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Activity of the *Enterococcus faecalis* EIIA^{gnt} PTS component and its strong interaction with EIIB^{gnt}

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ABSTRACT

Eubacteria can import and simultaneously phosphorylate a range of different carbohydrates by means of sugar specific phosphoenolpyruvate (PEP) dependent sugar phosphotransferase systems (PTSs). Here, we report the biochemical characterization of the gluconate specific PTS component EIIA^{gnt} from *Enterococcus faecalis* and its unexpectedly strong complex with EIIB^{gnt}. We analyze the activity of the complex regarding phosphoryl transfer using kinetic measurements and demonstrate by mutagenesis that His-9 of EIIA^{gnt} is essential for this process and represents most likely the phosphoryl group carrier of EIIA^{gnt}. With a combination of isothermal titration calorimetry (ITC), analytical ultracentrifugation (AUC), native gel electrophoresis and chemical crosslinking experiments we show that EIIA^{gnt} and EIIB^{gnt} form a strong 2:2 heterotetrameric complex, which seems to be destabilized upon phosphorylation of EIIB^{gnt}.

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Introduction

Eubacteria can import and simultaneously phosphorylate carbohydrates such as hexitols mono- and disaccharides by means of phosphoenolpyruvate (PEP) dependent sugar phosphotransferase systems (PTSs), thereby concentrating a sugar up to 10^6-fold in the cell [1–4]. The sugar phosphorylation occurs as last and irreversible step of a phosphorylation cascade that transfers a phosphate group from PEP via EI and HPr to the sugar specific EII^{sugar} components. There, EIIA accepts the phosphate group and passes it on to the hydrophilic domain EIIB which subsequently phosphorylates the sugar during its passage through the corresponding EIIC or EIIC/D membrane channel [1,5–8] (Fig. 1). For an efficient utilization of available carbohydrate sources, PTSs are connected to a number of cellular processes like chemotaxis, carbon catabolite repression/activation and signaling, including the direct regulation of other import systems as well as the regulation of gene expression via different mechanisms [1,9–13].

The general mechanism of the phosphoryl transfer appears to be similar for the various EII^{sugar} systems. However, differences are observed regarding the structure and organization of the individual components into single and/or multidomain proteins [1,14]. Thus, the EII^{sugar} systems are commonly divided into four (super)families (glucose/saccharose, mannitol/fructose, lactose/chitobiose and mannose) of which at least the EIIA and EIIB components adopt rather different folds [1,2,14,15] and oligomerization states [1].

Among the mannose family of PTS transporters [16] a gluconate specific EII system originating from the low GC content Gram positive bacterium *Enterococcus faecalis* was found. It consists of an EIIA, -B and -C component as well as a mannose family typical EIID component, all of which are present as separate polypeptide chains. This was also observed for the mannose family *Bacillus subtilis* levan- and *Klebsiella pneumoniae* sorbose specific EII components, but not for *Escherichia coli* EII^{man}, where EIIA and EIIB are present as single polypeptide chain. Despite the early discovery of the *E. faecalis* gluconate PTS in 1982 by Bernsmann et al. [17], no further characterization has been performed up to now. In

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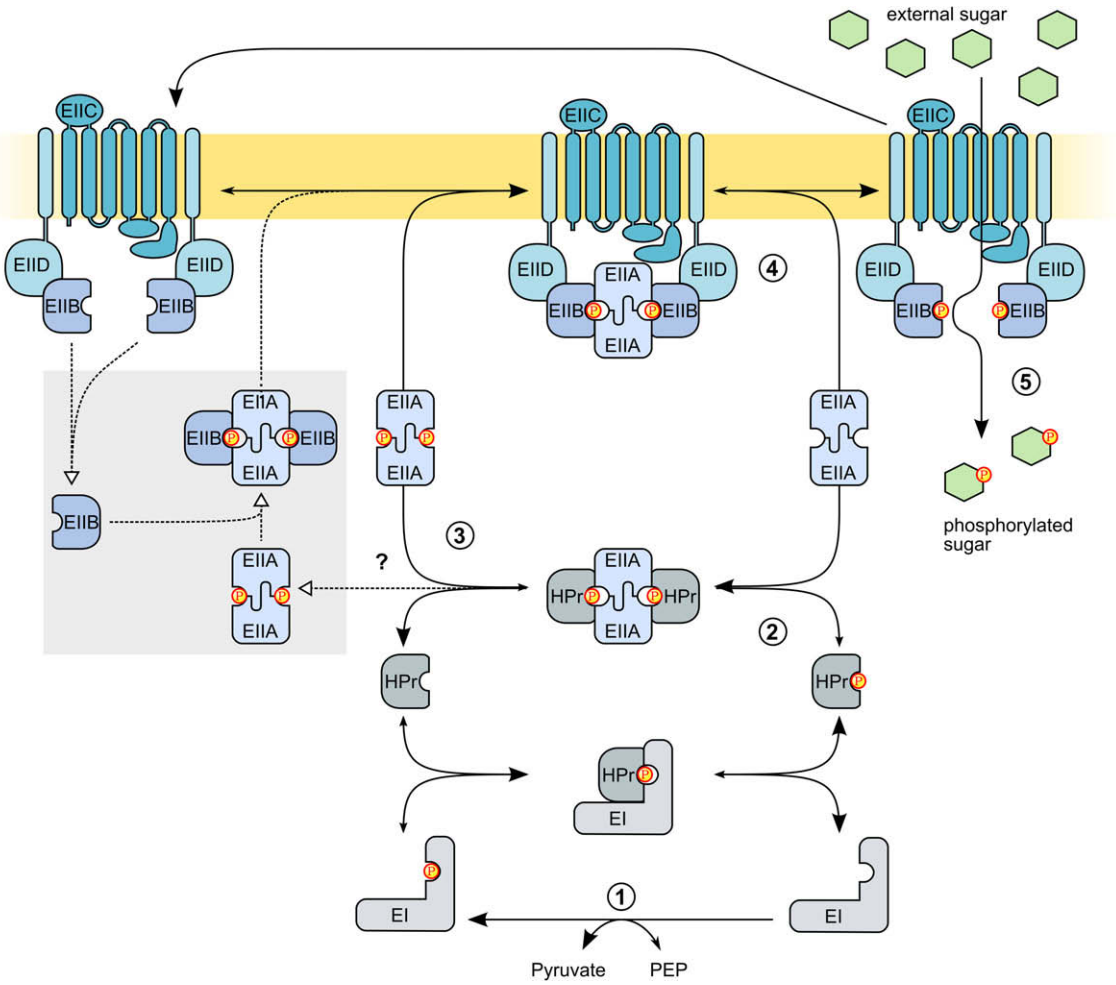

Fig. 1. Roles of EIIA and EIIB in the gluconate specific PTS from *E. faecalis*. The EIIC/D complex is shown according to the closely related mannose specific EIIC/D complex from *E. coli* [30]. Arrowheads are sized differently for clarity only. 1 – A phosphate group is transferred from PEP to EI; HPr binds P∼EI and becomes in turn His-15 phosphorylated. 2 – P∼HPr binds EIIA, transfers the phosphoryl group to His-9 and dissociates. 3 – P∼EIIA forms a complex with EIIB and transfers the phosphoryl group. It is not clarified yet if EIIB is transient (dotted arrows and area with gray background) or constantly associated with the membrane bound EIIC/D complex. 4 – The strongly bound EIIA dimer dissociates from EIIB, eventually due to EIIB being phosphorylated and bound to EIIC/D. 5 – EIIC imports sugar molecules from the outside of the cell, which are phosphorylated by P∼EIIB during the transfer.

particular, it is not clear whether EIIA^{gnt} and EIIB^{gnt} of the *E. faecalis* gluconate specific PTS (UniProtKB accession codes Q82ZC8 and Q82ZC7) form a stable complex or interact only transiently. Here we report the biochemical analysis of the *E. faecalis* EIIA^{gnt}:EIIB^{gnt} complex including its composition, stability and its kinetic properties. Based on the thermodynamic and kinetic data of our study, we can show that both EIIA^{gnt} and EIIB^{gnt} are catalytically active and form an unusually strong complex. Furthermore, we show that His-9 of EIIA^{gnt} is most likely the phosphoryl carrying residue and that the EIIA^{gnt}:EIIB^{gnt} complex appears to be destabilized upon phosphorylation.

Materials and methods

Cloning, expression and purification of E. faecalis EIIA^{gnt} H9A and EIIB^{gnt}. The purification of *E. faecalis* EIIA^{gnt} H9A was performed as described previously for EIIA^{gnt} [18]. In case of EIIB^{gnt}, following the anion exchange chromatography step, protein containing fractions were dialyzed for 3 h in LyB (50 mM Tris–HCl pH 7.5, 0.1 mM dithiothreitol (DTT), MWCO 10,000 kDa), diluted by a factor of three with LyB and applied to a P11 cellulose (Whatman) cation exchange chromatography column equilibrated with LyB. The column was then washed with 17 column volumes of LyB and the bound protein eluted with a linear NaCl gradient increasing from 0 to 1.0 M NaCl. Afterwards, the purification was continued as described before including concentration, desalting and lyophilization steps.

Native PAGE gels. Standard 12% native PAGE gels with a glycine/Tris buffer system were cast, several micrograms protein loaded as indicated and the electrophoresis performed with 30 mA/120 V at room temperature until the bromphenol band exited the gel. The protein bands were then stained with Coomassie Brilliant Blue R250 for visual inspection [19].

Analytical ultracentrifugation (AUC). For sedimentation and equilibrium centrifugation experiments a Beckmann Optima-XLA Ultracentrifuge was utilized. All measurements were performed at 20 °C and recorded at a wavelength of 280 nm, see Table 1a for further experimental parameters. Equilibrium centrifugation experiments were stopped once the concentration gradient was stable for a minimum of 12 h. In sedimentation centrifugation experiments, data sets were recorded at equidistant time intervals. Analysis of the data was done with the software package BPCFIT [20].

Isothermal titration calorimetry (ITC). The protein samples were degassed and equilibrated for 15 min at 25 °C. For the measurement a MCS-ITC calorimeter from Microcal was utilized and

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