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Intestinal oligopeptide transport

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It has been clear for the whole of this century that the gastrointestinal tract is a major site not only of protein metabolism but also of transport of α-amino-N and that in the small intestine this means transport of peptides rather than of free amino acids. In his scholarly review on protein and peptide digestion Matthews (1991), in reviewing the fascinating history of this field, emphasizes how for about 30 years in the middle of the 20th century the significance of peptide transport was nevertheless forgotten largely because transport systems for amino acids had been discovered. The emphasis has now been corrected with a vengeance following the recent cloning and expression of intestinal peptide transporters (Dantzig *et al.* 1994; Fei *et al.* 1994). These important papers will be discussed below; however, by way of background, there are some general issues which a modern review should emphasize.

TRANSCELLULAR *v.* PARACELLULAR PEPTIDE DELIVERY

Pappenheimer and colleagues (for example, see Madara & Pappenheimer, 1987) have controversially proposed that paracellular solute delivery across the small-intestinal epithelium is both widespread and regulated. In my opinion the jury is still out on this particular proposal but it has led to some interesting recent experiments relating to peptide absorption. Thus, when iodinated octapeptides were delivered orally, excretion of intact peptide was observed in the urine suggesting intact peptide uptake from the gastrointestinal tract (Pappenheimer *et al.* 1994); there was clear evidence of specificity in that not all peptides of equivalent size appeared in the urine suggesting that either they were not transported or they were degraded during translocation. This field has been reviewed recently by Gardner (1994) and the evidence is persuasive that peptides of molecular weight approximately 1–3 kD can enter the vascular system when taken orally. Two important points, however, remain unresolved; the route of entry and the quantitative significance of delivery.

TRANSCELLULAR TRANSPORT

Peptide entry

One feature which makes the transport of peptides interestingly different from other digestion products is the coupling of the peptide entry step to the electrochemical

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gradient of protons and not, as for many other organic solutes in the vertebrate gastrointestinal tract, to Na ions. This is an example of so-called tertiary active transport since the electrochemical gradient for protons is itself derived from (at least in part) Na–proton antiport activity in the brush border (Ganapathy & Leibach, 1985). More detail regarding the mechanism of coupling will be discussed later (p. 522).

Exit step

Although physiologically most peptides which enter the small-intestinal epithelium are rapidly hydrolysed before their release across the basal membrane as free amino acids, recent physiological and pharmaceutical studies (Inui *et al.* 1992; Bronk *et al.* 1993; Kramer *et al.* 1994) have established that under certain circumstances intact peptides can leave via transporters present in the basolateral membrane. Meredith & Boyd (1995) have proposed (on the basis of previously published data in the literature) that this is an electroneutral process able to show counter-transport and, therefore, is likely to be via anion exchange. The investigation of this step may be aided by the discovery that whereas the rate of peptide hydrolysis can be markedly reduced by incorporation of a D-amino acid at the amino terminal such peptides can both enter and leave the epithelium (Bronk *et al.* 1993). The molecular basis of this exit step, however, remains unknown.

PEPTIDE SUBSTRATE CHEMISTRY REQUIRED TO ALLOW INTERACTION WITH INTESTINAL TRANSPORT SYSTEMS

At present the field of peptide transport is bedevilled by our ignorance of the number of different transporters which are expressed in the apical surface of the small intestine. Recent molecular studies have suggested that at least two transporters may be found but at present the number and their individual substrate specificities are quite unknown. However, the following structural features appear to be required:
1. a peptide chain shorter than four amino acids (i.e. with limited tertiary structure),
2. the presence of a carboxy terminal amino acid in its L configuration,
3. the presence of at least one C–N (peptide) bond,
4. an overall charge of less than two positive units.

The implication of these structural features is that sites of protein cleavage by pancreatic and other proteases may be of great importance in determining the pattern of substrates available for subsequent transport. Particularly striking is the need for the charge on the peptide to be appropriate. Remarkably the side-chain configuration appears to be important only with regard to the affinity of binding so that both very small peptides, e.g. glycyl-glycine and a very large tripeptide, e.g. tryptophanyl-tryptophanyl-tryptophan are substrates.

A fuller understanding of the molecular features recognized by the peptide transporter(s) will be important for rational design of molecules which can enter the intestinal epithelium by this route. Recent studies have shown that it is possible to examine this process both in the intact *in vitro* small intestinal epithelium as well as in isolated membrane vesicles (Bronk *et al.* 1993; Temple *et al.* 1994). In the intact preparation the relative rate of entry, hydrolysis and exit can be determined and the following have emerged as structural features required if a peptide is to be a substrate.

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Dipeptides composed of D-amino acids at both amino and carboxyl terminals interact minimally with peptide transport systems of hydrolysing enzymes. In contrast naturally occurring LL-dipeptides, although transported rapidly are also hydrolysed so that only free amino acid appears in the serosal fluid. For the mixed (DL- and LD-) dipeptides a remarkable finding is that the presence of a terminal D-amino acid does not block transport but does very substantially reduce hydrolysis. Thus, these dipeptides are able to appear intact having been translocated across both the apical and basal surfaces of the small-intestinal epithelium. In contrast the presence of a D-amino acid at the carboxyl terminal is much less effective at reducing hydrolysis so that, as for the LL molecules, only free amino acid can be detected in the serosal component. The study of Bronk *et al.* (1993) also shows that the nature of the amino acids at the carboxyl and amino terminals is a feature recognized by the transporter; thus, D-Ala-L-Phe is transported more rapidly than is D-Phe-L-Ala; however, it is also hydrolysed faster than D-Phe-L-Ala making the latter molecule particularly attractive as a probe for investigation of transport.

Temple *et al.* (1994) have shown that the transport of D-Phe-L-Ala in epithelial brush-border-membrane vesicles is proton-gradient dependent and that the kinetics of transport are strongly influenced both by membrane potential and by proton gradient and concentration. The effect of membrane potential on V_{max} suggests that the transporter in the apical membrane carries a net negative charge in the unloaded form (i.e. when the transporter has not bound its substrate) and that the rate-limiting step is the return of the unloaded transporter to the external surface. That voltage has an effect on K_m is most readily interpreted (cf. Eleno *et al.* 1994) as suggesting that peptide binding at the external surface of the transporter involves residues on the membrane protein which are exposed to the lumen environment but which nevertheless sense the membrane field; this implies that they will lie within the transmembrane (TM) domains of the transporter, almost certainly towards the external end of such domains.

The exit step has not yet been investigated in such detail but it is clear that it is not identical to that found at the apical surface. For example, whereas the entry step is electrogenic (Boyd & Ward, 1982), exit appears to be electroneutral (Dyer *et al.* 1990).

Very recent work suggests that there may be regulation of peptide transport by phosphorylation. Brandsch *et al.* (1994) suggest that protein kinase C modulates (inhibits) peptide transport function. It is not clear whether this modulation works at the level of individual transporter turnover or at the level of insertion and/or retrieval from the brush-border membrane of pre-existing transporters (for discussion, see Meredith & Boyd, 1994). Physiologically this must be an important area of future experimental investigation along with study of the effect of dietary manipulation on peptide transporter expression.

MOLECULAR STUDIES ON PEPTIDE TRANSPORT

The field of peptide transport in the intestine has been revolutionized by the publication in 1994 of two papers (Dantzig *et al.* 1994; Fei *et al.* 1994) which reported the cloning, sequencing and expression of two different mammalian peptide transporters christened respectively PepT1 and HPT1. PepT1 was cloned (by expression) from rabbit small-intestinal mRNA (following the classical procedure by which SGLT1, the Na-dependent glucose transporter, was originally isolated (Hediger *et al.* 1987)). HPT1 was cloned using a functionally inhibitory monoclonal antibody raised against brush-border mem-

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brane proteins to immunoprecipitate the relevant protein and, thus, isolate the cDNA which led to its expression. HPT1 has sequence homology in its extracellular domain with members of the cadherin family of membrane proteins, and like these proteins it contains but a single transmembrane domain. Expression of HPT1 was observed in the gastrointestinal tract (duodenum, jejunum, ileum, colon and pancreatic ducts); it was absent from kidney, liver and lung. When expressed in the CHO cell line, HPT1 induced increased peptide (antibiotic) uptake and this was dependent on an inwardly directed proton gradient and was inhibited by addition of external peptide (Gly-Pro). This transporter (HPT1) had no sequence homology with PepT1 which was much more 'orthodox' as a candidate transporter in that it possessed twelve transmembrane domains in its 707 amino acids. PepT1 was strongly expressed in jejunum and also in the duodenum and ileum (but not colon). Moderate expression was detected in renal cortex and in liver. The very substantial transport induced by expression of the protein in *Xenopus* oocytes showed features characteristic of native intestinal peptide transport. It was electrogenic, proton-coupled, Na-independent and associated with intracellular acidification. In oocytes expressing PepT1 a wide range of peptides were able to depolarize the cell membrane and the affinity of peptides (in this assay of electrogenic transport) was in the low millimolar range (e.g. 1·9 mM for Gly-Sar). In an important experiment Fei *et al.* (1994) showed that when injected with rabbit intestinal total mRNA depleted of PepT1 by antisense oligonucleotide hybridization there was no detectable uptake of the peptide Gly-Sar over that observed in control oocytes. This was interpreted as implying that PepT1 is 'the only transporter responsible for transport of Gly-Sar in enterocytes. . . . The peptide transport in intestinal apical and basal membranes must therefore be due to the expression of PepT1'. This statement needs qualifying since for peptides other than Gly-Sar the critical experiment performed by Fei *et al.* (1994) has not been carried out. Thus, this important observation needs further work.

It is of considerable interest that PepT1 contains an overall net charge of −2, in complete agreement with observations on the voltage dependence of V_{max} made by others (Daniel *et al.* 1991; Temple *et al.* 1994); the authors themselves also show that the V_{max} of the expressed transporter is voltage-dependent. The observation that there are two histidine residues (in TM2 and 4) and that these lie within three residues of the external surface fits in with the known proton peptide stoichiometry and with the effect of voltage on K_m discussed previously. Another interesting feature revealed from the molecular structure of PepT1 is that it has within the cytoplasmic loop between transmembrane domain (TM) 8 and 9 sites for potential control by phosphorylation. Thus, PepT1 possesses many of the features which would be predicted from earlier functional studies and it seems likely that it is indeed the molecule responsible for uptake into the epithelium of the endproducts of protein digestion. Whether there are isoforms of PepT1 (for example, as for the Glut family of hexose transporters) and the molecular basis for the exit pathway for peptides at the basal membrane remains unclear. Moreover, the contrasting findings on HPT1 and its possession of but a single TM suggest that, in contrast, it may function as a regulatory molecule (perhaps as a hetero-oligomer with PepT1?) and experimental evidence for this must now be sought. It is remarkable that the field of peptide transport has suddenly caught up with both amino acid and carbohydrate transport and that the advent of molecular structures poses a whole set of new functional problems for future investigations. One of these will be the re-investigation of intestinal peptide transport activity in disease.

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