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Cloning, expression and structure determination of the major extracellular domain of the PepT1 oligopeptide transporter

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In the gastro-intestinal tract the PepT1 transporter is considered to provide the major route for absorption of the end-products of digestive proteolysis and also penicillin antibiotics. The three-dimensional structure of PepT1 has not yet been determined, although analyses of the primary structure of the protein suggest that it has twelve α-helical (Fei et al 1994) or perhaps sixteen β-strand (Barlow, personal communication) transmembrane regions, with both topological models showing a large glycosylated extracellular domain spanning residues 380 to 580. Such a large extracellular domain will undoubtedly serve some functional role in the transporter and, by analogy with the porin family of transporters (Cowan et al 1992), is likely to provide a key determinant of PepT1's substrate specificity and also play a part in its opening and closing.

We have undertaken the synthesis of the PepT1 major extracellular domain as an isolated protein with the intention of elucidating its three-dimensional structure in solution.

A gene encoding the native extracellular loop of the PepT1 transporter (PepT1-loop) has been synthesised using recursive polymerase chain reaction (PCR) (Prodromou and Pearl 1992). Twelve 80-mer oligonucloetides were designed to have unique restriction endonuclease (RE) sites and to anneal and extend during a single PCR in order to facilitate synthesis of the complete double stranded DNA sequence. The predicted base pair (bp) length of PepT1-loop (645 bp) was verified against a DNA base pair ladder, running the PCR products and ladder on a 2% w/v agarose gel (Fig. 1).

The PCR product was isolated from agarose gel, digested with the REs BamHI and Hin*d*III and ligated into the similarly digested plasmid pMTL2023. The ligation mix was used to transform *Escherichia coli* DH5α competent cells which were subsequenly grown on LB plates containing chloramphenicol.

Clones found to contain the recombinant plasmid on screening were cultured and harvested in order to prepare plasmid DNA for sequencing. The PepT1-loop was then subcloned into the expression vector pRSET-A, allowing the expression of the isolated protein extended at its N-terminus with a polyhistidine tag, permitting purification of the recombinant protein using nickel affinity chromatography.

Determination of the structure of the extracellular domain of the PepT1 transporter using multidimensional NMR in combination with molecular modelling will hopefully provide insight into the mechanism of action of this transporter, and could also give clues as to the structure and function of other organic solute transporter systems.

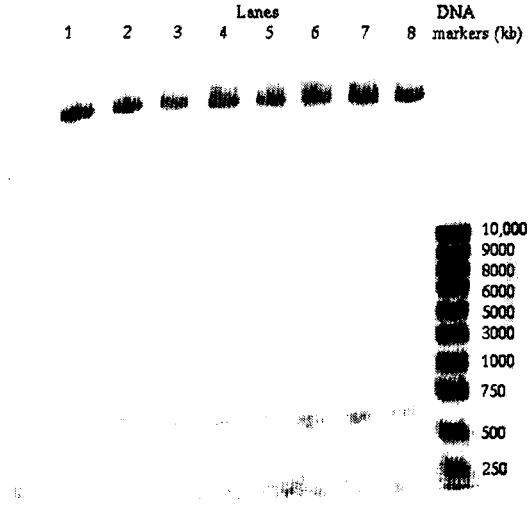

Figure 1. 0.8% Agarose gel of a digest of pMTL2023-PepT1-loop. The synthesised gene is absent in the native pMTL2023 (the control, lane 1).

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