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Physiological, Pharmacological and Toxicological Implications of Heterodimeric Amino Acid Transporters

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The heterodimeric amino acid transporter family is a subfamily of SLC7 solute transporter family which includes 14-transmembrane cationic amino acid transporters and 12-transmembrane heterodimeric amino acid transporters. The members of heterodimeric amino acid transporter family are linked via a disulfide bond to single membrane spanning glycoproteins such as 4F2hc (4F2 heavy chain) and rBAT (related to b^0, $^+$-amino acid transporter). Six members are associated with 4F2hc and one is linked to rBAT. Two additional members were identified as ones associated with unknown heavy chains. The members of heterodimeric amino acid transporter family exhibit diverse substrate selectivity and are expressed in variety of tissues. They play variety of physiological roles including epithelial transport of amino acids as well as the roles to provide cells in general with amino acids for cellular nutrition. The dysfunction or hyperfunction of the members of the heterodimeric amino acid transporter family are involved in some diseases and pathologic conditions. The genetic defects of the renal and intestinal transporters $b^{0,+}$AT/BAT1 ($b^{0,+}$-type amino acid transporter/$b^{0,+}$-type amino acid transporter 1) and y^+LAT1 (y^+L-type amino acid transporter 1) result in the amino aciduria with sever clinical symptoms such as cystinuria and lysin uric protein intolerance, respectively. LAT1 is proposed to be involved in the progression of malignant tumor. xCT (x-C-type transporter) functions to protect cells against oxidative stress, while its over-function may be damaging neurons leading to the exacerbation of brain damage after brain ischemia. Because of broad substrate selectivity, system L transporters such as LAT1 transport amino acid-related compounds including L-Dopa and function as a drug transporter. System L also interacts with some environmental toxins with amino acid-related structure such as cysteine-conjugated methylmercury. Therefore, these transporter would be candidates for drug targets based on new therapeutic strategies.

Key Words: Amino acid transporter, Epithelial transport, Cellular nutrltion, Oxldative stress, Transport mediated toxicity

Amino Acid Transporters and Their Molecular Identification

Plasma membrane amino acid transporters are essential to supply cells with amino acids for cellular nutrition. In the epithelia of kidney and small intestine, distinct transporters are developed in apical and basolateral membranes of epithelial cells to ensure the vectorial transport of amino acids through the epithelia (Silbernagl, 1979; Stevens et al, 1984). In brain, transporters for amino acids and related neurotransmitters function to terminate synaptic transmission and to protect neurons from the toxicity of excitatory amino acids (Kanai, 1997; Billups et al, 1998). A large number of amino acid transport systems in mammals distinguished based on substrate selectivity and ion-dependence have been charanged by molecular cloning approaches in the last decade (Christensen, 1990; Palacin et al, 1998). The transporters corresponding to each transport system have been identified so far (Table 1). Those transportes are classified into several transporter families based on the structural similarity. They include three Na^+-dependent families, SLC (solute carrier) 1, SLC6 and SLC38, and three Na^+-independent families, SLC7, SLC16 and SLC43 (Table 1).

The first molecular identification of amino acid transport systems was a serendipitous finding of Na^+-independent basic amino acid transporter CAT1 (cationic amino acid transporter 1) subserving system y^+ (MacLeod et al, 1994). CAT1 was originally cloned as an ecotropic retrovirus receptor. Because it exhibited sequence similarity to prokaryotic amino acid permeases, it was expressed in *Xenopus* oocytes to examine whether it functions as an amino acid

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ABBREVIATIONS: SLC, solute carrier; 4F2hc, 4F2 heavy chain; rBAT, related to $b^{0,+}$-amino acid transporter; LAT, L-type amino acid transporter; y^+LAT, y^+L-type amino acid transporter; Asc, asc-type amino acid transporter; xCT, x-C-type amino acid transporter; $b^{0,+}$AT/BAT, $b^{0,+}$-type amino acid transporter; AGT, aspartate/glutamate transporter; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid.

Table 1. Amino acid transport systems

Transport system	Substrate	Transporter	Family
Neutral amino acids			
Na^+-dependent			
A	Ala, Pro, N-methyl amino acids	ATA1, ATA2 , ATA3	[c]SLC38
G	Gly, Sar	GLYT1, GLYT2	SLC6
B^o	Broad substrate selectivity	B0AT1	SLC6
ASC	Ala, Ser, Thr, Cys,(Gln)	ASCT1, ASCT2	SLC1
N	Gln, Asn, His	SN1, SN2	SLC38
β-system	β-Ala, Tau	Taut	SLC6
y^+ L	[a]Neutral and basic amino acids	[b]y^+LAT1+4F2hc, y^+LAT2+4F2hc	SLC7
Na^+-independent			
L	Large neutral amino acids	[b]LAT1+4F2hc, LAT2+4F2hc	SLC7
		LAT3, LAT4	SLC43
asc	Ala, Ser, Thr, Cys	[b]Asc-1+4F2hc, Asc-2+?	SLC7
T	Aromatic amino acids	TAT1	SLC16
$b^{o,+}$	Neutral and basic amino acids	[b]$b^{0,+}$AT/BAT1+rBAT	SLC7
Basic amino acids			
Na^+-dependent			
$Bo,^+$	Neutral and basic amino acids	$ATBo,^+$	SLC6
Na^+-independent			
y^+	Basic amino acids	CAT1, CAT2, CAT2a, CAT3, CAT4	SLC7
$b^{o,+}$	Neutral and basic amino acids	[b] $b^{0,+}$AT/BAT1+rBAT	SLC7
y^+L	[a]Neutral and basic amino acids	[b]y+LAT1+4F2hc, y^+LAT2+4F2hc	SLC7
Acidic amino acids			
Na^+-dependent			
X_{AG}	L-Glu, L-/D-Asp	EAAC1, GLT-1, GLAST, EAAT4, EAAT5	SLC1
Na^+-independent			
x^-_c	Cystine/Glu exchange	[b]xCT+4F2hc	SLC7

[a]System y^+L is partially dependent on Na^+ for neutral amino acids and Na^+-independent for basic amino acids. [b]Heterodimeric transporters are composed of two subunits, ex. y^+LAT1+4F2hc is a heterodimer of y^+LAT1 (SLC7 family) and a type II membrane glycoprotein 4F2hc. [c]SLC (solute carrier family) is a naming of transporter families by Human Gene Nomenclature Committee.

transporter (Kim et al, 1991; Wang et al, 1991). Following CAT1, a taurine transporter with the properties of b-system (Uchida et al, 1992), a glycine transporter with the properties similar to those of system G (Smith et al, 1992a) and a brain specific proline transporter which could not be assigned to classically characterized amino acid transport systems (Fremeau et al, 1992) were identified as members of Na^+/Cl^--dependent neurotransmitter transporter family (SLC6) (Amara & Kuhar, 1993). Later on, a transporter with the properties of Na^+-dependent neutral and basic amino acid transport system $B^{0,+}$ was also isolated as a member of Na^+/Cl^--dependent transporter family (Sloan & Mager, 1999). Further recently, the transporter for system B0 whose genetic defect is responsible for Hartnup disease have been identified as a member of SLC6 (Broer et al, 2004). In 1992, three glutamate transporters with the properties of Na^+-dependent acidic amino acid transport system X-A,G were cloned so that a new family of amino acid transporters was established (Kanai &Hediger, 1992; Pines et al, 1992; Storck et al, 1992). This family was further expanded to include the transporters which exhibit functional properties of Na^+-dependent small neutral amino acid transport system ASC (SCL1 family) (Arriza et al, 1993; Shafqat et al, 1993; Kekuda et al, 1996; Utsunomiya-Tate et al, 1996; Kanai, 1997).

Four amino acid transporter families have subsequently been identified. In 1998, a heterodimeric amino acid transporter subserving Na^+-independent neutral amino acid transport system L was cloned (Kanai et al, 1998; Mastroberardino et al, 1998). Following this, molecular nature of several amino acid transport systems were revealed as heterodimeric amino acid transporters, as described in this review (SLC7 family). Transporters for Na^+-dependent neutral amino acid transport systems N and A were found as proteins structurally related to plant amino acid/auxin transporters and mammalian vesicular GABA transporters (SLC38 family) (Chaundhry et al, 1999; Hatanaka et al, 2000; Sugawara et al, 2000a; Sugawara et al, 2000b; Varoqui et al, 2000; Yao et al, 2000; Nakanishi et al, 2001). In 2001, a Na^+-independent transporter subserving system T which transports aromatic amino acids was identified by functional expression cloning (Kim et al, 2001; Kim et al, 2002b). Interestingly, the system T transporter exhibited the structural similarity to H^+/monocarboxylate transporters (SLC16). Most recently, the identification of a novel system L amino acid transporter structurally distinct from heterodimeric amino acid transporters of SLC7 family established a new transporter family (SLC43) (Babu et al, 2003).

Heterodimeric Amino Acid Transporter and its Discovery

The heterodimeric amino acid transporter family is a subfamily of SLC7. Structurally and functionally, the SLC7

family is divided into two major subgroups, the cationic amino acid transporters and the heterodimeric amino acid transporters that are also called light chains or catalytic units of the heterodimeric amino acid transporters (Fig. 1). In the heterodimeric amino acid transporters, 12-membrane-spanning light chains are linked with single-membrane-spanning heavy chains via a disulfide bond (Fig. 2). Transporters of this family exhibit interesting properties in their substrate selectivity. In general, substrate selectivity of amino acid transporters is relatively narrow, because amino acid transporters have to rely on three features of substrate molecules for their recognition: a positive charge conferred by the α-amino group, a negative charge conferred by the α-carboxyl group and the spacial and/or electric characteristic of the substrate-amino-acid side chain (Fig. 3). However, the transporters of the heterodimeric amino acid transporter family exhibits fairly broad substrate selectivity in which they can transport amino acids with variety of side chains. Because of this characteristics, amino acid-related compounds such as drugs and environmental or food-derived toxins could permeate the transporters so that they function as drug transporters which would contribute to the pharmacokinetics of amino acid-related drugs in the body. Another interest on the heterodimeric amino acid transporter family is that some of the members are closely related to the diseases or pathologic conditions such as amino aciduria (cystinuria and lysinuric protein intolerance), malignant tumors and oxidative stress, so that they can be the targets of therapeutic drugs.

Among the subunits of heterodimeric amino acid transporters, a heavy chain unit rBAT (related to $b^{0,+}$ amino acid transporter) was firstly discovered. When kidney cortex poly(A)$^+$RNA was expressed in *Xenopus* oocytes, it induced a high level of amino acid uptakes. A cDNA was isolated to account for this transport activity. The cDNA, however, encoded a protein designated rBAT with a single membrane spanning structure (Bertran et al, 1992b; Tate et al, 1992;

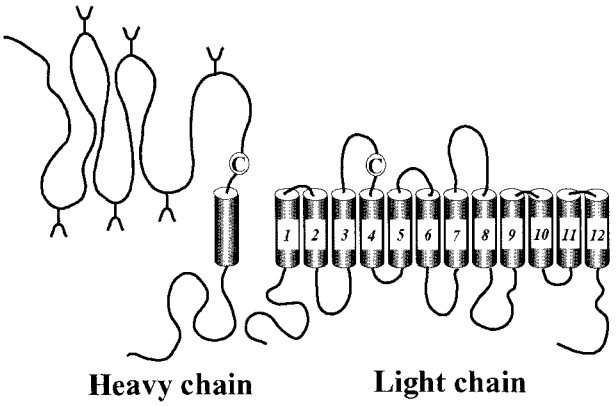

Fig. 2. Membrane topology of heterodimeric amino acid transporters. The heavy chain and its partner light chain are linked via a disulfide bond between a cysteine residue near the extracellular surface of the heavy chain and a cysteine residue of the light chain in the extracellular loop between predicted membrane spanning domains 3 and 4 indicated as "C" in the figure. N-liked glycosylation sites are predicted in the extracellular domain of the heavy chain indicated as "Y".

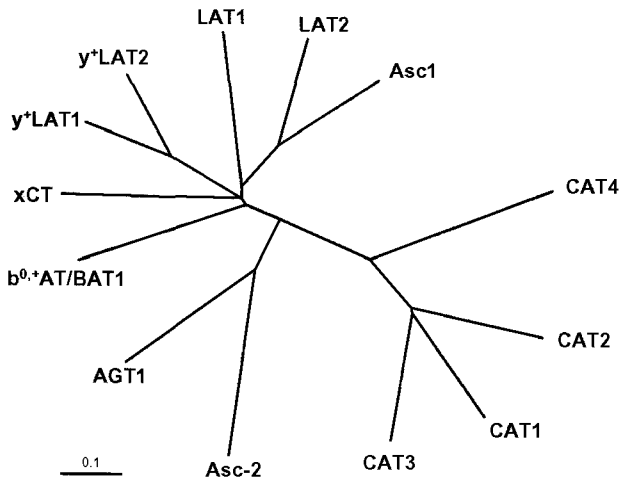

Fig. 1. Phylogenic relationship of the transporters of SLC7 family. SLC7 family consists of 14-membrane-spanning cationic amino acid transporters (CAT1, CAT2, CAT3 and CAT4) and 12-membrane-spanning heterodimeric amino acid transporters (LAT1, LAT2, Asc-1, y^+LAT1, y^+LAT2, xCT, $b^{0,+}$AT/BAT1, Asc-2 and AGT 1). LAT1, LAT2, Asc-1, y^+LAT1, y^+LAT2 and xCT are linked with a heavy chain 4F2hc to form the heterodimeric proteins, whereas $b^{0,+}$AT/BAT1 is linked to the other heavy chain rBAT. For Asc-2 and AGT1, however, associated heavy chains have not been determined. The branch lengths are a measure of the sequence divergence of the proteins and are approximately proportional to phylogenic distance..

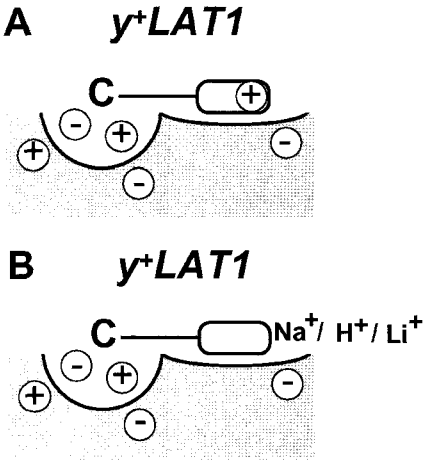

Fig. 3. A model for substrate-binding sites of y^+LAT1. The proposed mechanisms of substrate recognition are schematically shown for system y^+L transporter y^+LAT1. The binding site is proposed to be composed of two sites: one for the binding of charged a-amino and a-carboxyl moieties (indicated by + or - symbols near the a-carbon shown by "C"), and the other for the binding of the substrate amino acid side chains (indicated by the stub connected by a line). The side-chain-binding site of y^+LAT1 is proposed to be equipped with the machinery to accept a positive charge. Basic amino acids can interact with the binding site without Na^+ (A), whereas neutral amino acids require Na^+ for the interaction with the binding site (B). Li^+ or H^+ can substitute for Na^+ (B). The charged amino acid residues indicated by + or - symbols are proposed to be present at the substrate-binding sites.

Wells & Hediger, 1992). rBAT was regarded as an activator or a modulator of transporter proteins and not a transporter itself, because the single membrane structure is not typical for transporters usually with multiple membrane spanning domains (Palacin, 1994). In the nucleotide sequence data base searches, another single membrane spanning glycoprotein named 4F2 heavy chain (4F2hc) was found to possess structural similarity to rBAT. 4F2hc was originally identified by means of a monoclonal antibody 4F2 as a lymphocyte activation antigen (Haynes et al, 1981; Hemler & Strominger, 1982). It was shown that the glycoprotein 4F2hc (~85 kDa) links to a non-glycosylated light chain (~40 kDa) via a disulfide bond and forms a heterodimeric complex (Haynes et al, 1981; Hemler & Strominger, 1982). Because of the structural similarity to rBAT (~30% identity at amino acid level), 4F2hc was expressed in *Xenopus* oocytes and in fact shown to induce amino acid transport activity (Bertran et al, 1992a; Wells et al, 1992). Therefore, it was postulated that rBAT and 4F2hc are the regulatory subunits of proposed heterodimeric proteins and link to their catalytic subunits via a disulfide bond to form functional amino acid transporters.

The first molecular identification of the light chain component of 4F2 antigen was reported by two independent groups which relied on different approaches. We performed functional expression cloning using a *Xenopus* oocyte expression system in which a cDNA library prepared form C6 rat glioma cells was coexpressed with 4F2hc and screened for 14C-leucine transport activity (Kanai et al, 1998). At the end, we isolated a cDNA encoding a 512 amino-acid protein designated LAT1 (L-type amino acid transporter 1) with 12 putative membrane-spanning domains. LAT1 requires 4F2hc for its functional expression in *Xenopus* oocytes (Fig. 2). When co-expressed with 4F2hc, LAT1 exhibits Na^+-independent transport of neutral amino acids with branched or aromatic side chains which is sensitive to system L-specific inhibitor, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH), indicating that LAT1 is a transporter subserving system L. *In vitro* translation study revealed that LAT1 is a non-glycosylated membrane protein consistent with the property of 4F2 light chain (Fig. 2) (Kanai et al, 1998).

Verrey and coworkers identified a cDNA encoding a protein designated ASUR4b upregulated upon the stimulation of A6 epithelial cells by aldosterone (Spindler et al, 1997). Based on the sequence similarity between ASUR4b and prokaryote amino acid permeases, they postulated that ASUR4b is an amino acid transporter. They finally found that ASUR4b exhibited the functions of system L when coexpressed with 4F2hc (Mastroberardino et al, 1998). Immunoprecipitation studies using antibody against 4F2hc demonstrated that ASUR4b (*Xenopus* LAT1) is linked to 4F2hc via a disulfide bond (Mastroberardino et al, 1998). It was shown in *Xenopus* oocytes that the coexpression of

Table 2. Members of heterodimeric amino acid transporter family

Name of proteins	[a]Size (amino acids)	Associating type II membrane glycoproteins	Tissue distribution and cellular localization	[b]High affinity substrates
LAT1	507	4F2hc	brain, placenta, testis, bone marrow, fetal liver, tumor cells, brain capillary endothelial cells	L-Leu, L-Ile, L-val, L-Phe, L-Tyr, L-Trp,, L-Met, L-His, D-Leu, D-Phe, L-Dopa, D-Met, (triiodethyronine, thyroxine, melphalan), BCH is a selective inhibitor.
LAT2	535	4F2hc	brain, placenta, kidney, small intestine, testis, skeletal muscle, proximal tubules and small basolateral membrane of renal intestine epithelium	Gly, L-Ala, L-Ser, L-Thr, L-Cys, L-Gln, L-Asp, L-Leu, L-Ile, L-val, L-Phe, L-Tyr, L-Trp, L-Met, L-His, BCH is a selective inhibitor.
Asc-1	523	4F2hc	brain, lung, small intestine, placenta, kidney, heart	Gly, L-Ala, L-Ser, L-Thr, L-Cys, D-Ser, D-Ala, D-Cys, D-Thr, b-Ala, a-aminoisobutyric acid.
y^+LAT1	511	4F2hc	kidney, small intestine, placenta.	L-Lys, L-Arg, L-Orn, L-Gln, L-Leu, L-Ile, L-Met, L-His.
y^+LAT2	515	4F2hc	not determined	L-Lys, L-Arg, L-Orn, L-Gln, L-Leu, L-Ile, L-Met, L-His.
xCT	523	4F2hc	brain, spinal cord, activated macrophage, U87 glioma cells,	L-Cystine, L-Glu, L-homocysteate.
BAT1/$b^{0,+}$AT	487	rBAT	kidney, small intestine, liver	L-Cystine, L-Lys, L-Arg, L-Orn, L-Ala, L-Ser, L-Thr, L-Cys, L-Gln, L-Asp, L-Leu, L-Ile, L-val, L-Phe, LTyr, L-Trp, L-Met, L-His,
Asc-2	465	[c]ND	kidney, placenta, spleen, lung, skeletal muscle	Gly, L-Ala, L-Ser, L-Thr.
AGT1	478	[c]ND	kidney	L-Glu, L-Asp.

[a]Size of human proteins are listed. [b]The compounds with low Vmax values and those which exhibited strong inhibition whereas flux measurement were not performed are shown in parentheses. [c]The associating heavy chain has not been determined.

4F2hc is required for the surface expression of the light chains. Hemler and colleagues independently purified the 4F2 heavy chain/light chain complex using an anti-4F2hc monoclonal antibody. They collected the protein corresponding to the light chain and sequenced its *C*-terminus to reveal that it is identical to the *C*-terminal sequences of LAT1, confirming that LAT1 is the 4F2 light chain (Mannion et al, 1998). Minato and coworkers generated a monoclonal antibody against mouse 4F2 light chain and cloned the cDNA to find out that it encodes LAT1 (Nakamura et al, 1999). Thus it was establised that LAT1 is a light chain of 4F2 antigen.

Heterodimeric Amino Acid Transporter Family

Following the finding of LAT1, other structurally related light chains were rapidly identified. They include two system y^+L transporters y^+LAT1 and y^+LAT2, second isoform of system L transporter LAT2 and a system asc transporter Asc-1 (Fukasawa et al, 2000; Pfeiffer et al, 1999b; Pineda et al, 1999; Segawa et al, 1999; Torrents et al, 1998) (Table 2). System y^+L is a transport system which recognizes both neutral and basic amino acids (Deves et al, 1992). y^+LAT1 and y^+LAT2 exhibited similar transport properties with different tissue distribution of expression (Pfeiffer et al, 1999b; Torrents et al, 1998). LAT2 is a second isoform of system L transporter with different substrate selectivity compared with LAT1. LAT2 exhibits broad substrate selectivity covering most of the neutral amino acids (Pineda et al, 1999; Rossier et al, 1999; Segawa et al, 1999). In addition LAT2 is expressed more widely in the aminal body. Asc-1, in contrast, prefers small neutral amino acids such as alanine, serine, threonine and cysteine, consistent with the properties of system asc (Fukasawa et al, 2000). Bannai and co-workers independently performed functional expression cloning and isolated a cDNA encoding other 4F2 light chain (Sato et al, 1999) (Table 2). The encoded protein designated xCT exhibited the properties of system x-C which mediates cystine/glutamate exchange. Therefore, it was established that 4F2hc is associated with multiple transporters with different substrate selectivity (Table 2).

Because rBAT is structurally related to 4F2hc, it was reasonable to assume that the partner of rBAT would also be structurally related to the transporters associated with 4F2hc. As a partner of rBAT, a protein designated $b^{0,+}$AT or BAT1 ($b^{0,+}$-type amino acid transporter) was identified (Chairoungdua et al, 1999; International Cystinuria Consortium, 1999; Pfeiffer et al, 1999a) (Table 2). The $b^{0,+}$AT/BAT1 immunoreactivity was found in the apical membrane of proximal tubules in kidney where it was colocalized with rBAT immunoreactivity (Chairoungdua et al, 1999; Pfeiffer et al, 1999a; Mizoguchi et al, 2001). When expressed in COS-7 cells with rBAT, but not with 4F2hc, $b^{0,+}$AT/BAT1 exhibited a Na^+-independent transport of cystine as well as basic and neutral amino acids with the properties of system $b^{0,+}$ (Chairoungdua et al, 1999; Mizoguchi et al, 2001). The finding of $b^{0,+}$AT/BAT1 associated with rBAT together with the identification of multiple transporters associated with 4F2hc thus established a family of heterodimeric amino acid transporters whose members are linked with single membrane spanning glycoproteins via a disulfide bond (Table 2).

Further searches of nucleotide sequence data bases revealed at least two additional proteins Asc-2 (asc-type amino acid transporter 2) and AGT1 (aspartate/glutamate transporter 1) structurally related to heterodimeric amino acid transporters of SLC7 family (Chairoungdua et al, 2001; Matsuo et al, 2002). Asc-2 and AGT1 exhibit relatively low but significant sequence similarity to heterodimeric amino acid transporter light chains (24~29% identity). The cysteine residue responsible for the disulfide bond formation between light and heavy chain is conserved in Asc-2 and AGT1, although these proteins did not induce functional activity when co-expressed with the known heavy chains 4F2hc or rBAT (Chairoungdua et al, 2001; Matsuo et al, 2002). Therefore, it is proposed that these proteins are linked to (an) unknown heavy chain(s) by a disulfide bond via the conserved cysteine residue. By generating fusion proteins in which *C*-terminus of the transporters are connected with *N*-terminus of 4F2hc or rBAT to express the transporters in the plasma membrane, we were able to analyze their transport functions. Asc-2 was the second isofom of system asc transporter, whereas AGT1 was a Na^+-independent transporter for glutamate and aspartate, for which corresponding transport system was not described so far (Chairoungdua et al, 2001; Matsuo et al, 2002) (Table 2).

Roles of Heterodimeric Amino Acid Transporters in the Epithelial Transport of Amino Acids

Various amino acid transport systems contribute to the transepithelial transport of amino acids at renal proximal tubules and intestinal epithelia (Fig. 4). Among them, heterodimeric amino acid transporters play important roles in the transepithelial transport of neutral and basic amino acids as described below.

As for acidic amino acids, the amino acids are absorbed from the luminal fluid via the Na^+-dependent system X-A,G glutamate transporter EAAC1 (excitatory amino acid carrier 1) situated on the apical membrane of the epithelial cells (Fig. 4) (Shayakul et al, 1997). The defect of EAAC1 results in the acidic amino aciduria in which glutamate and aspartate are excreted in urine (Peghini et al, 1997). The absorbed glutamate has been proposed to be converted to neutral amino acids in the epithelial cells and leave the cells to the blood stream via neutral amino acid transport systems which include the system L. Because AGT1 with unknown heavy chains was found to be an acidic amino acid transporter present in the basolateral membrane of the proximal straight tubules and distal convoluted tubules in kidney, it is possible that this transporter participates in the exit of acidic amino acids from the basolateral membrane (Matsuo et al, 2002).

Neutral amino acids are absorbed from the luminal fluid via the Na^+-dependent system B0. Recently, a transporter designated B0AT1 corresponding to system B0 has been identified by positional cloning on the gene locus of Hartnup disease (Broer et al, 2004). The exit path for neutral amino acids to the blood stream has been proposed to be system L with a property of facilitated transporter (Fig. 4). A system L isoform LAT2 together with 4F2hc was shown to be present in the basolateral membrane of the epithelial cells of kidney and small intestine (Rossier et al, 1999). It is, however, still controversial as to whether LAT2

mediates facilitated transport or it is a purely obligatory exchanger. It is, therefore, proposed that additional system L transporters are present at the basolateral membrane with a facilitated transport mode which is more suited as the exit at the basolateral membrane (Verrey, 2003).

The absorption of cystine and basic amino acids are more complicated (Fig. 4). It has been proposed that, in the renal proximal tubules and small intestine, cystine and basic amino acids are absorbed from the luminal fluid via system $b^{0,+}$ transporter situated on the apical membrane of the epithelial cells (Chillaron et al, 1996; Palacin et al, 1998). Then, the basic amino acids pass through the basolateral membrane via system y^+L transporter into the extracellular fluid and blood stream (Chillaron et al, 1996; Palacin et al, 1998). At the apical membrane, the heterodimeric complex of $b^{0,+}$AT/BAT1 and rBAT functions as a system $b^{0,+}$ transporter. It exhibits the high affinity (100~500 μM) to cystine and basic amino acids corresponding to the renal proximal tubule transport system for these amino acids (Chairoungdua et al, 1999; Mizoguchi et al, 2001). The importance of system $b^{0,+}$ in the reabsorption of cystine and basic amino acids in kidney was verified by finding the mutations of $b^{0,+}$AT/BAT1 or rBAT in patients with cystinuria as discussed below and by generating a transgeninc knockout mouse in which $b^{0,+}$AT/BAT1 was disrupted (Palacin et al, 2001; Feliubadalo et al, 2003). Cystine is reduced to cysteine in the epithelial cells and leaves the cells via the system L transporter in the basolateral membrane. System $b^{0,+}$ is an amino acid exchanger which mediates the net influx of cystine and basic amino acids in exchange for neutral amino acids (Chillaron et al, 1996; Pfeiffer et al, 1999a; Mizoguchi et al, 2001).

The exit path for basic amino acids from the epithelial cells is the system y^+L. y^+LAT1 is present in the basolateral membrane of renal proximal tubules and small intestine with 4F2hc (Torrents et al, 1998; Pfeiffer et al, 1999b). y^+LAT1 transports both neutral and basic amino acids. The transport of basic amino acids by y^+LAT1 is Na^+-independent, whereas that of neutral amino acids, although not completely, is dependent on Na^+ (Pfeiffer et al, 1999b; Torrents et al, 1998; Kanai et al, 2000). In rat y^+LAT1 expressed in *Xenopus* oocytes, not only Na^+ but also H^+ was shown to support neutral amino acid transport (Kanai et al, 2000). Na^+ and H^+ augmented neutral amino acid transport by decreasing the apparent Km values, without affecting the Vmax values. It was proposed that a positive charge on the basic amino acid side chains or that conferred by inorganic monovalent cations such as Na^+ and H^+ is required for the substrate recognition by y^+LAT1 (Kanai et al, 2000). Therefore, the binding site of y^+LAT1 is constructed basically for the binding of positively charged basic amino acids. By the help of inorganic cations such as Na^+ and H^+, y^+LAT1 can accept neutral amino acids (Fig. 3A and B). It was also shown that Li^+ can substitute Na^+ (Kanai et al, 2000). This peculiar Na^+-dependence of y^+LAT1 is proposed to be quite beneficial for this transporter, which mediates an obligatory exchange of basic and neutral amino acids, to be an exit path for basic amino acids at basolateral membrane of epithelia (Kanai et al, 2000). It is proposed that neutral amino acids are not accepted efficiently by the intracellular substrate binding site of y^+LAT1 because of low Na^+ concentration inside the cells, so that basic amino acids are preferentially accepted to be efficiently transported out of the cells to the blood stream via the obligatory exchange. This is a mechanism through which basic amino acids are transported against electrical gradient through the plasma membrane. In fact, intracellularly loaded basic amino acids, but not neutral amino acids, efficiently move out of the cells via y^+LAT1 through the amino acid exchange, because the intracellular substrate-binding site prefers basic amino acids to neutral amino acids due to the low intracellular Na^+ concentrations (Kanai et al, 2000).

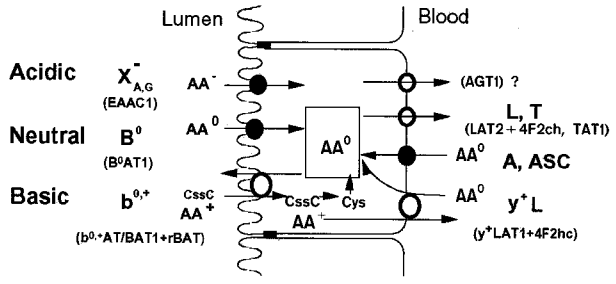

Fig. 4. Transepithelial transport of amino acids in the renal proximal tubules and small intestine. In renal proximal tubules and small intestine, transporters in the apical and basolateral membranes are in concert play critical roles in the absorption of amino acids from the luminal fluids. Systems $b^{0,+}$, L and y^+L are heterodimeric amino acid transporters composed of two subunits. Na^+-dependent transporters are shown in filled circles, whereas Na^+-independent transporters are shown in open circles in the figure. AA^-, acidic amino acids; AA^0, neutral amino acids; AA^+, basic amino acids; CssC, cystine.

Clinical and Therapeutic Implications

Cystinuria and lysinuric protein intolerance

Among the heterodimeric amino acid transporters which play critical roles in the reabsorption of amino acids from renal proximal tubules, systems $b^{0,+}$ and y^+L have been implicated in the aminoaciduria such as cystinuria and lysinuric protein intolerance (Palacin et al, 2001). The genetic defect of the apical membrane system $b^{0,+}$ transporter results in the disease called cystinuria in which the renal absorption of cystine and basic amino acids are de-

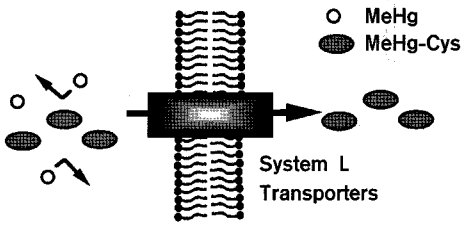

Fig. 5. Transport of methylmercury-cysteine conjugate via system L transporters. Because methylmercury-cysteine conjugate is structurally similar to neutral amino acid methionine, it can pass through the system L transporters which has high-affinity to methionine. Methylmercury itself is not transported by system L transporters.

fected. The patients with the disease suffer from recurrent renal stone formation because of low solubility of cystine in urine leading to severe renal dysfunctions (Palacin et al, 2001). Classically, cystinuria was classified into three types (I, II and III) based on the excretion of cystine and dibasic amino acids in obligate heterozygotes (Rosenberg et al, 1966). In type I cystinuria, only homozygotes are affected, while in non-type I (type II and type III) cystinuria, even heterozygotes exhibit high or moderate levels of hyperexcretion of cystine and basic amino acids into urine (Rosenberg et al, 1966). The analyses of cystinuria patients have revealed distinct cystinuria-related mutations in SLC3A1 gene encoding rBAT and *SLC7A9* gene encoding $b^{0,+}$*AT/BAT1* (Palacin et al, 2001). It was originally supposed that mutations of SLC3A1 and *SLC7A9* genes are responsible for type I and non-type I (type II and III) cystinuria, respectively. However, recent developments in the genetics and physiology of cystinuria have not supported such a traditional classification (Font et al, 2001; Dello Strologo et al, 2002; Leclerc et al, 2002). Although SLC3A1 is associated with the type I urinary phenotype, *SLC7A9* mutations were found in all three subtypes (Font et al, 2001; Leclerc et al, 2002). Therefore, a new cystinuria classification based on molecular analysis and not on urinary amino acid excretion patterns has been proposed: type A, due to two mutations of SLC3A1; type B, due to two mutations of *SLC7A9*; and type AB, with one mutation on each of the above-mentioned genes (Dello Strologo et al, 2002).

It is generally believed that apical membrane transporters constitute rate limiting steps of transepithelial transports so that the defect of basolateral membrane transporters would not result in sever symptoms. The genetic defect of basolaterally located y^+L transporter y^+LAT1 was, however, proved to be the cause of an aminoaciduria called lysinuric protein intolerance (Palacin et al, 2001). It is understandable when considering that y^+LAT1 plays an important role to transport out basic amino acids against electrical potential gradient at the basolateral membrane as discussed above. Lysinuric protein intolerance is an autosomal recessive multisystem disorder in which the patients suffer from sever symptoms including hepatosplenomegaly, osteoporosis and a life-threatening pulmonary involvement. Metabolic derangement is characterized by increased renal excretion of basic amino acids and reduced basic amino acid absorption from intestine. The involvement of y^+LAT1 in the disease confirms that the basolateral membrane transport systems are also critical in the transepithelial transport.

Amino acid transporters in malignant tumors

For continuous growth and proliferation, rapidly dividing tumor cells require more supply of sugars and amino acids. They are supported by the upregulation of transporters specialized for these nutrients (Christensen, 1990). Among the nutrient transporters, the transporters for essential amino acids are particularly important because they are indispensable for protein synthesis.

In the search for the genes upregultaed in rat hepatoma cells, Thompson and co-workers identified a tumor associated sequence designated TA1 exhibiting oncofetal pattern of expression (Faris et al, 1990; Sang et al, 1995). Although no expression was detected in normal liver, rat hepatomas expressed high levels of TA1 mRNA (Sang et al, 1995). TA1 expression was closely associated with progression in the rat hepatoma model, suggesting TA1 plays a role in the malignant phenotype. Now it has turned out that TA1 is a partial sequence of one of the 4F2 light chains LAT1 (Kanai et al, 1998). Because LAT1 is a system L amino acid transporter which transports large neutral amino acids including a lot of essential amino acids, LAT1 is proposed to be at least one of the amino acid transporters essential for tumor cell growth (Yanagida et al, 2001). Thompson and co-workers further generated antibodies against TA1 and showed that TA1 immunoreactivity was abundant in human colon cancer in vivo yet barely detected in surrounding normal colon tissues (Wolf et al, 1996), confirming the high level of expression of LAT1 protein in tumor cells. Expression of LAT1 in tumor cells was indicated in tumor masses of various tissue origins as well as various tumor cell lines (Sang et al, 1995; Wolf et al, 1996; Yanagida et al, 2001).

Beside TA1 (rat), partial or incomplete sequences of LAT1 were furthermore reported before LAT1 was identified as an amino acid transporter. E16 (human) was cloned as a sequence upregulated upon the mitogenic stimulation of lymphocytes (Gaugitsch et al, 1992). ASUR4b (*Xenopus*) was identified to be upregulated upon the stimulation of A6 epithelial cell line by aldosterone (Spindler et al, 1997). Because of this highly regulated nature as well as high level of expression in tumor cells, LAT1 is thought to be upregulated to support the high protein synthesis for cell growth and cell activation (Yanagida et al, 2001). It was reported that the monoclonal antibody against 4F2hc the partner of LAT1 suppressed the tumor cell growth, although it was not determined whether this was because of the inhibition of amino acid transport (Yagita et al, 1986). If LAT1 is an amino acid transporter essential for tumor cell growth, one can expect that the inhibition of LAT1 function would be a new rationale to anti-cancer therapy to suppress tumor growth.

Another possible application of LAT1 in cancer therapeutics is to generate LAT1-permeable anti-tumor agents to target tumor cells utilizing LAT1 upregultaed in tumor cells for efficient and selective drug delivery. It has been proposed that the phenylalanine mustard melphalan is transported by system L and accumulated in cancer cells (Cornford et al, 1992; Moscow et al, 1993; Harada et al, 2000). It was shown that melphalan competitively inhibits LAT1-mediated leucine transport and in fact transported by LAT1, although the rate of the transport is less than that for amino acid substrates (Yanagida et al, 2001; Kim et al, 2002a). It would be possible to generate LAT1- permeable anti-tumor drugs considering the broad substrate selectivity of LAT1. For cancer diagnosis LAT1-permeable would also be useful. Iodinated aromatic amino acid-related compounds such as 3-123I-iodo-[a]-methyl-L-tyrosine was developed as a functional imaging agent for neutral amino acid transport in the brain and pancreas and has been used clinically for SPECT of tumors. It was recently demonstrated that this compound is transported by LAT1 and accumulated in tumor tissues (Shikano et al, 2003a; b).

System L as a drug transporter

Because of the broad substrate selectivity, system L has

been implicated to the transport of amino acid related drugs such as L-Dopa, thyroid hormones, melphalan, gabapentin and cysteine-conjugates (Cornford et al, 1992; Mokrzan et al, 1995; Su et al, 1995; Gomes & Soares-da-Silva, 1999; Ritchie & Taylor, 2001). In fact, L-Dopa was shown to be transported by cloned rat LAT1 in *Xenopus* oocyte expression system (Yanagida et al, 2001; Uchino et al, 2002). For thyroid hormones, it was shown that ASUR4 (*Xenopus* LAT1) and LAT1 intrinsic to T24 human bladder carcinoma cells transported triiodothyronine and thyroxine, although the rate of the transport was less than that of amino acid substrates (Ritchie & Taylor, 2001). In addition, melphalan was shown to inhibit LAT1-mediated leucine transport and in fact transported by LAT1 although with low velocity (Yanagida et al, 2001; Kim et al, 2002a). Prevailing evidences, thus, favor the proposed roles of LAT1 as drug transporters.

An important contribution of system L in the pharmacokinetics of amino acid-related drugs is that it mediates the permeation of the drugs through the blood-tissue barriers. Recently it was shown that LAT1 together with 4F2hc is present in the brain capillary endothelial cells the major component of the blood-brian barrier (Duelli et al, 2000; Kageyama et al, 2000; Matsuo et al, 2000). In cultured brain capillary endothelial cells which expressed LAT1 at high level, it was demonstrated that L-Dopa is transported at the similar kinetics to that of LAT1, suggesting LAT1 mediates the transport of L-Dopa in these model systems (Kageyama et al, 2000; Kido et al, 2001). In placenta, LAT1 is present in the syncytio-trophoblasts of placenta, the major diffusion barrier of the placental barrier, so that LAT1 is proposed to be a placenetal barrier transporter (Ritchie & Taylor, 2001).

Toxicological Implications of System L Transporters

Because of the broad selective nature, system L transporters function as a path for the membrane permeation of drugs and toxic compounds occurring in the environment with amino acid-related structures. Methylmercury is widely known for its potent neurotoxicity and is the causal substance of Minamata disease. Because methylmercury easily forms conjugates with thiol compounds in vivo, the distribution of methylmercury in the body is closely related to the transport of the thiol compounds (Ballatori & Clarkson, 1982; Aschner, 1989). It was shown that methylmercury-cysteine conjugate is transported by system L transporters (Simmons-Willis et al, 2002)(Fig. 5).

Beside methylmercury-cysteine conjugate, amino acid-related neurotoxins such as *β-N*-methylamino-L-alanine (Weiss & Choi, 1988; Smith et al, 1992b), S-(1,2-dichlorovinyl)-L-cysteine (Patel et al, 1993) and 3-hydroxykynurenine (Okuda et al, 1998) are proposed to pass through system L transporters to exert their toxicity. Because the presence of such transporters is crucial for the manifestation of the organ toxicity, the inhibition of the transporters would be expected to be beneficial to prevent the disorders caused by the transporter-mediated toxicity.

Oxidative stress and xCT

It has been proposed that the transport of cystine through the plasma membrane is crucial to maintain intracellular glutathione levels (Christensen, 1990). Glutathione is a tripeptide radical scavenger synthesized intracellularly from glutamate, cysteine and glycine. Because cysteine is easily oxidized to form cystine in the extracellular environment, cystine transport mechanisms are essential to provide cells with cysteine for glutathione synthesis (Christensen, 1990). The amino acid transport system x^-C has been proposed to be responsible for the cystine transport through the plasma membrane. System x-C mediates an amino acid exchange and prefers cystine and glutamate as its substrates (Bannai & Kitamura, 1980; Christensen, 1990).

The expression of the cloned system x^-C transporter xCT was shown to be inducible in mouse macrophages, human fibroblasts, ARPE-19 human retinal pigment epithelial cells and U87 human glioma cells by oxidative stress or by the exposure to lipopolysaccharide (Sato et al, 1999; 2000; Bridges et al, 2001; Kim et al, 2001). The expression of xCT is, thus, regulated to maintain intracellular glutathione levels and to protect cells against oxidative stress.

In the central nervous system, however, the over-function of system x-C would be damaging neurons by increasing extracellular glutamate concentration because this exchanger releases glutamate through the cystine/glutamate exchange mechanism (Piani & Fontana, 1994). Therefore, the system x-C activity in glial cells needs to be regulated to meet just the requirement. It was recently shown that the increase in the xCT mRNA level by oxidative stress is transient in U87 glioma cells so that the over-production of xCT proteins would be prevented (Kim et al, 2001). It has been proposed that astrocytes and microglia are activated after brain ischemia to upregulate system x-C expression in these cells, which may exacerbate brain damage after ischemia by increasing the regional extracellular glutamate level (Piani & Fontana, 1994). Therefore, the suppression of x-C transporter might be effective to minimize the infarct area after brain ischemia.

CONCLUSION

The heterodimeric amino acid transporters are composed of 12-membrane-spanning light chains and single-membrane spanning heavy chain which are connected with each other via a disulfide bind. Among 9 light chains identified, six light chains are associated with a heavy chain subunit 4F2hc and one is associated with the other heavy chain subunit rBAT, whereas two of them are associated with unknown heavy chains. The members of heterodimeric amino acid transporter family exhibit diverse substrate selectivity and are expressed in variety of tissues. They play variety of physiological roles including epithelial transport of amino acids as well as a role in general cellular nutrition. The roles of the particular members in tumor cell growth, oxidative stress and transport-mediated toxicity provide us with an opportunity to investigate them as possible new therapeutic targets.

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