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Expert Review

Molecular Insights into the Structure–Function Relationship of Organic Anion Transporters OATs

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Abstract. The organic anion transporter (OAT) family encoded by SLC22A mediates the absorption, distribution, and excretion of a diverse array of environmental toxins, and clinically important drugs, including anti-HIV therapeutics, anti-tumor drugs, antibiotics, anti-hypertensives, and anti-inflammatories, and therefore is critical for the survival of mammalian species. Several OATs have been identified: OAT1 (SLC22A6), OAT2 (SLC22A7), OAT3 (SLC22A8), OAT4 (SLC22A11), OAT5 (SLC22A19) OAT6 (SLC22A20) and URAT1 (SLC22A12). The expressions of these OATs have been detected in key organs such as kidney, liver, brain and placenta. OAT dysfunction in these organs may contribute to the renal, hepatic, neurological and fetal toxicity and diseases. In this review, we summarize, according to the work done by our laboratory as well as by others, the most updated molecular studies on these OAT members, especially on the aspect of their structure–function relationships. The functional roles of *N*-glycosylation, transmembrane domains and individual amino acids, cell surface assembly, as well as associating proteins will be discussed. In addition, we will show the recent analyses of coding region polymorphisms of OATs, which give us information on the genetic variants of OATs and their potential effects on OAT functions.

KEY WORDS: drug transporter; structure–function relationship.

GENERAL FEATURES OF OATS

Organic anion transporters (OAT) play essential roles in the body disposition of clinically important anionic drugs including anti-HIV therapeutics, anti-tumor drugs, antibiotics, anti-hypertensives, and anti-inflammatories (1–7). Several OATs have been identified by different laboratories and have been shown to have distinct tissue and cellular localization (Table I). A number of detailed reviews have addressed the substrate specificity of these OATs and their pharmacokinetics (4, 5, 7–10). OAT1, originally cloned as NKT (11–14), and OAT3 are predominantly expressed in the kidney and the brain (11–25). In the kidney, these transporters utilize a tertiary transport mechanism to move organic anions across the basolateral membrane into the proximal tubule cells for subsequent exit/elimination across the apical membrane into the urine (12,14,20,26). Through this tertiary transport mechanism, Na^+K^+-ATPase maintains an inwardly directed (blood-to-cell) Na^+ gradient. The Na^+ gradient then drives a sodium dicarboxylate cotransporter, sustaining an outwardly directed dicarboxylate gradient that is utilized by a dicarboxylate/organic anion exchanger to move the organic anion substrate into the cell. This cascade of events indirectly links organic anion transport to metabolic energy and the Na^+ gradient, allowing the entry of a negatively charged substrate against both its chemical concentration gradient and the electrical potential of the cell. OAT2 is expressed in the liver and in the kidney (18,27–30). The transport studies performed in *Xenopus* oocytes indicated that human OAT2 (hOAT2) is a sodium-independent multi-specific organic anion/dimethyldicarboxylate exchanger (31). OAT4 is present mainly in the placenta and the kidney (32). In the kidney, OAT4 functions as an organic anion/dicarboxylate exchanger at the apical membrane of the proximal tubule and is responsible for the reabsorption of organic anions driven by an outwardly directed dicarboxylate gradient (33). OAT5 is predominantly expressed in the apical membrane of kidney proximal tubule (34,35). Study carried out in *Xenopus* oocyte showed that murine OAT5 (mOAT5)-mediated transport was neither cis-inhibited nor trans-stimulated by dicarboxylates, which suggested that mOAT5 is unlikely an organic anion/dicarboxylate exchanger (36); while studies with rat OAT5 (rOAT5) in *Xenopus* oocytes and S2 cells (derived from the S2 segment of the proximal tubular of transgenic mice harboring temperature-sensitive SV40 large T-antigen gene) showed that rOAT5-mediated transport was both cis-inhibited and trans-stimulated by dicarboxylates, indicating that rOAT5 is an organic anion/dicarboxylates exchanger (34). OAT6 is expressed predominantly in mouse olfactory mucosa and weakly in mouse testis (37). OAT6 is the only member of OATs, which has no expression in

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Table I. Tissue and Cellular Distribution of Human OATs

OAT Isoforms*	Accession Numbers	Tissue Localization	Cellular Localization	References
hOAT1	AB009697/AF057039/AF104038/AF097490 AF124373	Kidney	BM	(16,17,20–22,25,81)
		Skeletal muscle	ND	
		Brain	ND	
		Placenta	ND	
hOAT2	AF097518/AF210455/AY050498	Kidney	BM	(27,82)
		Liver	SM	
hOAT3	AF097491	Kidney	BM	(15,21,22,25,83)
		Brain	LM	
		Skeletal muscle	ND	
		Adrenal tissue	ND	
hOAT4	AB026116	Placenta	BM	(32,83,84)
		Kidney	AM	
		Adrenal tissue	ND	
hURAT1	AB071863	Kidney	AM	(39)

BM Basolateral membrane; *SM* sinusoidal membrane of hepatocytes; *LM* luminal membrane of choroid plexus; *AM* apical membrane; *ND* not determined
*Only human OAT isoforms are listed here

kidney. It was reported that murine OAT6 also acts as an organic anion/dicarbosylate exchanger when expressed in *Xenopus* oocyte and Chinese hamster ovary (CHO) cells (38). URAT1 is expressed on the apical membrane of kidney proximal tubular cells, functioning as an urate/organic anions exchanger (39).

OATs are subjected to acute regulation through multiple signaling pathways. Studies from our laboratory showed that OAT activity could be inhibited by phosphorylation at serine residue by an unknown protein kinase (40). OAT activity can also be inhibited by activation of protein kinase C (PKC) (20,40–44). However, PKC-induced down regulation of OAT1 activity is not due to the phosphorylation at the transporter protein but rather due to the carrier retrieval from the cell membrane into the intracellular compartment (40,43). In addition to the regulation by phosphorylation and PKC, OAT activity can also be regulated by epidermal growth factor (EGF) through MAPK pathway (41,45–47).

STRUCTURE–FUNCTION RELATIONSHIP OF OATS

OAT family and another closely related organic cation transporter (OCT) family belong to a large group of related proteins, the major facilitator superfamily (MFS). The members of MFS share common structural features, including 12 putative transmembrane-spanning domains and intracellular carboxyl and amino termini. Recent elucidation of high-resolution crystal structures of two other MFS members, LacY (48) and GlpT (49), suggests that all MFS members may share a common fold. Based on such assumption, three-dimensional structural models of rat OCT1 (50) and rabbit OCT2 (51) have been developed using the structural template of LacY and GlpT. In such a model, transmembrane-spanning domains 1, 2, 4, 5, 7, 8, 10, and 11 form a large hydrophilic cleft for substrate binding. This structural model provide useful tool in predicting substrate-binding sites of OATs. However, since the structural model mainly focused on the organization and alignment of residues within the 12 transmembrane-spanning domains, it offered no insight into the structure–function relationships of loops that connect transmembrane domains as well as the amino- and the carboxyl terminus of these transporters. Recent studies on the first extracellular loop of OATs, which bear mutiple *N*-glycosylation sites, indicated that this loop is as important as transmembrane domains in the function of OATs.

N-Glycosylation of OATs

Glycosylation has been demonstrated to play critical roles in regulation of intracellular targeting, protein folding, modulation of biological activity, maintenance of protein stability (resistance to proteolysis), and providing recognition structures for interaction with diverse external ligands. The processing of N-linked glycosylation of proteins occurs in several steps. First, a dolichol pyrophosphate precursor (Glc3Man9GlcNAc2) is added to Asn side chain of Asn-*X*-Ser/Thr consensus sequence for N-linked oligosaccharides in a nascent polypeptide in the endoplasmic reticulum (acquisition of N-linked oligosaccharides). Processing then begins by the removal of the three terminal glucose residues and at least one mannose residue in the endoplasmic reticulum. The partially processed polypeptide is then transported to Golgi apparatus, where mannose residues are further trimmed, and *N*-acetylglucosamine, galactose, and sialic acid residues are sequentially added. The newly synthesized glycoproteins then exit the Golgi and are transported to their final destination. Therefore, N-linked glycosylation consists of two major events: the acquisition/addition of oligosaccharides and the processing/modification of the oligosaccharides. One common feature for all members of the OAT family is the presence of multiple potential sites for N-linked glycosylation within the extracellular loop between transmembrane domains 1 and 2. Biochemical studies on hOAT1 (52) and hOAT4 (53) in cultured cells revealed that elimination of all the glycosylation sites in hOAT1 and hOAT4 either by mutation or tunicamycin treatment caused retention of the transporter in an intracellular compartment, thus preventing transport of its substrate. With the use of mutant Chinese

hamster ovary (CHO)-Lec cells lacking various enzymes required for glycosylation processing, it was shown that processing of glycosylation from a mannose-rich type to a complex type is associated with an increased affinity of OAT4 for its substrate. Glycosylation may be altered depending on the cell type and physiological states and therefore could serve as a means to specifically regulate OAT function *in vivo*.

Cell Surface Assembly of OAT1

Abundant evidence exists to suggest that many transporters function as oligomers (54–56). Therefore, it is legitimate to ask whether OATs might form oligomeric complexes. Such information is essential for understanding the regulation of these transporters. Recently, we investigated the quaternary structure of hOAT1 using combined approaches of chemical cross-linking, gel filtration chromatography, co-immunoprecipitation, cell surface biotinylation, and metabolic labeling (57). We demonstrated that hOAT1 exists in the plasma membrane of kidney LLC-PK1 cells as a homooligomer, possibly trimer.

Oligomerization plays a critical role in various aspects of transporter function. Each subunit in the oligomer may form a pore itself and allows the translocation of organic anions, a mechanism mimicking water channel CHIP28 (58). On the other hand, several subunits in the oligomer may be required to form a single pore, as in K+ channels (59). Based on recent structural model of OCT1 (50), which showed that substrate binding pocket consists of several transmembrane domains within the same subunit, oligomerization of OAT1 may not be essential for the formation of the translocation pathway for its substrates.

In addition to the functional role mentioned above, oligomerization is also believed to play a role in membrane trafficking and stability of the transporters. After synthesis in the endoplasmic reticulum (ER), proteins undergo a strict process of quality control. Newly synthesized transporters may contain retention signal and are thereby retained in the ER. Oligomerization may shield/hide such retention signal and therefore is essential for the egress of the transporters from ER for subsequent targeting to the plasma membrane (60). For example, mutations of gamma-aminobutyric acid transporter GAT-1 that failed to form oligomers resulted in retention in the ER (61). We are currently investigating the functional consequence of hOAT1 oligomerization.

The molecular determinants for OAT oligomerization are currently unknown. OATs contain, in their sequence, multiple structural motifs, which have been indicated to play critical roles in oligomerization of other transporters. One of such structural motifs is coiled-coil motif (62,63). The characteristic feature of coiled-coil motif is a heptad repeat pattern of primarily apolar residues (very often leucine residue). Another structural motif for oligomerization is GxxxG (glycine residue can also be replaced by a similar small residues: alanine, serine, and cysteine) (64,65). Whether any of these motifs or any nonclassical motifs dictate OAT oligomerization need to be examined.

It is important to emphasize that our study was carried out in kidney LLCPK1 cells. The cell culture-based system is an essential model system for the basic characterization of cloned transporters in isolation and may reflect the *in vivo* situation where OAT exists alone, such as OAT2 in the liver and OAT4 in the placenta. However, such a system might not completely reflect the native membrane environment of the tissue of interest in terms of (1) how highly the individual transport protein is expressed, and (2) any as yet unknown cell-type specific protein–protein interactions, especially when several OATs co-exist at the same membrane of the same cell type, such as OAT1 and OAT3 in the kidney and brain. Under such conditions, whether different OAT isoforms associate with one another to form heterooligomers is an important question we are trying to address.

Functionally Critical Amino Acid Residues

Since the initial cloning of OATs, amino acids critical for OAT function has been identified through mutagenesis studies (52,53,85–91). Many of these amino acids are conserved among all of the OATs and reside within transmembrane domains. Recently developed structural models for OCT1 (50) and OCT2 (51) showed that the binding pocket for organic cation is formed by the relative positions of transmembrane domains 1, 2, 4, 5, 7, 8, 10, and 11. Since OCTs and OATs belong to major facilitator superfamily, which may share common structural fold, by analogy, selected amino acids found in the corresponding helices of OATs may be involved in binding organic anion. In rOAT3 and fOAT1 for example, conserved aromatic residues (in transmembrane domains 7 and 8) and conserved basic residues (in transmembrane domains 1, 8, and 11) are required for transport activity as indicated in functional studies in *Xenopus* oocytes (87,89). These basic residues in OATs are localized in the positions corresponding to the acidic residues in OCTs, indicating the role of these basic residues in the charge specificity of OATs. However, not all the critical residues identified within these transmembrane domains are involved in substrate-binding. Leu-30 in transmembrane domain 1 of hOAT1 (85), Gly-241 and Gly-400 in transmembrane domains 5 and 8 of hOAT4 (90) were shown in transfected cells to participate in the targeting of the transporters to the plasma membrane. When interpreting the results from mutagenesis studies, it is important to consider that (1) point mutations could change directly or indirectly the binding affinity of the transporter for its substrate, which means that not all mutations that change binding affinity represent amino acids located within substrate-binding sites. Amino acids located outside the substrate-binding sites may affect substrate binding by transducing conformational changes over a long distance, (2) certain mutations may change the affinity of the inhibitors whereas other mutations may change the transport, and (3), given that more than one substrate-binding site was proposed in the structural model of OCT (50), mutations may change the binding affinity for one substrate without affecting the affinity for the other.

Interaction of OATs with Other Proteins

The key organs for drug disposition, such as kidney, brain, intestine, liver and placenta, are made of polarized epithelial cells. The capacity of any polarized epithelial cell

type to mediate a specific transport process is dependent on its capacity to deliver the appropriate transport proteins to its apical and basolateral surfaces. The same transport proteins may be called upon to serve as apical or basolateral proteins in order to fulfill the transport missions assigned to each of the many different epithelial cell types. The actual destination to which a given transporter is directed is chosen by the epithelial cell and is determined through protein–protein interactions between the transporter and the components of the sorting machinery that the epithelial cell expresses. In this manner, each type of transporting epithelial cell can differentially distribute transport proteins so as to achieve the localizations required by its particular physiologic role. Abnormal membrane sorting and trafficking of the transporters is the key cause for many clinical syndromes (66–68). After the sorting machinery delivers the transporter to a specific cell surface, maintenance of the correct localization at that surface requires interactions of the transporters with structural proteins located at or near the plasma membrane. It has been shown that the protein–protein interactions that orchestrate the polarized distributions of transport proteins may also regulate their functions. Certain transport proteins are not constitutive components of a particular cell surface domain. Instead, these proteins commute/recycle between cell surface and an intracellular storage compartment. In response to changes in intracellular second messenger concentrations, transporters are either inserted into or retrieved from the cell surface. By manipulating the surface populations of selected transport proteins, epithelial cells can precisely modulate their physiologic properties. Therefore, during their journey from endoplasmic reticulum, through secretary pathway, to cell surface, transporters interact with various accessory proteins. It is these interactions that determine their localization on the specific cell surface domain, their stability at the specific cell surface and their shuttling between the specific cell surface and the intracellular compartments when responding to stimuli.

OATs have been shown to be associated with other proteins to fulfill their function. PDZ proteins, for example, are one of the most common interacting partner with transporters. PDZ proteins contain PDZ domains ranging from 80 to 90 amino acids in length and bind typically to proteins containing PDZ consensus binding sites, the tripeptide motif (S/T)*Xö* (*X* = any amino acid and *ö* = a hydrophobic residue) at their C termini (69). These multidomain molecules not only target and provide scaffolds for protein– protein interactions but also modulate the function of receptors and ion channels, by which they associate (70,71). The disruption of the association between PDZ proteins and their targets contributes to the pathogenesis of a number of human diseases, most probably because of the failure of PDZ proteins to appropriately target and modulate the actions of associated proteins (72,73).

OAT4 and URAT1 were found to have PDZ consensus binding sites (74,75). Through a yeast two-hybrid screening, in vitro binding assay, coimmunoprecipitation and surface plasmon resonance analysis, it was revealed that the wild-type URAT1, but not its mutant lacking the PDZ consensus binding site, directly interacts with PDZ protein PDZK1 (74). The association of URAT1 with PDZK1 enhanced urate transport activity in HEK293 cells stably expressing URAT1 and transfected with PDZK1, and the deletion of the URAT1 C-terminal PDZ consensus binding site abolished this effect. The augmentation of the transport activity was accompanied by a significant increase in the maximum transport velocity Vmax of urate transport and was associated with the increased surface expression level of URAT1 protein. By similar analyses as that used in studying in the interaction of URAT1 and PDZK1, it was shown that OAT4 wild-type but not a mutant lacking the PDZ consensus binding site interacted directly with PDZ proteins PDZK1 and NHERF1 (75). OAT4, PDZK1, and NHERF1 proteins were co-localized at the apical membrane of renal proximal tubules. The association with PDZK1 or NHERF1 enhanced OAT4-mediated transport activity in HEK293 cells stably expressing OAT4 and transfected with PDZK1 or NHERF1, and the deletion of the OAT4 C-terminal PDZ consensus binding site abolished this effect. The augmentation of the transport activity was accompanied by an increase in maximum transport velocity Vmax of estrone sulfate transport, and was associated with the increased surface expression level of OAT4 protein.

OAT activity can also be modulated through interacting with another class of proteins: caveolins. Caveolins are major structural component of caveolae, the small flask-shaped and detergent insoluble structures in the plasma membrane. Various signaling molecules are found within caveolae and their functional interaction with the caveolin play important roles in transmembrane signaling (76).

The interaction of caveolins with OAT1 and OAT3 have been demonstrated (77,78). By Western blot analysis using the isolated caveolae-enriched membrane fractions or the immunoprecipitates by respective antibodies from the rat kidney, it was shown that rOAT1 and caveolin-2 co-localized in the same fractions and they formed complexes with each other (77). Similarly, rOAT3 and caveolin-1 co-localized in the same fractions and they formed complexes with each other (78). These results were confirmed by performing confocal microscopy with immunocytochemistry using the primary cultured renal proximal tubular cells. When the synthesized cRNA of rOAT1 along with the antisense oligodeoxynucleotides of *Xenopus* caveolin-2 were co-injected into *Xenopus* oocytes, the *p*-aminohippurate and methotrexate uptake was slightly, but significantly decreased (77). The similar results were observed when synthesized cRNA of rOAT3 along with the antisense oligodeoxynucleotide of *Xenopus* caveolin-1 were co-injected into *Xenopus* oocytes, the estrone sulfate uptake was significantly decreased (78). Therefore, the function of these transporters can be modulated by caveolins.

Genetic Polymorphisms of OATs

Characterization of genetic variation in transporter genes is an important step towards understanding the individual variation in drug response. Several groups have put efforts on characterizing OATs coding region polymorphisms and also explored the relationships between these OAT genetic variants and transporter functions. Table II listed those nonsynonymous variants of OATs, the function of which are affected by such mutation.

Table II. Nonsynonymous Coding Region Polymorphisms of Human OAT1–4

OAT Isoforms	Coding Region Variants	Functional Analysis	Kinetical Data and Protein Cells Surface Expression Analysis	Reference
hOAT1	R50H	Cidofovir ↑ Adefovir ↑ Tenofovir ↑ PAH —	Decreased K_m for cidofovir, adefovir and tenofovir, unchanged K_m for PAH	(92)
	K525I	Cidofovir — Adefovir — Tenofovir — PAH —	Unchanged K_m for cidofovir, adefovir, tenofovir and PAH	(92)
	L7P	N/A		(93)
hOAT2	T110I	N/A		(93)
	V192I	N/A		(93)
	G507D	N/A		(93)
hOAT3	F129L	ES, CIM —		(94)
	R149S	ES, CIM no uptake		(94)
	Q239Stop	ES, CIM no uptake		(94)
	I260R	ES, CIM no uptake		(94)
	R277W	ES ↓ , CIM ↓ (not significant)		(94)
	V281A	ES, CIM —		(94)
	I305F	ES ↓, CIM ↑ (slightly)	Increased K_m for ES (not significant) Decreased K_m for CIM	(94)
	A310V	ES, CIM —		(94)
	A399S	ES, CIM —		(94)
	V448I	ES, CIM —	Decreased K_m for CIM, Decreased V_{max} for CIM	(94)
	A389V	CL_r and CL_{sec} of pravastatin —		(95)
	I175V	N/A		(93)
hOAT4	V13M	N/A		(93)
	R48Y	N/A		(93)
	T62R	N/A		(93)
	V155M	N/A		(93)
	A244V	N/A		(93)
	E278K	N/A		(93)
	V399M	N/A		(93)
	T392I	N/A		(93)
hURAT1	W258Stop	No urate uptake	Impaired targeting to cell membrane	(39, 96–98)
	T217M	No urate uptake	Cell membrane expression not affected	(39, 98)
	E298D	No urate uptake	Cell membrane expression not affected	(39, 98)
	Q297Stop	N/A		(97)
	R90H	No urate uptake	Cell membrane expression not affected	(96, 97)
	V138M	No urate uptake	Cell membrane expression not affected	(97)
	G164S	Reduced urate uptake	Cell membrane expression not affected	(97)
	Q382L	Reduced urate uptake	Cell membrane expression not affected	(97)
	M430T	Reduced urate uptake	Impaired targeting to cell membrane	(97)
	Frame shift (IVS2+ 1G→A)	No urate uptake	Impaired targeting to cell membrane	(97)
	R477H	N/A		(96)

PAHpara-aminohippurate; *ES* estrone sulfate; *CIM* cimetidine; *N/A* data not available; CL_r Renal clearence; CL_{sec} clearence by renal secretion
↑ Increase; — no change; ↓ decrease

Knockout Mice Models of OATs

The structure–function studies in cultured cells proves to be a useful tool to thoroughly characterize each transporter in isolation. However, in a native environment such as kidney and brain, co-existing transporters within and outside the OAT family may share the same substrates as that of a particular OAT, this makes the dissection of the contribution of each transporter to overall transport capacity difficult. Such difficulty can be circumvented through generating knockout mouse model for specific transporter. The knockout mouse models of OAT1 (79) and OAT3 (80) have been recently reported. Although both knockout mice showed no morphological abnormalities, profound loss of organic anion transport was noticed in both models. In OAT1 knockout mice, substantial loss of *para*-aminuhippurate (PAH) transport in isolated renal slices and in urinary secretion was observed, whereas in OAT3 knockout mice, significant loss in transport of taurocholate, estrone sulfate, and PAH was identified in isolated kidney slices, and significant loss in transport of fluorescein was identified in intact brain choroid plexus. These studies provided experimental evidence for the crucial involvement of OAT1 and OAT3 in body disposition of these organic anions.

CONCLUSION REMARKS

The importance of OATs in drug disposition drew many attentions from researchers and made it a hot topic in the world. In this review, we presented many studies on the structure–function relationships of OATs, which led us to a better understanding of OATs. These information are also valuable data in helping us to solve the protein structure of OATs, the substrate features of OATs and finding more ways to regulate OATs in physiological conditions in order to get better clinical effects. Although there were only few data about the newly cloned OAT5 and OAT6, the discovery of new OAT members presents us a more expanded picture of OAT family and adds more targets for our clinical drug development. With the help of the data on OAT coding region polymorphisms, it is promising to establish personalized therapies for OAT deficient people in the future. But the wide substrate spectra and complicated regulation mechanisms of OATs still leave us a lot of problems unelucidated. More work need to be done in identifying the *N*-glycosylation status of OATs *in vivo*, exploring the roles of non-transmembrane regions of OATs such as the loops that connect transmembrane domains and the amino- and carboxyl termini, learning about the protein assembly statuses of other OATs and also studying the functional effects of more coding region polymorphisms of OATs. The answers to these questions will lead us to a new stage of understanding OATs and further applying these information into our clinical drug designs and evaluations.

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