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Crystal structure and mechanism of GlpT, the glycerol-3-phosphate transporter from *E. coli*

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Abstract The major facilitator superfamily represents the largest group of secondary active membrane transporters in prokaryotic and eukaryotic cells. They transport a vast variety of substrates, presumably via similar mechanisms, yet the details of these mechanisms remain unclear. Here we report the 3.3 Å resolution structure of a member of this superfamily—GlpT, the glycerol-3-phosphate transporter from the *E. coli* inner membrane, in the absence of a substrate. The antiporter mediates the exchange of glycerol-3-phosphate for inorganic phosphate across the membrane. Its N- and C-terminal domains exhibit a pseudo 2-fold symmetry along an axis perpendicular to the membrane. Eight of the twelve transmembrane α-helices are arranged around a centrally located substrate translocation pore that is closed off at the periplasmic surface. Present at the beginning of the pore are two arginine residues that presumably comprise the substrate-binding site which is accessible only from the cytosol, suggesting an inward-facing conformation for the transporter. The central loop connecting the N- and C-terminal domains is partially disordered and exhibits reduced susceptibility to trypsin in the presence of substrate, indicating conformational changes. We propose that GlpT operates via a single binding-site, alternating-access mechanism.

Keywords GlpT, transporter, membrane protein, crystallization, MFS

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Introduction

The transfer of solutes across the lipid bilayer of organelles or cell membrane is carried out by nanoscale biomachinery, namely transporters. While some active transporters use ATP to drive the translocation of substrate against its concentration gradient, others use an electrochemical gradient [1]. For antiporters, the downhill movement of one substrate along its electrochemical gradient drives the uphill transport of a second substrate against its concentration. A major question posed is: how does the electrochemical gradient drive this nanoscale biomachine?

GlpT is an antiporter that belongs to the largest family of gradient-driven transporters, the Major Facilitator Superfamily (MFS) [2]. Transporters in this family consist of twelve transmembrane segments. GlpT belongs to the subfamily of sugar–phosphate permeases [3]. It transports across the inner membrane of *Escherichia coli* (*E. coli*) an inorganic phosphate molecule 'downhill' with its concentration gradient. This drives the subsequent transport of glycerol-3-phosphate (G3P) 'uphill' against its concentration gradient [4,5].

In *E. coli*, the exchange of G3P for P_i by GlpT influences oxidative phosphorylation, lipid biosynthesis and pH regulation. A human homolog, glycerol-3-phosphate permease (G3PP) has been identified [6]. It is implicated in oxidative phosphorylation by supplying substrate for the glycerol-3-phosphate shuttle at the mitochondria. In addition, G3PP may affect lipid biosynthesis. Although G3PP has been

identified on the human chromosome 21, its connection to Down's Syndrome is unclear. This gene however does map to the autosomal deafness locus. Dysfunctional G3PP may have a similar phenotype to glycerol-kinase deficiency characterized by mental retardation, growth retardation, elevated urinary glycerol and pseudo-hypertrygliceridemia.

A structure of GlpT was sought in order to understand the mechanism of gradient-driven transport at the molecular level. An atomic model would reveal the substrate binding pocket and the substrate translocation pathway. In addition, the residues involved in the energy coupling may be identified. Lastly, the arrangement of the transmembrane helices has been of interest to many in the transport field. The structure of GlpT would provide a model for other MFS transporters.

The GlpT transporter was crystallized [7,8] and the structure solved to 3.3 Å using X-ray crystallography [9]. In this paper, the overall structure is described and its function via a single binding site with alternating access is discussed.

Methods

The methods for crystallization of GlpT and its structural determination were previously published in detail [7–9]. Briefly, *E. coli* GlpT was cloned into the expression vector pBAD-MycHis-A and subsequently expressed in LMG194 cells. Cells were induced at $OD_{600} = 1.0$ at a temperature of 25°C with 0.1% *L*-arabinose for 1.5 h. Cells were broken by French Press and membrane pelleted by ultracentrifugation at 100,000 *g*. Membrane fractions were solubilized in buffer consisting of 50 mM *Tris*, pH 8.0, 300 mM NaCl, 20% glycerol and 1.0% dodecylmatoside (DDM). GlpT was purified using a Ni–NTA affinity column. Following thrombin digestion to remove purification tags, GlpT was further purified using size-exclusion chromatography.

Crystallization was carried out using vapor diffusion. Crystals were grown at 6 mg ml^{-1} GlpT in 0.1–0.25% DDM and 0.04–0.1% $C_{12}E_9$, 25–27% PEG 2000MME, 0.1 M *Tris* pH 8.5–8.9, 20% glycerol, 5% MPD and 0–100 mM NaCl at 15–20°C. Experimental phases were obtained using single isomorphous replacement with anomalous signal from a tungsten cluster derivative collected to 3.9 Å. Phase extension was carried out using native data to 3.3 Å. Data collected from selenomethionine substituted GlpT was used to facilitate model tracing. Atomic coordinates were deposited in the Protein Data Bank under access code 1PW4 [9].

Results

The overall shape of GlpT is that of a Mayan temple (Fig. 1A). The protein is narrow at the periplasmic end, 35 Å × 45 Å, and wider at the cytoplasmic end, 35 Å × 60 Å. Except for the part of the long cytoplasmic loop connecting helices 6 and 7, the electron density was clearly seen for the majority of the molecule. The location of this loop and the N- and C-termini establishes the cytoplasmic from the periplasmic face of GlpT.

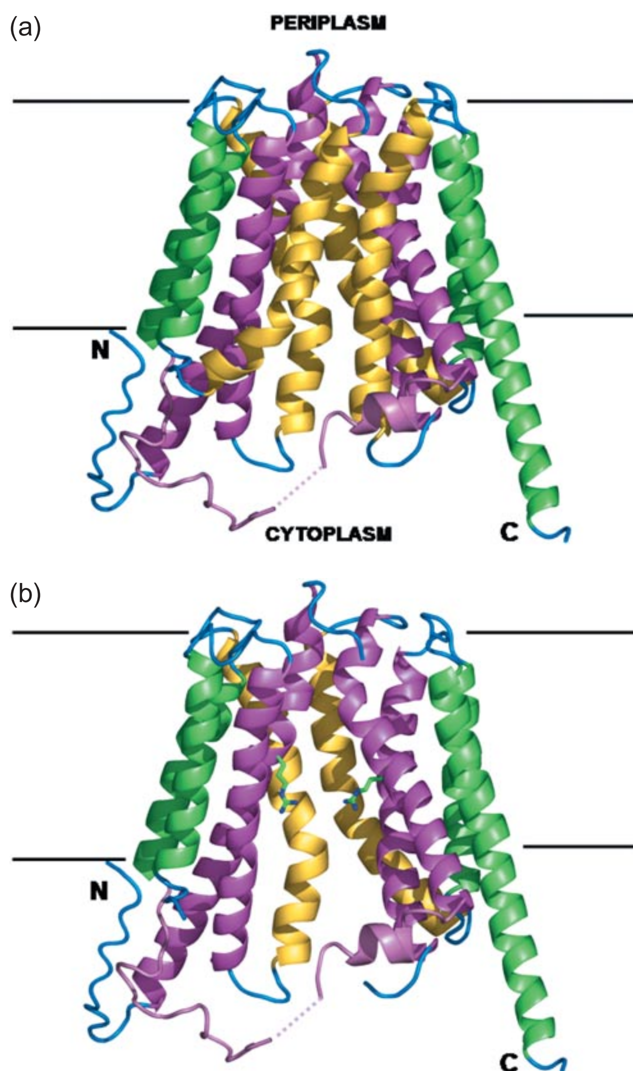

Fig. 1 (a) Ribbon representation of GlpT viewed from within the membrane. Transmembrane helices 3, 6, 9 and 12 are colored green. Helices 1, 4, 7 and 10 are colored magenta. Helices 2, 5, 8 and 11 are colored gold. Loops connecting transmembane helices are in marine with the exception of loops 6 and 7 colored in pink. Lack of density for the loop connecting transmembrane segments 6 and 7 is indicated by a dashed line. (b) Transmembrane helices 2 and 11 are removed to view two conserved arginine residues (green), present in the central cytoplasmic cleft. These two arginine residues are proposed to be involved in substrate binding. Adapted from [9].

There are twelve transmembrane segments in the GlpT structure (Fig. 1) as predicted by hydropathy analysis and fusion experiments [10]. The twelve helices form two domains of six-helix bundles. There is a pseudo 2-fold symmetry between the N- and C-terminal halves of the protein. On first examination, one can clearly note that there are helices with varied lengths. The shorter helices, such as TM3, 6 and 9, denote the hydrophobic boundary of the lipid bilayer. The longer helices extend into the cytoplasmic space and may interact with other peptides involved in G3P metabolism. Of particular interest are the short loops connecting the helices. These provide rigidity in the N- and

C-terminal domains of the structure, which has functional implications as discussed below.

GlpT is closed at the periplasmic face with an open pore at the cytoplasmic face (Fig. 1). Electrostatic charge potential on the surface of GlpT reveals neutral charge around the hydrophobic belt of GlpT and at the closed periplasmic face. In the cytoplasmic pore, there is a strong positive charge located at the deepest part near the center of the protein. This positive charge can be attributed to two arginine residues, R45 and R269, from TM1 and TM7, respectively (Fig. 1B). These two arginine residues are conserved among other sugar–phosphate transporters, such as in the homologous UhpT which is a hexose-6-phosphate transporter from *E. coli*. In UhpT, mutation of either arginine residue to a cysteine or lysine results in complete abrogation of function [11].

The conserved arginine residues are located on TM1 and TM7, two long helices each found in a six-helix bundle. The guanidinium groups of these two residues are 9.9 Å apart. The interaction of a phosphate ion with these two sidechains may result in a domain movement of each six-helix bundle. This rigid body movement of only 10° by each of the two halves of the protein would close the cytoplasmic pore and reveal an opening at the periplasmic end of GlpT. This 'rocker-switch' type of motion would explain the experimental evidence for a single substrate binding site with an alternating access to the cytoplasmic and periplasmic sides of the lipid bilayer.

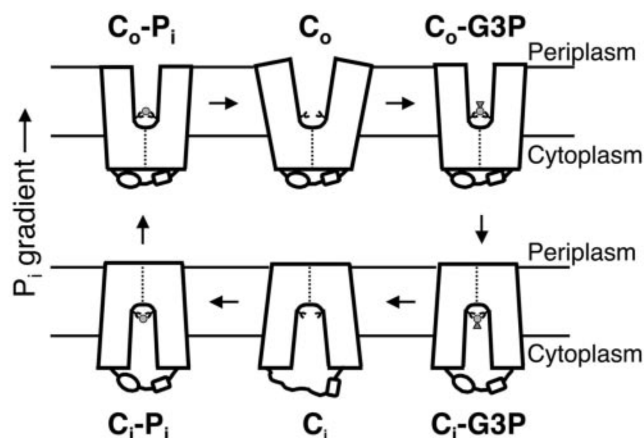

Fig. 2 Proposed mechanism for GlpT transport of P_i and G3P. A single binding site with alternating access mechanism is supported by the GlpT model. At least 6 different conformations of GlpT can be extrapolated from the structure of GlpT and supporting kinetic data for secondary active transport. C_o: outward facing conformation, C_i: inward facing conformation. Adapted from [9].

Discussion

The following model is proposed for a cycle of transport with GlpT [9] (Fig. 2)—GlpT in the inward facing conformation accepts a phosphate ion from the cytoplasm. The two six-helix bundles move closer resulting in a narrow pore at the cytoplasmic face and create a new pore at the periplasmic face with the arginines exposed. The presence of periplasmic G3P, with its higher affinity for GlpT, would then displace P_i from the outward facing conformation. The binding of G3P would allow GlpT to change to the inward facing conformation where G3P would be released. The binding of substrate to GlpT may lower the energy barrier between the inward and the outward facing conformation, facilitating their interconversion. While the binding of substrate is fast, the conformational change is most likely the rate-limiting step [9].

The structure of GlpT revealed much information sought by the many who work in the transporter field. However, there are still many questions to be answered: What are the structural changes upon substrate binding? A structure of GlpT in the outward facing conformation would give additional insight into its mechanism of transport. A structural model of GlpT in a complex with a substrate is being sought. In addition, mutagenesis is being carried out to characterize the residues implicated in substrate specificity and transport.

Concluding remarks

Our knowledge of MFS transporters was not only increased by the structure by GlpT but also by the recently published structure of lactose permease (LacY) [12,13]. Not surprisingly, considering that the two proteins belong to the MFS family of transporters, both have relatively similar structural features—12 transmembrane helices with two six-helix bundles. Both proteins were crystallized in the inward-facing conformation. Could the inward facing conformation be the low energy conformation for GlpT and LacY or other MFS transporters? Further structural analysis of MFS transporters in different conformations and complexed with substrate will reveal further details surrounding these nano-scale biomachines.

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