Comparison of Mammalian LAT1 and Bacterial BrnQ Transport Proteins Suggests Potential for Orally-Active Drug Uptake by Intestinal Bacteria

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Abstract

Transport proteins mediate the permeability of biological membranes for a variety of substrates. Individual classes of transport proteins are capable of discriminately recognizing specific substrates from a vast excess of structurally diverse molecules. Prokaryotic and eukaryotic cells generally share a broad range of transport capabilities. It is known that eukaryotic cells have transport proteins with the capacity to mediate drug transport. With the exception of antibiotics, it is not known whether prokaryotic cells share the capacity to mediate drug transport. A comparative study between transport proteins LAT1 from rabbit and BrnQ from the intestinal bacterium Escherichia coli *was conducted. Both proteins facilitate the transport of amino acids such as leucine, isoleucine, and valine, and LAT1 is known to mediate the transport of orally active drugs. Results of amino acid sequence analyses revealed that LAT1 displays a 42.1% amino acid sequence similarity to the BrnQ protein. Reliability estimates indicated that the likelihood that the two proteins belong to the same family of the proteins was approximately 99.6%. Secondary structural and topological modeling studies suggest that LAT1 and Brnq have similar structural characteristics. Thus, the results suggest that the two proteins are analogous; i.e., the two proteins share homologous functions but have heterologous evolutionary origins. The observations supported the hypothesis that intestinal bacteria may have the capacity to transport and accumulate orally active medications.*

The permeability of biological membranes is mediated by transport proteins. Transport proteins are integral membrane proteins which are embedded in the membrane. Membrane transport proteins play essential roles in cellular metabolism and activities. They function in the acquisition of nutrients such as simple sugars, amino acids, and metals, as well as, maintenance of ion homeostasis (1). A large family of proteins has been identified which mediate the permeability of biological membranes for a diverse variety of substrates (2, 3). Individual classes of transport proteins are capable of discriminately recognizing and binding specific substrate molecules from a vast excess of structurally diverse molecules.

Many eukaryotic and prokaryotic genomes encode proteins whose function is to facilitate the transport of common substrates. For example, both eukaryotic and prokaryotic cells have the ability to permit selective passage of the same types of substrates (e.g., simple sugars, amino acids, and metals). Yet, despite similarities in the chemical structures of the substrates, the proteins are evolutionarily unrelated and may have arisen through independent evolutionary events (4).

Many drugs are administered via the oral route (i.e., through the mouth), and certain types of orally active drugs utilize membrane transport proteins to facilitate absorption across the lining of the intestine tract (5, 6). Antibiotics are a well-known class of drugs that are transported into bacterial cells by membrane transport proteins (7-9). However, whether other classes of orally active drugs utilize bacterial transport proteins for entry into bacterial cells is unknown. Also the effects of orally active medications on the growth and metabolism of bacteria have not been studied. Many bacteria such as *Escherichia coli* are common inhabitants of the microbial flora commonly found in the lower intestines (10), and a question is whether orally-active medications potentially serve as substrates for bacterial transporters in the intestinal tract.

The Large-neutral Amino Acid Transporter 1 (LAT1) in mammalian cells is a membrane transport protein which mediates transport of large-neutral amino acids (e.g., leucine, isoleucine, phenylalanine, tyrosine, arginine and tryptophan) (11). LAT1 also transports several amino-acid-related drugs, such as the anti-Parkinsonian drug L-dopa, the anticonvulsant medication gabapentin, as well as, thyroid hormones such as triiodothyronine (12-14). A functionally equivalent transporter exists in the bacteria. The BrnQ transporter mediates uptake of the branched-chain amino acids leucine, isoleucine, and valine. (15-17). However, it is not known whether BrnQ has the potential for transporting amino-acid-related drugs similar to that of LAT1. We considered the hypothesis that if mammalian and bacterial transporters the share substrate specificity for naturally occurring substrates, then they might also share specificity for non-natural substrates such as orally-active medications. We reasoned that proteins which transport common molecules might also share similarities in manner in which the transport proteins recognized may recognize common molecules. Thus, we searched for similarities in protein sequences between mammalian and bacterial transporters which specificity for the same substrate molecules, using the intestinal bacterium Escherichia coli as a model organism.

Methods
Amino Acid Sequence Analysis
The source of data and computational tools used in the study are listed in Table 1. Amino acid sequences for LAT1 and BrnQ proteins were obtained from the Swiss-Prot database (18). Swiss-Prot is a comprehensive database that contains publicly available nucleotide and/or protein sequences, as well as, functional information. Amino acid sequences for the proteins were aligned using the Clustal W software (19). Identical amino acids were aligned, and gaps were introduced to improve similarity. Conserved amino acid substitutions, i.e., replacements of an amino acid residue with another one with similar properties, were considered. Also,

semi-conserved substitution amino acid, defined as replacement of an amino acid residue with another that has similar steric conformation, but does not share chemical properties were also considered. The one-letter code for amino acids is a useful way to display to represent amino acids. Amino acid replacement groups for conservative replacements in one-letter code are STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, and FYW. Semi-conservative amino acid replacements are CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, and HFY.

Protein Secondary Structure and Topological Studies.

Consensus secondary structure predictions, derived from several prediction algorithms, were performed using the Network Protein Sequence Analysis Tool (20). Secondary structural elements are indicated by solid color coils (α-helices), coils (random coils) and rods (extended strand) along the primary sequence, and where (---) indicate sequence interruptions. Topological models of protein structures were constructed using the TMPRED software to determine predictions of membrane-spanning regions and their orientation (21). The algorithm is based on the statistical analysis of naturally occurring transmembrane proteins. Results were used as input for the TOPO2 software which generates two dimensional topological representations of proteins (22).

Results and Discussion

LAT1 is an integral membrane protein that is 507 amino acids in length. The transporter is highly conserved in most mammalian species (23, 24). Conserved among bacteria, BrnQ is a integral membrane protein that is 439 amino acids in length (17). Sequence alignment studies revealed that LAT1 from rabbit was found displaying 42.1% overall similarity to BrnQ sequence from *E. coli* with 13.5% identical residues and where 28.6% constituted conservative or semiconservative sequence matches (Figure 1). To achieve optimal similarity between the proteins, 6 small gaps were introduced in the alignment. A reliability estimate, derived from a set of 58 classes of proteins among 1300 sequences, produced a probability of 99.6% for the likelihood that the two proteins belongs to the same family of the proteins (25).

Secondary structural predictions indicate the alpha helical characteristics of the BrnQ protein is 53.1% and is higher than that observed in LAT1 where alpha helical characteristics is 39.4% of the protein. The LAT1 transporter has a higher extended strand potential comprising 11.9% of the protein as compared to BrnQ with 6.8%. Both proteins have the same random coli potential. In LAT1, the random coli potential was 48.7% as compared to BrnQ with 40.1%. The overall distribution of structural characteristics was found to be similar through much of the two proteins. An analysis of the topology of the two protein suggest that LAT1 has 13 regions of the protein that are transmembrane domains, whereas, BrnQ has 12 predicted transmembrane domains. The predicted orientations of the two proteins differ at the carboxyl terminus where LAT1 is oriented on the outside face of the membrane, while in BrnQ the carboxyl terminus is oriented to the inside face of the membrane.

LAT1 and BrnQ proteins lack a high level of amino acid sequence similarity. However, the two proteins have similar secondary structural and topological features, and share the same substrate specificity. Such proteins are said to have analogous function. The implication is that analogous proteins followed evolutionary pathways from different origins to converge upon the same function. Thus, analogous proteins are considered a product of convergent evolution. Because LAT1 is involved in drug transport, it is postulated the BrnQ may also utilize orally-active medications as alternative substrates. This calls attention to the viewpoint that orally-active drugs may serve as alternative substrates for bacterial transporters. Absorption of orally-active drugs by bacterial cells might decrease absorption by mammalian hosts, thereby making drugs less effective to the hosts.

Alternatively, utilizing the property of bacterial transport proteins, such ingested drugs could alter the metabolism and growth of bacteria and may prove to be effective in inhibiting or killing intestinal bacteria. Future studies involving a survey of bacterial transporters with sharing substrate specificity with mammalian transport proteins could provide additional support for this view. Also, direct experimental observations of uptake of orally-active drugs by bacteria would provide additional support for the hypothesis.

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TABLE 1

Data Source and Computational Software Used in Study

| Tools	Description	Reference
Swiss-Prot Database	A central repository of protein data	18
Clustal W	Nucleotide and protein sequence alignment program	19
Network Protein Sequence Analysis Tool	A tool for secondary structure predictions	20
TMPRED	A program for prediction of membrane-spanning regions and their orientation	21
TOPO2	A software for creating transmembrane protein 2 dimensional topology images	22
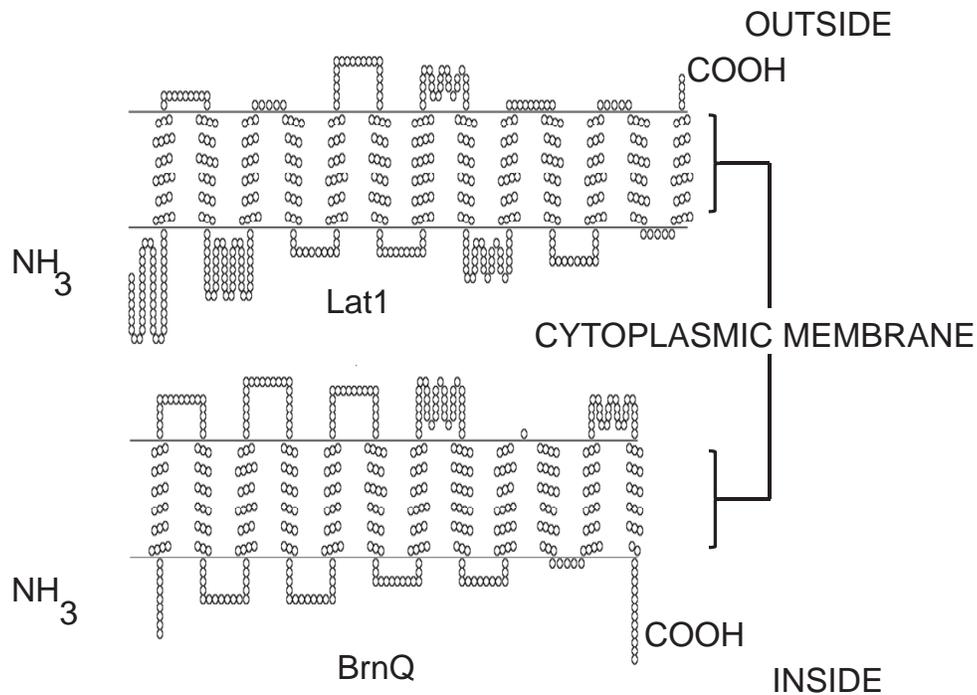
Figure 1: Predicted membrane topology of LAT1 and BrnQ proteins. Open circles: membrane protein structures were determined using the TOPO2, transmembrane protein display software (22).

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