

Minireview

Multiple functions of pro-parts of aspartic proteinase zymogens

Gerald Koelsch^a, Michael Mareš^b, Peter Metcalf^c, Martin Fusek^{a,*}

^aOklahoma Medical Research Foundation and University of Oklahoma Health Sciences Center, 825 N.E. 13th Street, Oklahoma City, OK 73104, USA

^bInstitute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, CZ 16610, Czech Republic

^cEuropean Molecular Biology Laboratory, Heidelberg, D-6900, Germany

Received 4 March 1994

Abstract

The importance of aspartic proteinases in human pathophysiology continues to initiate extensive research. With burgeoning information on their biological functions and structures, the traditional view of the role of activation peptides of aspartic proteinases solely as inhibitors of the active site is changing. These peptide segments, or pro-parts, are deemed important for correct folding, targeting, and control of the activation of aspartic proteinase zymogens. Consequently, the primary structures of pro-parts reflect these functions. We discuss guidelines for formation of hypotheses derived from comparing the physiological function of aspartic proteinases and sequences of their pro-parts.

Key words: Aspartic proteinase; Chaperon; Folding; Pro-part; Protein structure; Targeting

1. Introduction

Aspartic proteinases (EC 3.4.23) share a high degree of similarity which involves primary structures, extending through almost identical secondary structural motifs and manifesting a typical bilobal molecular shape [1,2]. Despite this overall similarity, substantial differences exist in the catalytic properties, the cellular localization, and consequently the biological function of this family of enzymes. Aspartic proteinases are usually divided into the following groups: pepsins and chymosins, cathepsins, renins, fungal aspartic proteinases and retroviral aspartic proteinases [3]. Eucaryotic aspartic proteinases are usually bilobal, monomeric molecules, while retroviral proteinases are functional as dimers of identical polypeptides [4]. Available evidence suggests that all eucaryotic aspartic proteinases are synthesized as zymogens. Retroviral aspartic proteinases, on the other hand, are synthesized as a part of a structural polyprotein [5]. In our review we focus on the roles of activation peptides, also referred to as pro-parts, in the function of aspartic proteinases. The participation of these peptides in the auto-activation of aspartic proteinases has been reviewed [6,7]. Several new features have been recognized for the function of pro-parts and are highlighted in our review of recent work.

The processing of newly synthesized aspartic pro-

teinases starts with the removal of their signal peptides during the passage into the endoplasmic reticulum. The complete activation of eucaryotic zymogens of aspartic proteinases is accomplished by a proteolytic removal of the N-terminal pro-part which is usually 44–50 amino acids long [6,7]. The known sequences of pro-parts of aspartic proteinases are summarized in the legend to Fig. 1. Numerous three-dimensional structures of activated aspartic proteinases are known [2,3] but for their zymogens only the tertiary structure of pig pepsinogen A is available [8,9] and preliminary data on the human progastricsin structure were recently reported by James et al. [10].

Recent evidence suggests that the pro-parts of aspartic proteinases participate in folding and in cellular sorting events. Furthermore, their sequences reveal conserved motifs correlated to the pH of physiological activation and to the mode of activation. Our review is organized accordingly.

2. Folding of zymogens

The pro-part of yeast proteinase A of *Saccharomyces cerevisiae* was shown to function as an intramolecular chaperon during the folding of the zymogen *in vivo* [11,12]. These studies suggest that the chaperon-like function depends on the N-terminal portion of the pro-part. Indirect evidence for such a function of pro-parts

*Corresponding author. Fax: (1) (405) 271 8568.

of aspartic proteinases follows from experiments on the expression of human procathepsin D in mammalian cells [13]. When this enzyme was expressed without the pro-

part, it was rapidly degraded intracellularly, probably as a result of improper folding. However, a different situation exists for renin where the enzyme expressed without the pro-part was correctly folded [14]. Successful folding of synthetic mature retroviral proteinases implies that no pro-part is, in this case, necessary for the folding step [15]. Accordingly, evidence of Lin et al. [16] indicates that the C-terminal domain of porcine pepsin may fold independently of the pro-part, although the N-terminal domain expressed without the pro-part folds poorly. These findings suggest that for some eucaryotic aspartic proteinase zymogens the pro-part may have co-evolved with the N-terminal domain following a gene-duplication event [1] in order to facilitate folding of this more recently specialized domain.

Similar observations of the dependence of folding upon the pro-part were made for the family of serine proteinases where an alternating pattern between charged and hydrophobic residues within pro-parts was suggested to play an important role in the intramolecular-chaperon function [17].

3. Sorting of aspartic proteinases

Aspartic proteinases function both extracellularly and intracellularly. Pepsins are secreted from specialized stomach chief cells after a signal transmitted by the hormone gastrin, whereas the secretion of prorenin is controlled by many renal factors, and an unknown signal initiates the secretion of fungal proteinases. Yeast and

	1P	10P	20P	30P
PIGPEPALVKVLVLRK.KSLRNLIK.NGK...	LKDF.LKTHKH..		
HUMPEPAIMYKVLIRK.KSLRRTL.SERGL...	LKDF.LKKHNL..		
TUNPEPALLQVPL.EKGQSAREYL.QEGL...	WEQYRLK.YPN..		
CHIEPEASIHRVPL.KKGKSLRKQL.KDHGL...	LEDF.LKKHPYN..		
BOVPEPBLVKTP.L.KKFKSIRETM.KKGL...	LZBF.LRTYKH..		
HUMPEPCAAVVKVPL.KKFKSIRETM.KKGL...	LGEF.LRTHKYD..		
GPGPEPCTQIKVPL.KKIKSIREVL.REKGL...	LGDF.LKNHKPQ..		
BOVCHYAETRIPL.YGKSLRKAL.KHGL...	LEDF.LQKQYV..		
OVICHYAETRIPL.YGKSLRKAL.KHGL...	LEDF.LQKQYV..		
HUMREN	TFGLPTDTTTPKRIPL.KRMPVSRESL.KERG...	DMARLQ..PEWS..		
MOUREN	TFSLPTRTATFERIPL.KKMPVSREIL.EERG...	DMTRLQ..AERG..		
HUMCEQGSILHRVPL.RRHPSLKKKL.RARSQ...	LSEF.WKSHNLD..		
GPGCEALHVRPLSRR.ESLRKKL.RAQGQ...	LLEL.WKSNLNL..		
HUMCDLVRIPL.HKFTSIRRTMSEVGGSV...	EDL.IA..K.G..		
INSCDDFVRVQL.HKTESARQHF...	RNVDTIHK..		
CELCDIQRIKLEK..	TYTRE..QYKFGSIQ.EH..		
PEP4AKV..HK..AKIYKHELSD.EMKEVTPEQH.LAH..			
BARPEGLVRIALKKR..PIDRN.SRVATGLSGGEEQPL..	LSGAN..		
EPAP	ALSSPTKQHVGPVNASPEVGPCKYSFKQVRNPYKFGN..	PL.SV.KKTY..		
MPAPLTSRRPV..	S.KQ.SDADDKLLALPLTSVNR..		
CAAPTPT..TTKR..SAG.FVALDFSVV.KTP..			
YAP3KIIPAANKRDDSDNSKFVKLPFKHLYGDSLENVGSDKKPE			

	40P	1	9
PIGPEPA	..**..PASKYFPE..A...AAL..GDEPLENY..	*
HUMPEPAPARKYFPQWEA..	PTLV..	DEQPLENY..
TUNPEPAPMAKF..	DPSFAVA..	GEPMND..
CHIEPEAPASKYHPV..	TESY..	EPMTNY..
BOVPEPBPAEKY..	RF..GDFIVAT..	EPMD.Y..
HUMPEPCPAWKY..	RF..GDLVTV..	EPMA.Y..
GPGPEPCHARKFFRNRIAK..	TGDFVLY..	EPMS.Y..
BOVCHYISSKY..	SG.FEE.VASV..	PLTNY..
OVICHYVSSEY..	SG.FEE.VASV..	PLTNY..
HUMRENQPKKRLT..	LGNTSSVI..	LTNY..
MOURENVFKKRPS..	LINITSPVV..	LTNY..
HUMCEM...QTE..	SCSMD..	QSAKEPLINY..
GPGCEMQ...CSTI..	QSANEPILNY..
HUMCDPVSQY..	SQ..AV..	PAVTEGPIPEVLKNY..
INSCDLRLKYN..	AVS.GPVPEPLSNY..
CELCDLKAKYVPGYIPN..	KD..	AFNEG..LSDY..
PEP4LGQKYLQFEKA..	NPE.VVFSREH..	PFFTEGGHDVPLTNY..
BARPP.LR..	SE..	EEGDIVA.LKNY..
EPAPLKY..	GVPIPAWLEDAVONSTSGLAERSTG.SATTTPTDSL..	
MPAPKYSQTK..	HGQAAEKLGKIKAF..	GDG.S.VDTP.GLY..
CAAPKAF..	PVTN..GQEGKTS..	KR..QAVPVTLHNEQVTY..
YAP3	VRLLRK...DGY..	EETITNQSSFY..

Fig. 1. Multiple sequence alignment of activation peptides of aspartic proteinases. Each sequence includes the activation peptide plus subsequent amino acids of the mature amino terminus including the residue which aligns with Tyr9 of porcine pepsinogen (PIGPEPA). The numbering is according to PIGPEPA. Mature amino termini are shaded; the positions of the Lys36P, Tyr37P, and Tyr9 of PIGPEPA are marked with an * above their positions. The Lys36P, Tyr37P, Tyr9 triad interacts with the active site in the pepsinogen crystal structure [8,9]. Vertebrate sequences were aligned first using GCG PILEUP [45] with minor alterations made to the alignment. Remaining fungal sequences were aligned to that group. The sequences were obtained from either GENBANK (GB), EMBL (EM), PIR, or SWISSPROT (SW) data banks using GCG TFASTA or FASTA with the N-terminal 77 amino acids of human cathepsin D as a query sequence. Sequences are listed with species, proteinase name, keyword as appears in the alignment, and databank accession number. Appearing in the alignment are: Porcine pepsin A, **PIGPEPA**, PIR:A32455; Human pepsin A, **HUMPEPA**, PIR:A00980; Tuna pepsin 1, **TUNPEPA**, SW:P20139; Chicken pepsin A, **CHIEPEA**, PIR:A00984; Bovine pepsin B, **BOVPEPB**, PIR:A23457; Human pepsin C, **HUMPEPC**, PIR:A31811; Guinea pig pepsin C, **GPGPEPC**, PIR:B43356; Bovine chymosin A, **BOVCHY**, PIR:A00985; Ovine chymosin, **OVICHY**, PIR:S10996; Human renin, **HUMREN**, PIR:A21454; Mouse renin, **MOUREN**, PIR:JH0083; Human cathepsin E, **HUMCE**, PIR:A34401; Guinea pig cathepsin E, **GPGCE**, PIR:A43356; Human cathepsin D, **HUMCD**, PIR:A25771; Mosquito aspartic proteinase, **INSCD**, GB:A45117; *C. elegans* clone homologous to cathepsin D, **CELCD**, GB:M88822; *S. cerevisiae* proteinase A, **PEP4**, PIR:A25379; Barley aspartic proteinase, **BARP**, PIR:S19697; *E. parasitica* endothiapepsin, **EPAP**, PIR:S26871; *M. pusillus* aspartic proteinase, **MPAP**, PIR:A25767; *C. albicans* aspartic proteinase, **CAAP**, SW:PS8871; *S. cerevisiae* aspartic proteinase, **YAP3**, PIR:S20150. Other sequences obtained from the databank search, but not included in the alignment are listed here as above: Bear pepsin A, PIR:A28859; Bovine pepsin A, PIR:A92157; *C. elegans* clone homologous to pepsin, GB:M89231; Embryonic chicken pepsin A, PIR:A41443; Rhesus macaque pepsin A, PIR:JT0309; Japanese macaque pepsin A, PIR:S19681; Rabbit pepsin A, PIR:B38302; Rabbit pepsin F, PIR:A38302; Rat pepsin A, PIR:A24608; Tuna pepsin 2, SW:P20140; Bullfrog pepsin C, PIR:A39314; Japanese macaque pepsin C, PIR:A00986; Rat pepsin C, PIR:A05145; Bovine cathepsin D, SW:P80209; Chicken cathepsin D, GB:S49650; Mouse cathepsin D, PIR:S14704; Rat cathepsin D, PIR:S13111; Rat renin, PIR:A29991; Rat cathepsin E, PIR:A34657; *S. aureus* cathepsin E, PIR:C34643; Feline chymosin, PIR:A20146; Human chymosin pseudogene, GB:M57258 (exon 1 of 9); *A. awamori* aspergillopepsin A, PIR:PS0140; *C. albicans* intracellular aspartic proteinase, SW:P10977; *C. parapsilosis* aspartic proteinase 1, GB:Z11919; *C. parapsilosis* aspartic proteinase 2, GB:Z11918; *C. tropicalis* aspartic proteinase, SW:Q00663; *M. miehei* aspartic proteinase, PIR:A29039; *R. chinensis* rhizopus-pepsin, GB:M63451; *R. niveus* aspartic proteinase I, GB:M19100; *R. niveus* aspartic proteinase II, GB:X56964; *R. niveus* aspartic proteinase III, PIR:JU0343; *R. niveus* aspartic proteinase IV, GB:X56992; *R. niveus* aspartic proteinase V, GB:X56993; *S. cerevisiae* barrier protein, PIR:A34084; *S. fibulgeria* acid proteinase, **PEPI**, PIR:JT0334; Bovine aspartic proteinase, PIR:JT0399; Bovine pregnancy-specific antigen, PIR:B41545; Ovine pregnancy-specific antigen, PIR:A41545; Rice aspartic proteinase, PIR:JS0732.

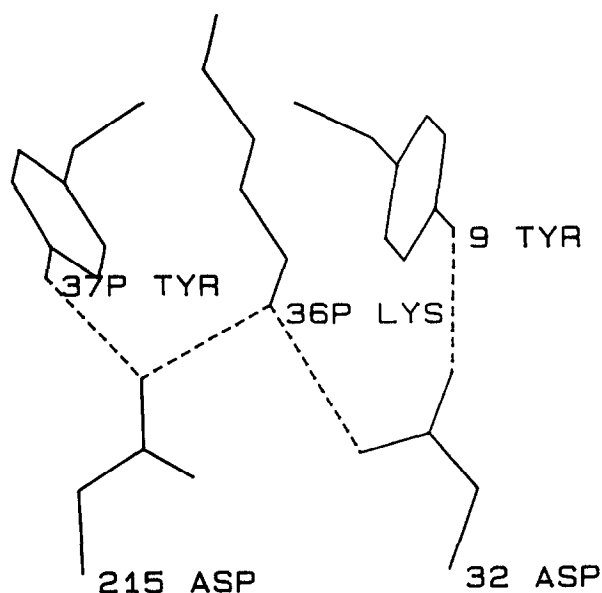


Fig. 2. Hydrogen bond interactions (dashed lines) of active site aspartic acids 32 and 215 with Lys36P, Tyr37P, and Tyr9 of porcine pepsinogen [9].

mammalian proteinases with prohormone processing activity were suggested to be localized within secretory vesicles [18,19]. Human cathepsin D is localized in lysosomes (reviewed by Hasilik, [20]) and yeast proteinase A is localized in vacuoles (vacuoles in yeast are equivalent to lysosomes) [21] and both are major intracellular aspartic proteinases, while cathepsin E is localized intracellularly but extra-lysosomally [22].

Human cathepsin D is targeted to lysosomes by the mannose-6-phosphate (M6P) targeting pathway using the M6P marker and also by an alternative targeting pathway (reviewed by Kornfeld and Mellman, [23]). There are several reports on M6P-independent association of procathepsin D with intracellular membranes of mammalian cells (summarized in [25]), suggesting interaction of the pro-part with yet unknown receptor(s). A 60 kDa protein specifically interacting with procathepsin D within the cell has been identified which may be the receptor [26]. It was recently shown that rat procathepsin D, expressed in yeast, is correctly targeted to the acidic vacuoles suggesting an interaction with the same targeting system as the zymogen of proteinase A [24]. In yeast, the C-terminal part of the pro-part of proteinase A seems to play a crucial role in the targeting of the zymogen to vacuoles [11].

The alignment shown in Fig. 1 indicates a similarity between part of the pro-part of proteinase A and the pro-parts of procathepsin D molecules (sequence Pro-Ala-Val-Thr-Glu-Gly, at the C-terminus of procathepsin D pro-part). A three-dimensional model of human procathepsin D suggests that this region of procathepsin D may form a surface exposed structure [27]. We propose that this sequence might be an evolutionarily related

region between pro-parts of proteinase A and procathepsin D which can interact with a receptor. This hypothesis has recently been supported by the identification of a sequence of the pro-part of procathepsin L which interacts with an intracellular receptor [28]. Similarity between the sequences of non-homologous lysosomal and vacuolar proteins procathepsin L and yeast procaryboxypeptidase Y was also identified [28].

4. Mode of processing

The activation of zymogens of aspartic proteinases is presumably initiated by a dramatic conformational rearrangement of the first 60 amino acids of the zymogen [8,9]. This process is often triggered by low pH and results in proteolytic cleavage of a pro-part [6,7].

The following three mechanisms have been observed for the degree to which zymogens of aspartic proteinases may undergo self-processing of their pro-parts during activation: complete self-processing, self-processing which is partially assisted by a different proteinase, or fully assisted processing. An example of the first type is stomach pepsinogens A (reviewed by Tang, [6]) for which both intramolecular and intermolecular autoactivation may contribute to the activation of these zymogens [29]. Lysosomal and vacuolar proteinases are representatives of the second type. Human procathepsin D is capable of only partial self processing and activation [30,31] requiring lysosomal cysteine proteinase(s) for the final processing of its pro-part [32]. Similarly, serine vacuolar proteinase B is responsible for the final proteolytic maturation of the zymogen of proteinase A of yeast [33]. The third type of processing of the pro-part is typical for the activation of prorenin. Either thiol [34] or serine proteinase [35] was shown to be required in this process, depending on the source of the prorenin.

The activation of retroviral aspartic proteinases is more complicated as most are synthesized as a subunit of *gag-pol* derived polyprotein precursors with resulting N- and C-terminal extensions [5]. Autoprocessing of the precursor of HIV proteinase was demonstrated with a shortened form of the precursor [36]. The N-terminal, p6 peptide extension of HIV proteinase is involved in the inhibitory regulation of proteinase autoprocessing reminiscent of the function of pro-parts [37]. Constructs of HIV aspartic proteinase with partial N-terminal extensions with an introduced mutation which prevented the self-cleavage of the proteinase were found as intrinsically active and displayed a pH-optimum shift to neutral pH [38]. Possible roles of these terminal extensions in the viral replication process remains to be elucidated.

A prediction of the type of processing of proenzymes of aspartic proteinases might be sought from their primary structures (Fig. 1). It is reasonable to propose that for zymogens which are capable of self-activation, the

cleavage site at their mature termini should be in agreement with their specificity. The two hydrophobic residues, preferred at P₁ and P₁' positions by pepsins [39], are found in the cleavage sites of the zymogen. On the contrary, cathepsin D, which shares analogous general specificity, has the sequence Glu–Gly as the final assisted maturation cleavage site. A similar feature is found in other aspartic proteinases; for example renin or proteinase A for which activation is assisted by different processing enzymes.

The data on aspartic proteinases with only partially processed pro-parts indicate that these forms might have different biological properties compared to fully activated enzymes. The importance of these forms remains to be elucidated.

5. The pH of physiological activation

Intact pro-parts of human procathepsin D and chicken pepsinogen inhibit most aspartic proteinases with K_i in the nanomolar range and with significant pH dependence [40]. Screening of synthetic fragments of the pro-part of prorenin identified two shorter peptides inhibiting renin: the peptide 10P–20P and the C-terminal peptide 32P–43P [41]. It was also shown that N-terminal fragments of the pro-part of pig pepsinogen (1P–12P and 1P–16P) are inhibitory [29,42]. The sequence alignment of prorenin and pepsinogen shows similarity between region 5P–15P of pig pepsinogen and 10P–20 of human prorenin (Fig. 1) which, together with the inhibition data, may reflect similarity in function.

The fact that two independent motifs within the pro-parts are inhibitory can be explained from the X-ray structure of pig pepsinogen. The N-terminal portion (1P–9P) of the pro-part participates in a beta-sheet and interacts with residues of the S₃ binding subsite [2] while the structure of the doublet Lys36P–Tyr37P and the highly conserved hydrophobic residue Tyr9 forms hydrogen bonds with the active site aspartates blocking S₁ and S₁' subsites (Fig. 2). Inhibition of aspartic proteinases by these two segments of the pro-part may be explained by interactions seen in the native zymogen structure. As both of these regions contain titrable residues it is plausible that their ionization states contribute to the inhibitory function optimally at pH for which ionic interactions may be maintained [8,9].

At low pH, protonation of acidic amino acids involved in ionic interactions of the pro-part is proposed to trigger the conformational change which is followed by the cleavage of the pro-part [7]. An interesting coincidence is found when zymogens of aspartic proteinases are grouped according to the pH of their physiological compartmentation and according to the presence of the Lys36P–Tyr37P sequence (Fig. 1). Those proteinases which are activated at lower pH (pepsin, cathepsin D,

yeast vacuolar proteinase A) apparently have the Lys–Tyr mechanism for locking the active site. On the other hand, those proteinases which are activated at higher pH (renin, cathepsin E, *Candida* yeast extracellular aspartic proteinases) evidently employ a different device for blocking and/or protection of the active site. There are reports on the detection of proteolytic activity of intact pro-renin (summarized in [43]), which supports the idea that the presence of the Lys–Tyr pair in the primary structure of the pro-part influences the pH of physiological activation.

6. Conclusions

The pro-parts of zymogens of aspartic proteinases have multiple functions. Some of these functions have a plausible explanation based on a multiple sequence alignment and on the tertiary structure of porcine pepsinogen and three conclusions can be made. First, the presence or absence of the Lys–Tyr sequence in the pro-part can be used as an indicator of pH of physiological activation when only the sequence of a zymogen is known. Second, the sequence near the maturation cleavage site (Pro-Ala-Val-Thr-Glu-Gly in procathepsin D) seems to be a potential candidate for the interactions of some aspartic proteinases with cellular receptors for targeting. Third, a sequence of the maturation cleavage site which is not in agreement with the specificity of that particular proteinase indicates control of the maturation process by the action of a different proteinase. The nature of multiple roles for pro-parts of aspartic proteinase zymogens described here is probably a more general feature common also to pro-parts of other classes of proteolytic enzymes [44].

Acknowledgements: The authors are thankful to Dr. J. Tang for his support and for valuable comments on the manuscript. Part of the study was supported by Grant 455105 from the Czech Academy of Sciences.

References

- [1] Tang, J., James, M., Hsu, I.N., Jenkins, J.A. and Blundell, T.L. (1978) *Nature* 271, 618–621.
- [2] Davies, R.D. (1990) *Annu. Rev. Biophys. Biophys. Chem.* 1990, 189–215.
- [3] Szecsi, P.B. (1992) *Scand. J. Clin. Lab. Invest.* 52 Suppl. 210, 5–22.
- [4] Miller, M., Jaskolski, M., Rao, J.K.M., Leis, J. and Wlodaver, A. (1989) *Nature* 337, 576–579.
- [5] Krausslich, H.-G. and Wimmer, E. (1988) *Annu. Rev. Biochem.* 57, 701–754.
- [6] Tang, J. and Wong, R.N.S. (1987) *J. Cell. Biochem.* 33, 53–63.
- [7] Foltmann, B. (1988) *Biol. Chem. Hoppe-Seyler* 369 Suppl. 311–314.
- [8] James, M.N.G. and Sielecki, A.R. (1986) *Nature* 319 33–38.
- [9] Hartsuck, J.A., Koelsch, G. and Remington, S.J. (1992) *Proteins Structure, Function and Genetics* 13, 1–25.

- [10] Moore, S., Sielecki, A., Tarasova, N., Chernaiia, M., Andreeva, N. and James, M. (1993) Presentation at the 5th International Conference on Aspartic Proteinases, Gifu, Japan.
- [11] Klionsky, D.J., Banta, L.M. and Emr, S.D. (1988) *Mol. Cell. Biol.* 8, 2105–2116.
- [12] van den Hazel, H.B., Kiellandt-Brandt, M.C. and Winther, J.R. (1993) *J. Biol. Chem.* 268, 18002–18007.
- [13] Conner G.E. (1992) *J. Biol. Chem.* 267, 21738–21745.
- [14] Norman, J.A., Baska, R., Hadjilambris, O., Youngsharp, D. and Kumar, R. (1991) in: *Structure and Function of Aspartic Proteinases* (Dunn, B.M., Ed.) pp 373–377, Plenum Press, New York.
- [15] Schneider, J. and Kent, S.B.H. (1988) *Cell* 54, 363–368.
- [16] Lin, X., Lin, Y., Koelsch, G., Gustchina, A., Wlodawer, A. and Tang, J. (1992) *J. Biol. Chem.* 267, 17257–17263.
- [17] Shinde, U. and Inouye, M. (1993) *Trends Biochem. Sci.* 18, 442–446.
- [18] Egel-Mitani, M., Flygenring, H.P. and Hansen, M.T. (1990) *Yeast* 6, 127–137.
- [19] Loh, P.Y., Parish, D.C. and Tuteja, R. (1985) *J. Biol. Chem.* 260, 7194–7205.
- [20] Hasilik, A. (1992) *Experientia* 48, 130–151.
- [21] Leney, J.F., Matile, P., Wiemken, A., Schellenberg, M. and Meyer, J. (1974) *Biochem. Biophys. Res. Commun.* 60, 1378–1383.
- [22] Lapresle, C. (1971) in: *Tissue Proteinases* (Barrett, A.J. and Dingle, J.T., Eds.) pp. 135–155, North-Holland, Amsterdam.
- [23] Kornfeld, S. and Mellman I. (1986) *Annu. Rev. Biochem.* 55, 167–193.
- [24] Nishimura, Y., Fujita, H., Kato, K. and Himeno, M. (1993) Presentation at the 5th International Conference on Aspartic Proteinases, Gifu, Japan. Plenum Press.
- [25] von Figura, K. (1991) *Curr. Opin. Cell Biol.* 3, 642–646.
- [26] Grassel, S. and Hasilik, A. (1992) *Biochem. Biophys. Res. Commun.* 182, 267–282.
- [27] Koelsch, G., Vetvicka, V., Metcalf, P. and Fusek, M., (1993) Proceedings of the 5th International Conference on Aspartic Proteinases, Gifu, Japan. Plenum Press, in press.
- [28] McIntyre, G.F., Gobold, G.D. and Erickson, A.H. (1994) *J. Biol. Chem.* 269, 567–572.
- [29] Nielsen, F.S. and Foltmann, B. (1993) *Eur. J. Biochem.* 217, 137–142.
- [30] Conner, G.E. (1989) *Biochem. J.* 263, 601–604.
- [31] Larsen, L.B., Boisen, A. and Petersen, T.E. (1993) *FEBS Lett.* 319, 54–58.
- [32] Samarel, A.M., Ferguson, A.G., Decker, R.S. and Lesch, M. (1989) *Am. J. Physiol.* 257, C1069–C1079.
- [33] Hazel, B.H., Kielland-Brandt, M.C. and Winther, J.R. (1992) *Eur. J. Biochem.* 207, 277–283.
- [34] Shinagawa, T., Yung, S.D., Baxter, J.D., Carylli, C., Shilling, J. and Hsueh, W.A. (1990) *Proc. Natl. Acad. Sci. USA* 87, 1927–1931.
- [35] Kim, W.-S., Hatsuzawa, K., Ishizuka, Y., Hashiba, K., Murakami, K. and Nakayama, K. (1990) *J. Biol. Chem.* 265, 5930–5933.
- [36] Co, E., Koelsch, G., Lin, Y., Ido, E., Hartsuck, J.A. and Tang, J. (1994) *Biochemistry* 33, 1248–1254.
- [37] Partin, K., Zybarth, G., Ehrlich, L., DeCromburghe, M., Wimmer, E. and Carter, C. (1991) *Proc. Natl. Acad. Sci. USA* 88, 4776–4780.
- [38] Phylip, L.H., Mills, J.S., Parten, B.F., Dunn, B.M. and Kay, J. (1992) *FEBS Lett.* 314, 449–454.
- [39] Fruton, J. (1970) *Adv. Enzymol.* 33, 401–433.
- [40] Fusek M., Mares M., Vagner J., Voburka Z. and Baudys M. (1991) *FEBS Lett.* 287, 160–162.
- [41] Richards, A.D., Kay, J., Dunn, B.M., Bessant, C.M. and Charlton (1991) *Int. J. Biochem.* 24, 297–301.
- [42] Dunn, B.M., Lewitt, M. and Pham, C. (1983) *Biochem. J.* 209, 355–362.
- [43] Derkx, F.H.M., Deinum, J., Lipowski, M., Verhaar, M., Fischli, W. and Schalkenkamp, M.A.D.H. (1992) *J. Biol. Chem.* 267, 22837–22842.
- [44] Sorensen, P., Winther, J.R., Kaarsholm, N.C. and Poulsen, F.M. (1993) *Biochemistry* 32, 12160–12166.
- [45] Genetics Computer Group (1991) 575 Science Drive, Madison, WI 53711, USA.