View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

FEBS 29274

FEBS Letters 579 (2005) 1285–1290

Recombinant human procathepsin S is capable of autocatalytic processing at neutral pH in the presence of glycosaminoglycans

Olga Vasiljeva, Marko Dolinar, Jerica Rozman Pungerčar, Vito Turk, Boris Turk*

Department of Biochemistry and Molecular Biology, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

Received 2 December 2004; revised 21 December 2004; accepted 28 December 2004

Available online 22 January 2005

Edited by Horst Feldmann

Abstract **Cathepsin S is unique among mammalian cysteine cathepsins in being active and stable at neutral pH. We show that autocatalytic activation of procathepsin S at low pH is a bimolecular process that is considerably accelerated (~20-fold) by glycosaminoglycans and polysaccharides such as dextran sulfate, chondroitin sulfates A and E, and dermatan sulfate through electrostatic interaction with the proenzyme. Procathepsin S is also shown to undergo autoactivation at neutral pH in the presence of dextran sulfate with $t_{1/2}$ of ∼20 min at pH 7.5. This novel property of procathepsin S may have implications in pathological conditions associated with the appearance of active cathepsins outside lysosomes.**

© 2005 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Cathepsin; Lysosomal cysteine protease; Processing; Autocatalytic activation; Glycosaminoglycan; Ionic strength

1. Introduction

Cathepsin S (EC 3.4.22.27) is a single chain, non-glycosylated papain-like lysosomal cysteine protease that is, unusually, highly active and stable at neutral pH [1–3]. A number of studies have indicated that cathepsin S is indispensable for MHC class II-mediated antigen presentation (reviewed in [4,5]). Moreover, inhibition of cathepsin S in vivo has been shown to alter autoantigen presentation and development of organ-specific autoimmunity, and therefore cathepsin S is considered to be a potential target for autoimmune- and hyperimmune-disease therapy [6–9]. Due to its high elastinolytic activity, it has been suggested that cathepsin S is involved in atherogenesis [10], vascular matrix remodeling during angiogenesis [11] and promotion of cilia motility in the lung [12]. In addition, increased expression of cathepsin S has also been associated with the pathogenesis of Alzheimer's disease [13], tumor invasion [14,15] and muscular dystrophy [16].

Like other papain-like cysteine proteases, cathepsin S is synthesized as an inactive zymogen, and is converted to the mature form by limited proteolysis at low pH by other proteases, or by autocatalytic processing which is a bimolecular process. During activation, which is believed to occur in the acidic environment of late endosomes or lysosomes, the N-terminal propeptide is removed from the body of the enzyme thereby unmasking the active site of the enzyme (reviewed in [1]). Polysaccharides, with dextran sulfate as the most frequently used model compound, were found to substantially accelerate processing of papain-like cathepsins, including cathepsin S [17–20].

Due to the high stability and activity of the enzyme at neutral pH, we investigated the pH-dependence of autocatalytic activation of procathepsin S and the influence of polysaccharides and ionic strength on the processing. Here, we show that the proenzyme can be autoactivated at neutral pH, making possible a role for cathepsin S in pathological conditions associated with the extra-lysosomal activity of papain-like cathepsins.

*Corresponding author. Fax: +386 1 257 35 94.
E-mail address: boris.turk@ijs.si (B. Turk).

2. Materials and methods

2.1. Materials

Bz-Phe-Val-Arg-AMC (7-amido-4-methylcoumarin) and Z-Phe-Arg-AMC were from Bachem (Bubendorf, Switzerland) and E-64 [*trans*-epoxysuccinyl-L-leucyl-amido-(4-guanidino)butane] from Peptide Research Foundation (Osaka, Japan). Iodoacetic acid and polyethylene glycol 6000 were from Serva (Heidelberg, Germany) and dimethyl sulfoxide from Merck (Darmstadt, Germany). Heparin, dextran sulfate, heparan sulfate, chondroitin sulfate, polylysine, ADA [*N*-[2-acetamidol]-2-iminodiacetic acid], TES [*N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], HEPES [2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulfonic acid] and PIPES [piperazine-1,4-bis(2-ethanesulfonic acid)] were from Sigma (St. Louis, USA).

2.2. Expression and purification of recombinant human procathepsin S

Recombinant procathepsin S was expressed in the form of inclusion bodies in *Escherichia coli* strain BL21[DE3]pLysS, solubilized, refolded and reoxidized as described previously [19]. Protein concentrations were determined from absorbance at 280 nm (Perkin–Elmer λ-18 spectrometer, USA) using the molar absorption coefficients of 72 685 and 35 225 for procathepsin S and active cathepsin S, respectively, calculated by the method of Pace et al. [21]. The active concentration of processed cathepsin S was determined by titration with stefin A [22].

2.3. Processing and activation of procathepsin S

Autocatalytic activation of procathepsin S was studied by incubation (final concentration 1–5 μM) at 37 °C in 0.5 ml of the appropriate buffer (see below) containing 2.5 mM dithiothreitol, in the absence and presence of dextran sulfate, as described previously [20,23]. Aliquots of 10 μl were taken from the reaction mixture at the times indicated and mixed with 2.5 ml of substrate solution (10 μM Bz-FVR-AMC in 0.1 M phosphate buffer, pH 6.5, containing 1 mM EDTA and 0.1% (w/v) polyethylene glycol 6000). Fluorescence of the released AMC was then monitored continuously for 1 min in a Perkin–Elmer LS-50 spectrofluorimeter (Norwalk, CT, USA) at excitation and emission

0014-5793/$30.00 © 2005 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2004.12.093

wavelengths of 370 and 460 nm. The concentration of dimethylsulfoxide in the assay was adjusted to 4% (v/v). The following processing buffers were used: 0.1 M acetate buffer between pH 3.0 and 5.5; 0.1 M phosphate buffer between pH 6.0 and 8.0 and 0.1 M Tris–HCl buffer between pH 8.0 and 9.0. All processing buffers contained 1 mM EDTA.

Autocatalytic processing of procathepsin S was followed by 12.5% SDS–PAGE under reducing conditions. 12 μl aliquots of the reaction mixture (see above), with or without dextran sulfate, was taken at appropriate times and processing was stopped by adding 4 μl of 4× SDS–PAGE loading buffer containing 4% (v/v) β-mercaptoethanol and 1% (w/v) SDS, and heating at 100 °C for 5 min.

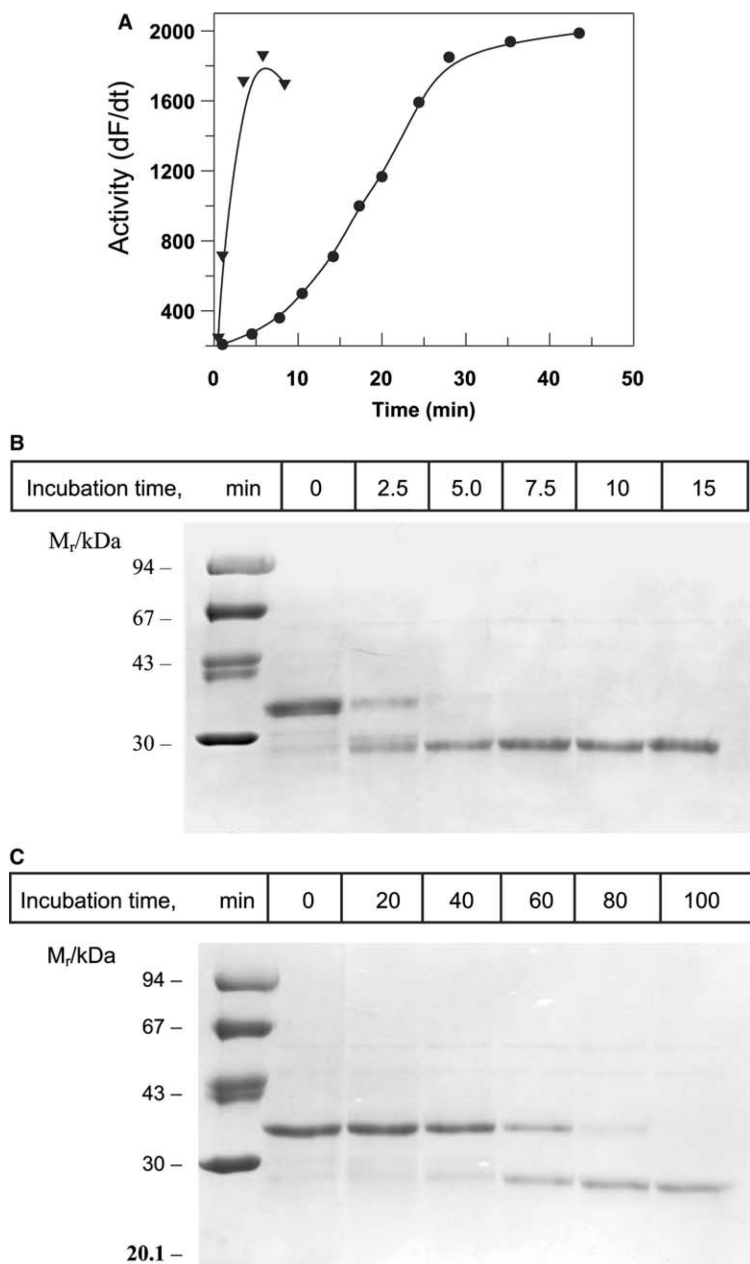

Fig. 1. Autocatalytic processing of recombinant human procathepsin S. Proteolytic activity was assayed against 10 μM Bz-FVR-AMC and is expressed in arbitrary fluorescence units per unit time (d*F*/d*t*). (A) Processing mixtures were incubated in the presence of 25 μg/ml DS at 37 °C in 25 mM Tris–HCl buffer, pH 7.5 (●) and in 25 mM citrate buffer, pH 5.0 (▼). (B) Time dependence of the cleavage of procathepsin S at acidic pH followed by SDS–PAGE. Procathepsin S was incubated in 25 mM citrate buffer, pH 5.0, containing 1 mM EDTA at 37 °C in the presence of 25 μg/ml DS. Low molecular mass standards are shown in the first lane. Lane 0 contains procathepsin S before activation. (C) SDS–PAGE analysis of procathepsin S processing at alkaline pH. Procathepsin S was incubated in 0.1 M Tris–HCl buffer, pH 8.0, containing 1 mM EDTA at 37 °C in the presence of 25 μg/ml DS and samples were taken every 20 min. Low molecular mass standards are shown in first lane. Lane 0 contains procathepsin S before activation.

Table 1
Effect of pH and dextran sulfate on the rate of procathepsin S processing at 37 °C

pH	Half-time of procathepsin S processing ($t_{1/2}$)	
	$t_{1/2}$ (−DS), min	$t_{1/2}$ (+DS), min
5.0	78 ± 5.0	4 ± 0.5
7.5	nd	18 ± 3.0

Half-times ($t_{1/2}$) (±S.E.M.) of procathepsin S processing, in the absence (−DS) and in the presence (+DS) of 2.5 μl/ml DS, were estimated by the discontinuous method. The procathepsin S concentration was 3 μM in all experiments. nd – no reaction detected.

2.4. N-terminal protein sequence determination

The N-terminal amino acid sequences of the samples, subjected to SDS–PAGE under non-reducing conditions and blotted to a PVDF membrane, were determined using an Applied Biosystems (USA) PROCISE 492A protein sequencing system.

3. Results

3.1. Processing of procathepsin S

In initial experiments, a dextran sulfate (DS) concentration of 25 μg/ml was found to be optimal for the processing of procathepsin S at acidic pH (data not shown), in agreement with previous data on cysteine cathepsins [19,20,24,25], and was therefore used in all subsequent experiments. Activation of procathepsin S was then investigated as a function of pH in the presence of dextran sulfate. Since our preliminary experiments suggested that procathepsin S could be activated both at acidic and at neutral pH, we investigated activation of the enzyme at pH 5.0 and 7.5. The process was substantially faster at pH 5.0, with a half-life of ∼4 min compared to ∼18 min at pH 7.5 (Fig. 1A; Table 1). A sigmoidal increase of cathepsin S activity was observed at pH 7.5, suggesting a bimolecular mechanism, in agreement with previous data on procathepsins B and K activation [20,26]. At pH 5.0, activation was too rapid to allow the initial lag phase to be detected by the discontinuous method. In the absence of dextran sulfate, activation was 20-fold slower, and no activation was detected at pH 7.5 (Table 1). The final activity of the processed cathepsin S was unaffected by DS (data not shown), in agreement with related results with chondroitin sulfate [27]. Moreover, these results also indicated that the active enzyme was stable and did not undergo further degradation, as observed for recombinant cathepsin H [23].

In order to verify that activation was accompanied by limited proteolysis, processing of the enzyme was followed by SDS–PAGE in the presence of DS at acidic and neutral pH. At pH 5.0 rapid cleavage was observed, paralleling the enzyme activity experiments (Fig. 1B). Processing was complete, even at pH 8.0, although minor degradation of the protein was observed (Fig. 1C). However, no processing intermediates were observed at either pH value. N-terminal amino acid sequences were determined on the same samples. A single sequence, AQLHK, was obtained for procathepsin S, identical to that deduced from the cDNA sequence [28,29]. The sequence of active cathepsin S started with SNPNR, an extension of 6 residues over the sequence of natural cathepsin S [30]. Such extensions of 2–6 amino acid residues are common for most recombinant cysteine cathepsins. However, they do not affect the catalytic efficiency of the enzymes [31].

3.2. pH-activity profile of recombinant human procathepsin S processing

In order to investigate the pH-profile of procathepsin S autocatalytic activation, we compared the activities of cathepsin S after 30 min of incubation at 37 °C in the pH range between 3.0 and 9.0, in the absence and presence of DS (Fig. 2). The 30 min time point was selected as the time, in the presence of DS, by which activation of cathepsin S at both acidic and neutral pH was complete (Fig. 1A). The activity profile in the absence of DS showed a single maximum between pH 4.0 and 5.0, typical for papain-like cathepsins [1,31]. In contrast, the activity profile in the presence of DS exhibited two maxima: a sharp peak at pH 5.0 and a broader peak at neutral pH (7.0–8.0) (Fig. 2).

To exclude any artifacts of the buffer systems and to investigate any role of buffer composition on the efficiency of procathepsin S activation at neutral pH, the processing in the presence of DS was analyzed at pH 7.2 using several buffer systems: ADA, TES, TRIS, PIPES, HEPES and BES. All the buffers contained 1 mM EDTA and NaCl was added to the buffers to achieve an ionic strength (I) of 0.025. No significant differences in the rate of processing were observed

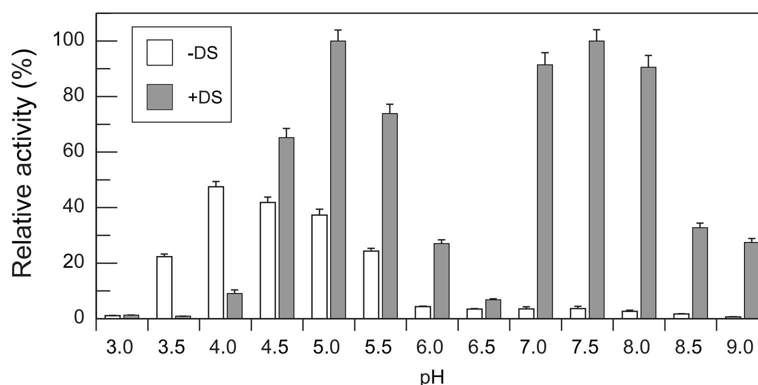

Fig. 2. pH profile of recombinant human procathepsin S processing in the absence and presence of DS. Procathepsin S was incubated for 30 min at 37 °C in 0.1 M acetate buffer (pH 4.0–5.0), 0.1 M phosphate buffer (pH 6.0–8.0) and 0.1 M Tris–HCl buffer (pH 8.0–9.0), followed by the activity measurements as described in Section 2. Results are presented as relative activity.

Table 2
Effect of polysaccharides on procathepsin S processing at pH 5.0 and 37 °C

Polysaccharide	$t_{1/2}$ (min)
None	78 ± 5.0
Dextran sulfate	4 ± 0.5
Heparin	31 ± 7.9
Heparan sulfate	76 ± 10.8
Chondroitin sulfate A	<4.0
Chondroitin sulfate E	<4.0
Hyaluronic acid	65 ± 11.3
Dermatan sulfate	<4.0

Half-times ($t_{1/2}$) (±S.E.M.) of procathepsin S processing, in the presence of different polysaccharides at concentrations of 2.5 μl/ml, were estimated by the discontinuous method. The procathepsin S concentration was 10 μM in all experiments.

in any of the buffer systems, suggesting/indicating that processing rate is independent of buffer composition (data not shown).

3.3. *Autocatalytic processing of recombinant human procathepsin S in the presence of glycosaminoglycans*

Since dextran sulfate accelerates the processing of cathepsin S it was important to investigate the possible effect of more physiologically relevant glycosaminoglycans. Activation experiments were therefore performed at pH 5.0 in the presence of 25 μg/ml concentrations of heparin, heparan sulfate, chondroitin sulfates A and E, hyaluronic acid, dermatan sulfate, with DS as the control. Chondroitin sulfates A and E, and dermatan sulfate were found to be at least as efficient as DS, although more precise values could not be obtained since the processing was too fast. Heparin was only moderately efficient (~2.5-fold acceleration), whereas heparan sulfate and hyaluronic acid had no effect (Table 2).

3.4. *Effect of ionic strength on the rate of procathepsin S processing*

Due to their negative charge, glycosaminoglycans and other charged polysaccharides interact with their target proteins primarily via electrostatic interactions. Therefore, we investigated the influence of ionic strength on the rate of procathepsin S processing. The incubation time selected was the time needed, in the absence of NaCl, for activation of at least 50% of the procathepsin S. Thus activity was measured after 15 min in the presence of DS (at pH 5.0 and 7.0), and after 60 min (pH 4.0) in its absence. As shown in Fig. 3, the effect of ionic strength is dramatic, especially at neutral pH, where processing within the initial 15 min was almost completely abolished at an ionic strength of 0.1. A moderate effect was observed at pH 5.0 (60% activity reduction), whereas only a minor effect was seen in the absence of DS, indicating a crucial role of charge–charge interactions between DS and procathepsin S in the rate of cathepsin S autocatalytic activation.

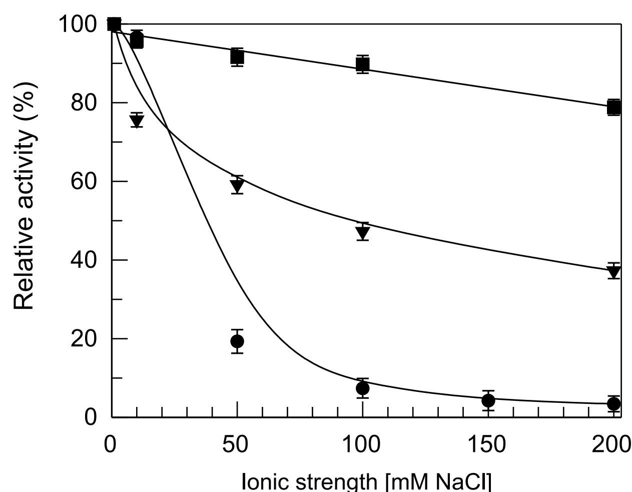

Fig. 3. Effect of ionic strength on the rate of procathepsin S processing at different pHs, in the presence and absence of dextran sulfate. Procathepsin S was incubated for 15 min in 25 mM Tris–HCl buffer, pH 7.0 (●) and in 25 mM citrate buffer, pH 5.0 (▼), both in the presence of 25 μg/ml DS, and for 60 min in 25 mM citric buffer, pH 4.0 (■) in its absence. Ionic strengths of solutions are based on the computer program "Java Buffer Calculator" and varied by addition of NaCl. All other experimental details were as described under Section 2.

4. Discussion

Glycosaminoglycans and dextran sulfate have long been known to be able to accelerate activation of cysteine cathepsins [17–20]. The same was found to be true for cathepsin S with dextran sulfate, chondroitin sulfates A and E, and dermatan sulfate. Their interaction was shown to be primarily ionic and can be completely abolished at even moderate ionic strengths (Fig. 3). Since they bind to mature enzymes with only moderate affinity [32,33], it suggests that the glycosaminoglycan binds to the propeptide of procathepsin S and possibly of other cathepsins. The crystal structure of procathepsin S is not known but the propeptide contains a large number of positively charged residues – eight histidines, nine lysines and five arginines – all positioned in clusters, strongly supporting the proposal that this is the primary site of interaction with glycosaminoglycans. Their binding to the propeptide thus appears to be important for the initial step of procathepsin S processing. The differences in catalytic efficiency observed for different glycosaminoglycans and dextran sulfate (Table 2), as for the interactions of glycosaminoglycans with cathepsin B and papain [32,33], presumably reflect the differences in backbone structure of different polysaccharides and only to a minor extent the differences in their charge density. This could possibly explain only a minor effect of heparin as compared with chondroitin sulfates A and E. The location of glycosaminoglycans in lysosomes as the result of proteoglycan recycling, in the extracellular matrix, at the cell surface and at basement membranes [34], supports the proposal that they are involved in in vivo processing, not only of cathepsin S, but also of other cysteine cathepsins both inside and outside lysosomes.

For a long time lysosomal cysteine proteases were believed to be produced autocatalytically at acidic pH only. However, procongopain, a parasitic cysteine protease from *Trypanosoma congolense*, was recently found to be capable of autocatalytic activation at neutral pH [35]. Here, we have shown that mammalian procathepsin S can be autocatalytically activated at neutral pH in the presence of dextran sulfate and related glycosaminoglycans, a feature not observed before for any of the related cathepsins. An amino acid sequence alignment of the propeptides of cysteine cathepsins [1] shows that the num-

bers of arginines and lysines in the propeptides of the related cathepsins K, L, V and F are similar (5–9), whereas the numbers of histidines are considerably smaller (1–6) than the eight histidines in cathepsin S propeptide. It is likely that these additional histidines are involved in interactions with the negatively charged residues of the mature part of the enzyme. Histidines become deprotonated at pH values around neutral and would thereby weaken propeptide binding to the mature enzyme part. This could facilitate glycosaminoglycan binding to the propeptide and, consequently, zymogen activation. The appearance of the second peak of activation of procathepsin S around neutral pH in the presence of glycosaminoglycans could be explained by this mechanism. It would appear that cathepsin S is therefore unique among the mammalian cysteine cathepsins.

However, the mechanism involving histidine deprotonation does not account for the zymogen processing at acidic pH in the absence of glycosaminoglycans. This could be explained by the substantially weaker binding of propeptide to the mature part of the enzyme at lower pH, which is in agreement with the data for binding of synthetic propeptides to cathepsin B [36] and cathepsin L [37].

Due to the high stability of cathepsin S at neutral pH [38], its high elastinolytic potential [39,40], and its ability to activate other cysteine cathepsins [41] cathepsin S can be extremely harmful in the extracellular environment. Our finding that procathepsin S can undergo autoactivation at neutral pH further supports this idea. In fact, cathepsin S has been observed in the extracellular environment in various pathological conditions, from cancer progression [14,15], to rheumatoid arthritis [42], atherogenesis [10] and muscular dystrophy [16]. It is possible therefore that cathepsin S participates in extracellular proteolytic cascades, directly by cleavage of extracellular substrates and/or indirectly through activation of other lysosomal proteases, thereby amplifying their degradative capacity, potentially leading to pathological damage, including facilitating the penetration of tissues by cancer cells [43].

In conclusion, our results show that procathepsin S is capable of rapid autocatalytic activation, not only at acidic pH but also at neutral pH in the presence of glycosaminoglycans, which could be an important mechanism for regulating its activity and that of other cysteine cathepsins, not only inside lysosomes but also in the cytosol or extracellular space where cathepsin S is often found in various pathologies.

Acknowledgments: The work was supported by the Ministry of Education, Science and Sports of Republic of Slovenia. We thank Roger Pain for critical reading of the manuscript.

References

[1] Turk, B., Turk, D. and Turk, V. (2000) Lysosomal cysteine proteases: more than scavengers. Biochim. Biophys. Acta 1477, 98–111.
[2] Turk, V., Turk, B. and Turk, D. (2001) Lysosomal cysteine proteases: facts and opportunities. EMBO J., 20–33.
[3] Turk, B., Turk, D. and Salvesen, G.S. (2002) Regulating cysteine protease activity: essential role of protease inhibitors as guardians and regulators. Curr. Pharm. Des. 8, 1623–1637.
[4] Riese, R.J. and Chapman, H.A. (2000) Cathepsins and compartmentalization in antigen presentation. Curr. Opin. Immunol. 12, 107–113.
[5] Honey, K. and Rudensky, A.Y. (2003) Lysosomal cysteine proteases regulate antigen presentation. Nat. Rev. Immunol. 3, 472–482.
[6] Riese, R.J., Mitchell, R.N., Villadangos, J.A., Shi, G.P., Palmer, J.T., Karp, E.R., De Sanctis, G.T., Ploegh, H.L. and Chapman, H.A. (1998) Cathepsin S activity regulates antigen presentation and immunity. J. Clin. Invest. 101, 2351–2363.
[7] Nakagawa, T.Y., Brissette, W.H., Lira, P.D., Griffiths, R.J., Petrushova, N., Stock, J., McNeish, J.D., Eastman, S.E., Howard, E.D., Clarke, S.R., Rosloniec, E.F., Elliott, E.A. and Rudensky, A.Y. (1999) Impaired invariant chain degradation and antigen presentation and diminished collagen-induced arthritis in cathepsin S null mice. Immunity 10, 207–217.
[8] Saegusa, K., Ishimaru, N., Yanagi, K., Arakaki, R., Ogawa, K., Saito, I., Katunuma, N. and Hayashi, Y. (2002) Cathepsin S inhibitor prevents autoantigen presentation and autoimmunity. J. Clin. Invest. 110, 361–369.
[9] Wiendl, H., Lautwein, A., Mitsdorffer, M., Krause, S., Erfurth, S., Wienhold, W., Morgalla, M., Weber, E., Overkleeft, H.S., Lochmuller, H., Melms, A., Tolosa, E. and Driessen, C. (2003) Antigen processing and presentation in human muscle: cathepsin S is critical for MHC class II expression and upregulated in inflammatory myopathies. J. Neuroimmunol. 138, 132–143.
[10] Sukhova, G.K., Zhang, Y., Pan, J.H., Wada, Y., Yamamoto, T., Naito, M., Kodama, T., Tsimikas, S., Witztum, J.L., Lu, M.L., Sakara, Y., Chin, M.T., Libby, P. and Shi, G.P. (2003) Deficiency of cathepsin S reduces atherosclerosis in LDL receptor-deficient mice. J. Clin. Invest. 111, 897–906.
[11] Shi, G.P., Sukhova, G.K., Kuzuya, M., Ye, Q., Du, J., Zhang, Y., Pan, J.H., Lu, M.L., Cheng, X.W., Iguchi, A., Perrey, S., Lee, A.M., Chapman, H.A. and Libby, P. (2003) Deficiency of the cysteine protease cathepsin S impairs microvessel growth. Circ. Res. 92, 493–500.
[12] Chapman, H.A., Riese, R.J. and Shi, G.P. (1997) Emerging roles for cysteine proteases in human biology. Annu. Rev. Physiol. 59, 63–88.
[13] Lemere, C.A., Munger, J.S., Shi, G.P., Natkin, L., Haass, C., Chapman, H.A. and Selkoe, D.J. (1995) The lysosomal cysteine protease, cathepsin S, is increased in Alzheimer's disease and Down syndrome brain. An immunocytochemical study. Am. J. Pathol. 146, 848–860.
[14] Kos, J., Sekirnik, A., Kopitar, G., Cimerman, N., Kayser, K., Stremmer, A., Fiehn, W. and Werle, B. (2001) Cathepsin S in tumours, regional lymph nodes and sera of patients with lung cancer: relation to prognosis. Br. J. Cancer 85, 1193–1200.
[15] Flannery, T., Gibson, D., Mirakhur, M., McQuaid, S., Greenan, C., Trimble, A., Walker, B., McCormick, D. and Johnston, P.G. (2003) The clinical significance of cathepsin S expression in human astrocytomas. Am. J. Pathol. 163, 175–182.
[16] Fang, J., Shi, G.P. and Vaghy, P.L. (2000) Identification of the increased expression of monocyte chemoattractant protein-1, cathepsin S, UPIX-1, and other genes in dystrophin-deficient mouse muscles by suppression subtractive hybridization. J. Cell. Biochem. 79, 164–172.
[17] Mason, R.W. and Massey, S.D. (1992) Surface activation of procathepsin L. Biochem. Biophys. Res. Commun. 189, 1659–1666.
[18] Ishidoh, K. and Kominami, E. (1994) Multi-step processing of procathepsin L in vitro. FEBS Lett. 352, 281–284.
[19] Kopitar, G., Dolinar, M., Štrukelj, B., Pungerčar, J. and Turk, V. (1996) Folding and activation of human procathepsin S from inclusion bodies produced in *Escherichia coli*. Eur. J. Biochem. 236, 558–562.
[20] Rozman, J., Stojan, J., Kuhelj, R., Turk, V. and Turk, B. (1999) Autocatalytic processing of recombinant human procathepsin B is a bimolecular process. FEBS Lett. 459, 358–362.
[21] Pace, C.N., Vajdos, F., Fee, L., Grimsley, G. and Gray, T. (1995) How to measure and predict the molar absorption coefficient of a protein. Protein Sci. 4, 2411–2423.
[22] Turk, B., Križaj, I., Kralj, B., Dolenc, I., Popovič, T., Bieth, J.G. and Turk, V. (1993) Bovine stefin C, a new member of the stefin family. J. Biol. Chem. 268, 7323–7329.
[23] Vasiljeva, O., Dolinar, M., Turk, V. and Turk, B. (2003) Recombinant human cathepsin H lacking the mini chain is an endopeptidase. Biochemistry 42, 13522–13528.
[24] Barlič-Maganja, D., Dolinar, M. and Turk, V. (1998) The influence of Ala205 on the specificity of cathepsin L produced by dextran sulfate assisted activation of the recombinant proenzyme. Biol. Chem. 379, 1449–1452.

[25] Quraishi, O. and Storer, A.C. (2001) Identification of internal autoproteolytic cleavage sites within the prosegments of recombinant procathepsin B and procathepsin S. Contribution of a plausible unimolecular autoproteolytic event for the processing of zymogens belonging to the papain family. J. Biol. Chem. 276, 8118–8124.

[26] McQueney, M.S., Amegadzie, B.Y., D'Alessio, K., Hanning, C.R., McLaughlin, M.M., McNulty, D., Carr, S.A., Ijames, C., Kurdyla, J. and Jones, C.S. (1997) Autocatalytic activation of human cathepsin K. J. Biol. Chem. 272, 13955–13960.

[27] Li, Z., Yasuda, Y., Li, W., Bogyo, M., Katz, N., Gordon, R.E., Fields, G.B. and Bromme, D. (2004) Regulation of collagenase activities of human cathepsins by glycosaminoglycans. J. Biol. Chem. 279, 5470–5479.

[28] Shi, G.P., Munger, J.S., Meara, J.P., Rich, D.H. and Chapman, H.A. (1992) Molecular cloning and expression of human alveolar macrophage cathepsin S, an elastinolytic cysteine protease. J. Biol. Chem. 267, 7258–7262.

[29] Wiederanders, B., Brömme, D., Kirschke, H., von Figura, K., Schmidt, B. and Peters, C. (1992) Phylogenetic conservation of cysteine proteinases. Cloning and expression of a cDNA coding for human cathepsin S. J. Biol. Chem. 267, 13708–13713.

[30] Ritonja, A., Čolić, A., Dolenc, I., Ogrinc, T., Podobnik, M. and Turk, V. (1991) The complete amino acid sequence of bovine cathepsin S and a partial sequence of bovine cathepsin L. FEBS Lett. 283, 329–331.

[31] Brömme, D., Nallaseth, F.S. and Turk, B. (2004) Production and activation of recombinant papain-like cysteine proteases. Methods 32, 199–206.

[32] Almeida, P.C., Nantes, I.L., Rizzi, C.C., Judice, W.A., Chagas, J.R., Juliano, L., Nader, H.B. and Tersariol, I.L. (1999) Cysteine proteinase activity regulation. A possible role of heparin and heparin-like glycosaminoglycans. J. Biol. Chem. 274, 30433–30438.

[33] Almeida, P.C., Nantes, I.L., Chagas, J.R., Rizzi, C.C., Faljoni-Alario, A., Carmona, E., Juliano, L., Nader, H.B. and Tersariol, I.L. (2001) Cathepsin B activity regulation. Heparin-like glycosaminogylcans protect human cathepsin B from alkaline pH-induced inactivation. J. Biol. Chem. 276, 944–951.

[34] Kjellen, L. and Lindahl, U. (1991) Proteoglycans: structures and interactions. Annu. Rev. Biochem. 60, 443–475.

[35] Serveau, C., Boulange, A., Lecaille, F., Gauthier, F., Authie, E. and Lalmanach, G. (2003) Procongopain from *Trypanosoma congolense* is processed at basic pH: an unusual feature among cathepsin L-like cysteine proteases. Biol. Chem. 384, 921–927.

[36] Fox, T., de Miguel, E., Mort, J.S. and Storer, A.C. (1992) Potent slow-binding inhibition of cathepsin B by its propeptide. Biochemistry 31, 12571–12576.

[37] Carmona, E., Dufour, E., Plouffe, C., Takebe, S., Mason, P., Mort, J.S. and Menard, R. (1996) Potency and selectivity of the cathepsin L propeptide as an inhibitor of cysteine proteases. Biochemistry 35, 8149–8157.

[38] Kirschke, H., Wiederanders, B., Bromme, D. and Rinne, A. (1989) Cathepsin S from bovine spleen. Purification, distribution, intracellular localization and action on proteins. Biochem. J. 264, 467–473.

[39] Xin, X.Q., Gunesekera, B. and Mason, R.W. (1992) The specificity and elastinolytic activities of bovine cathepsins S and H. Arch. Biochem. Biophys. 299, 334–339.

[40] Yasuda, Y., Li, Z., Greenbaum, D., Bogyo, M., Weber, E. and Bromme, D. (2004) Cathepsin V, a novel and potent elastolytic activity expressed in activated macrophages. J. Biol. Chem. 279, 36761–36770.

[41] Dahl, S.W., Halkier, T., Lauritzen, C., Dolenc, I., Pedersen, J., Turk, V. and Turk, B. (2001) Human recombinant pro-dipeptidyl peptidase I (cathepsin C) can be activated by cathepsins L and S but not by autocatalytic processing. Biochemistry 40, 1671–1678.

[42] Hou, W.S., Li, W., Keyszer, G., Weber, E., Levy, R., Klein, M.J., Gravallese, E.M., Goldring, S.R. and Bromme, D. (2002) Comparison of cathepsins K and S expression within the rheumatoid and osteoarthritic synovium. Arthritis Rheum. 46, 663–674.

[43] Podgorski, I. and Sloane, B.F. (2003) Cathepsin B and its roles in cancer progression. Biochem. Soc. Symp. 70, 263–276.