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Crystal structure of a phosphorylation-coupled saccharide transporter

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Abstract

Saccharides play a central role in the nutrition of all living organisms. Whereas several saccharide uptake systems are shared between the different phylogenetic kingdoms, the phosphoenolpyruvate-dependent phosphotransferase system exists almost exclusively in bacteria. This multi-component system includes an integral membrane protein EIIC that transports

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Supplementary Information accompanies the paper.

Author Contributions J.L., M.P., B.R. and W.A.H identified ChbC homologues in the database. J.L. carried out the cloning and the initial expression studies. Y.C., H.H., E.J.L., J.W., and M.Z. performed protein expression, purification, crystallization, and X-ray diffraction data collection and analysis. X.J., Y.Z., E.J.L., and M.Z. solved and refined the structures. M.Q., Y.C., Y.P., J.A.J., and M.Z. characterized ChbC function. K.R.R. advised on data collection and crystallography. E.J.L. and M.Z. wrote the manuscript with input from all authors.

saccharides and assists in their phosphorylation. Here we present the crystal structure of an EIIC from *Bacillus cereus* that transports diacetylchitobiose. The EIIC is a homodimer, with an expansive interface formed between the N-terminal halves of the two protomers. The C-terminal half of each protomer has a large binding pocket that contains a diacetylchitobiose, which is occluded from both sides of the membrane with its site of phosphorylation near the conserved His250 and Glu334 residues. The structure shows the architecture of this important class of transporters, identifies the determinants of substrate binding and phosphorylation, and provides a framework for understanding the mechanism of sugar translocation.

Bacterial phosphoenolpyruvate-dependent phosphotransferase systems (PTS)¹ transport saccharides across the cell membrane and phosphorylate them before their release into the cytosol (reviewed by²⁻⁷). Phosphorylation of the incoming saccharide primes it for subsequent utilization as a nutrient in cellular metabolism and also prevents its efflux. While the system can transport a cognate sugar by slow facilitated diffusion *in vitro* in the absence of phosphorylation, phosphorylation greatly speeds up the overall rate of sugar uptake^{2,3}, allows concentration of intracellular substrate relative to the environment, and is necessary for growth of the host bacteria when the PTS sugar is provided as the sole carbon source. Unlike the primary ABC-type transporters that hydrolyse ATP^{8,9} or the secondary transporters that harness a sodium or proton gradient¹⁰⁻¹² to drive transport, PTS systems therefore use covalent modification of their substrate during transport to ensure its uni-directional flux.

PTS systems are composed of three components: enzyme I (EI), the heat-stable phosphocarrier protein (HPr), and enzyme II (EII). Both EI and HPr are general energy coupling-proteins and are not sugar specific, while EII is sugar specific and is itself a protein complex composed of the cytosolic EIIA and EIIB proteins and the integral membrane protein EIIC. In certain EIIs, EIIA or EIIB or both are translated with EIIC as a single polypeptide chain. Bacteria often possess several different types of EIIs that are induced by the presence of their substrate^{13,14}. The phosphate group originates from phosphoenolpyruvate, and is transferred sequentially to EI, HPr, EIIA, EIIB, and eventually to the incoming sugar substrate bound to EIIC, the component responsible for translocating the sugar^{2,3}.

Of the four EIIC superfamilies, the largest is the Glc family, which has subfamilies each specialized in transporting glucose, several β -glucosides, mannitol, fructose, or lactose^{6,15}. All Glc family EIICs have an almost universally conserved glutamate residue (Supplementary Figure 1) essential for substrate transport and phosphorylation^{16,17}. This conserved glutamate is located within a conserved motif, which was first identified as GITEP in the glucose- and β -glucoside-specific EIICs^{2,6}. To understand further the mechanism of sugar selectivity, translocation, and phosphorylation, we initiated structural studies on a group of EIICs that are members of the lactose subfamily of the Glc superfamily. These members have an ortholog in *Escherichia coli*, ChbC, which was shown to transport N,N'-diacetylchitobiose ((GlcNAc)₂), a β -1,4-linked N-acetylglucosamine disaccharide^{18,19}. We crystallized and solved the structure of a ChbC homolog from *Bacillus cereus* (Supplementary Fig.1).

Functional characterization

The *B. cereus chbC* gene was heterologously expressed in *E. coli* and the resulting protein purified to homogeneity (Supplementary Fig. 2a). The molecular weight of detergent-solubilised ChbC was estimated at approximately 100 kDa by combining size-exclusion chromatography with light scattering and refractive index measurements. Since the molecular weight of an individual protomer is ~47 kDa, detergent-solubilised ChbC is

therefore a homodimer. This dimer is stable and monodisperse in a number of detergents, but partially dissociates in shorter chain detergents such as octylmaltoside (Supplementary Fig. 2b–e). These results are consistent with earlier reports of a dimeric assembly for EIICs from other members of the Glc superfamily^{20–24} and suggest that the purified ChbC retains its native quaternary assembly in long-chain detergents.

We reconstituted purified *B. cereus* ChbC into proteoliposomes and measured its ability to transport sugars by monitoring uptake of ¹⁴C-labelled N-acetylglucosamine (GlcNAc), which is the monosaccharide that is condensed to form (GlcNAc)₂. Addition of ¹⁴C-GlcNAc resulted in a time-dependent accumulation of the radiotracer within the lumen of the proteoliposomes that stabilized after ~15 minutes (Fig. 1a), consistent with a facilitated diffusion process that dissipates the initial concentration gradient of the radiotracer. Control liposomes lacking ChbC showed little accumulation even after 30 minutes. Further experiments showed that ¹⁴C-GlcNAc uptake was significantly inhibited in the presence of non-labelled GlcNAc or (GlcNAc)₂, while the same concentration of glucose had no significant effect (Fig. 1b). This experiment indicates that the reconstituted ChbC is capable of translocating a sugar, and that it is selective for GlcNAc and (GlcNAc)₂ over glucose.

Structure determination

After extensive refinement of crystallization conditions, a data set was collected on a crystal grown in the presence of 4 mM (GlcNAc)₂. The crystal had P4₃2₁2 symmetry and diffracted to 3.3 Å (Supplementary Table 1). Initial phases were estimated from a Ta₆Br₁₂ derivative diffracting to 4.5 Å, and the phases were gradually extended to the native data set (Methods, Supplementary Fig. 3). There are four ChbC protomers in the asymmetric unit, and the building and refinement of an accurate atomic model were aided by the use of four-fold non-crystallographic symmetry (NCS) restraints, which were maintained throughout the refinement until the last few rounds (Methods). The final models of all four protomers in the asymmetric unit contain the full-length ChbC except for 2 residues at the N-terminus that are not resolved. In addition, each chain contains one (GlcNAc)₂ and two nonylmaltosides (NM) that were used to solubilise ChbC. One of the NM molecules is only partially resolved.

Tertiary and quaternary structure of ChbC

Each ChbC protomer contains 10 transmembrane helical regions (TM 1–10), including one (TM8) that is tilted at a roughly 45° to the membrane norm and is split into two short hydrophobic helices joined by a hydrophilic loop (Fig 1c and Supplementary Fig 4). It also contains two re-entrant hairpin-like structures (HP1 and HP2) with opposite orientations in the membrane, and two horizontal amphipathic α-helices (AH1 and AH2). AH1 and AH2 most likely lie along the inner and outer boundaries of the hydrophobic core of the lipid bilayer, which is marked in Fig. 1d and e. To the best of our knowledge, ChbC has a novel fold.

The protein is a homodimer and the two protomers are oriented parallel in the membrane, related by a two-fold axis perpendicular to the membrane (Fig. 1d, e and Supplementary Fig. 5). Both the N- and C-termini likely reside on the cytoplasmic side as inferred from the “positive-inside” rule²⁵, and from the experimentally-determined topology of ChbC from *E. coli*²⁶ (Supplementary Figure 4). This assignment is also consistent with the location of the termini determined experimentally in other members of the Glc superfamily^{6,27–29}. When viewed from within the membrane with the extracellular side on the top, the dimer is roughly 50 Å thick along the twofold axis and has the shape of a capsized canoe, with a concave surface facing the intracellular side (Fig. 1d, e). β-hairpins from each protomer protrude an extra 20 Å into the extracellular space, although the hairpins mediate a key

crystal contact and may be perturbed slightly from their native conformation (Supplementary Fig. 6). When the dimer is viewed looking down the two-fold axis from the intracellular side, the two dimensions of the concave surface are ~ 60 Å and 100 Å (Fig. 1f, g). Stereo views of the ChbC dimer in three orientations are shown in Supplementary Fig. 5.

The extensive dimer interface is formed primarily by the N-terminal half of ChbC: TM 1, 2, 3 and 5 from each protomer line the interface with a buried surface area of 2746 Å² per protomer (Fig. 1h and Supplementary Fig. 7). The long loop between TM4 and 5 also contributes to the interface by extending along the cytoplasmic face of the neighboring subunit (Fig. 1i). This large and mostly hydrophobic interface is expected because EIICs are known to function as a dimer in the membrane^{20–24}. An extensive dimer interface was also observed in an electron microscopy projection map of a Glc family EIIC that transports mannitol³⁰, suggesting that this feature is conserved among subfamilies of the Glc family transporters.

Substrate binding site

The C-terminal half of each protomer (TM6–10) contains a deep, electronegative cleft on its intracellular side (Fig 2a). Although the cleft is located on the intracellular face of each protomer, it is not solvent-exposed because part of TM 5 and the preceding TM4–5 loop from the neighboring protomer extend beneath it (Fig 1i). This cleft is lined partly by the re-entrant hairpin loops HP1 and HP2. HP1 harbours the glutamate residue (Glu334) in the Glc family conserved motif, which is NINEP in this ChbC (Supplementary Fig. 1). The tips of HP1 and HP2 meet in the middle of the membrane (Fig 2b and Supplementary Fig. 8a). The arrangement of these two loops is strongly reminiscent of two re-entrant hairpins in the otherwise dissimilar structure of the glutamate transporter Glt_{ph}³¹.

A large non-protein electron density is present in the deep cleft (Fig. 2b), and although we cannot unambiguously determine its identity due to the modest resolution of the data set, this electron density is consistent with the size and shape of a (GlcNAc)₂ molecule. The shape of the ligand density allows for two orientations of (GlcNAc)₂, with the non-reducing sugar either closer to or farther away from the intracellular side (Supplementary Fig 9). It is known that *E. coli* ChbC phosphorylates (GlcNAc)₂ on the 6th position hydroxyl of the non-reducing sugar (C6-OH)¹⁹. When (GlcNAc)₂ is oriented with its non-reducing sugar ring closer to the intracellular side (Fig. 2c and Supplementary Fig. 9a), the C6-OH would be accessible for phosphorylation. This orientation would also place the C6-OH within hydrogen-bonding distance of the conserved residues Glu334 and His250, whose importance for sugar binding and phosphorylation has been demonstrated in an EIIC that transports mannitol^{16,17,32–34}. In contrast, the alternate orientation would position the C6-OH in the protein interior (Supplementary Fig. 9b) where it would not seem accessible for the required phosphorylation. In light of these observations, we deemed the former orientation to be more plausible and used it in the final model. After refinement, the hydroxyl oxygen of C6-OH is 2.6 – 2.8 Å from the carboxylate of Glu334 on HP1, and 2.7 – 3.1 Å from the ε-nitrogen on His250, which is part of the loop between TM6–7 (Fig. 2c and Supplementary Fig. 8b). We speculate that Glu334, His250, and the C6-OH may form part of an active site where transfer of a phosphate group from EIIB takes place, although the precise mechanism of catalysis is currently unknown.

Although the substrate selectivity of ChbC has not been measured systematically, it appears that the binding pocket is well suited for (GlcNAc)₂. In addition to Glu334 and His250, the side chains of conserved Trp245 from TM7, Asp290 from TM8a, and Asn333 from HP1 are able to form hydrogen bonds with the (GlcNAc)₂ when it is modelled in the orientation shown in Fig 2c. Trp382 from HP2 provides stacking interactions with the ring of the

reducing sugar. The backbone carbonyl oxygen atom of Gly297 from the conserved TM 8 loop also makes a hydrogen bond with the nitrogen atom of the N-acetyl group, and the methyl group from the acetyl group is 3.5 Å away from Tyr294, which is also from the TM8 loop. When a glucose is modelled in the binding site by aligning it with the non-reducing sugar of (GlcNAc)₂, the interactions between the acetyl group on (GlcNAc)₂ and residues Trp245 and Tyr294 are both missing, suggesting that these two interactions are important for sugar selectivity (Fig. 1b). Curiously, the substrate-binding cavity is substantially larger than necessary to accommodate a (GlcNAc)₂ molecule (Fig. 2a). In *E. coli*, ChbC was shown to also transport the trisaccharide of GlcNAc¹⁹, and the large size of this cavity suggests that *B. cereus* ChbC is able to accommodate a trisaccharide as well.

Implications for mechanism of transport

In the observed conformation, the binding pocket for (GlcNAc)₂ faces the cytoplasmic side, but the bound (GlcNAc)₂ cannot diffuse to either side of the membrane without changes in protein conformation. The crystal structure therefore likely corresponds to what is referred to as the occluded state in the terminology of the alternate access model proposed for sodium-coupled secondary transporters^{35–38}. In this framework, the full transport cycle should include at minimum two additional states: an outward-open state capable of binding substrate from the periplasm, and an inward-open state that interacts with EIIB to phosphorylate and release the substrate into the cytoplasm. Based on the known structure, we will briefly speculate on possible conformational changes leading to the other states.

The substrate-binding cavity is sealed off to the intracellular side by residues in the loop between TM4 and 5 from the neighbouring protomer (Fig. 3a). The TM4–5 loop has little interaction with the rest of the protein and could potentially be moved away to expose the bound substrate by a straightening of a kink near the N-terminus of TM5. Therefore, the TM4–5 loop seems a reasonable candidate for the intracellular gate. Once the substrate is released the strong electronegativity of the binding cavity (Fig 2a) may assist in preventing the phosphorylated sugar from rebinding to the transporter and effluxing from the cell. Although it is impossible to determine from the crystal structure alone, the involvement of structural features from both protomers in forming the binding site, along with the considerable size of the dimer interface, raises the interesting possibility that binding or release of substrate may be cooperative. Further functional and structural studies, and in particular the structure of ChbC in complex with its corresponding EIIB ChbB in the phosphorylated state, will be necessary to reveal the nature and sequence of the conformational changes leading to phosphorylation and release of the bound carbohydrate.

Because the substrate-binding site is located nearer to the cytoplasmic side of the membrane, it would require a more substantial conformational change to form the outward open state. However, similarities between ChbC and the unrelated transporter Glt_{ph} provide one possible clue. In that transporter, a rigid-body motion of a transport domain containing the substrate-binding pocket relative to an immobile oligomerization domain is responsible for conversion between the outward and inward-facing states. The oppositely-oriented re-entrant loops on the transport domain form both the substrate-binding site and the moving interface with the oligomerization domain³⁷. The parallels between the architecture of the transport domain in Glt_{ph} and the C-terminal region of ChbC containing HP1, HP2, and TM8–10 raise the intriguing possibility that a similar rigid-body motion in ChbC could be responsible for converting the inward-occluded state observed in the crystal structure to an outward-open state with an exposed binding site for external sugar (Fig 3b and Supplementary Fig. 10). This large motion would be facilitated by the generous length of the 12-residue extracellular loop between TM7 and TM8. Further structural studies will be

necessary to resolve the nature of the outward-facing state, possibly with apo-ChbC, which likely favours a conformation capable of binding periplasmic substrate.

Methods Summary

Bacillus cereus ChbC was cloned into a modified pET plasmid (Novagen) with a C-terminal polyhistidine tag connected by a TEV protease recognition site³⁹. The protein was synthesized in BL21(DE3) cells and purified on an IMAC column. After cleavage of the tag by TEV protease, the protein was then exchanged into buffer containing 150 mM NaCl, 20 mM HEPES, pH 7.5, 5 mM β -mercaptoethanol and 12 mM *n*-nonyl- β -D-maltoside, and concentrated to 6 mg/mL. Crystals in the P4₃2₁2 space group were grown by the sitting drop method in solution containing 4 mM N,N'-diacetylchitobiose, 30% polyethyleneglycol (PEG) 400, 100 mM Li₂SO₄, 0.5% polyvinylpyrrolidone, and 100 mM sodium citrate, pH 5.6. Diffraction data were collected and phased by SAD using a Ta₆Br₁₂-derivatized P4₃2₁2 crystals. Experimental phases were obtained to 4.5 Å and improved by solvent flattening and averaging, and iterative rounds of model building and refinement were then carried out to obtain the final model.

For the functional assays, ChbC was reconstituted in liposomes following a method described for a K⁺ channel⁴⁰. Uptake of ¹⁴C-GlcNAc (45 Ci/mmol; Moravek) was measured in buffer containing 100 mM potassium phosphate, pH 7.5 for varying periods of time. The reactions were quenched with ice-cold buffer containing 100 mM potassium phosphate, pH 6.0 and 100 mM LiCl, and immediately filtered through GF/F filters (Advantec MFS, Inc.). GlcNAc uptake was quantified by comparing scintillation counts of the filters with standard curves from known amounts of ¹⁴C-GlcNAc.

Full Methods

Target selection, cloning and initial protein production—ChbC was established as a pipeline target for structural studies by a bioinformatics analysis^{39,41}. A total of 25 ChbC genes from 13 prokaryotic genomes were identified, amplified by PCR from the genomic DNAs, and cloned into a modified pET plasmid (Novagen) with a C-terminal deca-histidine tag and a TEV protease recognition site. ChbC genes were then over-expressed in *E. coli* BL21(DE3) cells in small scale cultures (~1 mL), and the translation level examined using Western blots. The target selection, cloning, and protein production screening were performed at the central facility of the New York Consortium on Membrane Protein Structure (NYCOMPS) as described in Love *et al.*³⁹.

Protein purification and crystallization—Seven western-positive clones received from the NYCOMPS were scaled up for mid-scale (1 liter) purification studies. ChbCs from *Salmonella enterica* and *Bacillus cereus* yielded higher than 0.25 mg/liter cell culture. While both proteins exhibited a mono-dispersed profile in size-exclusion chromatography, only ChbC from *Bacillus cereus* (ChbC) produced diffracting crystals and thus became the focus of crystallization efforts. After cleavage of the deca-histidine tag, the protein contains the full length ChbC protein, residues 1 to 434, plus nine residues (AAAENLYFQ) at the C-terminus due to addition of a cloning site and the TEV protease recognition site.

For large-scale (10–20 L) production and purification of native ChbC, cells were grown in Luria broth at 37°C and induced with 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) after the OD_{600nm} reached ~1.0. ChbC extraction and purification followed a protocol described in ref⁴². After removal of the His tag with TEV protease, the protein was concentrated to ~6 mg/ml and subjected to size exclusion chromatography with a Superdex 200 10/300 GL column (GE Health Sciences) equilibrated in 150 mM NaCl, 20 mM HEPES

pH 7.5, 5mM β -mercaptoethanol and 12 mM *n*-nonyl- β -D-maltoside (NM). Purified ChbC protein was concentrated to ~10 mg/ml as approximated by ultraviolet absorbance.

ChbC crystals with $P4_32_12$ symmetry were grown over a period of two weeks or longer by vapor diffusion in sitting drops mixed from 2–3 μ l of the protein solution supplemented with 4 mM N,N'-diacetylchitobiose and an equal volume of well solution containing 30% polyethyleneglycol (PEG) 400, 100 mM Li_2SO_4 , 0.5% polyvinylpyrrolidone, and 100 mM sodium citrate pH 5.6. Tantalum-derivatized crystals were prepared by adding $\text{Ta}_2\text{Br}_{12}$ powder into sitting drops containing ChbC crystals. The crystals gradually turned green after 24–48 hours, and were directly flash-frozen in liquid nitrogen for X-ray diffraction. The $P4_32_12$ crystals diffracted to resolutions of up to 3.3 Å and the Tantalum-derivatized crystals to 4.5 Å.

Transport measurements in proteoliposomes—Purified ChbC was reconstituted at a 1:100 (w/w) ratio into lipid vesicles composed of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (Avanti Polar Lipids) in a ratio of 3:1 (w/w) in 100 mM potassium phosphate, pH 7.5 as described previously⁴⁰. Prior to the uptake reaction frozen ChbC-containing proteoliposomes and control liposomes (at 10 mg lipid/mL) were subjected to three freeze/thaw cycles followed by extrusion through a 400 nm polycarbonate membrane (Avestin). Uptake of 92 μ M ^{14}C -GlcNAc (45 Ci/mmol; Moravsek) was measured at 23°C in assay buffer composed of 100 mM potassium phosphate buffer, pH 7.5 for the indicated periods of time. The reactions were stopped by quenching the samples with ice-cold 100 mM potassium phosphate, pH 6.0/100 mM LiCl, followed by rapid filtration through GF/F filters (Advantec MFS, Inc.) and scintillation counting of the filters. Known amounts of ^{14}C -GlcNAc were used to convert the amount of internalized radioactivity into nmol per mg of protein. The amount of ChbC in the proteoliposomes used for the uptake reaction was determined⁴³.

Data collection and structure solution—Diffraction data were collected on beamlines X25 and X29 at the National Synchrotron Light Source and 24ID-C and 24ID-E at the Advanced Photon Source. Due to the long c-axis, crystals were re-oriented using mini-kappa to avoid overlaps. The data were indexed, integrated and scaled using HKL2000⁴⁴. The experimental phases were determined to 4.5 Å by SAD using a dataset collected at the tantalum L-III absorption edge on a crystal derivatized with $\text{Ta}_6\text{Br}_{12}$. The positions of 12 Ta sites corresponding to two clusters were located using SHELXD⁴⁵ and refined with SHARP⁴⁶. The experimental phases were calculated using SHARP and improved by solvent flattening with DM⁴⁷. The resultant density-modified map allowed for identification of molecular boundaries for four chains in the asymmetric unit by manual inspection. Within the boundaries of each of the four chains in the asymmetric unit, C α traces for several helical fragments were positioned manually at equivalent regions. The C α coordinates of these fragments were then used to calculate NCS operators. Subsequent four-fold NCS averaging, solvent flattening, and histogram matching were done with DM to extend phases to a 3.3 Å native dataset. After several rounds of refinement with a polyalanine model, sufficient side chain density became apparent to assign a sequence register. Manual model building was done with COOT⁴⁸, and structure refinement was done using PHENIX⁴⁹ with four-fold NCS restraints. At the final round of refinement, (GlcNAc)₂ molecules were modelled into the clear electron density features in the Fo-Fc map. In the final refined model, all four protomers contain the full length ChbC except for the two N-terminus residues that are not resolved. The additional nine residues added during the cloning process are not resolved for three of the protomers, and partially resolved (435–438) in the fourth protomer. In addition, each asymmetric unit has four (GlcNAc)₂ molecules, four partially resolved NM molecules built with only the maltose head group, four NM molecules, and 6 citrates.

Electrostatic potentials were calculated with DelPhi⁵⁰ by solving the nonlinear Poisson-Boltzmann equation at physiological ionic strength (0.145 M). The calculations used a 1.4 Å probe radius, interior dielectric constant of 4, solvent dielectric constant of 80, Debye-Hückel boundary conditions with a grid size of 251.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Function and structure of ChbC

(a) Time course of the uptake of 92 μM ^{14}C -labelled GlcNAc in proteoliposomes reconstituted with *B. cereus* ChbC (red square) or in control liposomes (black circle). (b) Accumulation after 30 minutes of ^{14}C -labelled GlcNAc in ChbC-containing proteoliposomes in the absence or presence of 10 mM unlabelled GlcNAc, (GlcNAc)₂, or glucose. (c) A cartoon representation of a ChbC protomer is shown from two orientations. (d–g) The structure of the ChbC dimer is shown in cartoon and surface representations as viewed from (d, e) within the plane of the membrane, represented as a gray rectangle, and (f, g) the intracellular side of the membrane. (h–i) The same views of the ChbC dimer, but with the back protomer shown as an opaque surface and as an outline in the front. Helices TM1–5 are shown as either a darker surface (back) or as colored cylinders (front).

Figure 2. The C-terminal sugar binding domain

(a) Cross-section of the solvent-accessible surface of the ChbC dimer, colored by electrostatics as calculated by the program DelPhi⁵⁰. Bound (GlcNAc)₂ molecules are shown in cyan. (b) The C-terminal domain and bound (GlcNAc)₂ molecule viewed from the plane of the membrane. The green mesh corresponds to F_o-F_c density calculated in the absence of (GlcNAc)₂ and contoured at 2.5 sigma. The inset on the upper left shows the location of the C-terminal domain in the dimer. (c) The sugar-binding pocket viewed from the intracellular side. A (GlcNAc)₂ molecule is shown modelled in the orientation placing the C6-OH of the non-reducing sugar (red arrow) closest to the cytoplasm, along with residues potentially forming hydrogen bonds or hydrophobic interactions with the sugar.

Figure 3. Proposed conformational changes in sugar transport

(a) On the intracellular face of ChbC (left panel), helix TM5 and the TM4–5 loop from one protomer cover the binding pocket for (GlcNAc)₂ (shown as a pink surface) on the opposite protomer. The right panel corresponds to the region marked on the left with a rectangle, zoomed-in and rotated to view from within the plane of the membrane. Straightening of a kink in TM5 (red star) could potentially expose the substrate-binding site to the cytoplasm.

(b) The substrate is occluded (left panel, crystal structure) from the periplasmic side by the highlighted region containing TM8–TM10 (green), HP1 (orange) and HP2 (brown), connected to the remainder of the protein by the TM7–8 loop (red). A rigid-body rotation of this region could potentially move and expose the substrate-binding site to the periplasmic space (right panel, model). The short helix between TM1 and TM2 is omitted for clarity.