Perspectives in Pharmacology

Structures and Models of Transporter Proteins

Svein G. Dahl, Ingebrigt Sylte, and Aina Westrheim Ravna
Department of Pharmacology, Institute of Medical Biology, University of Tromsø, Tromsø, Norway
Received December 22, 2003; accepted February 23, 2004

ABSTRACT
Transporter proteins in biological membranes may be divided into channels and carriers. Channels function as selective pores that open in response to a chemical or electrophysiological stimulus, allowing movement of a solute down an electrochemical gradient. Active carrier proteins use an energy producing process to translocate a substrate against a concentration gradient. Secondary active transporters use the movement of a solute down a concentration gradient to drive the translocation of another substrate across a membrane. ATP-binding cassette (ABC) transporters couple hydrolysis of adenosine triphosphate (ATP) to the translocation of various substrates across cell membranes. High-resolution three-dimensional structures have now been reported from X-ray crystallographic studies of six different transporters, including two ATP-binding cassette (ABC) transporters. These structures have explained the results from many previous biochemical and biological studies and shed new light on their functional mechanisms. All these transporters have α-helical structures of the membrane-spanning domains, as suggested from many previous studies, and some of the helices have irregular shapes with kinks and bends. Together these crystal structures demonstrate the large flexibility of transporter proteins and that substantial movements take place during the substrate translocation process, which to a certain extent may distinguish active carriers from channel proteins. These structures and other low-resolution structures of membrane proteins have served as a basis for construction of three-dimensional protein models that have provided insight into functional mechanisms and molecular structures and enabled formulation of new hypotheses regarding transporter structure and function, which may be experimentally validated.

Twelve years ago a review article described the growth of information on transporters as a “transporter explosion” (Uhl and Hartig, 1992). Since then, genome sequencing has made information on transporters expand in magnitude at an even more rapid rate, and the amount of information on their structures and functional mechanisms seems to have grown exponentially. A transporter protein classification system (TC) proposed in 1999 was based on phylogenetic analysis and functional properties of about 250 families of sequence-related transporters identified in bacteria, archaea, and eukaryotes (Saier, 1999). Three years later, nearly 400 families were included in the TC classification system, which then had been adopted by the International Union of Biochemistry and Molecular Biology (Busch and Saier, 2002).

Phylogenetic analysis of integral membrane transport protein sequences has shown that families of transport proteins of similar topology have evolved independently of each other, at different times in evolutionary history, using different routes (Saier, 1999, 2000; Busch and Saier, 2002). Now that the detailed three-dimensional molecular structures of some transporter proteins have been reported, the possible similarity in protein-folding patterns between these and other transporters is of crucial importance. Similar folding patterns between any protein and one for which the crystal structure is known would enable construction of a fairly

ABBREVIATIONS. TC, transporter protein classification system; MFS, major facilitator superfamily; 5-HTT, serotonin transporter; ABC, ATP-binding cassette; AcrA, membrane fusion protein; AcrB, resistance-nodulation-cell division transporter; DAT, dopamine transporter; ECM, electron cryo microscopy; EmrE, bacterial multidrug transporter; GlcV, ATP-binding cassette-ATPase subunit of glucose transporter; GlpT, glycerol-3-phosphate transporter; LacY, lactose permease; MDR, multidrug resistance; MsbA, lipid flipase; NBD, nucleotide-binding domain; NET, norepinephrine transporter; NhaA, Na+/H+ antipporter; OxlT, oxalate transporter; P-site, auto-phosphorylation site; P-gp, P-glycoprotein; RND, resistance-nodulation-cell division; SERCA1a, skeletal muscle sarcoplasmic reticulum calcium ATPase; TMH, transmembrane α-helix; ToIC, outer membrane channel of multidrug exporter system.
accurate three-dimensional protein model of the unknown structure using the related crystal structure as a template and modern computational techniques.

However, although atomic resolution crystal structures of soluble proteins have been reported in an amazingly increasing number, such progress has not been made in terms of transporters and other membrane proteins, which have proven extremely difficult to crystallize for two main reasons. One is related to the amphipathic nature of their surface, with a hydrophobic area in contact with membrane phospholipids and polar surface areas in contact with the aqueous phases on both sides of the membrane (Ostermeier and Michel, 1997). Second, the majority of medically important membrane proteins are present in tissues at very low concentrations, making overexpression a prerequisite for structural studies, and this often has proven to be very difficult. In spite of recent advances in overexpression technology, bacterial membrane proteins still are the most amenable for structure determination, simply because they often can be easily expressed in large quantities (Tate, 2001). As shown in Table 1, five of the six currently reported high-resolution transporter structures are from *Escherichia coli.*

Their inherent conformational flexibility may also contribute to the difficulties in obtaining stable crystals of transport proteins. Using inhibitors to rigidify their structure has therefore been proposed as a possible strategy for crystallization of flexible receptors and transporters (Tate, 2001). Lactose permease was only crystallized in a mutant form (Abramson et al., 2003), which shows greater thermal stability that the wild type and markedly reduced ligand-induced conformational changes (Smirnova and Kaback, 2003).

The experimental obstacles involved in crystallization and structure determination of membrane proteins are illustrated by the fact that before determining the crystal structure of a lipid flippase (MsbA) from *E. coli,* the group cloned, overexpressed, and purified more than 20 different bacterial ABC transporters, and tested 96,000 different crystallization conditions for several multidrug resistance (MDR)-ABC transporters using about 20 different detergents (Chang and Roth, 2001).

## Classification of Transport Proteins

In the TC classification system, transporters are divided into two main classes: channels and carriers (Saier, 2000; Busch and Saier, 2002), as indicated in Fig. 1. Channel proteins function as selective pores that open in response to a chemical or electrophysiological stimulus, allowing movement of a solute down an electrochemical gradient. Active carrier proteins translocate substrates across a biological membrane against a concentration gradient. To do this, the transporter must couple the carrier process to another energy-producing process and also be able to prevent the two processes from occurring uncoupled in their energetically favorable directions.

Secondary active transporters use the movement of a solute down a concentration gradient to drive the translocation of an other substrate across a membrane against a concentration gradient, by symport or antiport mechanisms. Symporters (cotransporters) catalyze the transport of two or more molecular species in the same direction, and antiporters (exchange transporters) catalyze the exchange of one or more

### Table 1

<table>
<thead>
<tr>
<th>Protein Organism</th>
<th>Other Domains</th>
<th>Structure Determination</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NhaA</strong></td>
<td>E. coli</td>
<td>1 12 12 ECM</td>
<td>Williams, 2000</td>
</tr>
<tr>
<td><strong>O. formigenes</strong></td>
<td>E. coli</td>
<td>1 12 12 ECM</td>
<td>Hirai et al., 2002</td>
</tr>
<tr>
<td><strong>EmrE</strong></td>
<td>E. coli</td>
<td>2 4 8 ECM</td>
<td>Ubarretxena-Belandia et al., 2003</td>
</tr>
<tr>
<td><strong>MDR1 P-gp</strong></td>
<td>Chinese hamster ovary cells</td>
<td>2 ECM</td>
<td>Rosenberg et al., 2001</td>
</tr>
<tr>
<td><strong>MDR3 P-gp</strong></td>
<td>Mouse MDR1 P-gp expressed in Pichia pastoris</td>
<td>2 ECM</td>
<td>Lee et al., 2002</td>
</tr>
<tr>
<td><strong>Multidrug efflux transporter</strong></td>
<td>S. cerevisiae</td>
<td>2 6 12 X-ray</td>
<td>Abramson et al., 2003</td>
</tr>
<tr>
<td><strong>GlpT</strong></td>
<td>E. coli</td>
<td>2 6 12 X-ray</td>
<td>Huang et al., 2003</td>
</tr>
<tr>
<td><strong>AcrA, 3 TolC</strong></td>
<td>S. cerevisiae</td>
<td>3 12 36 3</td>
<td>Murakami et al., 2002</td>
</tr>
<tr>
<td><strong>BtuCD</strong></td>
<td><em>E. coli</em></td>
<td>2 6 12 X-ray</td>
<td>Ferreira-Pereira et al., 2003</td>
</tr>
<tr>
<td><strong>BtuC</strong></td>
<td>E. coli</td>
<td>2 6 12 X-ray</td>
<td>Chami et al., 2002</td>
</tr>
<tr>
<td><strong>LacY</strong></td>
<td>E. coli</td>
<td>2 6 12 X-ray</td>
<td>Abramson et al., 2003</td>
</tr>
<tr>
<td><strong>GlpT</strong></td>
<td>E. coli</td>
<td>2 6 12 X-ray</td>
<td>Huang et al., 2003</td>
</tr>
<tr>
<td><strong>AcrB</strong></td>
<td>E. coli</td>
<td>2 6 12 X-ray</td>
<td>Chami et al., 2002</td>
</tr>
<tr>
<td><strong>SERCA1a</strong></td>
<td>Skeletal muscle sarcoplasmic reticulum</td>
<td>1 10 10 1 NBD, 1 P-site X-ray</td>
<td>Toyoshima and Nomura, 2002</td>
</tr>
<tr>
<td><strong>Calcium ATPase</strong></td>
<td>1 10 10 1 NBD, 1 P-site X-ray</td>
<td>3.1 Å</td>
<td></td>
</tr>
</tbody>
</table>

ECM, electron cryo microscopy; X-ray, X-ray crystallography.
molecular species for another in opposite directions (Busch and Saier, 2002). Uniporters (facilitated diffusion carriers) transport a single molecular species down its concentration gradient. The uniporters, symporters, and antiporters were previously regarded as members of one superfamily of transporters (Goswitz and Brooker, 1995). As more data have become available, these carrier proteins have since been classified in a large group of “porters” containing several transporter superfamilies (Busch and Saier, 2002). Secondary active transporters form a major part of the porter group.

The major facilitator superfamily (MFS) includes more than 1000 evolutionarily related proteins and is implicated in the transport of a variety of solutes and metabolites across the membranes of organisms ranging from bacteria to humans (Busch and Saier, 2002). The MFS includes both uniporters, symporters, and antiporters. Many members of the MFS superfamily have medical or pharmacological relevance, as discussed by Huang and colleagues in their recent report on the glycerol-3-phosphate transporter (GlpT) structure (Huang et al., 2003). It should be noted that a classification system based on transport mechanisms may not always be completely clear-cut. For example, the neurotransmitter transporters 5-HTT (serotonin transporter), DAT (dopamine transporter), and NET (norepinephrine transporter), classified as neurotransmitter/sodium symporters (Saier, 2000), not only mediate the transport of their substrates and sodium in the same direction, but also transport some other ion (K⁺ or H⁺) in the opposite direction.

ABC transporters are found in all taxa and form one of the largest transporter superfamilies, which contains both uptake and efflux transport systems (Saier, 2000). The ABC transporters are ubiquitous membrane proteins that couple hydrolysis of ATP to the translocation of various substrates across cell membranes. Also the ABC transporter superfamily has more than 1000 members with known sequences. Members of this superfamily, which includes both importers and exporters, recognize substrates ranging from single ions to entire protein toxins. ABC transporters have a number of highly conserved ABC cassette motifs, many of which are involved in the binding and hydrolysis of ATP. It is generally assumed that all ABC cassettes bind and hydrolyze ATP in a similar way and use a common mechanism to provide energy for substrate transport through the membrane-spanning domains (Locher et al., 2002). When the substrate has traversed the membrane, the transporter returns to the resting state through dissociation of ADP and inorganic phosphate. Conformational changes that may have a role in the mechanism of energy transduction and/or allosteric control of the ABC-ATPase activity have been reported from X-ray crystal-
lographic studies of the ABC-ATPase subunit (GleV) of a transporter mediating glucose uptake in the Sulfolobus solfataricus bacterium (Verdon et al., 2003).

Three-Dimensional Transporter Structures

A list of the structures discussed below is given in Table 1, and their location in the TC classification system is indicated in Fig. 1.

Low-Resolution Structures

NhaA Antiporter (NhaA). Na⁺/H⁺ antiporters play a major role in the pH and Na⁺ homeostasis of cells from bacteria to mammals (Padan et al., 2001). The E. coli Na⁺/H⁺ antiporter (NhaA), located in the bacterial inner membrane, uses an inwardly directed H⁺ electrochemical gradient to expel Na⁺ from the cytoplasm. A three-dimensional electron density projection map of the E. coli NhaA, determined by electron cryo microscopy, shows twelve cylindrical density features, assumed to represent membrane-spanning domains (Williams, 2000). These results were significant because this was the first reported experimental three-dimensional structure of an ion-coupled transporter protein, confirming the conclusion from previous biological studies and sequence analysis that NhaA has 12 membrane-spanning domains. However, the reported electron density map did not indicate the order of the helices in the protein sequence, nor which sides of the map that represented the periplasmic and cytoplasmic sides of the transporter. Although being a low-resolution structure, it provided a basis for molecular modeling of the transporter when combined with results from other biochemical studies (Ravna et al., 2001).

Oxalate Transporter (OxlT). The bacterial OxlT is an MFS antiporter that catalyzes the exchange of oxalate for formate across the cytoplasmic membrane. A three-dimensional electron density projection map of OxlT from Oxalobacter formigenes, determined by electron cryo microscopy, indicates that OxlT has 12 membrane-spanning helices surrounding a single central cavity, presumably along the pathway of substrate transport (Hirai et al., 2002). In the oxalate-bound state, the 12 helices form a symmetrical structure that displays a well-defined pseudo 2-fold axis perpendicular to the membrane plane and two less pronounced, mutually perpendicular pseudo 2-fold axes in the plane of the membrane, passing through the center of the molecule.

Bacterial Multidrug Transporter (EmrE). The three-dimensional structure of the E. coli multidrug transporter EmrE, an H⁺ antiporter involved in antibiotic drug resistance in bacteria, was determined to 7.0 Å resolution by electron cryo microscopy of two-dimensional crystals (Uhreretxena-Belandia et al., 2003). The EmrE structure shows an asymmetric homodimer forming a bundle of eight transmembrane α-helices, with one substrate molecule bound near the center. The substrate-binding chamber is formed by six α-helices and is accessible from the aqueous phase and laterally from the lipid bilayer.

MDR1 P-glycoprotein (P-gp). The P-glycoprotein encoded by the mdr1 gene hydrolyzes ATP and extrudes cytotoxic drugs from mammalian cells. A low-resolution structure of the mammalian MDR1 P-glycoprotein shows two hydrophobic transmembrane domains, each spanning the membrane six times, and two hydrophilic nucleotide-binding domains (NBD) located at the cytoplasmic face of the membrane (Rosenberg et al., 2001). The 12 membrane-spanning segments were assumed to be α-helices.

MDR3 P-gp. A low-resolution structure of the mouse MDR3 P-gp shows two domains related by a pseudo 2-fold symmetry axis through the middle of the molecule, perpendicular to the membrane plane. The two domains were interpreted as the N- and C-terminal halves of the molecule (Lee et al., 2002).

Multidrug Efflux Transporter (Pdr5p). Pdr5p is a multidrug exporter in the yeast Saccharomyces cerevisiae and a member of the ABC superfamily. Pdr5p shares similar mechanisms of substrate recognition and transport with the human MDR1 P-gp. The Pdr5p structure determined at 25 Å resolution showed a dimeric organization (Ferreira-Pereira et al., 2003). Each monomer was composed of three subregions corresponding to a membrane region that joins two well-separated protruding stalks, each ending with a cytoplasmic NBD lobe. The two NBDs appeared oriented perpendicularly within a monomer.

Multidrug Transporter (YvcC). A low-resolution structure of a bacterial multidrug resistance transporter, YvcC, showed a homodimeric organization with each subunit composed of a transmembrane region, a “stalk” of about 40 Å in height and 20 Å in diameter, and a cytoplasmic lobe identified as the nucleotide-binding domain (Chami et al., 2002). Three-dimensional reconstruction of YvcC embedded in a membrane revealed an asymmetric organization of the two NBD sites within the homodimer, and a dimeric interaction between two homodimers.

High-Resolution Structures

Lactose Permease Symporter (LacY). Lactose permease belongs to the MFS superfamily and is a member of the oligosaccharide/proton symporter family (Fig. 1). LacY mediates the coupled cotransport of lactose and H⁺, using the movement of H⁺ down a concentration gradient as energy source. A high-resolution crystal structure of the protonated Cys154 → Gly mutant of LacY from E. coli, in an inward-facing conformation and with bound substrate, shows two almost identical protein domains related by an approximate 2-fold symmetry axis (Abramson et al., 2003). Each domain has six membrane-spanning α-helices, and the substrate-binding site was located at their interface at a similar distance from each side of the membrane. This structure and the GlpT structure from E. coli that was published at the same time (Huang et al., 2003) both support the “alternating access” mechanism of transport, in which the protein undergoes conformational transitions such that the ligand-binding site is alternatively accessible to one side of the membrane or the other but not to both sides simultaneously. Each of the eight helices that form the surface of the hydrophilic substrate-binding cavity in LacY contains several proline and glycine residues and was heavily distorted by kinks and bends.

GlpT. A member of the MFS transporter superfamily and the organophosphate/Pi antiporter family (Fig. 1), GlpT, catalyzes the exchange of glycerol-3-phosphate for inorganic phosphate using the movement of phosphate down a concentration gradient as energy source. A high-resolution crystal structure of GlpT from E. coli in an inward-facing conforma-
tion shows two similar protein domains related by a central pseudo 2-fold symmetry axis perpendicular to the membrane plane and a substrate-binding site at their interface. Each domain has six membrane-spanning α-helices (Huang et al., 2003), as also observed for LacY.

**Multidrug Efflux Transporter (AcrB).** Multidrug efflux pumps cause serious problems in cancer chemotherapy and treatment of bacterial infections. AcrB is a resistance-nodulation cell division (RND) transporter that cooperates with a membrane fusion protein (AcrA) and an outer membrane channel (TolC), to form a multidrug exporter system that transports cationic, neutral, and anionic substances out of bacteria. The crystal structure of AcrB from *E. coli* (Murakami et al., 2002), determined by X-ray crystallography, shows a homodimer with each subunit containing six transmembrane α-helices and a nucleotide-binding domain at the cytoplasmic face of the membrane (Chang and Roth, 2001). An asymmetric distribution of charged residues lining a central chamber suggests a general mechanism for substrate translocation by MsbA and other MDR-ABC transporters. The structure of MsbA from *E. coli*, determined by X-ray crystallography, shows a homodimer with each subunit containing six transmembrane α-helices, each with 10 membrane-spanning α-helices (Locher et al., 2002). The BtuC subunits thus have a total of 20 transmembrane helices, which are grouped around a translocation pathway that is closed to the cytoplasm by a gate region. This arrangement of the transmembrane domain is distinct from that observed for the *E. coli* lipid flippase MsbA (Chang and Roth, 2001). A prominent cytoplasmic loop of BtuC forms the contact region with the ATP-binding cassette and appears to represent a conserved motif among the ABC transporters.

The authors suggest that the variability in the number of transmembrane passes among different ABC transporters may reflect diverse architectures of the membrane-spanning domains, where the sequence similarities are generally low between different transporters or the need for more transmembrane passes to translocate larger substrates such as vitamin B₁₂ (Locher et al., 2002). Alternatively, that difficulties in predicting membrane-spanning segments may have led to an underestimation of their number for other ABC transporters (Locher et al., 2002). The authors also note...
that the 10 transmembrane helices found per BtuC subunit are packed together in a rather intricate way that does not resemble the arrangement of the six helices in an MsbA monomer.

**Calcium ATPase (SERCA1a).** In skeletal muscle cells, calcium ions are pumped from the cytoplasm into the sarcoplasmic reticulum against a concentration gradient by a P-type calcium ATPase (SERCA1a). P-type phosphorylases are ion pumps that are auto-phosphorylated during the reaction cycle, and the group also includes Na\(^+\)K\(^-\)-ATPase and gastric H\(^+\)-K\(^-\)-ATPase. The crystal structure of SERCA1a has been determined with two Ca\(^{2+}\) ions bound in the transmembrane domain, after growing crystals in the presence of 10 mM Ca\(^{2+}\) (Toyoshima et al., 2000). The structure of the transporter in a calcium-free state was later reported from the same group (Toyoshima and Nomura, 2002). The transporter has ten membrane spanning \(\alpha\)-helices, some of which were unwound or kinked at the middle of the membrane. The cytoplasmic region consists of three well separated domains, with the phosphorylation site in the central catalytic domain and the adenosine-binding site on another domain. The auto-phosphorylation site was located more than 25 Å away from the bound nucleotide. This and other studies (Huang and Squier, 1998) indicate that binding of Ca\(^{2+}\) induces largescale domain motions and that the nucleotide-binding and phosphorylation sites get closer to each other during ATP hydrolysis.

**Relevance of Three-Dimensional Transporter Structures.** Two of the most widely prescribed drugs in the world, fluoxetine and omeprazole, have a transporter protein as site of action, and high-resolution transporter structures have a large potential value in drug discovery. As pointed out in a commentary to the reported LacY and GlpT structures (Locher et al., 2003), one prerequisite for the solving of these high-resolution transporter structures has been the access to synchrotron X-ray sources and their staffs to surmount the limitations of modest diffraction quality and substantial crystal-to-crystal variability. Due to the availability of such techniques, high-resolution structures of more than 90 membrane proteins have now been reported (http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html), and the number is increasing steadily.

In their report of the high-resolution SERCA1a structure, the authors note that their crystal structure “explains many more experimental results than we can describe here” (Toyoshima et al., 2000). This may indeed have been the case for all the recently reported high-resolution transporter structures, which have been preceded by a wealth of biochemical and biological data.

It interesting to note, for example, that all high-resolution transporter structures have confirmed the \(\alpha\)-helical structures of the membrane-spanning domains (Table 1), suggested from many previous studies. The high-resolution structures also show that \(\alpha\)-helices lining the presumed substrate translocation pathway often have irregular shapes with kinks and bends.

Together these crystal structures demonstrate the large structural flexibility of transporter proteins and that substantial movements take place during the substrate translocation process. Recent data indicate that this feature may not be unique to transporters but may be a property shared with certain ion channels (Jiang et al., 2003a,b).

**Molecular Modeling of Transporter Proteins**

Molecular modeling may be used to simulate three-dimensional structures for proteins where no high-resolution structural data are available and dynamic structural rearrangements related to functional mechanisms. Model building of proteins with an unknown three-dimensional structure may be performed by using a traditional homology modeling approach, or by using indirect and low-resolution structural knowledge from experimental studies. The latter approach is referred to as “knowledge-based modeling” in the present article.

Knowledge-based modeling usually starts with an initial model of conserved regions based on information from a low-resolution structure, amino acid sequence alignment, and information from biochemical and biophysical studies. The accuracy and reliability of such protein models depend on the accuracy of the structural data that the model is based on and the resemblance of the modeled structure with the one or those used as a template for the initial model.

In homology modeling, the initial model is based on a high-resolution structure of a homolog protein and amino acid sequence alignments. In general, a higher accuracy may be expected by homology-based than by knowledge-based modeling; however, the accuracy of homology-based models is limited by the validity of the sequence alignment of the modeled molecule to the template, the accuracy of the template, and by the accuracy of the modeling of inserted or nonaligned regions. This is particularly important when a protein from a bacteria is used as template to model a related mammalian membrane protein, which may have substantially larger intra- and extracellular loops between the transmembrane \(\alpha\)-helices. This is illustrated in Fig. 2, which shows the crystal structures of LacY, GlpT, and AcrB from *E. coli* and a molecular model of the human DAT.

**ABC Transporters.** ABC transporters are associated with many different diseases including intrahepatic cholestasis, cystic fibrosis, and failure of cancer chemotherapy, and represent potential targets for therapeutic drugs in various therapeutic areas. Molecular modeling of ABC transporters therefore has considerable interest in drug discovery.

ABC transporters may undergo major conformational changes during the transport cycle, as demonstrated by biochemical assays and visualized at low resolution for the human multidrug resistance P-glycoprotein encoded by the *mdr1* gene (Rosenberg et al., 2001). The most significant rearrangement is believed to occur as the substrate is shuttled across the membrane, and ATP is hydrolyzed. Molecular modeling of ABC transporters would, in principle, provide an excellent tool for simulation of such rearrangements, working hand in hand with experimental biochemical studies.

Some ABC transporters, however, translocate hydrophobic substances whereas others translocate highly hydrophilic substances including sugars and inorganic ions. As discussed in a commentary to the reported MsbA structure (Higgins and Linton, 2001), it is not apparent, therefore, how for instance the crystal structure of MsbA, which transports hydrophobic substances from the bacterial inner membrane leaflet, can be extrapolated to other ABC transporters.

Comparative sequence analyses, motif search, and secondary structure prediction indicated that each of the two halves of the human MDR1 P-glycoprotein is structurally similar to...
the monomer structure of the E. coli MshA lipid transporter and suggested that the open dimer structure may be used as a valid model for the P-glycoprotein (Seigneuret and Garnier-Suillerot, 2003). Homology modeling was therefore used to predict the structure of P-glycoprotein, using MshA as a template. Significant differences between P-glycoprotein and MshA in size and charges of the internal chamber open to the inner leaflet and the inner medium were ascribed to the differences of the transported substrates. The authors note that the modeled structure provided insight into structural relationships between the transmembrane and nucleotide-binding domains that seem important for transport. The interactions between the intracellular domain and the nucleotide-binding domain of the modeled structure suggested that these contacts are involved in mediating the coupling between conformational changes of the nucleotide-binding domain and reorientation of transmembrane α-helices during the transport process (Seigneuret and Garnier-Suillerot, 2003).

The crystal structure of BtuCD (Locher et al., 2002) may be combined with previous biochemical and structural data to simulate the structural rearrangements associated with vitamin B12 transport; however, judging from the diversity of the structural architecture of the membrane-spanning and other domains of the ABC transporters included in Table 1, it appears that the BtuCD structure may not readily be extrapolated to a larger part of the ABC transporter superfamily.

**MFS Transporters.** The reported OxlT electron density map did not permit direct assignment of the helices to specific membrane segments; however, analysis of extensive biochemical data on this and related proteins indicated that helices 3, 6, 9, and 12 most likely are located at the periphery of the transporter and facing the lipid bilayer (Hirai et al., 2002). Information from the 6.5 Å resolution OxlT structure was later combined with sequence information from other members of this protein family to construct models of the arrangement of the transmembrane α-helices in the MFS superfamily (Hirai et al., 2003). A probable model for the relative spatial arrangement for the 12 helices was proposed, consistent both with the structural findings from the electron cryo microscopic study and with previous biochemical studies on members of this superfamily (Hirai et al., 2003).

**Glut1 Uniporter.** A three-dimensional model of the human facilitative glucose transporter (Glut1) has been constructed by knowledge-based methods, since its structure and function have been extensively studied, and no detailed three-dimensional template was available (Zuniga et al., 2001). The Glut1 models had two channels, one traversing the structure completely, lined by many solvent-accessible residues, which may serve as the substrate transport pathway. The structure was consistent with previous mutagenesis and biochemical studies.

**NhaA Cation Antipporter.** A three-dimensional molecular model of the NhaA antipporter was constructed by interactive molecular graphics and energy calculations, based on the three-dimensional electron density projection map from E. coli NhaA and information from previous biochemical and biophysical studies (Ravna et al., 2001). The model suggests that the pH dependent activity of NhaA may be explained by charge changes in the intracellular loop between TMH8 and TMH9 that alter the positions of TMH4, TMH5, and TMH11 relative to each other, such that a pore area of the transporter protein is opened.

**The Neurotransmitter/Sodium Symporter Family.** Members of the neurotransmitter/sodium transporter family are the target of action of many currently used psychotropic drugs and several substances of abuse. The serotonin transporter (5-HTT) and the norepineprine transporter (NET) are the main sites of action of antidepressant drugs, and the dopamine transporter (DAT) is the main site of action of cocaine and other substances of abuse. We have used knowledge-based methods to construct molecular models of DAT, NET, and 5-HTT, since no detailed crystal structure of any homolog protein has been available (Ravna et al., 2003a,b). There are examples that a single point mutation may convert a symporter into a uniporter (Busch and Saier, 2002), which suggests a similarity in their overall three-dimensional molecular structures. Although many data have indicated that there is a certain structural similarity between the antiporter NhaA on one side, and the symporters DAT, 5-HTT, and NET on the other side, it has not been proven that these share a common architecture of the membrane-spanning domains.

Based on the hypothesis that this was indeed the case, modeling of the 12 membrane-spanning α-helices in DAT, NET, and 5-HTT were based on the low-resolution NhaA structure (Williams, 2000) like our previous NhaA model, in addition to site-directed mutagenesis and other biological data on DAT, 5-HTT, NhaA, and LacY (Ravna et al., 2003b). Shortly after publication of the DAT and 5-HTT models, the high-resolution LacY structure was reported (Abramson et al., 2003). A relatively good resemblance between the membrane-spanning domains of LacY and our NhaA model, as shown in Fig. 3, demonstrates that knowledge-based modeling may give a reasonably good model of a membrane protein, provided sufficient biochemical data are available in addition to a low-resolution structure. Neurotransmitter transporter models may therefore predict ligand-binding mechanisms and provide new insight into the structure and functional mechanisms of such transporters. Computational methods based on accurate molecular transporter models represent one among other useful tools in the discovery of safer and more efficient drugs acting on membrane transporters.
References


Address correspondence to: Dr. Svein G. Dahl, Department of Pharmacology, I.M.B., University of Tromsø, N-9037 Tromsø, Norway. E-mail: sgd@fagmed.uit.no