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Review

Structural insight into the PTS sugar transporter EIIC☆

Jason G. McCoy, Elena J. Levin, Ming Zhou *

Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA

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ABSTRACT

Background: The enzyme IIC (EIIC) component of the phosphotransferase system (PTS) is responsible for selectively transporting sugar molecules across the inner bacterial membrane. This is accomplished in parallel with phosphorylation of the sugar, which prevents efflux of the sugar back across the membrane. This process is a key part of an extensive signaling network that allows bacteria to efficiently utilize preferred carbohydrate sources.
Scope of review: The goal of this review is to examine the current understanding of the structural features of the EIIC and how it mediates concentrative, selective sugar transport. The crystal structure of an N,N′-diacetylchitobiose transporter is used as a structural template for the glucose superfamily of PTS transporters.
Major conclusions: Comparison of protein sequences in context with the known EIIC structure suggests that members of the glucose superfamily of PTS transporters may exhibit variations in topology. Despite these differences, a conserved histidine and glutamate appear to have roles shared across the superfamily in sugar binding and phosphorylation. In the proposed transport model, a rigid body motion between two structural domains and movement of an intracellular loop provide the substrate binding site with alternating access, and reveal a surface required for interaction with the phosphotransfer protein responsible for catalysis.
General significance: The structural and functional data discussed here give a preliminary understanding of how transport in EIIC is achieved. However, given the great sequence diversity between varying glucose-superfamily PTS transporters and lack of data on conformational changes needed for transport, additional structures of other members and conformations are still required. This article is part of a Special Issue entitled: Structural biochemistry and biophysics of membrane proteins.

1. Introduction

The PTS is a multiple component carbohydrate uptake system that drives specific saccharides across the bacterial inner membrane while simultaneously catalyzing sugar phosphorylation. The proteins composing the PTS include a series of soluble phosphotransferases, and an integral membrane protein responsible for transport of the sugar into the cell. The phosphorylation state of the soluble components is indicative of intracellular carbohydrate levels and provides a mechanism for regulating carbohydrate metabolism [1–3]. In this review we discuss the structural basis for the functions of the transmembrane component of the PTS, including recognition of the cognate substrate, the mechanism of transport, and its role in catalyzing the phosphotransfer reaction. We focus on the glucose superfamily of PTS transporters, including the recently solved structure of the N,N′-diacetylchitobiose transporter, bcChbC, as well as the *Escherichia coli* transporters PtsG (glucose transporter), MtlA (mannitol transporter), and BglF (β-glucoside transporter) as the best-characterized representatives of their respective families.

1.1. Classification of PTS transporters

Phylogenetic analysis indicates that PTS transporters originate from at least four independent sources [4,5]. Of these four superfamilies, the glucose superfamily of PTS transporters is the largest and the primary focus of this review [4,6]. Five distinct subfamilies of proteins have been identified within the glucose superfamily: the lactose family, the glucose family, the β-glucoside family, the mannitol family, and the fructose family [6]. It has been suggested that the fructose-specific transporter is the oldest, followed by the mannitol-specific transporter [5]. The glucose and β-glucoside transporters show greater similarity to each other than to the fructose and mannitol transporters and the lactose transporters are the most divergent [5]. Though homologous, the PTS transporters of different family members show a great deal of sequence diversity. For example, the transport domains of bcChbC, PtsG, MtlA, and BglF only share between 17 and 19% identity between them. Although the families are named for specific sugars, the selectivity

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* Corresponding author.

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of individual members within each family can vary considerably. For instance, the bcChbC protein is a member of the lactose family, but is selective for N,N′-diacetylchitobiose.

1.2. Components and organization of the phosphotransferase system

The PTS is composed of several proteins that serially transfer a phosphate moiety until it is ultimately attached to a sugar molecule (Fig. 1A). The glycolytic intermediate phosphoenolpyruvate provides the initial phosphate [7] which is then transferred to enzyme I (EI), heat-stable phosphocarrier protein (HPr), and subsequently to enzyme II (EII). EII is composed of three separate domains named EIIA, EIIB, and EIIC. The phosphate is serially transferred from HPr to EIIA, and then to EIIB. The number of phosphate inversions as measured through isotopic substitution indicates that the phosphate is then transferred directly from EIIB to the sugar without formation of a covalent EIIC-phosphate intermediate; however, this transfer only occurs when the sugar is bound to EIIC [8]. Bacteria typically contain multiple PTS systems for uptake of different sugars. EI and HPr are not selective and are shared by different PTS systems. In contrast to EI and HPr, EIIA and EIIB have been shown to have different folds between different subfamilies of the PTS glucose superfamily and are not interchangeable [9–16].

The EIIA, EIIB, and EIIC domains can be expressed as a single protein or as distinct polypeptides (Fig. 1B). The order of connected domains varies as well. While bcChbC consists of just the EIIC domain, the β-glucoside transporter BglF is composed of an N-terminal EIIB followed by EIIC and then EIIA [17], the mannitol transporter MtlA contains EIIC followed by EIIB and EIIA [18], and the glucose transporter PtsG contains an EIIC domain followed by an EIIB [19]. This variability occurs even within individual subfamilies. For example, the *E. coli* glucose family member TreB (trehalose transporter) has the EIIB domain before the EIIC. The connectivity and arrangement of these domains appears to have effects on function. For the glucose transporter PtsG, separation of the EIIB and EIIC components leads to a 50-fold decrease in phosphotransfer [20], and scrambling the order of domains in BglF from BCA to CBA prevents phosphotransfer to the sugar [21].

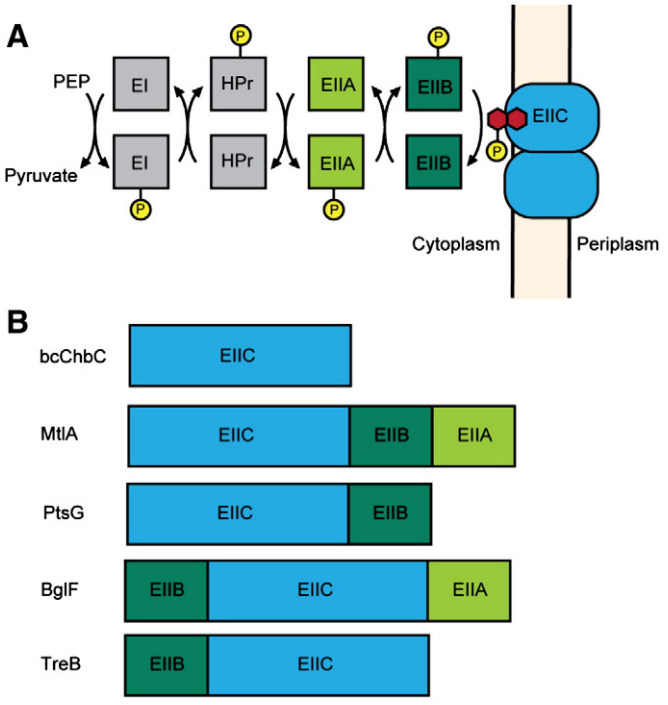

Fig. 1. Organization of the PTS. A. Flow diagram illustrating sequence of phosphorylation events leading to the addition of phosphate (yellow) to the EIIC transporter-bound sugar molecule (red). B. The EIIC domain can be translated individually or as a multidomain protein containing EIIB and/or EIIA.

2. The structure of EIIC

The membrane-embedded EIIC domain forms a dimer [22–24] and is responsible for selective binding and transport of sugar molecules [18,25,26]. While many NMR and crystallographic structures of EIIA and EIIB domains have been reported, including the isolated domains [9–16], complexes with each other [27–29], and complexes with other transporters [30]; obtaining high resolution structures of the EIIC domain has proven difficult. Previous efforts have resulted in EM projection maps of MtlA (5 Å) and PtsG (12 Å), both of which confirmed the dimeric assembly of the EIIC but were of insufficient resolution to provide atomic level information about the protein [31,32]. The first 3D crystal structure of an EIIC domain was reported in 2011 [24]. The structure of bcChbC, an N,N′-diacetylchitobiose transporter from *Bacillus cereus* (PDB ID: 3QNQ), was solved to a resolution of 3.3 Å and is described below [24].

2.1. Overall fold of bcChbC

The bcChbC protein contains just the EIIC component, and its corresponding EIIB and EIIA are encoded by separate genes [33]. The protein was purified in detergent and eluted from a gel filtration column as a dimer. Each monomer consisted of 10 transmembrane (TM) helices with the N and C termini facing the cytoplasmic side of the protein. These transmembrane domains form two separate domains: the N-terminal oligomerization domain made up of TM1–5, and a C-terminal transport domain containing TM6–10 (Figs. 2 and 3). These two domains are connected by an amphipathic helix (AH2) on the periplasmic side of the membrane. The C-terminal domain also contains two reentrant helical hairpins. The first reentrant loop is located on the cytoplasmic side between TM8 and 9 and contains two helices, HP1a and HP1b. The second reentrant loop is between TM9 and 10 on the periplasmic side, and contains only one helix (HP2). TM8 is split into two helices connected by a short, flexible loop (TM8a and TM8b).

The bcChbC dimerization interface has an expansive buried surface area of 2746 Å^2 (Fig. 2A). The dimer has approximate 2-fold symmetry along an axis perpendicular to the plane of the membrane. Most of the interface is mediated by TM helices 1, 2, 3, and 5 from the N-terminal domain of each monomer. Unlike the extensive interface between the two protomers, the interface between the N-terminal oligomerization and C-terminal transport domains is quite small due to the presence of a large cavity in both monomers lined by TM 1, 6, 7, and 8, HP1b, the loop preceding HP2, and the TM4–TM5 loop from the other monomer. This cavity contains a bound N,N′-diacetylchitobiose molecule in each protomer. Consequently the oligomerization and sugar transport/phosphorylation functionality appear to be segregated to the N-and C-terminal domains respectively. One exception to this is the TM4–TM5 loop, which extends across the N-terminal domain of its dimeric partner to make contact with both N,N′-diacetylchitobiose and HP1b within the other protomer (Fig. 2A).

2.2. The ligand binding site in bcChbC

The N,N′-diacetylchitobiose binding site is formed by helices TM6, TM7, HP1b, TM8, and the reentrant loop containing HP2 in both bcChbC monomers (Fig. 2B). The nonreducing monomer of the N,N′-diacetylchitobiose disaccharide has extensive interactions with the protein. OH-6 forms hydrogen bonds with E334 on HP1b and H250 on the cytoplasmic loop connecting TM6 and TM7, while OH-3 and OH-4 form hydrogen bonds with the sidechain and backbone amide of N333 from HP1b. N-2 forms a hydrogen bond with the backbone carbonyl of G297 on TM8. OH-7 forms a hydrogen

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