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Critical role of a conserved transmembrane lysine in substrate recognition by the proton-coupled oligopeptide transporter YjdL

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ABSTRACT

Proton-coupled oligopeptide transporters (POTs) utilize an electrochemical proton gradient to accumulate peptides in the cytoplasm. Changing the highly conserved active-site Lys117 in the *Escherichia coli* POT YjdL to glutamine resulted in loss of ligand affinity as well as inability to distinguish between a dipeptide ligand and the corresponding dipeptide amide. The radically changed pH_{Bulk} profiles of Lys117Gln and Lys117Arg mutants indicate an important role of Lys117 in facilitating protonation of the transporter; a notion that is supported by the close proximity of Lys117 to the conserved ExxERFxYY POT motif previously shown to be involved in proton translocation. These results point toward a novel dual role of Lys117 in direct or indirect interaction with both proton and peptide.

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1. Introduction

Proton-coupled oligopeptide transporters (POTs) are responsible for facilitating the intracellular accumulation of di- or tripeptides, thereby providing the organism with vital nutrients that are readily converted into amino acids, nitrogen and carbon sources. As typical members of the major facilitator superfamily (MFS), POTs display a structure that harbors a substrate-binding cavity, located in between two 6-helix bundles, that can exist in three overall conformational states: (i) the outward-facing conformation allowing the substrate and a proton to bind; (ii) the occluded conformation closing off both ends of the active site; and (iii) the inward-facing conformation releasing both substrate and proton into the cytoplasm (Fig. 1A) (Doki et al., 2013). Four different bacterial POT structures, $PepT_{So}$ and $PepT_{So_2}$ from *Shewanella oneidensis* (Guettou et al., 2013; Newstead et al., 2011), $PepT_{St}$ from *Streptococcus thermophilus* (Solcan et al., 2012), and GkPOT from *Geobacillus kaustophilus* (Doki et al., 2013) have been determined so far in the overall inward-facing conformation (Fig. 1B). The two other overall structural conformations have been reported for other MFS members, e.g. EmrD (occluded) (Yin et al., 2006) and FucP (outward-facing) (Dang et al., 2010).

POTs have very broad substrate specificity, and for some organisms it has been shown to span almost all possible amino acid combinations (Fei et al., 1994; Theis et al., 2001). Although di- and tripeptides are the most commonly recognized substrates for POTs, certain POT family members have diversified their substrate specificity toward related molecules such as single amino acids, e.g. the human peptide/histidine transporter, hPHT (Bhardwaj et al., 2006), or NRT1.1 of *Arabidopsis thaliana* which transports the unrelated nitrate ion (Tsay et al., 2007). A wealth of mutagenesis and functional data have accumulated through studies on mammalian POTs (Bolger et al., 1998; Chen et al., 2000; Fei et al., 1997; Hauser et al., 2005; Kulkarni et al., 2003; Pieri et al., 2009; Terada et al., 1996; Uchiyama et al., 2003; Xu et al., 2009) and several bacterial POTs (Doki et al., 2013; Jensen et al., 2012a, 2012b; Malle et al., 2011; Solcan et al., 2012). These studies generally support the peptide binding mode observed independently from the structure of GkPOT (Doki et al., 2013) and $PepT_{So_2}$ (Guettou et al., 2013) in complexes with the dipeptide analog alafosfalin, which is a substrate of prototypical POTs (Fig. 1C). In GkPOT, alafosfalin binding is mediated by hydrogen bonds between the phosphonate group, tyrosine residues 40 and 78 that are highly conserved among POTs with experimentally proven peptide-translocating abilities (Jensen

Abbreviations: AMCA, 7-amino-4-methylcoumarin-3-acetic acid; IPTG, isopropyl β-D-1-thio-galactopyranoside; MFS, major facilitator superfamily; POTs, proton-coupled oligopeptide transporters.

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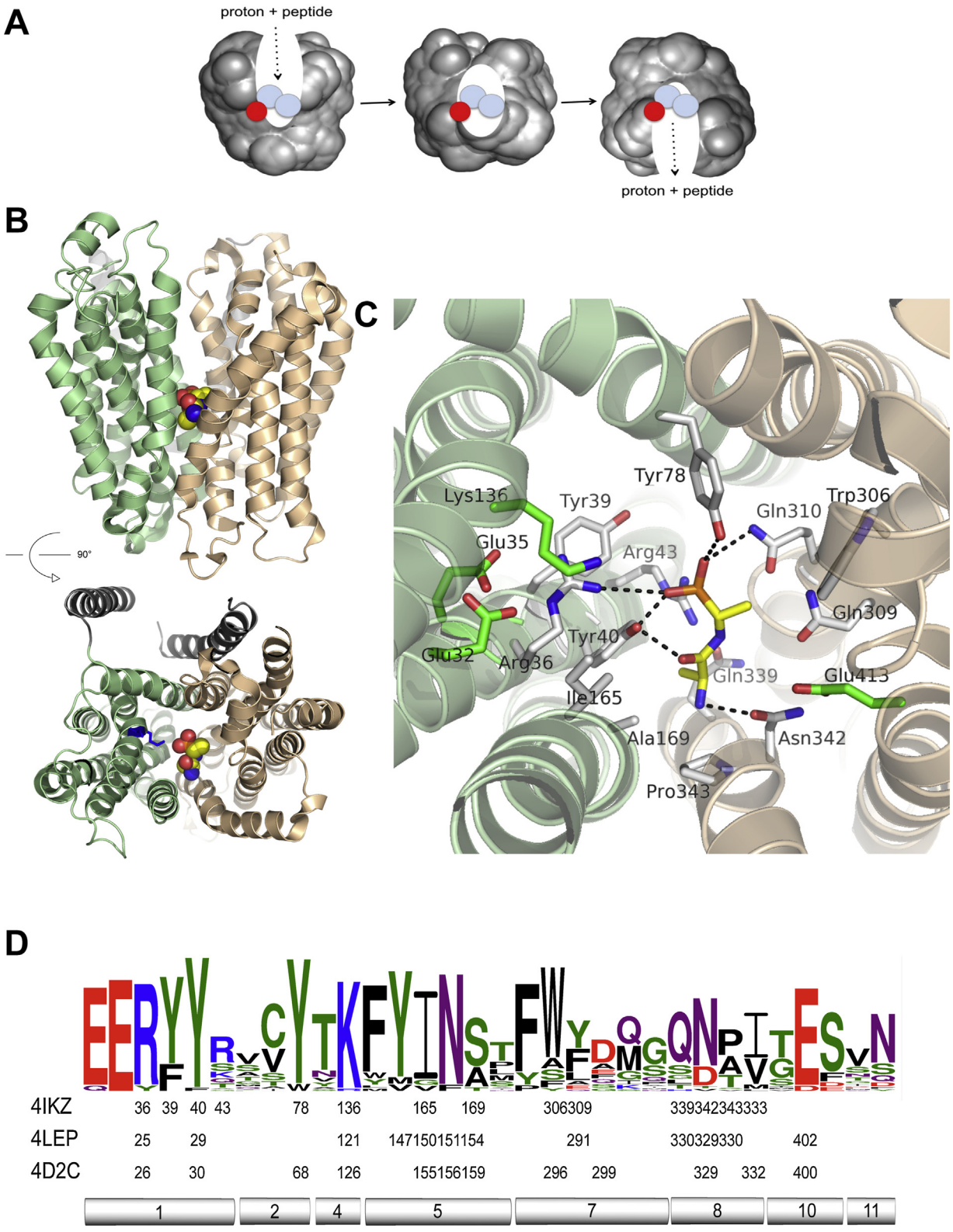

Fig. 1. (A) Schematic view of the overall structural changes in the POT transport cycle; the proton is represented as a red sphere while the peptide is represented by two light blue spheres. (B) Cartoon representation of the GkPOT (PDB ID: 4IKZ) with the N-terminal domain colored green and the C-terminal domain colored light brown. The bound alafosfalin is shown in vdW spheres representation and Lys136 is shown as blue sticks. (C) A close-up view of the peptide-binding site of GkPOT (PDB ID: 4IKZ). Residues in proximity (<5 Å) of the bound alafosfalin (C-atoms yellow) are in the sticks representation shown as white sticks. Adjacent residues with functional importance, but more than 5 Å away from alafosfalin, are shown in green. (D) Sequence logo based on an alignment of experimentally verified peptide-translocating POTs (Fig. S1). Only residues within 8 Å of alafosfalin have been included. Residue numbering below the logo indicates the actual residue in contact with alafosfalin from GkPOT (PDB ID: 4IKZ), $PepT_{So_2}$ (PDB ID: 4LEP) or $PepT_{St}$ (PDB ID: 4D2C), respectively. The numbered horizontal bars indicate which helix the logo residues belong to. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

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