit of the transporter is likely a dimeric unit [35].

PHYSIOLOGICAL ROLES

Tissue Distribution

SVCT1 is expressed primarily in epithelial cells of the small intestine and the proximal tubule of the kidney [54]. It is also expressed in the liver, the lungs, and skin [3,5,6]. SVCT1 is responsible for maintaining whole-body ascorbic acid levels (Figure 3.3), facilitating intestinal uptake from the diet and renal reabsorption of L-ascorbic acid [4]. In contrast, SVCT2 is expressed in different tissues and organs that require vitamin C, such as the brain, the lungs, the liver, skin, the spleen, muscles, the adrenal glands, the eyes, the prostate, and the testis [2,6,12,14]. SVCT2 is the predominant transporter that delivers ascorbate into tissues that are in high demand of the vitamin, to support specific metal ion-dependent enzymatic activities and to protect cells against oxidative stress [1].

SVCT2 is not expressed in endothelial cells of the blood-brain barrier (BBB) but rather in choroid plexus (Figure 3.3). Thus, ascorbic acid is not transported across the BBB. DHA, the oxidized form of vitamin C, is delivered across the BBB. The latter is facilitated by the GLUT1/SLC2A1 transporter that is expressed at the luminal and abluminal membranes of the BBB [55]. Under physiological conditions, however, primarily ascorbic acid, the reduced form of vitamin C, is present in human plasma, and only 5%–10% of vitamin C exists in the oxidized form of DHA.

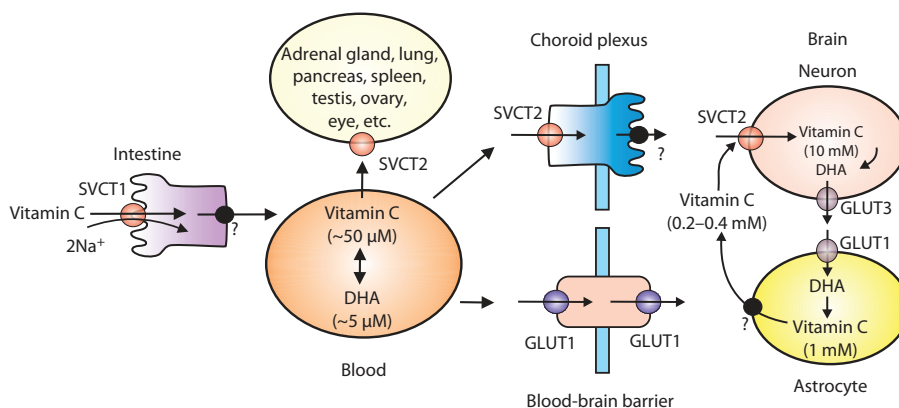


Figure 3.3. Vitamin C transport and tissue accumulation. SVCT1 absorbs vitamin C across the intestinal brush border membrane. The way it leaves the epithelial cells is yet unknown. To enter the brain, there are two possible routes. Either through the choroid plexus (blood-cerebrospinal fluid barrier) via SVCT2 or, in the form of DHA, through the blood-brain barrier via GLUT1. Since DHA is present at relatively low concentrations in the blood compared to L-ascorbic acid, the SVCT2 route across the choroid plexus may be the predominant pathway. In neurons, vitamin C is oxidized to DHA, which is released by GLUT3. Astrocytes import DHA via GLUT1 and reduce it to vitamin C. The exit pathway of vitamin C from astrocytes is currently unknown. The neurons import ascorbic acid via SVCT2, thereby closing the vitamin C recycling pathway.

Therefore, it is likely that DHA transport via GLUT1 across the BBB is not a major route for uptake of vitamin C into the brain. In contrast, in the epithelial cells of the choroid plexus, forming the blood–cerebrospinal fluid barrier, SVCT2 is highly expressed [6], indicating that ascorbic acid is mainly absorbed via this pathway [18]. In red blood cells, the main entry pathway of vitamin C is the uptake of DHA, which is immediately reduced to ascorbic acid after entering the cell. Red blood cells do not express SVCT2. GLUT1 and GLUT3 are responsible for the transport of DHA [56].

Knockout Mice

As already noted, all plants and most animals are capable of synthesizing ascorbic acid from glucose. Humans and other species, however, lost the ability to synthesize ascorbic acid. While mice are able to synthesize ascorbic acid [1,2], humans cannot synthesize it because the gene for the enzyme gulonolactone oxidase is mutated, resulting in a nonfunctional protein [1,2,57]. *Gulo*^{-/-} mice lacking this gene were used as model organisms for research on vitamin C metabolism [2].

Studies with SVCT1 or SVCT2 knockout mice revealed that *Slc23a1*^{-/-} mice have high ascorbic acid levels in the urine, implying that SVCT1 is involved in reabsorption of ascorbic acid from urine. However, these knockout mice were able to absorb ascorbic acid from the intestinal lumen. This indicates that another transporter makes up for the lack of SVCT1 in the intestine [1]. Interestingly, *Slc23a2*^{-/-} mice lacking SVCT2, the predominant ascorbic acid transporter, die within minutes after birth. All fetal cells except the liver, which is the site of ascorbic acid synthesis, show very low ascorbic acid levels [58]. The immediate cause of death is respiratory failure and hemorrhage in the brain [2].

Role of SVCT in Human Health and Ascorbic Acid Recycling

Ascorbic acid is an essential nutrient involved in important processes in the whole body. It acts as a cofactor for many enzymes and is an antioxidant, which can scavenge reactive oxygen species (ROS). The two transporters of ascorbic acid, SVCT1 and SVCT2, are responsible for the uptake from food and distribution in the body [1,2,59].

The mechanisms of vitamin C delivery into the brain have already been discussed. Neurons require

relatively high amounts of vitamin C, since they have a high rate of oxidative metabolism compared to other cells, leading to the oxidation of ascorbic acid to DHA. The mechanisms of ascorbic acid recycling are presented in Figure 3.3. DHA leaves the neurons, avoiding toxic effects of DHA accumulation. DHA efflux is facilitated via the GLUT3 transporter. Via the GLUT1 transporter, DHA is then imported into astrocytes. Astrocytes do not express SVCT2, but they take up DHA via GLUT1, which is then converted back into ascorbic acid [2,18]. Mechanisms for conversion into vitamin C involve glutathione and reducing enzymes. Indeed, the glutathione level of astrocytes is four times higher than that in neurons [18]. How ascorbic acid leaves astrocytes is still under investigation. The released ascorbic acid is then delivered back into neurons via SVCT2 [2,18]. In the extracellular space between the neurons and astrocytes, the ascorbic acid concentration is between 200 and 400 μ M, while in neurons it is 10 mM and in astrocytes 1 mM [2].

REGULATION OF THE EXPRESSION OF SVCT1 AND SVCT2

The substrate ascorbic acid of SVCT1 and SVCT2 is an important regulator for these transporters. In *Gulo*^{-/-} mice, which depend on ascorbic acid uptake from the diet, ascorbic acid starvation led to increased mRNA and protein expression of SVCT1 and SVCT2 in the liver, increased expression of SVCT2 in the cerebellum, and increased expression of SVCT1 in the small intestine. Also during development in mice, expression levels and ascorbic acid levels change. In late embryonic and early natal stages, the cortex and cerebellum of these mice have high ascorbic acid levels and low SVCT2 mRNA and protein levels. During adolescence, the expression of SVCT2 mRNA and protein is increased, and the ascorbic acid levels decrease. A development in a similar direction occurs in the liver of the mice: at birth, the expression levels of SVCT1 and SVCT2 are low, increasing over time [2]. It was shown that the hepatocyte nuclear transcription factor HNF1 α (hepatocyte nuclear factor 1 homeobox alpha) increases the SVCT1 promoter activity (Figure 3.4) [4,59,60]. HNF1 α transcription is inhibited when the NF- κ B (nuclear factor–kappa light chain enhancer of activated B cells) signaling pathway is activated. The activated NF- κ B pathway leads to increased levels of inflammatory cytokines and cell death [4]. SVCT2 is also regulated in the

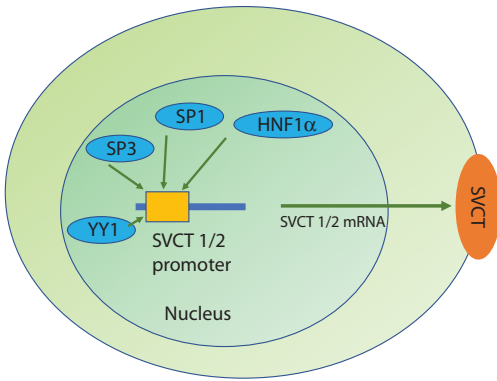


Figure 3.4. The promoter region of SVCT1 and SVCT2 is regulated by different transcription factors, Sp1, Sp3, HNF1 α , and YY1. Binding of these transcription factors to the promoter of SVCT1 or SVCT2 leads to transcription of mRNA.

same manner by HNF1 α . SVCT1 and SVCT2 are both regulated on the transcriptional level by transcription factor Sp1. A decrease in Sp1 leads to decreased mRNA and protein levels of SVCT1 and SVCT2 [60]. Other transcription factors such as SP3 and YY1 are also reported to regulate the expression of these transporters [61]. The euchromatin markers H3K4me3 [62,63] and H3K9ac also increase the expression of SVCT1 and SVCT2 [62]. The expression of SVCT1 and SVCT2

depends on many factors, and it is currently an active research topic because of their important role in vitamin C homeostasis.

Regulation of the cell surface distribution: SVCT1 and SVCT2 are responsible for the uptake of ascorbic acid from the extracellular space, fulfilling their task as plasma membrane transporters. For successful cell surface targeting and cell membrane incorporation of SVCT1 and SVCT2, intact C- and N-termini are required. The cell surface expression of SVCT2 also depends on microtubules and microfilaments. Nocodazole, a microtubule depolarizing agent, disturbs the transport of SVCT2 to the plasma membrane. The localization of SVCT2 is also increased by inhibitors of the myosin-II ATPase. It was furthermore shown that prostaglandin E2 increases the localization of SVCT2 in the plasma membrane [2].

PATHOLOGICAL ROLE

Inflammation, Infection

Prolonged, severe infections are associated with disturbance in the ascorbic acid homeostasis, resulting in low ascorbic acid levels (Figure 3.5a). It is not only infections that can disturb ascorbic acid homeostasis, but also inflammatory diseases such as inflammatory bowel disease are linked to low

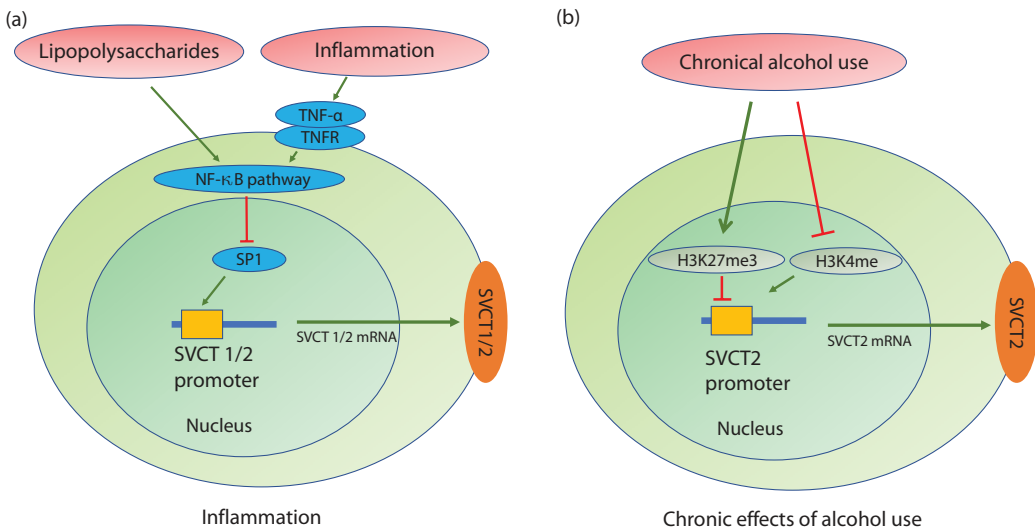


Figure 3.5. (a) Chronic inflammation or lipopolysaccharides lead to the activation of the NF- κ B pathway. This pathway inhibits the transcription factor Sp1, leading to a decreased expression of SVCT1/2. (b) Chronic alcohol use leads to increased levels of H3K27me3, which leads to heterochromatin in the promoter region of the SLC23A2 gene. At the same time, the levels of H3K4me are decreased, which leads to euchromatin in the promoter region of the SLC23A2 gene. The activity of the promoter region is decreased, resulting in a decreased expression of SVCT2.

levels of ascorbic acid. It is known that ascorbic acid deficiency is linked to delayed immune responses by natural killer cells and suppression of cytotoxic T-cell activity [4]. This has led to the assumption that the uptake of ascorbic acid is decreased during inflammation. Lipopolysaccharides are released by various bacteria such as the pathologic *Salmonella*. These molecules bind to toll-like receptors, which leads to activation of inflammatory signaling pathways, for example, NF- κ B and p38 mitogen-activated protein kinase (MAPK) (Figure 3.5a). Activation of these pathways leads to decreased ascorbic acid uptake in Caco-2 cells and mouse jejunum [60]. Increased levels of the cytokine TNF- α also lead to decreased ascorbic acid uptake in Caco-2 cells. TNF- α seems to regulate the ascorbic acid uptake the same way as lipopolysaccharides, because it also activates the NF- κ B pathway. It was shown that treating cells with TNF- α leads to a decreased expression of HNF1- α , which interacts with the SVCT promoter region. In Caco-2 cells and mouse enteroids, treatment with TNF- α leads to decreased mRNA and protein levels of SVCT1 [4]. Experiments with Caco-2 cells and mouse jejunum treated with lipopolysaccharides revealed decreased levels of TNF- α and SPI, both interacting with the promoters of SLC23A1 and SLC23A2, resulting in a decreased expression of SVCT1 and SVCT2 at the mRNA and protein levels [60].

Chronic Alcohol Abuse

In developed countries, severe vitamin C deficiency leading to scurvy is relatively rare. Moderate vitamin C deficiency, however, is common and occurs especially in the elderly, smokers, and alcoholics [59,60]. As already highlighted, ascorbic acid is an important scavenger of ROS, and it is able to reduce alcohol-induced oxidative damage. Chronic alcohol abuse is linked to lower plasma ascorbic acid levels. In humans and mouse pancreatic acinar cells (PACs), SVCT2 is highly expressed (Figure 3.5b). PACs chronically exposed to alcohol exhibited reduced ascorbic acid uptake. Both SVCT2 mRNA and protein levels were decreased, indicating inhibition at the transcriptional level. Changes in chromatin modeling could be shown, with an increase of H3K27me3, leading to heterochromatin in the SLC23A2 promoter region. At the same time, H3K4me, which leads to euchromatin in the promoter region of the SLC23A2 gene, was

decreased. Due to these changes, the expression level of SVCT2 was reported to be decreased [63].

Cancer

Cancer patients are frequently ascorbic acid deficient [3]. The effect of ascorbic acid on cancer is discussed controversially. In the 1970s, it was shown by Ewan Cameron and Linus Pauling that high oral doses of ascorbic acid lead to survival benefits in patients with advanced cancer [64–66]. A follow-up double-blind placebo-controlled study could not confirm the results, and ascorbic acid was dismissed as an anticancer agent [66–68]. New findings revealed that oral doses of ascorbic acid can be increased to 80-fold without great changes in the plasma levels because of limited intestinal uptake, tissue saturation, renal reabsorption, and excretion. This may explain the different outcomes of the studies: Cameron used 10 g intravenous ascorbic acid, while in the studies in which ascorbic acid failed as an anticancer agent, ascorbate was administered orally [66].

SVCT2, as the predominant tissue transporter of ascorbic acid, has a large influence on the efficacy of ascorbic acid as an anticancer drug. A study with different human colon cancer cell lines revealed different outcomes of ascorbic acid treatment on cancer cells, depending on the expression level of SVCT2. Specifically, there is an antiproliferative effect of ascorbic acid in cancer cells, which is dependent on the expression level of SVCT2. High expression resulted in high intracellular levels of ascorbic acid, slowing down proliferation. The cells with low expression of SVCT2 exhibited a “hormetic” proliferation response to ascorbic acid, meaning that low concentrations of ascorbic acid increased proliferation of colon cancer cells, whereas high concentrations still had an anticancer effect due to the accumulation of vitamin C–altering proliferation pathways. Specifically, in low SVCT2-expressing cells, ascorbic acid at a moderate dose of 10 μ M increased the expression of c-myc and cyclin D and, due to this, cell proliferation. In contrast, high doses of ascorbic acid >1 mM decreased the expression of c-myc and cyclin D [69]. This likely explains the different outcomes of the studies described earlier. The plasma level of ascorbic acid by oral administration was probably not high enough to trigger the anticancer effect of ascorbic acid in cancer cells with low SVCT2 expression. The expression level of SVCT2,

however, was not measured in this study, highlighting the need for additional experiments to verify this hypothesis.

There are other studies underlining the importance of SVCT2 in anticancer treatment. Colon cancer cell lines with KRAS mutations were shown to be resistant to the anticancer drug cetuximab. Cotreatment with ascorbic acid was able to overcome cetuximab resistance but only in colon cancer cells expressing SVCT2. In contrast, cotreatment in colon cancer cells not expressing SVCT2 had no effect. Expression of SVCT2 in these cells restored the anticancer effect. Furthermore, in SVCT2-expressing colon cancer cells, knock down of SVCT2 with siRNA led to resistance against the cotreatment. The cotreatment caused cetuximab-triggered apoptotic and necrotic cell death only in colon cancer cells expressing SVCT2 [70]. Whether vitamin C alters signaling pathways or affects enzymes needed for the action of cetuximab remains to be determined.

Generating vitamin C in cancer cells by a “bystander effect” has been proposed to be a mechanism of how tumor cells in an oxidative state accumulate ascorbic acid without the expression of SVCT1 or SVCT2. According to this mechanism, superoxide generated in these cells is released to the extracellular space in an oxidative burst. Extracellular superoxide then converts ascorbic acid to DHA, which is imported into the cells via GLUTs, followed by the reduction of DHA to ascorbic acid [18].

Huntington Disease

Huntington disease is a genetic disorder caused by a mutation of the gene coding for Huntingtin. Mutation of Huntingtin leads to involuntary movements, cognitive deterioration, dementia, and weight loss. This protein is responsible for the intracellular trafficking of vesicles, organelles, and proteins to the cell surface. In immortalized striatal neurons expressing mutated Huntingtin, it was shown that SVCT2 is no longer able to translocate to the plasma membrane in response to increased extracellular ascorbic acid levels. Huntingtin is known to be associated to vesicles and microtubules, suggesting a role of this protein in the transport of SVCT2-containing vesicles, in order to deliver SVCT2 to the plasma membrane of neurons. Huntington disease is connected to increased oxidative damage in lipids, proteins, and

DNA, highlighting the protecting role of vitamin C as an antioxidant in neurons. These results also highlight the possible role of SVCT2 in other neurodegenerative diseases such as Alzheimer and Parkinson diseases [71].

Single Nucleotide Polymorphisms

In human SVCT1 and SVCT2, there are several single nucleotide polymorphisms (SNPs) linked to diseases.

SVCT1 SNPs: Several studies revealed SNPs in *SLC23A1* that are associated with a specific phenotype. The intronic SNP rs6596473-C is linked to increased risk of follicular lymphoma but showed no link to gastric cancer and advanced colorectal adenoma [3]. Another intronic SNP, rs10063949-G, is associated with increased risk of Crohn disease [72]. The SNP rs33972313 is the only known polymorphism that affects the coding region (see Figure 3.2a). It causes missense variations at position V264, with substitution by the bulkier side chains Leu or Met. These may cause steric clashes that compromise transporter structure and function. These SNPs were associated with low circulating concentrations of ascorbic acid [73].

SVCT2 SNPs: SNP rs6139591 in the *SLC23A2* gene is connected to a higher risk for spontaneous preterm delivery [74]. Two SNPs localized in the intron of the gene *SLC23A2* were associated with increased risk for chronic lymphocytic leukemia. Different studies in different cohorts of patients showed SNPs linked with increased risk for gastric cancer and chemoradiotherapy-induced toxicity [3]. The proposed impact of SNPs on diseases and ascorbic acid homeostasis has been discussed in detail in recent reviews [75,76].

Pharmacological Relevance

SVCT1 and SVCT2 are promising drug transporters. SVCT2 may be exploited to facilitate the delivery of certain drugs to the brain via the choroid plexus. For example, nipecotic acid, a γ -aminobutyric acid (GABA) uptake inhibitor, was reported to be transported by SVCT2 when conjugated to 6-Br-ascorbic acid [2]. Saquinavir is a protease inhibitor used in the treatment of HIV. It has a high anti-HIV potency but a low bioavailability. A new prodrug, ascorbyl-succinic-saquinavir, was shown to increase absorptive permeability and

metabolic stability. This prodrug was transported by SVCT2 in Caco-2 cells, which could be a potent drug delivery mechanism for anti-HIV protease inhibitors [77]. Ascorbic acid in combination with SVCT2 can also be used as a targeting agent for nanocarriers to transport drugs [2]. Overall, especially SVCT2 with its broad tissue distribution is a promising drug transporter for drugs linked to ascorbic acid or 6-Br-ascorbic acid.

SVCT2 is not well established as a direct drug target. In one clinical trial, sepsis was treated with a combination of hydrocortisone, vitamin C, and thiamine. There was no change in mortality, but there were significant reductions in the requirement for vasopressin [78]. Hydrocortisone belongs to the glucocorticoids, a class of steroid hormones, which upregulate the expression of SVCT2 [79,80]. As already discussed, SVCT2 expression is reduced in sepsis, leading to reduced ascorbic acid levels.

SVCT2 is more interesting as a drug transporter than SVCT1, because it is expressed in many different tissues and organs. SVCT1 is only expressed in epithelial cells. The expression of SVCT2 in the choroid plexus is especially interesting for drug delivery to the brain.

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