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A new family of serine-type peptidases related to prolyl oligopeptidase

The deduced amino acid sequence of a prolyl endopeptidase from pig brain (EC 3.4.21.26) was recently reported (Rennex *et al.*

al., 1991), and essential catalytic residues were identified as Ser⁵⁵⁴ and His⁶⁸⁰ by reaction with active-site-directed reagents (Rennex *et al.*, 1991; Stone *et al.*, 1991). No significant resemblance was noted of either the sequence as a whole or the segments containing the catalytic residues to other known peptidases. Moreover,

Fig. 1. Alignment of the C-terminal segments of the amino acid sequences of pig brain prolyl oligopeptidase and the related peptidases

The sequences are: *pop*, prolyl oligopeptidase (Rennex *et al.*, 1991) (residues 459–710); *acph*, rat liver acylaminoacyl-peptidase (Kobayashi *et al.*, 1989) (residues 486–732); *3p21*, human protein 3p21 (Naylor *et al.*, 1989) (residues 383–606); *dppiv*, rat liver dipeptidyl-peptidase IV (Ogata *et al.*, 1989) (residues 529–767); *dapb*, yeast dipeptidyl-peptidase B (Roberts *et al.*, 1989) (residues 575–814, the C-terminus being 841). The numbering is that of prolyl oligopeptidase (Rennex *et al.*, 1991), and residues identical to those of prolyl oligopeptidase are shown in white on black. Active site Ser and His residues previously identified in prolyl oligopeptidase are marked ‘*’, and Asp residues that may form part of a catalytic triad are marked ‘?’.

Polgar (1991) has demonstrated that the catalytic mechanism is significantly different from those of the serine endopeptidases of the chymotrypsin and subtilisin families. Because EC 3.4.21.26 hydrolyses oligopeptides but not proteins, unlike the prolyl endopeptidases from various species of bacteria that degrade immunoglobulin A, we propose that it should be renamed prolyl oligopeptidase.

It now appears that prolyl oligopeptidase can be placed in an evolutionary family of serine-type peptidases. The sequence of prolyl oligopeptidase shows a moderate similarity to that of dipeptidyl-peptidase IV (EC 3.4.14.5), but there is a still clearer relationship to acylaminoacyl-peptidase (EC 3.4.19.1). The strongest resemblance between the sequences is seen in the C-terminal 250 residues or so, which contain the identified catalytic residues of prolyl oligopeptidase (Fig. 1).

The statistical significance of the relationships between prolyl oligopeptidase and the other enzymes was tested with the RDF2 program from the FASTA package of Pearson & Lipman (1988). The KTUP value was set to 1, and 100 random uniform shuffles of the test sequences were performed. Values of six standard deviation units or more are considered significant in this test, and the following sequence pairs met this requirement (standard deviation values in parentheses): dipeptidyl-peptidase IV and yeast dipeptidyl-peptidase B (80.34); prolyl oligopeptidase and acylaminoacyl-peptidase (8.73); and dipeptidyl-peptidase B and acylaminoacyl-peptidase (8.21). It therefore follows that all four enzymes are members of a single homologous family of proteins.

The sequence of pig acylaminoacyl-peptidase (Mitta *et al.*, 1989) is closely similar to that of rat and it has been reported that the (unpublished) sequence of yeast dipeptidyl-peptidase A is related to that of dipeptidyl-peptidase B (Roberts *et al.*, 1989). Another homologous sequence is that of the human protein 3p21. The gene for 3p21 is located on the short arm of human chromosome 3, deletion of which is associated with small cell lung cancer (Naylor *et al.*, 1989). Protein 3p21 shows marked sequence similarity to the acylaminoacyl-peptidases; it is shorter at both N- and C-termini (Jones *et al.*, 1991), but contains the putative catalytic site residues we identify here. These proteins can therefore be considered additional members of the new family.

By analogy with prolyl oligopeptidase, we would predict that the catalytic residues in the other enzymes are the aligned Ser⁵⁸⁷ and His⁷⁰⁷ (acylaminoacyl-peptidase), Ser⁶³¹ and His⁷⁴¹ (rat dipeptidyl-peptidase IV), and Ser⁶⁷⁸ and His⁷⁸⁸ (yeast dipeptidyl-peptidase B). Other known serine-dependent peptidases contain a 'catalytic triad' of essential residues: serine, histidine and aspartic acid. There is no direct evidence as to the identity of any essential aspartic acid residue in the prolyl oligopeptidase family, but candidates are the conserved Asp⁵²⁹ and Asp⁶⁴² residues (or Asp⁶⁴¹, in a slightly shifted alignment).

Like prolyl oligopeptidase, dipeptidyl-peptidase IV and acylaminoacyl-peptidase are inhibited by di-isopropylfluorophosphate, and acylaminoacyl-peptidase also is inactivated by a chloromethane (Kobayashi & Smith, 1987). The other enzymes do not show the marked thiol-dependence of prolyl oligopeptidase, however.

From the point of view of the catalytic activities of the component enzymes, this is one of the most disparate families of peptidases so far recognized, containing an endopeptidase that is confined to action on oligopeptides (prolyl oligopeptidase), an exopeptidase that cleaves dipeptides from the N-termini of polypeptides only when the N-terminus is free (dipeptidyl-peptidase IV), and an omega peptidase that preferentially cleaves N-terminal acetyl-aminoacyl residues from polypeptides (acylaminoacyl-peptidase). The enzymes therefore fall into three distinct sub-sections of the Enzyme Nomenclature list (IUB,

1984). Prolyl oligopeptidase and dipeptidyl-peptidase IV show marked preference for the cleavage of prolyl bonds, but no such specificity is seen with acylaminoacyl-peptidase (McDonald & Barrett, 1986). Moreover, prolyl oligopeptidase and acylaminoacyl-peptidase are soluble, cytosolic enzymes, whereas dipeptidyl-peptidase IV is a heavily glycosylated enzyme that is predominantly membrane-associated in both rat and yeast, containing a transmembrane segment near the N-terminus (Ogata *et al.*, 1989).

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More von Willebrand factor type A domains? Sequence similarities with malaria thrombospondin-related anonymous protein, dihydropyridine-sensitive calcium channel and inter- α -trypsin inhibitor

von Willebrand factor is an adhesive glycoprotein involved in various binding interactions (for recent review see [1]). The subunits of this oligomeric complex are large in size and have a typical mosaic structure containing a number of repeated domains. As a result of the 'exon shuffling' process [2] such domains may occur in apparently unrelated proteins involved in very different extracellular pathways [3–6]. Indeed, different types of domains observed in von Willebrand factor were also