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Minireview

The cystatins: protein inhibitors of cysteine proteinases

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The last decade has witnessed enormous progress of protein inhibitors of cysteine proteinases concerning their structures, functions and evolutionary relationships. Although they differ in their molecular properties and biological distribution, they are structurally related proteins. All three inhibitory families, the stefins, the cystatins and the kininogens, are members of the same superfamily. Recently determined crystal structures of chicken cystatin and human stefin B established a new mechanism of interaction between cysteine proteinases and their inhibitors which is fundamentally different from the standard mechanism for serine proteinases and their inhibitors.

Cystatin; Stefin; Kininogen; Mechanism of interaction; Structure; Amino acid sequence

1. INTRODUCTION

During the last decade, our understanding of cellular processes involving protein degradation has reached the level at which we can explain with more certainty the regulation and mechanism controlling proteinase activities. Among four classes of proteinases, the aspartic, the serine, the metallo and cysteine proteinases, more recently attention has been paid to the last group of enzymes of mammalian origin. Current evidence suggests that lysosomal cysteine proteinases cathepsins B, H, L and S are papain-like enzymes. They are well-characterized proteins (reviewed in [1,2]) with known primary structures [3–7]. Very recently, the crystal structure of human liver cathepsin B has been solved by X-ray crystallography indicating that the overall folding pattern of this enzyme and the arrangement of the active-site residues are similar to the related cysteine proteinases papain and actinidin [8].

Within the last ten years it also has become evident that newly discovered protein inhibitors of cysteine proteinases, named cystatins, offer a new insight into the processes in which the inhibitors participate (reviewed in [9,10]). These inhibitors might protect the cells from inappropriate endogenous or external proteolysis and/or could be involved in the control mechanism responsible for intracellular or extracellular protein breakdown. The cystatins are tight and reversibly binding inhibitors of the papain-like cysteine proteinases. They form a superfamily of sequentially homologous proteins subdivided into three families, the stefins, the cystatins and the kininogens [11]. The recently discovered cathelin, a protein inhibitor of cathepsin L, indicates that a new family of cysteine proteinase inhibitors may exist [12].

In this minireview it is impossible to cite all of the authors who have contributed information to the present status of cystatins. Therefore, additional references will be found in reviews cited in this paper. This minireview will stress some of the more important features of cystatins including their recently determined structures and the proposed mechanism of interaction with their target enzymes.

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2. STEFIN FAMILY

The protein inhibitors of this family are single-chain proteins with M_r of about 11 000, which lack disulfide bonds and carbohydrates [9]. Human stefin A and rat cystatin α are found in high concentrations in various types of epithelial cells and in polymorphonuclear leucocytes. In contrast, human stefin B and rat cystatin β are found to be widely distributed amongst different cells and tissues [2,9,13]. Although it was suggested that stefins are intracellular proteins, they have been found also in extracellular fluids [14]. The stefins have been isolated from different mammalian tissues including humans, and characterized. Whereas human stefin A occurs in multiple isoelectric forms, with pI values in the range 4.5–5.0 [9,15,16], human stefin B is a more neutral protein with pI values in the range 5.9–6.5

[9,17]. Stefins are stable in the neutral and alkaline pH range as well as heat stable. Human stefin B forms disulfide-linked dimers with M_r of about 25 000. The dimeric form is inactive and can be converted to the inhibitory active monomer under reducing conditions [17]. Dimeric bond formation is consistent with similar observation for rat cystatin β [18]. It has been suggested that the inhibitory activity of rat cystatin β can be regulated with covalent modification by forming a mixed disulfide with glutathione. Residue Cys-3 of rat cystatin β is involved in the binding of glutathione in such a manner that the free form of the inhibitor is active and the complex with glutathione is inactive [18,19].

Stefins are potent reversible and competitive inhibitors of cysteine proteinases. The inhibition constant for papain with human stefin A is 1.9×10^{-11} M. Cathepsin B inhibition (7.3×10^{-8} M) is weaker than that for papain, cathepsin H and cathepsin L.

Recently, a stretch of DNA containing the coding sequence for human stefin A was synthesized and expressed in *E. coli* [20,21] and the resulting recombinant stefin A exhibits similar biochemical properties as the native protein. Also a gene coding for human stefin B was synthesized and expressed [22,23]. In order to avoid oxidation of Cys-3 which causes dimer formation, the mutant [Ser^3]stefin B was prepared and the resulting recombinant protein was used for determination of the crystal structure of stefin B in a complex with papain [24].

In addition to stefins of animal origin, in rice there are two different inhibitors of cysteine proteinases, designated as oryzacystatin I and II [25,26]. Besides papain and other cysteine proteinases [25] oryzacystatin also inhibits insect digestive cysteine proteinases [27]. Both plant inhibitors do not contain cysteine (see Fig. 1), similar to other members of the stefin family. Although it was originally proposed that oryzacystatins represent a new photocystatin family [26], both the rice inhibitors should be included at least temporarily in the stefin family. The photostefins may act as host plant resistance factors for insects and other agricultural pests.

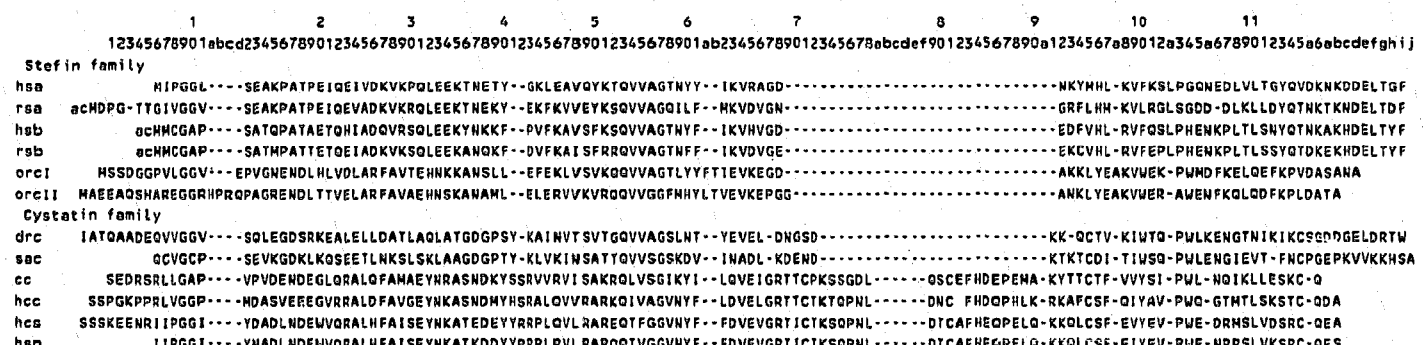

Fig. 1. Alignment of amino acid sequences of stefins, cystatins and the homologous domains of kininogens. The alignment has been prepared mainly by use of the ALIGN programme [81]. Numbering is according to the chicken cystatin sequence using the single letter code for amino acids. Key: (hsa) human stefin A; (rsa) rat cystatin α; (hsb) human stefin B; (rsb) rat cystatin β; (orcI) oryzacystatin I [81]; (orcII) oryzacystatin II [25]; (drc) *Drosophila* cystatin [83]; (sac) sarcocystatin A [84], (hcc) human cystatin C; (cc) chicken cystatin; (rcc) rat cystatin C [85]; (rcs) rat cystatin S [86]; (hcs) human cystatin S; (hsn) human cystatin SN; (hsa) human cystatin SA; (bcc) bovine colostrum cystatin; (pac) African puff adder venom cystatin [87]; (hk1, hk2 and hk3) three human kininogen segments; (bk1, bk2 and bk3) three bovine kininogen segments; (rk1, rk2 and rk3) three rat kininogen segments; (rt11, rk12, rk13) three rat T-kininogen 1 segments; (rt21, rt22, rt23) three rat T-kininogen 2 segments. References are in brackets. Other references are in [9,80].

3. CYSTATIN FAMILY

The name cystatin was first used by Barrett [28] to describe an inhibitor that had been discovered and partially characterized from chicken egg-white of papain, ficin and other related cysteine endopeptidases [29]. When other protein inhibitors of cysteine proteinases were characterized and their amino acid sequences determined, it became apparent that they are related to chicken cystatin, and thus they are members of the cystatin superfamily [11]. At the same time it was decided that the second family within the cystatin superfamily may also be called the cystatin family. The main characteristic of the inhibitors of the cystatin family is the presence of two disulfide bonds located towards the carboxyl-terminus. They are composed of about 115 amino acid residues with M_r of about 13 000. These proteins occur at relatively high concentrations in many biological fluids such as human cystatin C in human seminal plasma (51 mg/l) and cerebrospinal fluid (5.8 mg/l), and at lower concentrations in other fluids such as plasma, saliva and urine (reviewed in [9,14,30]).

Chicken cystatin, the best known representative, was isolated as a mixture of two major isoelectric forms of identical amino acid sequences [31,32], a non-phosphorylated form **1** with pI of 6.5 and a phosphorylated form **2** with pI of 5.6 [33]. Both variants can be further separated into full-length chains with 116 amino acid residues (Ser-forms) and several amino terminal-truncated forms including the Gly- and Ala-forms with 108 and 107 amino acid residues, respectively [34]. The shorter Gly-9- and the Ala-10-forms (cystatin numbering) of chicken cystatin bind almost 10 000-fold weaker to papain compared to the longer forms (K_i = 6 pM) [34,35]. Chicken cystatin is a powerful protein inhibitor of papain ($K_i = 5 \times 10^{-12}$ M) and other lysosomal cysteine proteinases (reviewed in [9,74]. Recently, the inhibitor has been cloned [36,37] and expressed [36]. The recombinant protein is active and similar in properties to those of the natural inhibitor. Chicken cystatin also has been crystallized and its three-dimensional structure determined [38].

The most investigated inhibitor of human origin is cystatin C. It was isolated from serum of patients with autoimmune diseases [39]. The inhibitor occurs in several multiple forms with pIs in the alkaline pH range. When its partial amino acid sequence was determined [39], we discovered that this inhibitor is identical to γ-trace, a basic protein, of hitherto unknown biological function [40]. Due to its high degree of homology with chicken cystatin [31], we proposed that the newly discovered inhibitor of cysteine proteinases should be named human cystatin [19] and soon thereafter the name human cystatin C was suggested [41]. Cystatin C is also a very potent inhibitor of lysosomal cysteine proteinases and papain [9]. With the exception of the rat cystatin C [42], all other members of this family described so far are not glycosylated.

It was reported that the human cystatin C variant (former γ-trace) is a major constituent of the amyloid fibrils in patients from Iceland with hereditary cerebral hemorrhage with amyloidosis [43]. The protein consists of 110 amino acid residues beginning at its 11th amino-terminal residue Gly. It has an amino acid substitution glutamine for leucine at position 68 (in human cystatin C numbering) suggesting that a point mutation has occurred. Human cystatin C was cloned and expressed [44–46]. The recombinant protein displayed full biological activity against its target enzymes [46].

There are many additional papers published by M. Abrahamson, A. Grubb and I. Olafsson indicating the biological importance of cystatin C. Due to the length of this minireview, it is not possible to include all of their important contributions. Several other cystatins were isolated from human saliva [47] and other species and their sequences determined (reviewed in [9]; see Fig. 1).

4. KININOGEN FAMILY

Kininogens have long been known as the precursor proteins of the vasoactive kinins and as participants in the blood coagulation cascade [48]. In addition, their role in the acute phase response [49,50], inflammation [51], and the inhibition of papain-like cysteine proteinases [52–54] demonstrate their remarkable multifunctional nature. There are three distinct types of kininogens, designated as high molecular weight kininogen (H-kininogen) with M_r of about 120 000, low molecular weight kininogen (L-kininogen) with M_r of about 68 000, and T-kininogen (also known as 'major acute phase protein') with M_r of about 68 000 (reviewed in [9,55–58]). They are all single-chain proteins. They share the heavy chains preformed at the N-terminus and the consecutive kinin fragment, but differ in their light chain at the C-terminus. Both L- and H-kininogen also exist as oligomers, as well as monomers. Kininogens are acidic proteins with pI values for multiple isoelectric forms in the pH range 4.0–5.2. They have been found in plasma and other secretions of mammalian species. The concentrations of kininogens in human plasma are 109–272 μg/ml for L-kininogen and 69–116 μg/ml for H-kininogen [59]. The inhibitory activities of kininogens are stable at neutral and alkaline pH range, but labile below pH 4.0. They are thermally stable (at 90°C) for short periods of time.

The kininogens (L- and H-) are strong inhibitors of papain and cathepsin L and weaker inhibitors of cathepsin H and particularly cathepsin B (reviewed in [9,52,53,55,60]). Strong inhibitory capacity against papain and cathepsin L with T-kininogen was also found [49,61]. Our findings of the presence of cleavage products from the kininogen heavy chain, in pathologic

human plasma and synovial fluid [62,63], and human placenta [64] released by unknown proteinases, as smaller-sized inhibitors with M_r of about 20 000 clearly suggested that proteolytic processing of kininogens can also occur in vivo, possibly affecting the coagulation system. Similar fragments were obtained when human L-kininogen was subjected to limited proteolysis with trypsin, chymotrypsin, elastase and other proteinases [65,66]. Several independent cleavage sites distinct from the typical kallikrein cleavage sites flanking the kinin region were found. All of the identified cleavage sites (in proteinase sensitive regions) cluster in two stretches of 11–12 residues of the kininogen heavy chain. By sequence analysis three domains corresponding to the individual cystatin-like domains (segments) were identified and numbered 1–3 from the N-terminus (see Fig. 1). Only domain 1 was not inhibitory for cysteine proteinases. Domain 2 was an inhibitor of chicken calpain, papain and cathepsin L, whereas domain 3 did not inhibit calpain, but inhibited papain and cathepsin L strongly. T-Kininogen (also called thiostatin) is an exception to the general rule for kininogens, since it is not susceptible to kallikrein hydrolysis. However, cathepsin D can liberate T-kinin (Ile-Ser-bradykinin) from T-kininogen [67,68]. Cathepsin D also inactivates the third domain of human kininogen and cystatin C [64], as well as human stefins [69] indicating that it has a biological role in the regulation of cysteine proteinase activity. Similarly, leucocyte elastase rapidly cleaves Val-10–Gly-11 bond of human cystatin C, thus decreasing the inhibitory capacity of the truncated inhibitor for human cathepsins B and L by three orders of magnitude [69a].

In T-kininogen four proteinase sensitive regions were found [61]. Two were close to the junction between the heavy chain cystatin-like domains as noted in [65,66], the third was in the kinin-containing region, and the fourth was close to the C-terminus of the T-kininogen light chain.

5. STRUCTURE OF CYSTATINS AND THE MECHANISM OF THEIR INTERACTION WITH CYSTEINE PROTEINASES

The sequences of the three families of cystatin superfamily: the stefins, the cystatins and the cystatin-like domains of the kininogens, are aligned in Fig. 1, according to the chicken cystatin numbering system. The presented alignment is based on the known tertiary structure of human stefin B [24] which confirms the relationship between the stefin and cystatin families. In particular, conserved residues of stefin B occur in positions equivalent to those of chicken cystatin. However, there are also some important differences in alignment which are difficult to predict. Thus, the published sequence alignment [9,55] has been found to be incorrect, largely due to a deletion of 23 residues with respect to the chicken cystatin sequence, which is a source of a high gap penalty in most alignment algorithms. The sequences (Fig. 1) are most highly conserved between residues 53 and 57 (QVVAG region) in the stefins and in the second and the third domains of the kininogens. The corresponding region in the cystatin family shows that only Gln-53 and Gln-57 are highly conserved. The only other residue conserved in all of the inhibitory cystatins (but not in the non-inhibitory first domain of the kininogens) is Gly-9, once proposed to be a part of a substrate-like inhibitory reactive site of cystatins [35].

Fig. 2 is a ribbon-like stereo representation of the chicken cystatin molecule based on the crystal structure [38]. The molecule consists mainly of a straight five turn α-helix, a five stranded antiparallel β-pleated sheet which is twisted and wrapped around this α-helix, and an appended segment of partial α-helical geometry. The

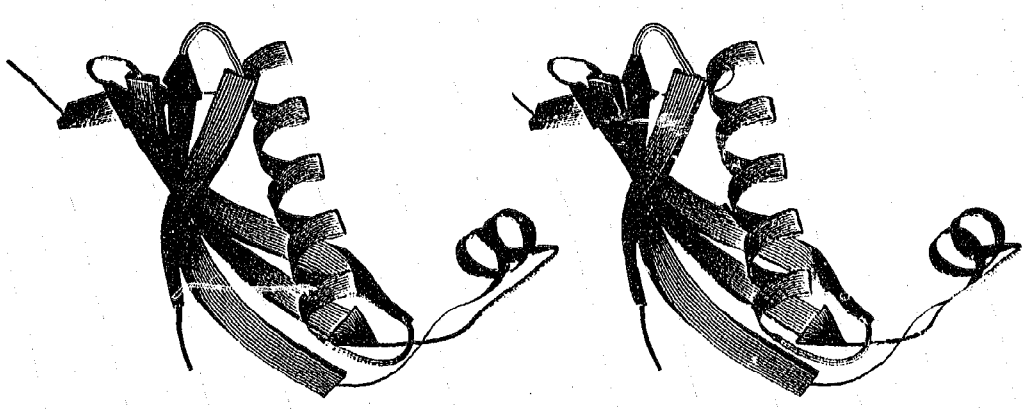

Fig. 2. Ribbon-like representation (made with RIBBON, kindly provided by Dr. Priestle) of chicken egg-white cystatin. α-Helical segments, extended β-strands and irregular intervening segments are shown as regularized helical and arrow-like elements and connecting laces. The view is approximately along the wedge-shaped edge placed at the left upper side, with (from back to front) amino-terminus (from Gly-9 onwards), first and second hairpin loop. The carboxy-terminus is at the bottom left, the appending helix on the right-hand side.

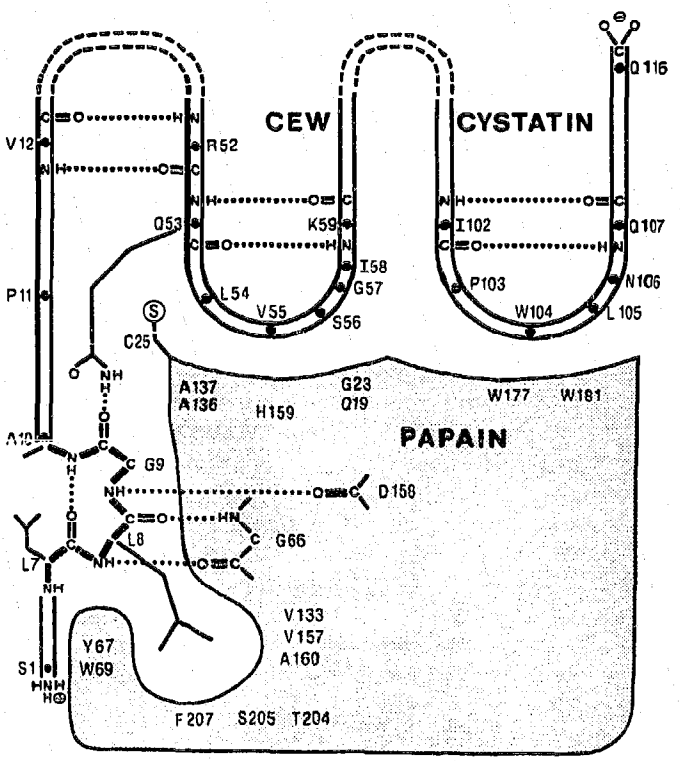

Fig. 3. Scheme of the proposed 'trunk model' for the interaction of chicken egg-white cystatin (cew cystatin) and papain.

peptide segment from Gln-53 to Gly-57 folds into a tight 'first' β-hairpin loop which is on opposite sides flanked by the amino-terminal segment and by a 'second' β-hairpin loop that contains Pro-Trp residues. These residues are highly conserved in the cystatin family and the third domain of the kininogens. Both hairpin loops and the N-terminus form a hydrophobic wedge-shaped 'edge' which is highly complementary to the active site cleft of papain as shown by docking experiments [38]. The essential interactive elements of this hypothetical complex are shown in Fig. 3 and discussed in detail in [34,38,70,71]. In this 'trunk model', both the first and the second hairpin loops have precisely the appropriate shape and size to fill the more open part of the active site cleft of papain which represents the S1'-S2' subsites. The amino-terminal segment of cystatin (Gly-9–Ala-10) is directed towards the substrate subsite S2, but in an inappropriate conformation and too far away to be attacked by the reactive site Cys-25. Due to the rigid positioning of Ala-10 in cystatin away from Cys-25 in papain, a cleavage of the Gly-9–Ala-10 bond is impossible. The important role of Trp-104 as part of the proteinase-binding site in the second hairpin loop was recently confirmed [72–74]. The proposed model was recently verified by the crystallized stoichiometric complex of human stefin B and papain [24]. The conserved residues form a tripartite wedge, which penetrates into the papain active site as proposed earlier [38]. The main interactions are provided by the amino-terminal 'trunk' and the first hairpin loop containing the highly conserved QVVAG region, with minor contributions coming from the second hairpin loop. The carboxyl-terminus provides an additional interaction with respect to chicken cystatin. The data presented clearly show that the cystatin inhibition mechanism differs substantially from the standard mechanism generally observed for serine proteinase-inhibitor complexes [75].

Structural analysis of stefins, cystatins and three domains of the kininogens revealed that they share extensive sequence homology, enough to be classified as members of one superfamily [11]. A scheme for the evolution of mammalian cystatins was proposed predicting that the kininogens arose from the cystatins by gene triplication via intermediates containing two cystatin-like structural domains [76]. From difference matrix analyses a phylogenetic dendrogram was constructed that attempts to depict the progress of evolution for the cystatins [9,77]. The finding that α_2-HS glycoprotein [78] and histidine-rich glycoprotein (both inactive as proteinase inhibitors) contain two cystatin-like domains initiated further studies of the evolutionary relationships for this superfamily. Most recently a new scheme for the evolution of the cystatin superfamily was proposed [80] based on the numbers of copies of cystatin-like domains and the presence or absence of disulfide bonds and following the evolution of the superfamily among four main lineages. Continuing investigations of the cystatins from a variety of species of animal and plant origins will undoubtedly lead to a more definitive scheme for the evolution of these interesting and physiologically important proteins.

6. CONCLUSIONS

In the field of cystatin and their target enzymes we have achieved in the last decade enormous progress concerning their structures, functions and evolutionary relationships. There is also rapidly growing information about the importance of these biologically active substances in many pathological events as inflammation, muscle dystrophy, Alzheimer's disease, multiple sclerosis, viral diseases, hereditary diseases, tumor malignancy and other health-related disorders. The potential for cystatins playing a role in host plant resistance is also apparent. Therefore, further studies are required to obtain more knowledge of the biochemistry and molecular biology of cysteine proteinase and their inhibitors, in general, in order to better understand protein degradation under normal and pathological conditions. Further advances in the structure and function of cystatins might be useful for the design of new therapeutic and pest control agents.

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REFERENCES

[1] Barrett, A.J. and Kirschke, H. (1981) Methods Enzymol. 80, 535-561.
[2] Katunuma, N. and Kominami, E. (1985) Curr. Top. Cell. Reg. 27, 345-360.
[3] Takio, K., Towatari, T., Katunuma, N., Teller, D.C. and Titani, K. (1983) Proc. Natl. Acad. Sci. USA 80, 3666-3670.
[4] Ritonja, A., Popovič, T., Turk, V., Widenmann, K. and Machleidt, W. (1985) FEBS Lett. 181, 169-172.
[5] Ritonja, A., Popovič, T., Kotnik, M., Machleidt, W. and Turk, V. (1988) FEBS Lett. 228, 341-345.
[6] Meloun, B., Baudys, M., Pohl, J., Pavlik, M. and Kostka, V. (1988) J. Biol. Chem. 263, 9089-9093.
[7] Ritonja, A., Čolić, A., Dolenc, I., Ogrinc, T., Podobnik, M. and Turk, V. (1991) FEBS Lett. 283, 329-331.
[8] Musil, D., Zucic, D., Turk, D., Engh, R.A., Mayr, I., Huber, R., Popovič, T., Turk, V., Towatari, T., Katunuma, N. and Bode, W. (1991) EMBO J. (in press).
[9] Barrett, A.J., Rawlings, N.D., Davies, M.E., Machleidt, W., Salvesen, G. and Turk, V. (1986) in: Proteinase Inhibitors (Barrett, A.J. and Salvesen, G. eds.) pp. 515-569, Elsevier, Amsterdam.
[10] Turk, V. (1986) Cysteine Proteinases and their Inhibitors, Walter de Gruyter, Berlin/New York.
[11] Barrett, A.J., Fritz, H., Grubb, A., Isemura, S., Järvinen, M., Katunuma, N., Machleidt, W., Müller-Esterl, W., Sasaki, M. and Turk, V. (1986) Biochem. J. 236, 312.
[12] Ritonja, A., Kopitar, M., Jerala, R. and Turk, V. (1989) FEBS Lett. 255, 211-214.
[13] Davies, M.E. and Barrett, A.J. (1984) Histochemistry 80, 373-377.
[14] Abrahamson, M., Barrett, A.J., Salvesen, G. and Grubb, A. (1986) J. Biol. Chem. 261, 11282-11289.
[15] Brzin, J., Kopitar, M., Turk, V. and Machleidt, W. (1983) Hoppe-Seyler's Z. Physiol. Chem. 264, 1475-1480.
[16] Turk, V., Brzin, J., Lenarčič, B., Ločnikar, P., Popovič, T., Ritonja, A., Babnik, J., Bode, W. and Machleidt, W. (1985) in: Intracellular Protein Catabolism (Khairallah, E.A., Bond, J.S. and Bird, J.W.C. eds.) pp. 91-103, Alan R. Liss, New York.
[17] Lenarčič, B., Ritonja, A., Šali, A., Kotnik, M., Turk, V. and Machleidt, W. (1986) in: Cysteine Proteinases and their Inhibitors (Turk, V. ed.) pp. 473-487, Walter de Gruyter, Berlin.
[18] Wakamatsu, N., Kominami, E., Takio, K. and Katunuma, N. (1984) J. Biol. Chem. 259, 13832-13838.
[19] Katunuma, N. (1989) in: Intracellular Proteolysis Mechanisms and Regulations (Katunuma, N. and Kominami, E., eds.) pp. 3-23, Japan Sci. Soc. Press, Tokyo.
[20] Strauss, M., Stollwerk, J., Lenarčič, B., Turk, V., Jany, K.D. and Gassen, H.G. (1988) Biol. Chem. Hoppe-Seyler 369, 1019-1030.
[21] Kaji, H., Kumagai, I., Takeda, A., Miura, K. and Samejima, T. (1989) J. Biochem. 105, 143-147.
[22] Jerala, R., Trstenjak, M., Lenarčič, B. and Turk, V. (1988) FEBS Lett. 239, 41-44.
[23] Thiele, U., Auerswald, E.A., Gebhard, W., Assfalg-Machleidt, I., Popovič, T. and Machleidt, W. (1988) Biol. Chem. Hoppe-Seyler 369, 1167-1178.
[24] Stubbs, M.T., Laber, B., Bode, W., Huber, R., Jerala, R., Lenarčič, B. and Turk, V. (1990) EMBO J. 9, 1939-1947.
[25] Kondo, H., Abe, K., Nishimura, I., Watanabe, H., Emori, Y. and Arai, S. (1990) J. Biol. Chem. 265, 15832-15837.
[26] Kondo, H., Abe, K., Emori, Y. and Arai, S. (1991) FEBS Lett. 278, 87-90.
[27] Liang, C., Brookhart, G., Feng, G.H., Reeck, G.R. and Kramer, K.J. (1991) FEBS Lett. 278, 139-142.
[28] Barrett, A.J. (1981) Methods Enzymol. 80, 771-778.
[29] Sen, L.C. and Whitaker, J.R. (1973) Arch. Biochem. Biophys. 158, 623-632.
[30] Grubb, A.O., Weiber, H. and Löfberg, H. (1983) Scand. J. Clin. Invest. 43, 421-425.
[31] Turk, V., Brzin, J., Longer, M., Ritonja, A., Eropkin, M., Borckart, W. and Machleidt, W. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 1487-1496.
[32] Anastasi, A., Brown, M.A., Kembhavi, A.A., Nicklin, M.J.H., Sayers, C.A., Sunter, D.C. and Barrett, A.J. (1984) Biochem. J. 211, 129-138.
[33] Laber, B., Krieglstein, K., Henschen, A., Kos, J., Turk, V., Huber, R. and Bode, W. (1989) FEBS Lett. 248, 162-168.
[34] Machleidt, W., Thiele, U., Laber, B., Assfalg-Machleidt, I., Esterl, A., Wiegand, G., Kos, J., Turk, V. and Bode, W. (1989) FEBS Lett. 243, 234-238.
[35] Abrahamson, M., Ritonja, A., Brown, M.A., Grubb, A., Machleidt, W. and Barrett, A.J. (1987) J. Biol. Chem. 262, 9688-9694.
[36] Auerswald, E.A., Genenger, G., Assfalg-Machleidt, I., Kos, J. and Bode, W. (1988) FEBS Lett. 243, 186-192.
[37] Collela, R., Sakaguchi, Y., Nagase, H. and Bird, J.W.C. (1989) J. Biol. Chem. 264, 17164-17169.
[38] Bode, W., Engh, R., Musil, D., Thiele, U., Huber, R., Karshikov, A., Brzin, J., Kos, J. and Turk, V. (1988) EMBO J. 7, 2593-2599.
[39] Brzin, J., Popovič, T., Turk, V., Borchart, U. and Machleidt, W. (1984) Biochem. Biophys. Res. Commun. 118, 103-109.
[40] Grubb, A. and Löfberg, H. (1982) Proc. Natl. Acad. Sci. USA 79, 3024-3027.
[41] Barrett, A.J., Davies, M.E. and Grubb, A. (1984) Biochem. Biophys. Res. Commun. 120, 631-636.
[42] Esnard, F., Esnard, A., Faucher, D., Capony, J.P., Deraucourt, J., Brillard, M. and Gauthier, F. (1990) Biol. Chem. Hoppe-Seyler 371, Suppl. 161-166.
[43] Ghiso, J., Jensson, O. and Frangione, B. (1986) Proc. Natl. Acad. Sci. USA 83, 2974-2978.
[44] Strauss, M., Bartsch, F.O., Stollwerk, J., Trstenjak, M., Böhming, A., Gassen, H.G., Machleidt, W. and Turk, V. (1988) Biol. Chem. Hoppe-Seyler 369, 209-218.
[45] Abrahamson, M., Grubb, A., Olafsson, I. and Lundwall, A. (1987) FEBS Lett. 216, 229-233.
[46] Abrahamson, M., Dalbøge, H., Olafsson, I., Carlsen, S. and Grubb, A. (1988) FEBS Lett. 236, 14-18.
[47] Isemura, S., Saitoh, E. and Sanada, K. (1987) J. Biochem. 102, 693-704.
[48] Kato, H., Nagasawa, S. and Iwanaga, S. (1981) Methods Enzymol. 80, 172-198.
[49] Esnard, F. and Gauthier, F. (1983) J. Biol. Chem. 258, 12443-12447.
[50] Cole, T., Inglis, A., Roxburgh, C.M., Howlett, G.J. and Schreiber, E. (1985) FEBS Lett. 182, 57-61.
[51] Okamoto, H. and Greenbaum, L.M. (1983) Life Sci. 32, 2007-2013.
[52] Müller-Esterl, W., Fritz, H., Machleidt, W., Ritonja, A., Brzin, J., Kotnik, M., Turk, V., Kellermann, J. and Lottspeich, F. (1985) FEBS Lett. 182, 310-314.
[53] Sueyoshi, T., Enjyoji, K., Shimada, T., Kato, H., Iwanaga, S., Bando, Y., Kominami, E. and Katunuma, N. (1985) FEBS Lett. 182, 193-195.
[54] Ohkubo, I., Kurachi, K., Takasawa, T., Shiokawa, H. and Sasaki, M. (1984) Biochemistry 23, 5691-5697.
[55] Barrett, A.J. (1987) Trends Biochem. Sci. 12, 193-196.

[56] Müller-Esterl, W., Iwanaga, S. and Nakanishi, S. (1986) Trends Biochem. Sci. 11, 336–339.
[57] Müller-Esterl, W. (1987) Sem. Thromb. Hemostas. 13, 115–126.
[58] Müller-Esterl, W. (1989) Thromb. Hemostas. 61, 2–6.
[59] Adam, A., Albert, A., Calay, G., Closset, J., Damas, J. and Franchimont, P. (1985) Clin. Chem. 31/3, 423–426.
[60] Higashiama, S., Ohkubo, I., Ishiguro, H., Kunimatsu, M., Sawaki, K. and Sasaki, M. (1986) Biochemistry 25, 1669–1675.
[61] Moreau, T., Gutman, N., Faucher, D. and Gauthier, F. (1989) J. Biol. Chem. 264, 4298–4303.
[62] Brzin, J., Trstenjak, M., Ritonja, A., Turk, V. and Machleidt, W. (1986) in: Cysteine Proteinases and their Inhibitors (Turk, V. ed.) pp. 443–453, Walter de Gruyter, Berlin/New York.
[63] Lenarčič, B., Gabrijelčič, D., Rozman, B., Drobnič-Košorok, M. and Turk, V. (1988) Biol. Chem. Hoppe-Seyler 369, Suppl., 257–261.
[64] Lenarčič, B., Krašovec, M., Ritonja, A., Olafsson, I. and Turk, V. (1991) FEBS Lett. 280, 211–215.
[65] Salvesen, G., Parkes, C., Abrahamson, M., Grubb, A. and Barrett, A.J. (1986) Biochem. J. 234, 429–434.
[66] Vogel, R., Assfalg-Machleidt, I., Esterl, A., Machleidt, W. and Müller-Esterl, W. (1988) J. Biol. Chem. 263, 12661–12668.
[67] Okamoto, H. and Greenbaum, L.M. (1983) Biochem. Biophys. Res. Commun. 112, 701–708.
[68] Sakamoto, W., Satoh, F., Nagasawa, S. and Handa, H. (1988) Biochem. Biophys. Res. Commun. 150, 1199–1206.
[69] Lenarčič, B., Kos, J., Dolenc, I., Lučovnik, P., Krišaj, I. and Turk, V. (1988) Biochem. Biophys. Res. Commun. 154, 765–772.
[69a] Abrahamson, M., Mason, R.W., Hansson, H.,Buttle, D.J., Grubb, A. and Ohlsson, K. (1991) Biochem. J. 273, 621–626.
[70] Bode, W., Musil, D., Engh, R., Huber, R., Brzin, J., Kos, J. and Turk, V. (1990) in: Intracellular Proteolysis - Mechanisms and Regulations (Katunuma, N. and Kominami, E. eds.) pp. 297–304, Japan Sci. Press, Tokyo.
[71] Bode, W., Engh, R., Musil, D., Laber, B., Stubbs, M., Huber, R. and Turk, V. (1990) Biol. Chem. Hoppe-Seyler 371, 111–118.
[72] Nycander, M. and Björk, I. (1990) Biochem. J. 271, 281–284.
[73] Björk, I. and Ylinenjärvi, K. (1990) Biochemistry 29, 1770–1776.
[74] Lindahl, P., Alriksson, E., Jornvall, H. and Björk, I. (1988) Biochemistry 27, 5074–5082.
[75] Laskowsky, M., Jr. and Kato, I. (1980) Annu. Rev. Biochem. 49, 593–626.
[76] Müller-Esterl, W., Fritz, H., Kellermann, J., Lottspeich, F., Machleidt, W. and Turk, V. (1985) FEBS Lett. 191, 221–226.
[77] Šali, A. and Turk, V. (1987) Biol. Chem. Hoppe-Seyler 368, 493–499.
[78] Elzanowski, A., Baker, W.C., Hunt, L.T. and Seibel-Ross, E. (1988) FEBS Lett. 227, 167–170.
[79] Koide, T. and Odani, S. (1987) FEBS Lett. 216, 17–21.
[80] Rawlings, N.D. and Barrett, A.J. (1990) J. Mol. Evol. 30, 60–71.
[81] Dayhoff, M.O., Barker, W.C. and Hunt, L.T. (1983) Methods Enzymol. 91, 524–545.
[82] Abe, K., Emori, Y., Kondo, H., Suzuki, K. and Arai, S. (1987) J. Biol. Chem. 262, 16793–16797.
[83] Delbridge, M.L. and Kelly, L.E. (1991) FEBS Lett. 274, 141–145.
[84] Saito, H., Suzuki, T., Ueno, K., Kubo, T. and Natori, S. (1989) Biochemistry 28, 1749–1755.
[85] Cole, T., Dickson, P.W., Esnard, F., Averill, S., Risbridger, G.B., Gauthier, F. and Schreiber, G. (1989) Eur. J. Biochem. 186, 35–42.
[86] Shaw, P.A., Cox, J.L., Barka, T. and Nayto, Y. (1988) J. Biol. Chem. 263, 18133–18137.
[87] Ritonja, A., Evans, H.J., Machleidt, W. and Barrett, A.J. (1987) Biochem. J. 246, 799–802.