Structure and Activity of Aspartic Proteinases

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Aspartic proteinases and their inhibitors

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Proteolytic enzymes are classified on the basis of their catalytic mechanism as belonging to one of four groups: the serine, cysteine, metallo and aspartic proteinases (Kay, 1982). In contrast to the detailed sequence information and three-dimensional structures that have been produced for many enzymes belonging to the first three groups, relatively little is known about the aspartic proteinases.

These enzymes have been isolated from five major sources:
(a) The stomachs of a number of species. Three different types of gastric enzyme have been resolved, the pepsins, chymosins and gastricsins, together with a less-well-characterized component, which has been termed slow-moving protease because of its electrophoretic mobility (Samloff et al., 1985). Gastricsin is also produced by the prostate which is the source of the aspartic protease of seminal fluid (Reid et al., 1984).
(b) The lysosomes of many cell types contain cathepsin D (and, sometimes, cathepsin E).
(c) Tissues such as kidney and submaxillary gland produce renin.
(d) Micro-organisms (but not bacteria), e.g. the proteinases from Endothia parasitica, Penicillium janthinellum and Mucor pusillus; yeast protease A.
(e) Plants such as squash and cucumber (Wilimowska-Pelc et al., 1983).

In order to be classified as an aspartic proteinase, an enzyme must, by definition, be susceptible to inhibition by pepstatin and by the active-site-directed affinity labels, diazoacetyl-norleucine methyl ester and epoxy \(-\)-nitrophenylalanine; Sta, statine [(35, 48)-4-amino-3-hydroxy-6-methylheptanoic acid].

Abbreviations used: NO\textsubscript{2}-Phe, \textit{p}-nitrophenylalanine; Sta, statine [(35, 48)-4-amino-3-hydroxy-6-methylheptanoic acid].

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and cathepsin D contain higher percentages of carbohydrate. Renin may be a glycoprotein depending on the species and tissue of origin.

A further difference between mammalian and microbial aspartic proteinases is that, whereas the microbial enzymes do not appear to have to be synthesized in precursor form in order to facilitate folding, all of the mammalian enzymes are produced in the form of well-documentedzymogens such as pepsinogen, prochymosin, progastricin and prorennin (Kay, 1980). A precursor for cathepsin D has also been observed (Puizdar & Turk, 1981).

Naturally occurring inhibitors of aspartic proteinases are relatively uncommon and are found in only certain specialized locations:

(1) proteins (Mr approx. 17000) from Ascaris lumbricoides;

(2) the inhibitor peptide (containing 16/17 residues) released on activation of (pig/cow) pepsinogen(s);

(3) acylated pentapeptides (pepstatins) from various species of actinomycetes;

(4) renin-binding proteins;

(5) the inhibitor of proteinase A in yeast.

While neither of the last two examples, none of these naturally occurring inhibitors can be considered to be of physiological importance to the host cell as regulators of the activity of its own aspartic proteinases (Kay, 1982). It is likely that modulation of this type of proteinase has to be achieved through regulation of the pH or of the rate of supply of substrate proteins.

Furthermore, the susceptibility of individual aspartic proteinases to inhibition by these compounds varies considerably. The Ascaris proteins, for example, are effective inhibitors of human (Abu-Errish & Peanasky, 1974), pig and chicken pepsins, pig gastricsin and (rabbit) cathepsin E with a weaker influence on human gastricsin and little or no effect on other aspartic proteinases (Valler et al., 1985).

The selectivity of inhibition is also observed with the pepsin inhibitor peptide obtained upon activation of pepsinogen. It has been known for almost 50 years that one of the peptides released on activation of (pig) pepsinogen binds to pepsin above pH 4.5 to stabilize the enzyme at higher pH values and to inhibit it (measured traditionally in a milk-clotting assay at pH 5.3). This inhibitor has been identified as the pentapeptide released in the sequential activation of pig (and cow) pepsinogen(s) and is derived from the first 16/17 residues in the zymogens (Kay et al., 1983). There is considerable sequence homology in this region of the propeptide aromatic amino acid residues known to be present in the naturally occurring substrate for the enzyme. In addition, whereas the enzyme is inhibited by these compounds whereas and the identity of the inhibitor peptide is essential. However, the naturally occurring I–16 propeptide from (pig) pepsinogen is able to inhibit certain enzymes only so that, given the extensive homologies in propeptide sequences, it seems likely that the features that determine susceptibility to inhibition must lie within the enzymes themselves.

Detailed investigations have revealed that only those enzymes which have a glutamic acid residue in position 13 in the protein sequence are affected by the inhibitor peptide (Dunn, 1982; Kay et al., 1983; Dunn et al., 1985).

At pH values above about 4.5, this will be ionized and the inhibitor peptide can no longer bind to pepsin above pH 4.5 to stabilize the enzyme at higher pH values and to inhibit it. This differential susceptibility to inhibition by naturally occurring compounds appears to be of the best-known category, the pepstatins. Two forms of the acyl-Val-Val-Sta-Ala-Sta pentapeptide structure predominate in Actinomycetes and these differ only in the nature of their acyl substituent. These are the isovaleryl and acetyl derivatives and both are very poorly soluble in aqueous solution. However, by modification of the naturally occurring pepstatins, it is possible to introduce a hydrophilic lactoyl residue as the acylating group and the resultant (shorter) lactoyl-Val-Val-Sta-Ala-Sta (lactoyl-pepstatin) is much more soluble in water (Kay et al., 1982; Valler et al., 1985). Lactoyl-pepstatin is as effective as isovaleryl- and acetyl-pepstatins in inhibiting pig and human pepsins and pig gastricsin but it interacts much less tightly than its hydrophobic counterparts with other aspartic proteinases (Valler et al., 1985). This suggests that the residue occupying the S3 subsite of the inhibitor peptide is an important determinant of the efficiency of inhibitor binding. Furthermore, whereas the isovaleryl- and acetyl-pepstatins are equally potent towards all of the mammalian enzymes, microbial aspartic proteinases (e.g. from Endothia parasitica, Mucor pusillus and Penicillium janthinellum) appear to be more susceptible (by approx. one order of magnitude) to isovaleryl-pepstatin.

Thus, the different nature of the subsites of the active site of individual enzymes is reflected by their distinctive susceptibility to inhibition. Nevertheless, some degree of inhibition is always observed with each pepstatin with every enzyme and this probably results from the energy of interaction derived from the first statine residue in the acyl-Val-Val-Sta-Ala-Sta sequence being bound in close proximity to the two catalytic aspartic acid residues. The –CHOH–CH2– structure intrinsic to statine has been suggested to be an analogue of the tetrahedral intermediate (or transition state) for the enzymatic reaction.

On the basis of these observations with naturally occurring inhibitors, it would seem to be possible to design synthetic counterparts that should be specific for individual enzymes. The rationale behind such inhibitors has been to synthesize a peptide of an appropriate length which contains the amino acid residues known to be present in the naturally occurring substrate for the enzyme but with the introduction of statine in place of the scissile peptide bond of the substrate. An approach has led to the synthesis of highly potent inhibitors of human renin (Boger et al., 1983) and calf chymosin (Powell et al., 1985).

In a somewhat different strategy, a chemical modification of the scissile peptide bond is used to introduce a non-hydrolysable analogue of the tetrahedral transition state formed during hydrolysis (Szeleke et al., 1982). Thus, instead of using the naturally occurring statine residue as the centrepiece around which the inhibitor is constructed, the complete amino acid sequence of the substrate is retained in the inhibitor except that the scissile peptide bond –CO–NH– between residues P3–1/1 of the substrate is replaced by, for example, the synthetic secondary amine

\[ \text{CH}_2\text{NH} \]

as the non-hydrolysable analogue of the transition state. Using this approach, a tight-binding inhibitor of human renin has been developed which is a synthetic analogue based on the sequence of residues known to occur on either side of the scissile –Leu–Val– peptide bond of human angiotensinogen but with a reduced –\( \text{CH}_2\text{NH} \)– isostere in place of the –\( \text{CONH} \)– of the substrate.

It would thus appear that while naturally occurring inhibitors of aspartic proteases may have little physiological significance in regulating their target enzymes in vivo, nevertheless such compounds together with their synthetic counterparts have proved of inestimable value in
facilitating distinction among the different types of aspartic proteinases.

Thus, while considerable progress has been made in elucidating the structure, activity and importance (biological and commercial) of the aspartic proteinases, much remains to be learned about the distinctions in molecular architecture and how these are reflected in the functions of the various enzymes. Inhibitors (naturally occurring and synthetic) have permitted detailed biochemical and crystallographic investigations to be made but an understanding of the selectivity of such inhibitors may be of just as much importance for the design and synthesis of specific inhibitors for use therapeutically in controlling individual aspartic proteinases (e.g. renin). These and other aspects will be discussed in the articles which follow this introductory review.

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Human renin inhibitors

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Renin is an aspartic proteinase synthesized mainly in the juxtaglomerular cells of the kidney. It cleaves its substrate angiotensinogen to give the decapeptide angiotensin I, which is then converted to the effector hormone of the renin–angiotensin system, angiotensin II, by a carboxy-dipeptidase, converting enzyme. Angiotensin II has many effects concerned with the control of electrolyte balance and blood pressure.

The specificity of renin for one peptide bond in its substrate and the fact that it is active at pH 7.4 makes it unusual among aspartic proteinases, although it has features in common with other members of the group. Certain regions of primary sequence are homologous, especially around the two active-site aspartic acid residues. Knowledge of the primary structure, deduced from cDNA cloning of the human renin gene, has allowed predictions to be made concerning the three-dimensional structure of the enzyme (Blundell et al., 1983; Imai et al., 1983; Soubrier et al., 1983).

Like peptidase D, renin is synthesized as an enzymically inactive precursor which is cleaved, probably by a serine proteinase, to give the active enzyme. The prosequence shows differences compared with that of pepsinogen and this may be related to the different mechanism of activation (Imai et al., 1983; Soubrier et al., 1983).

The substrate specificity of renin was first investigated at a molecular level by Skeggs et al. (1957), who showed that it requires the (6–13)octapeptide at the N-terminus of angiotensinogen in order to cleave the scissile bond, which is Leu-10–Leu-11 in horse angiotensinogen and Leu-10–Val-11 in the human protein (Tewskbury et al., 1978). After the discovery of the sequence, substrate analogue inhibitors of renin were synthesized by substitution of amino acid residues in the octapeptide sequence

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