# *SESSION 4: Peptide transport*

## Towards a structural understanding of drug and peptide transport within the SLC15 proton dependent oligopeptide transporter (POT) family

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The principle route by which organisms obtain bulk quantities of amino acids is through the transport of di- and tri-peptides across cellular membranes. This process is mediated through a large and diverse family of integral membrane proteins called the proton dependent oligopeptide transporter (POT) family, also referred to simply as the peptide transporter or PTR2 family [1]. POT family transporters are found in all organisms, with the notable exception of the archaea, and use the proton electrochemical gradient to drive the uptake of di- and tri-peptides across cell membranes. The human genome contains four members of the POT family, which belong to the SLC15A1-A4 gene family and are called PepT1, PepT2, PHT2 and PHT1. The PepT1 gene was first identified in 1994, and was quickly established as the principle route through which peptides are absorbed across the enterocyte brush border membrane in the small intestine [2]. The closely related PepT2 protein was later identified in kidney cells, and is commonly referred to as the renal peptide transporter, although the tissue distribution of PepT2 is significantly broader, with confirmed reports of expression in the liver, lung, testis, and central nervous system [3]. The physiological role of PepT2 in these diverse organs is however still unclear. Early work on peptide transporter physiology discovered that peptide transporters were primarily responsible for the oral bioavailability of many commonly prescribed drug molecules, most notably the beta-lactam antibiotics and certain angiotensin converting enzyme inhibitors. This additional function was ascribed to the similar stereochemical properties these drug molecules have to naturally occurring peptides, with main consensus being that these drugs were simply ‘carried along for the ride’. This realisation led to the development of specifically engineered peptide pro-drugs; molecules that can be attached to peptide scaffolds and subsequently utilise the widespread tissue distribution of peptide transporters to improve their pharmacokinetic properties, most notably oral bioavailability. There are currently a number of peptide pro-drugs being routinely prescribed to patients, such as the antiviral valacyclovir and the vasopressor midrodrine, all of which take advantage of endogenous peptide transporters to improve both efficacy and bioavailability over the original parent molecule [4].

Although the primary structure, tissue distribution and substrate specificity have all been investigated for mammalian peptide transporters, there is currently no structural information on these proteins, which has severely hampered further investigation into the molecular mechanism of peptide transport and the utilisation of these proteins as ‘Trojan horses’ for drug delivery. To gain much needed insight

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into the molecular mechanism of transport we determined the crystal structure of a prokaryotic member of the POT family, from the bacterium *Shewanella oneidensis*, $PepT_{So}$ [5]. $PepT_{So}$ shows a high degree of sequence conservation within the transmembrane region (~30 % identity) to the mammalian PepT1 and PepT2 proteins and associated functional data reveal similar kinetic properties supporting a common mechanism of peptide transport. $PepT_{So}$ contains 14 transmembrane helices, of which helices H1-H12 adopt the overall fold observed previously in other members of the MFS transporter superfamily, with the N- and C-terminal six helical bundles, formed by helices H1-H6 and H7-H12, coming together to form a V-shaped transporter, with both halves related by a pseudo-two fold symmetry axis running normal to the membrane plane. $PepT_{So}$ is the largest member of the MFS family for which a crystal structure is available, with the additional two helices being formed from the large intracellular loop connecting the end of helix H6 to the start of helix H7. This raises interesting questions as to the role these additional helices in the biochemistry of bacterial peptide transporters and more generally within the MFS.

The structure revealed a previously unobserved ligand bound occluded state, with an unidentified ligand bound within a large central cavity that is closed to both side of the membrane. Many of the residues conserved between $PepT_{So}$ and the mammalian peptide transporters cluster around this cavity. The cavity is striking for the number and placement of charged residues that cluster to opposite ends to create a potential macro dipole across the two halves of the transporter. This orientation of charged residues within the binding site suggests a possible mechanism of peptide recognition, with the amino terminus of peptides interacting with the conserved negative glutamic acid on the C-terminal six helical bundle and the carboxy termini interacting with the conserved cluster of positively charged arginines and lysine residues on the opposing, N-terminal bundle. The additional placement of three conserved tyrosine residues is also reminiscent of many antigen recognistion sites, where tyrosines play pivotal roles in providing a flexible, hydrophobic environment for side chain accommodation. These characteristics could also explain the specificity we observe within the POT family for di- and tri-peptides, as the dimensions of the cavity are only sufficient to accommodate peptides of these lengths, with larger tetra-peptides being sterically restricted and single amino acids unable to interact with both halves of the transporter and so presumably fail to trigger the required conformation changes to re-orientate the binding site across the membrane.

The occluded conformation reveals many intriguing new features concerning possible mechanisms for transport, both generally within the MFS superfamily and more specifically within the POT peptide transporters. The most obvious of which is the potential significance of the extracellular cavity we observe opening out towards the extracellular side of the membrane. The cavity causes $PepT_{So}$ to adopt an overall asymmetric state, breaking the pseudo two-fold symmetry observed in previous MFS structures. We are currently working to determine the significance of this with respect to specific

conformational changes within the C-terminal helical bundle during transport. The structure however does suggest that within the POT family at least, it is the C-terminal six helical bundle that may contain the mobile gates, both extracellular and intracellular, that alternately open and close around peptides to move them across the membrane. Interestingly, observed at the base of the extracellular cavity is a conserved histidine residue, shown to be protonated during the transport cycle in PepT1. Mutating this residue in $PepT_{So}$ also abolishes transport; raising the question as to what role both this residue and the additional cavity play in the mechanism of peptide transport.

There is a pressing requirement to understand the molecular mechanism of the human peptide transporters, PepT1 and PepT2. Their central role in human health, dietary absorption and their effect on drug disposition make them promising targets to improve the pharmacokinetic properties of many drug molecules. The structure of $PepT_{So}$ provides the first crystal structure model with which to further investigate the underlying biochemistry of these processes and gain further insight into the transport of peptides and drugs across cellular membranes.

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# Functional studies of the bacterial PepT1 homologue YjdL: insights into the POT mechanism

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**Introduction:** Proton-dependent oligopeptide transporters (POTs) are secondary active symporters that utilize the proton gradient to facilitate the inward translocation of di- and tripeptides. These transporters, present in a wide range of organisms, are of particular interest due to the medicinal potential of the human intestinal POT hPepT1 as a drug uptake facilitator.

Different models have been proposed to account for peptide binding and translocation through POTs [1, 2]. It is generally believed that the charged peptide N-terminus plays an essential role in peptide binding and that it interacts with one of two highly conserved glutamate residues.

**Aim**: We have studied the two highly conserved glutamate residues (Glu20 and Glu388) in the *E. coli* POT YjdL by mutational analyses to probe their possible role as counter-ions to the charged peptide N-terminus.

**Methods**: Glu20 and Glu388 were mutated into Asp, Gln, and Ala and over-expressed in *E. coli*. To probe the functional role of each residue competition assays were performed using a series of ligands with lack of or varying N-terminal length.

**Results and conclusions** from these experiments suggested that the carboxyl group at Glu388 can sense the change in N-terminal position and hence acts as a counter-ion to the peptide N-terminal positive charge. pH-profiling suggested that Glu20 plays a possible role in proton translocation.

Work is in progress to investigate the role of other POT residues important for peptide binding and translocation.

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# The initial pathways of small peptides assimilation in small intestine

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**Introduction**. There are two main mechanisms for the dipeptide assimilation across the intestinal enterocyte brush border (apical membrane): coupled with proton transfer of intact small oligopeptides (PepT1) and coupled with ion (f. e. sodium) transport of monomers (amino acids), formed as a result of hydrolysis of dipeptides.

**Aim**. At different pH in the presence and absence of sodium to assess the contribution of these two mechanisms for the initial stages of nutrients assimilation.

**Methods**. $Na^+$-dependent transport of nutrients (dipeptides and amino acids) was studied by short-circuit current (SCC) technique. Magnitude of SCC response on addition to the washing solution of dipeptides and amino acids was recorded on isolated segments of rat small intestine; that parameter characterizes the rate of $Na^+$-dependent absorption of these nutrients.

**Results**. In the presence of sodium in Ringer's solution SCC responses on glycyl-glycine and carnosine (β-alanyl-L-histidine) in contrast to the glycyl-DL-methionine, glycyl-L-leucine and glycyl-L-alanine with an increase in pH tend to decrease. When removing sodium from solutions (pH 8.5) SCC responses on glycyl-L-alanine ($p < 0.1$) and glycyl-L-leucine ($p < 0.05$) decrease, but remain at a fairly high level. More importantly, with increasing pH dynamic of SCC responses on glycyl-L-alanine and glycyl-L-leucine dramatically changed – now these responses have a tendency to decrease with increasing pH. At pH 8.5 the researcher will observe more efficient dipeptide transport, as SCC responses to the dipeptides more than SCC responses on a mixture of amino acids. In contrast, at pH 5.5 one can observe an inverse relationship –SCC responses to a mixture of amino acids are more than SCC responses on dipeptides.

**Conclusion**. Increases with pH sodium-dependent component of the stimulating effect of easily hydrolysable dipeptides is due to the membrane digestion, and possibly due to the operation of the enzymatic transport ensemble, as the SCC responses to the dipeptides are more than SCC responses on a mixture of amino acids, of which they consist. Sodium independent transport component of dipeptides decreasing with pH apparently caused by functioning in the apical membrane of enterocytes of proton-dependent transporter PepT1.