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Molecular Modeling of PepT1 — Towards a Structure

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Abstract. The proton-coupled uptake of di- and tri-peptides is the major route of dietary nitrogen absorption in the intestine and of reabsorption of filtered protein in the kidney. In addition, the transporters involved, PepT1 (SLC15a1) and PepT2 (SLC15a2), are responsible for the uptake and tissue distribution of a wide range of pharmaceutically important compounds, including β-lactam antibiotics, angiotensin-converting enzyme inhibitors, anti-cancer and anti-viral drugs. PepT1 and PepT2 are large proteins, with over 700 amino acids, and to date there are no reports of their crystal structures, nor of those of related proteins from lower organisms. Therefore there is virtually no information about the protein 3-D structure, although computer-based approaches have been used to both model the transmembrane domain (TM) layout and to produce a substrate binding template. These models will be discussed, and a new one proposed from homology modeling rabbit PepT1 to the recently crystallized bacterial transporters LacY and GlpT. Understanding the mechanism by which PepT1 and PepT2 bind and transport their substrates is of great interest to researchers, both in academia and in the pharmaceutical industries.

Key words: PepT1 — PepT2 — Peptide transporter — Structure — Modeling

Introduction

The proton-coupled transport of di- and tripeptides is now well established as the major route for absorption of dietary nitrogen across the intestinal epithelium, as well as for reabsorption from the glomerular filtrate in the kidney (*see* Meredith & Boyd, 2000 and Daniel, 2004 for reviews). The peptide transporters also hold considerable interest for pharmacologists, as they transport various peptide-like drugs ('peptidomimetics') such as the β-lactam antibiotics, angiotensin-converting enzyme (ACE) inhibitors for hypertension, anti-virals such as valacyclovir, and anti-cancer agents such as bestatin (*see* Terada & Inui, 2004 for a review).

The gene encoding the membrane transport protein responsible for this uptake has been cloned, first from rabbit small intestine and named PepT1 (Fei et al., 1994), and subsequently from other species, as well as a second related gene product PepT2 (reviewed in Daniel & Kottra, 2004). The rabbit PepT1 gene encodes a protein (rPepT1) of 707 amino acids predicted to have 12 transmembrane-spanning domains (12TMs, Fei et al., 1994, and *see* Fig. 1). Human PepT1 (hPepT1) is very similar to that of rabbit, with 708 amino acids and 81% identity, and there is no direct structural information for either except some confirmation of the membrane topology. Covitz et al. (1998) used EE epitope tagging to show that the C-terminal of hPepT1 is intracellular, and that the loops between TMs 3 & 4 and 9 & 10 are extracellular, which is in line with the hydropathy plots (Fei et al., 1994). Indirect approaches to elucidate the protein structure have taken two complementary paths: first, the proposal of a template for transported substrates based on modeling the 3-D substrate shape and correlating that to the function of PepT1 (i.e., the binding affinity, Bailey et al., 2000, Gebauer et al., 2003, Bailey et al., 2006); and second, computer modeling of the predicted transmembrane regions of hPepT1 (Bolger et al., 1998, Yeung et al., 1998) to produce models of how the TMs might be arranged in the membrane. Both types of studies have used site-directed mutagenesis of individual residues (e.g., Meredith, 2004) and cysteine scanning of TMs

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Abbreviations: GlpT, *E.coli* glycerol-3-phosphate/inorganic phosphate antiporter; hPepT1, human PepT1; LacY *E.coli*, proton-coupled lactose permease; rPepT1, rabbit PepT1; TM, transmembrane domain?

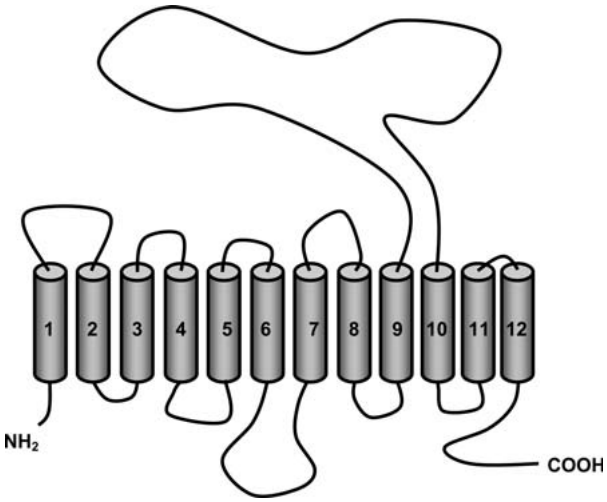

Fig. 1. Cartoon of PepT1 showing its 12-TM topology (redrawn from Fei et al., 1994).

(e.g., Kulkarni et al., 2003a, 2003b) to infer which amino acid residues might be important.

In this review article we will evaluate the current computer models of the PepT1 protein before putting forward an alternative model based on homology modeling to two known membrane transporter crystal structures. Finally, we will consider whether it is possible to correlate computer models with substrate template models, and look to the future.

Computer Modeling of PepT1

Having recognized that crystallization of a membrane protein such as PepT1 was unlikely to occur in the near future, Bolger et al. (1998) and Yeung et al. (1998) used a two-approach computer modeling method to generate a model of hPepT1, based on minimizing the interactions of the faces of TMs in a pairwise fashion. In their papers, they drew helical wheels from the TM sequences, each of which had 7 faces, so giving 49 ways in which two TMs can interact. By minimizing these pairwise interactions, calculating the amphipathicity of the helices, and then minimizing and modeling the 12-TM PepT1 structure, they were able to predict an arrangement of the TMs and a putative 'channel' (Fig. 2*A*). Furthermore, by overlaying the structures of the di- and tri-peptides Gly-Gly and Gly-Gly-Gly, amino acid residues in the hPepT1 channel that would potentially interact with substrates were identified, shown in Table 1. Many of these residues have been mutated by us in rabbit PepT1, or by others, and indeed the predictions have proved remarkably accurate. The model was further refined and reviewed by Lee et al. (1999), including predicting that there would be a vestibule (or 'bubble', Fig. 2*B*) in the centre of the protein when viewed side on, large enough to hold a dipeptide.

One drawback of the methodology used by Lee's group is that there is an implicit assumption that TMs that are adjacent in the sequence are also physically adjacent, i.e., TM1 is next to TM2, TM2 next to TM3, but TM3 does not interact with TM1, and so on. While this may be true in 12-TM proteins which have little in the way of intra- and extra-cellular loops and thus may be a natural restriction of 3-D arrangement (although *see* structures of LacY and GlpT later), PepT1 has large loops on both sides of the membrane (Fig. 1). Thus the arrangement of the TMs of PepT1 may not be subject to such constraints. Secondly, for simplicity of modeling the helices are predicted to cross the lipid bilayer perpendicular to the surface. While both of these assumptions will be challenged later on in this article, the approach of Lee's laboratory was both groundbreaking and with considerable merit.

Challenging the Hydropathy Plot

In the original cloning paper, Fei et al. (1994) used a hydropathy plot based on the Kyte-Doolittle algorithm to predict that rPepT1 had 12 TM domains. Covitz et al. (1998) used insertion of EE epitope tags into the hPepT1 protein to try to map the loops between the TMs, and thus confirm the topology. However, although the C-terminal portion of hPepT1 was relatively straightforward to map, the N-terminal TMs proved more difficult due to failure of the epitope-tagged constructs to express in the heterologous cell system used. Although the EE tag between TMs 3 and 4 produced a functional protein that was immunoreactive to an anti-EE antibody from the extracellular medium, TMs 1 and 2 could not be mapped by similar methodology. Insertion of the EE tag in the putative loop between TMs 1 and 2 did not produce a successfully expressed protein, and so their exact position remained unclear.

The positioning of the 12 TMs as in the Fei et al. (1994) model has been uncontroversial; however, we ran the rPepT1 sequence through the prediction program MEMSAT3 (McGuffin et al., 2000), the most recently released version of a transmembrane domain prediction program that claims a high percentage (78%) of accuracy. MEMSAT3 predicted that TM1 would be later in the amino acid sequence, formed from residues 24 to 42, rather than 7 to 25 as previously suggested, although other TM prediction programs still predict the latter position, highlighting that there is a low degree of confidence for TM1. All other TMs predicted by MEMSAT3 were in the position predicted by Fei et al. (1994). However, if TM1 were formed from residues 24 to 42, this could explain why when

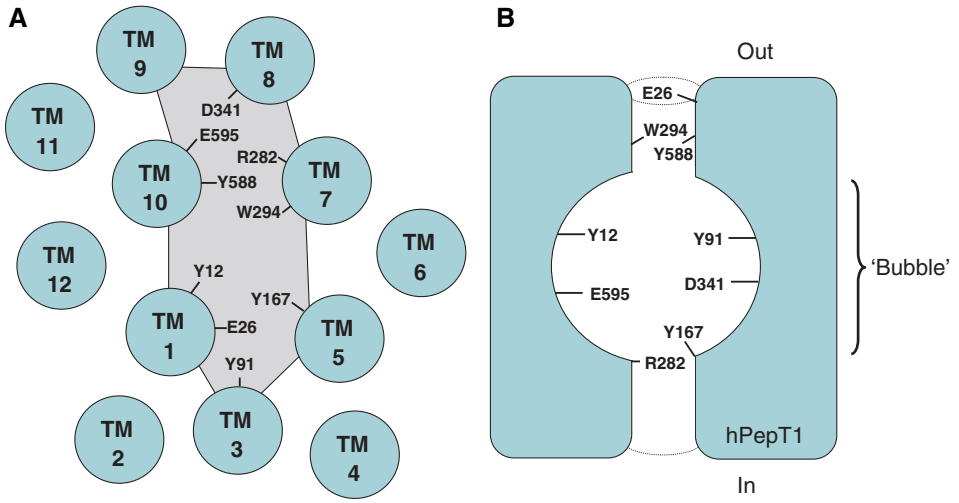

Fig. 2. (***A***) 12-TM model for hPepT1 proposed by Bolger et al. (1998) and Yeung et al. (1998), adapted from Lee et al. (1999). The putative hydrophilic channel, seen from above, through which the substrate passes through the transporter is shown in grey, with amino acid residues in TMs predicted to be involved in hPepT1 function labeled. (***B***) Schematic diagram (side view) of hPepT1, showing the transmembrane channel with vestibule ('bubble') and amino acid residues predicted to be important in transporter function (adapted from Lee et al., 1999).

Table 1. Amino acid residues in TMs predicted by Bolger et al. (1998) to be involved in hPepT1 transport activity, and the expected effect of mutation (adapted from Bolger et al., 1998), and the actual effect of mutating these residues where known (*ND* = not determined).

Amino acid (hPepT1)	TM location	Expected function in dipeptide uptake	Major expected result of mutation	Actual result of mutation	Reference
W294	7	Participates in initial binding	Affects K_m	W294A-hPepT1 Km ↑ Vmax ↓	Bolger et al., 1998 Panitsas et al., 2006 Unpublished[a]
				W294F-rPepT1 no transport	
				W294A/C-rPepT1 no transport	
Y588	10	Participates in initial binding	Affects K_m	Y587F-rPepT1 no apparent effect	Pieri et al., 2005
E26	1	Facilitates binding	Affects K_m	ND	
Y12	1	Regulates passage	Affects V_{max}	Y12A-hPepT1 Vmax ↓ by 25%	Bolger et al., 1998
E595	10	Regulates passage	Affects V_{max}	E595A-hPepT1 no transport	Bolger et al., 1998 Meredith, 2003
				E594D-rPepT1 no transport	
D341	8	Regulates passage	Affects V_{max}	D341R-rPepT1 like wildtype	Pieri et al., 2004
Y91	3	Interacts with proton	Affects pH dependence	Y91F-rPepT1 transport ↓ by 80% (pH dependence normal)	Pieri et al., 2005
Y167	5	Regulates passage	Affects V_{max}	Y167A-hPepT1 no transport	Bolger et al., 1998 Kulkarni et al., 2003b
				Y167C-hPepT1 transport ↓ by 75%	
				Y167F-rPepT1 transport ↓ by >90%	Pieri et al., 2005
R282	7	Regulates translocation	Affects V_{max}	R282A-hPepT1 like wildtype R282E-rPepT1 not proton-stimulated with simultaneous non-specific cation channel activity	Bolger et al., 1998 Meredith, 2004

[a]T. Bowden, K.E. Panitsas & D. Meredith

Covitz et al. (1998) inserted an EE epitope tag at position 39 it resulted in a non-expressed protein. In order to test this hypothesis we inserted a FLAG epitope tag at position 49, which in the MEMSAT3 model would be in the loop between TMs 1 and 2, and indeed we got expression of a functionally normal PepT1 (D. Meredith, unpublished data) which was immunoreactive with extracellular antibody (using the luminometry method of Panitsas et al., 2006). Insertion of a FLAG tag at residue 108 also gave a functional protein with the epitope tag available to external antibody (Panitsas et al., 2006), confirming the finding of Covitz et al. (1998) that the loop predicted to be between TMs 3 and 4 is indeed extracellular. The combination of these two findings suggests that the MEMSAT3 prediction may be correct, as will be discussed later.

We are currently in the process of making rPepT1 constructs that have truncations up to and including TM1, and the generation of progressively more severe N-terminal truncations will hopefully reveal which parts of the protein are necessary for correct trafficking and function.

Crystal Structures

In 2003, the simultaneous publication of two crystal structures for the *E.coli* proton-coupled lactose permease (LacY, Abramson et al., 2003) and the glycerol-3-phosphate/inorganic phosphate antiporter (GlpT, Huang et al., 2003) opened a window onto the 3-D structure of 12-TM transporters. One of the most striking findings was that despite their different functions, the two structures were remarkably similar (Fig. 3). Indeed, when all the TMs were overlaid, only TMs 2 and 8 (which are symmetrically related) were displaced, which could be due to either basic differences in the transporters or their substrates, or the fact that LacY was crystallized with substrate bound, whereas GlpT was not (Abramson et al., 2004).

The solving of the crystal structure for LacY has given insight into the reliability of site-directed mutagenesis experiments on this protein, due to previous extensive studies (*see* Kaback et al., 2001 for a review). In the previous mutation studies, only six residues were found to be irreplaceable with respect to lactose transport (E126, R144, E269, R302, H322 and E324), and the first three of these were found to participate in the substrate binding of thiodigalactoside with the LacY transport protein when they were co-crystallized (Aramson et al., 2003). Thus, at least for LacY, there is a good correlation between the interpretation of site-directed mutagenesis experiments and their effect on transport function, and the crystal structure (reviewed in Kaback, 2005).

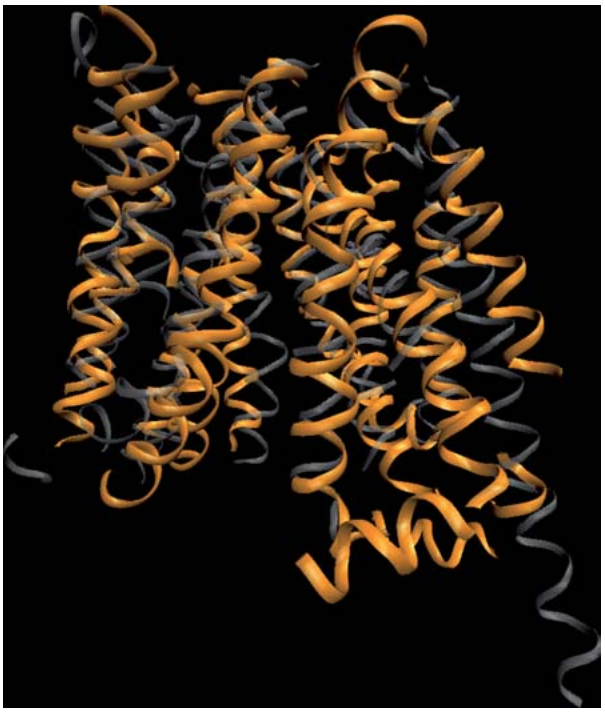

Fig. 3. rPepT1trunc sequence mapped onto the crystal structures of LacY (*orange*) and GlpT (*grey*) using 3D-JIGSAW (Bates et al., 1999 & 2001; Contreras-Moreira & Bates, 2002), showing how close the structures are in 3-D; this is despite LacY and GlpT not having any significant sequence homology. The sequence alignment (O'Donoghue & Luthey-Schulten, 2003) and image were made with VMD software support. VMD is developed with NIH support by the Theoretical and Computational Biophysics group at the Beckman Institute, University of Illinois at Urbana-Champaign, IL.

Both LacY and GlpT are members of the major facilitator superfamily (MFS, Saier et al., 2006), a large family of over 1000 proteins that have been identified to have signature motif(s) that identify them as transporters. It has been suggested that members of the MFS may have the same overall 3-D structure (Abramson et al., 2004), and therefore it is of note that PepT1 is also a member of the MFS (Chang et al., 2004; Saier et al., 2006) and thus might be expected to share structural similarity with LacY and GlpT . With this in mind we decided to investigate whether there was any merit in homology modeling PepT1 to the known crystal structures.

In order to do this, we attempted to thread the full-length rPepT1 sequence onto those of the previously crystallized bacterial membrane transporters using the program Cn3D (Wang et al., 2000; http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3dtut.shtml). Such threading is a relatively straightforward procedure for proteins that are of a similar length (e.g., it has been done for the monocarboxylate transporter MCT4, Wilson et al., 2005). However, due to the much longer rPepT1 sequence (approximately 300 extra residues that form large intra- and

extra-cellular loops, especially those between TMs 7 and 8 and between 9 and 10, respectively), this proved not to be reliable, as judged by the failure of the program to predict a structure with 12 TMs. Therefore, it was necessary to adopt an indirect approach instead:

i. the *E.coli* lactose permease (LacY) sequence (accession number P02920) was aligned to the rat PHT1 sequence (accession number BAA20489, a member of the same POT gene family as PepT1, Daniel et al., 2006)

ii. regions of rat PHT1 that did not have corresponding regions in LacY were deleted to create a new sequence, rat PHT1trunc

iii. rPepT1 (accession number P36836) was then aligned to rat PHT1trunc and regions of rPepT1 that were not represented in rat PHT1trunc were deleted to give rPepT1trunc (Fig. 4)

iv. finally, the rPepT1trunc sequence was then threaded onto the merged structures of *E.coli* lactose permease (LacY, PBD accession number 1PV6) and the *E.coli* glycerol-3-phosphate/inorganic phosphate antiporter (GlpT, 1PW4) to give a predicted structure (Fig. 5, showing rPepT1trunc sequence overlain on the crystal structure of LacY)

Although rPepT1trunc is obviously not a naturally occurring protein (*see* Fig. 4 for a comparison of the rPepT1 and rPepT1trunc sequences), there are several pieces of evidence that support its use in this modeling. First, if the rPepT1trunc sequence is put through the MEMSAT3 TM prediction program, the 12 TMs are predicted to lie in virtually the same places as in the wild type (Fig. 4). Second, truncation of the majority of the large extracellular loop between TMs 9 and 10 (rPepT1Δ426-571) does give a functional protein (albeit with a much higher affinity (i.e., a much lower *K*t) than the wild type (S. Williams & D. Meredith, unpublished data), indicating that the presence of this loop is not essential for transporter function. Indeed, oligopeptide transport proteins from lower organisms are not as large as PepT1, and their smaller size is accounted for by their not having the large connecting loops between the TMs (Daniel et al., 2006). Third, the truncated protein sequence still contains the POT superfamily signature consensus regions (Paulsen & Skurray, 1994).

If one accepts that the structure in Fig. 5 is broadly correct, then several interesting features become apparent. First, when the plan of how the TMs line up in the membrane (Fig. 6*A* and *B*) is considered, it is immediately apparent that the homology model could not have been predicted from a pairwise alignment method as used by Lee and colleagues (Fig. 6*C* vs Fig. 2*A*). As can be seen in Fig. 6*C*, in fact many of the TMs that are adjacent in the sequence show little or no apparent interaction. In some cases this is due to the prediction that second, many of the TMs do not lie perpendicular to the plane of the membrane, but are diagonally orientated, this being especially true for TMs 1, 4, 5, 7, 8 and 10. Third, as a result of being diagonally orientated, most of the TMs are predicted to be longer than the minimum number of amino acid residues needed to cross a lipid bilayer as an α-helix. The need for an unusually long TM1 (from residues 13 to 42) may help to explain the low level of confidence of the TM prediction programs in identifying TM1, as discussed above. If one draws helical wheels for the TMs in Cn3D and arranges them in the same layout as the homology model (Fig. 6*D*), most of the residues shown to be important for PepT1 function can face the central 'pore' as in the Bolger et al./Yeung et al. models. The one residue that does not seem to do this is Y91 (TM3), the relatively conservative mutation of which to a phenylalanine (Y91F-PepT1) gives a protein with a very low rate of transport compared to the wild type (Pieri et al., 2005). Conversely, in the homology model, TM2 is now part of the pore: this TM contains a histidine residue (H57) which has been shown to be essential for PepT1 function (Meredith & Boyd, 1996; Terada et al., 1996) and has been proposed, when protonated, to bind the carboxy terminus of the peptide substrate (Bailey et al., 2000). This hypothesis is supported by the finding that mutating the neighboring residue, Y56, gives a transporter (Y56F-rPepT1) with a greatly increased affinity for its substrate, possibly due to Y56 stabilizing H57 in the unprotonated form (Pieri et al., 2006). In addition, TM4, which contains the functionally important H121 (Terada et al., 1996), is also now part of the pore. It should be mentioned that an obvious limitation of such a helical wheel model as in Fig. 6*D* is that the helices are once again represented as being perpendicular to the membrane plane; however, it is intriguing that the residues shown by site-directed mutagenesis studies to be functionally important in PepT1 still largely appear to be on TMs involved in lining the central pore.

Another feature apparent in the homology model is the prominent role that TM7 appears to play. This TM has been subjected to cysteine-scanning mutation analysis (Kulkarni et al., 2003b), and three residues were identified that were intolerant to cysteine replacement: F293, L296 and F297. These residues were proposed to be structurally important for PepT1, possibly due to their hydrophobic packing interaction. Interestingly, W294 was tolerant of cysteine replacement in human PepT1 with transport rates virtually unchanged, although transport activity was significantly reduced when W294 was mutated to an alanine (Bolger et al., 1998), and again a structural role was proposed. In our hands, rabbit W294 is an essential residue, with neither W294F- (Panitsas et al., 2006), W294A- or W294C-rPepT1 (T.Bowden, K. Panitsas & D. Meredith, unpublished data) having any detectable transport activity. The finding that

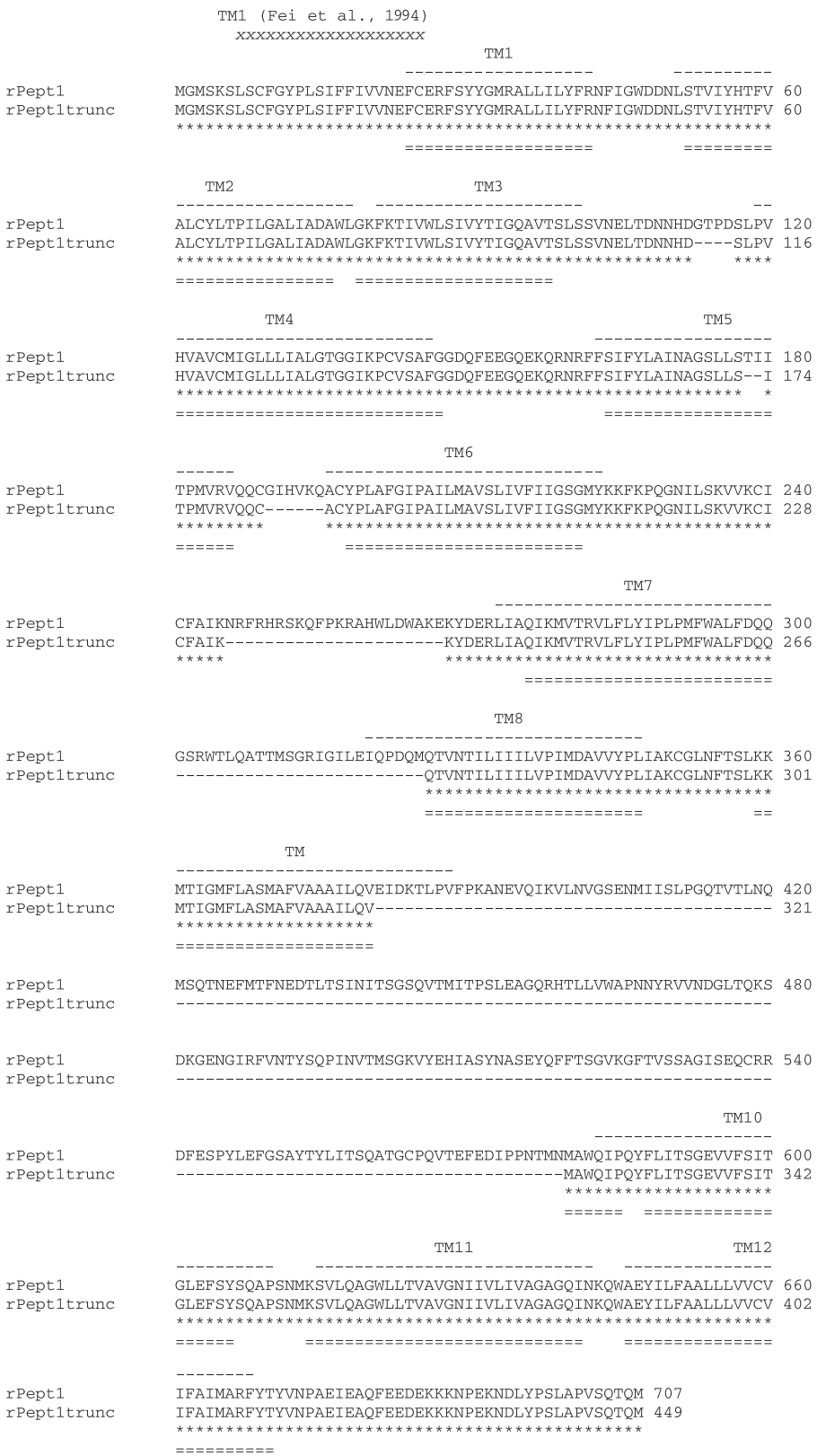

Fig. 4. Sequence line-up of rPepT1 vs rPepT1trunc, showing MEMSAT3 (McGuffin et al., 2000) predicted TMs for both (– – – – and = = = =, respectively), and Fei et al. (1994) TM1 position (××××).

most of the cysteine-substituted amino acid residues in TM7 were accessible to [2-(trimethylammonium) ethyl] methanethiosulfonate bromide (MTSET) suggests that TM7 is relatively solvent-accessible along its length (Kulkarni et al., 2003b), consistent with it lining the pore as proposed by the homology model.

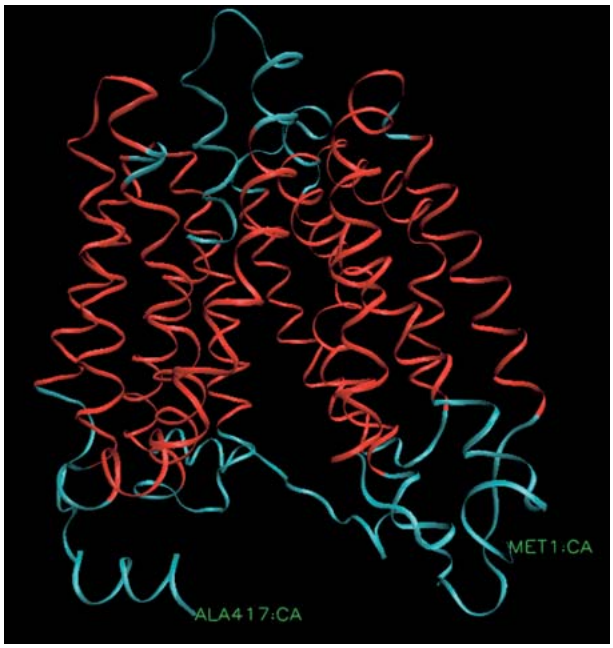

Fig. 5. The sequence of rPepT1trunc overlain on the structure of LacY chain A, with the rPepT1(trunc) TMs as predicted by MEMSAT3 (McGuffin et al., 2000) highlighted in red. The N- and C-terminal residue numbering refers to the rPepT1trunc sequence. This image was made with VMD software support. VMD is developed with NIH support by the Theoretical and Computational Biophysics group at the Beckman Institute, University of Illinois at Urbana-Champaign, IL.

The same group also used cysteine-scanning mutation to analyze TM5 in hPepT1 (Kulkarni et al., 2003a). As well as identifying a number of important residues for transport function (Y167, N171, S174 and P182), they predicted that TM5 would be ".....slightly tilted from the vertical axis of the channel, with the exofacial half forming a classical amphipathic α-helix and the cytoplasmic half being highly solvent-accessible." (Kulkarni et al., 2003a). When one looks at the homology model, then it appears that TM5 does contribute to the pore at its cytoplasmic end, but the extracellular end is more embedded in the membrane/other parts of the protein. Mutations of Y167 in both hPepT1 and rPepT1 have shown the importance of this TM5 residue (Bolger et al., 1998; Kulkarni et al., 2003a; Peiri et al., 2005; Table 1).

Finally with regard to the homology model, it is tempting to see if there is any pattern to the residues that we and others have been shown by site-directed mutagenesis to be important in the function of PepT1 (Fig. 7). It is interesting that the two locations where we have inserted FLAG epitope tags, at amino acids 49 and 108, seem to be on the extremities of extracellular loops between TMs 1 and 2 and between 3 and 4, respectively, in keeping with their both being extracellularly accessible to immunolabeling (Fig. 7). Using molecular modeling programs such as VMD (developed with NIH support by the Theoretical and Computational Biophysics group at the Beckman Institute, University of Illinois at Urbana-Champaign, IL) it is possible to estimate intramolecular distances between specified atoms in a given structure: for example, H57 and E594, which have been proposed to bind the carboxyl and amino termini of rPepT1 substrates (Bailey et al., 2000) are approximately the correct distance apart in the homology model to fulfill this role if they both were facing each other. One approach to test such hypotheses would be the use of known-length cross-linker molecules to probe the distances between particular residues.

What Does the Future Hold for PepT1 Modeling?

It is clear that in attempting to move from a model such as that in Fig. 1 to one like that in Fig. 3, the exact position in the sequence of the TMs becomes an important consideration. Therefore for modeling to be advanced, it is going to be necessary to definitively confirm the TM locations in PepT1. The PepT1trunc sequence is a theoretical form of PepT1, although given credence by the fact that PepT1 minus the large extracellular loop is functional, and so it would be interesting to know whether a PepT1trunc gene would produce a functional protein. The model as it is presented is relatively crude, as we have yet to try to refine it by, for example, energy minimization of the TM side chains to see whether the packing changes, or minimizing interactions between the physically adjacent TMs.

Obviously, to relate this back to one of the major reasons for being interested in PepT1, namely, its potential as a drug delivery route, requires the identification of the residues that are binding the substrate so that a substrate binding template such as that of Bailey et al. (2000) can be superimposed onto the model, and this used to predict binding affinity. It is worth remembering that crystal structures are static and reveal little about transport mechanism (for example, one of the reasons that LacY could be crystallized was that a mutant form C154G of the protein was used, that was effectively locked in one conformation, thought to be inward-facing; Abramson et al., 2003), and it is possible for compounds to bind to transporters but not be translocated. This fact has been highlighted for PepT1 by a recent paper by Vig et al. (2006), who showed that contrary to general belief, some naturally occurring dipeptides were not translocated substrates for hPepT1 (although they did bind), and previous studies have identified peptidomimetic compounds that bind to rPepT1 but are not translocated (e.g., Meredith et al., 1998).

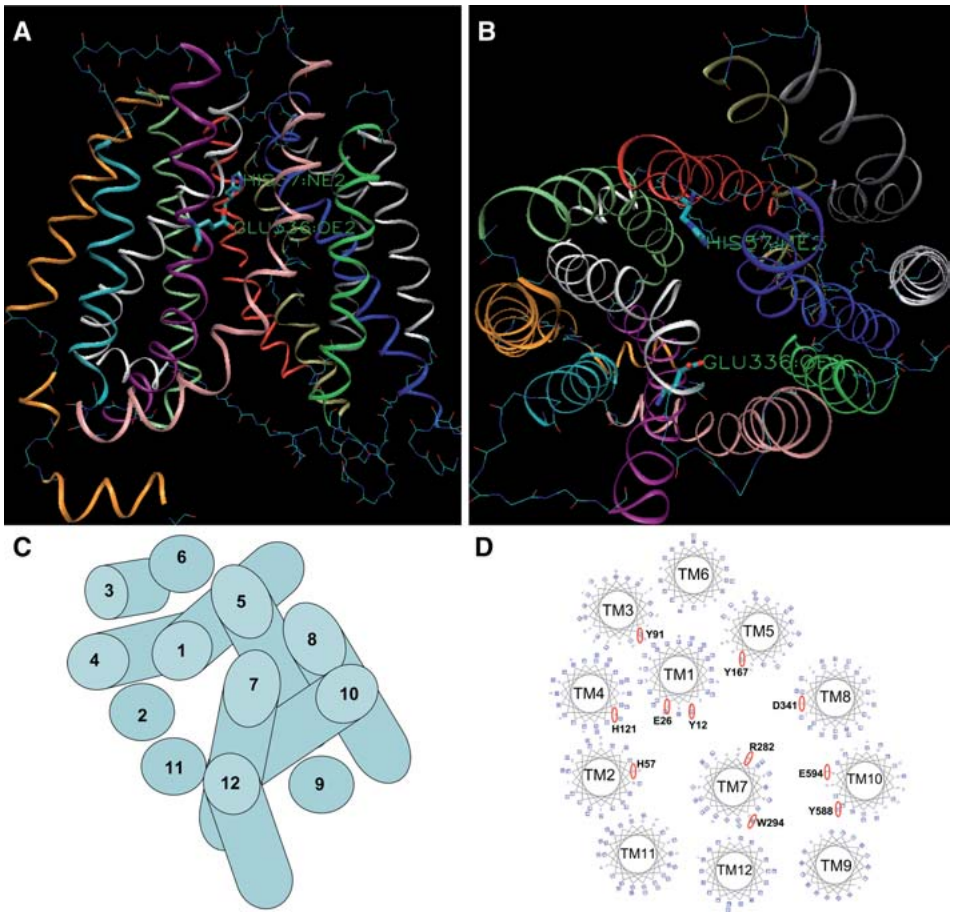

Fig. 6. (*A*) Side view of rPepT1trunc overlaid on the LacY chain A structure, with residues His57 and Glu336 (Glu594 in full-length rPepT1) highlighted. This image and the one in Fig. 6*B* were made with VMD software support. VMD is developed with NIH support by the Theoretical and Computational Biophysics group at the Beckman Institute, University of Illinois at Urbana-Champaign, IL. (***B***) Plan view of rPepT1trunc overlaid on LacY chain A, with residues His57 and Glu336 (Glu594 in full length rPepT1) highlighted. (***C***) Cartoon of the position of TMs of rPepT1trunc when overlain on the LacY crystal structure, drawn from Fig. 6*B*, and reorientated to match the TM model in Fig. 2*A*. (***D***) Helical wheel plan of the TMs of rPepT1trunc drawn from Fig. *6B* using the freely available EMBOSS program Pepwheel (e.g., through www.tcdb.org/analyze.php), with residues in square boxes being hydrophobic. The helical wheels were arranged so that where possible the residues that were predicted to face into the putative central channel by Bolger et al. (1998) still do so and are circled and labeled, along with the two histidine residues that have been proposed to play a functional role in PepT1, H57 and H121 (Meredith & Boyd, 1996; Terada et al., 1996).

Finally, it should also be noted that PepT1 is a multimer, probably a tetramer: this has been shown by radiation size inactivation for peptide transporters in renal brush-border membrane vesicles (but most likely PepT2 due to the substrate used; Boll & Daniel, 1995), and more recently by using a dominant negative rPepT1 mutant in *Xenopus* oocytes (Panitsas et al., 2006). While this could mean that any model of PepT1 based on a single protein may be compromised, it seems probable that each subunit of the multimer will have a complete substrate binding site. For example, the bacterial multidrug resistance protein EmrE has been shown to be a homodimer, with each subunit crystallized with a tetraphenylphosphonium ion (TPP^+) substrate bound to it (Ubarretxena-Belandia & Tate, 2004), and LacY was crystallized as a dimer (Abramson et al., 2003).

Conclusion

In this article we have reviewed the published modeling of PepT1, and proposed a new model based on homology with the crystal structures of LacY and GlpT. Our approach has produced a model that is quite different from those before, and these differences may simply represent the inadequacies of trying to model large proteins based on relatively little hard evidence and starting with different assumptions. Only subsequent experimental testing will allow for this first attempt at full molecular modeling of PepT1 to be tested and refined. Of course, no modeling can replace the solving of a crystal structure of a proton-coupled peptide transporter; while this seems unlikely to be possible for a mammalian PepT1 due to the large protein size with its intra/extracellular loops,

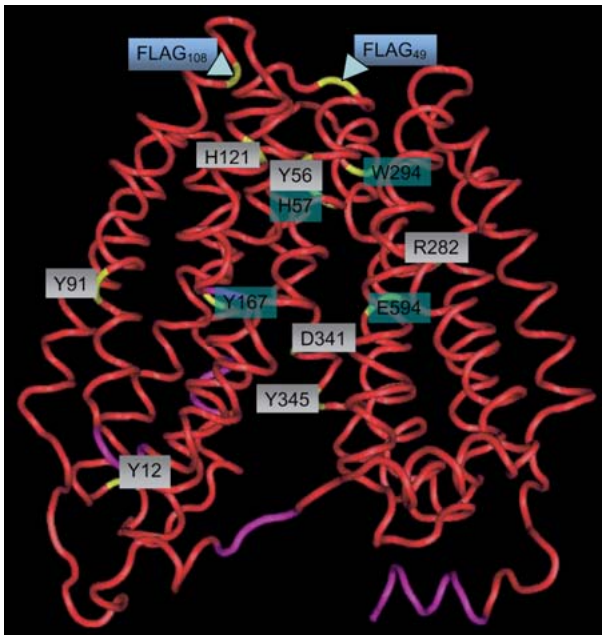

Fig. 7. Residues in rPepT1 that have been shown to have either significant effect on transport (identified in *grey*) or be essential (in *blue*) for PepT1 function, plus the FLAG tag epitope positions, mapped onto the LacY structure using the Cn3D program (Wang et al., 2000). Y345 has been shown to play a role in rPepT1 function, as when mutated to Y345F transport was significantly reduced (Pieri et al., 2005).

plus the difficulty in expressing large amounts of protein (over 1g was needed for LacY; Abramson et al., 2004), valuable information may be obtained from a POT family member from a lower organism. However, in the absence of such a crystal structure, computer/homology modeling backed up by functional experiments remains a valid approach for trying to elucidate the 3-D structure of PepT1.

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