Characteristics of Activation of Cathepsin B by Sodium Salicylate and Comparison of Catalytic Site Properties of Cathepsins B and H

Kenji YAMAMOTO, Mitsue TAKEDA and Yuzo KATO
Department of Pharmacology, Nagasaki University School of Dentistry, Nagasaki 852, Japan
Accepted June 24, 1985

Abstract—Attempts to gain greater understanding of characteristics of catalytic site properties of two homologous lysosomal cysteine proteinases, cathepsins B and H, were made by using sodium salicylate (SA) and several specific inhibitors, which extends the studies on the structure-activity relationship and kinetics for the activation of cathepsin B by SA in the previous paper (Yamamoto, K., Takeda, M. and Kato, Y.: Japan. J. Pharmacol. 38, 215–218, 1985). The half-maximal activation of cathepsin B by SA was observed at around a molar ratio of 10^4 : 1 (SA/cathepsin B). No preincubation time was needed for the SA-stimulated reaction, but the rate of activation was more rapid as pH values in the preincubation mixture decreased. The extent of inactivation of cathepsin B by leupeptin and E-64 significantly decreased in the presence of SA. Catalytic site properties of cathepsins B and H were also distinguished by differences in the extent of inhibition by cysteine proteinase inhibitors. Cathepsin B was more sensitive than cathepsin H to inhibition by antipain, chymostatin, iodoacetic acid and mercuric chloride as well as leupeptin and E-64. Despite the similarity in inhibitory effects of iodoacetic acid and E-64, the rat spleen cathepsin H was characterized by insensitivity to mercuric chloride that had a considerable inhibitory effect on the corresponding enzyme from rat liver and the rat spleen cathepsin B.

Cathepsins B and H are lysosomal cysteine proteinases which contain an essential cysteine residue in the active center (1). These two enzymes are believed to be important in physiological protein degradation (2, 3) and are suggested to be related to the development of various pathological conditions (4–7) such as inflammation. However, since they are similar in some physical properties and enzymic activities (8) and since these two enzymes are strikingly homologous to each other in amino acid sequences including the essential catalytic site regions (9), it is difficult to distinguish between these two enzymes with respect to their biological functions. It remains difficult to determine what part they play in pathophysiological protein degradation or what is the nature of the factors which regulate them. To understand the role of each proteinase in a variety of biological processes and to identify with confidence enzymes that catalyze particular reactions, we need detailed information on the catalytic site characteristics of each enzyme and on the relation of catalytic site properties to structural changes induced by intra- and extracellular compounds.

In previous papers (10, 11), we reported similarities in a number of enzymic properties between rat spleen cathepsin B and the corresponding enzyme from rat liver and between cathepsin H from the two sources. Also, in view of the immunological identity between the respective enzymes from the two sources (12) and the similarity in amino acid sequence between rat liver cathepsin B and the corresponding enzyme from human...
liver (13), we have assumed that rat spleen cathepsins B and H were similar to the respective enzymes from rat liver, even in the primary structures. The results obtained with the rat spleen enzymes are of great use in inferring the catalytic site characteristics of cathepsins B and H.

In the course of testing the ability of anti-inflammatory agents to affect the activities of lysosomal cysteine proteinases, we have found that flufenamic acid and indomethacin are selective inhibitors of cathepsin B from rat spleen (14). The preliminary study in this laboratory has also shown that sodium salicylate (SA) specifically accelerates the rate of activation of cathepsin B, but not cathepsin H, in vitro (15). These findings suggest the variation in therapeutic effectiveness among anti-inflammatory agents (14) and the differences between cathepsins B and H in drug-induced conformational changes. This paper deals with a more precise investigation of the SA-stimulated reaction of cathepsin B, and we also describe the discriminative effects of several specific inhibitors on cathepsins B and H.

**Materials and Methods**

**Materials:** Leupeptin, N-[N-(L-3-trans-carboxyoxirane-2-carbonyl)-L-leucyl]-agmatine (E-64), antipain, chymostatin, L-leucine 2-naphthylamide (Leu-NA), L-arginine-4-methyl-7-coumarylamide (Arg-MCA) and carbobenzoxy-L-phenylalanyl-L-arginine-4-methyl-7-coumarylamide (Z-Phe-Arg-MCA) were obtained from the Protein Research Foundation, Osaka, Japan. Sodium salicylate was a product of Sanko Seiyaku Co., Tokyo. All other chemicals were analytical grade reagents.

**Enzyme preparation:** The purification of cathepsins B and H from rat spleen was performed as described earlier (10, 11), but was slightly modified. This modification primarily improved the yield and accomplished the simultaneous purification of cathepsins B and H. Sodium phosphate buffer (10 mM), pH 6.5, containing 5 mM cysteine and 1 mM EDTA was used as the standard buffer. The non-adsorbed pool from the pepstatin-Sepharose column (3.2×5.0 cm), which provided a rapid and efficient method for removing almost all the contaminating acid proteinases such as cathepsins D (16) and E (17), was concentrated and dialyzed overnight against the buffer on ice. The dialyzed supernatant was centrifuged to remove insoluble materials and applied to a column (2.5×20 cm) of DEAE-Sepharose equilibrated with the standard buffer. A typical elution profile is shown in Fig. 1. Most of the activity toward Leu-NA was detected in the non-adsorbed fraction and referred to as the crude cathepsin H fraction. The fraction eluted with 50 mM NaCl contained most of the cathepsin B. Each enzyme fraction from DEAE-Sepharose was concentrated by ultrafiltration on an Amicon Diaflo YM 2 membrane and was separately applied to a chromatofocusing column (1.0×27 cm) packed with PBE 94 (Pharmacia) that had been equilibrated with 25 mM triethanolamine-HCl buffer, pH 8.3 (for cathepsin H) or with 25 mM bis-Tris HCl buffer, pH 6.3 (for cathepsin B). Each enzyme was eluted with polybuffer 74, pH 4.0. Cathepsins B and H each migrated as a single peak of activity between pH 5.0 and 5.4 and between pH 7.0 and 7.5, respectively. The purification of cathepsin B was accomplished by gel filtration on Sephadex G-75. Cathepsin H was purified by further chromatography on CM-Toyopearl and concanavalin A-Sepharose (10). Since cathepsins B and H consisted of two and three isoenzymes, respectively, the enzymes used in this study were the respective major forms (B, form I; H, form II). Both enzymes gave single protein bands on nondenatured and denatured polyacrylamide gel electrophoresis. Rat liver cathepsin H was also purified to homogeneity according to the method used in the isolation of cathepsin H from rat spleen. The rat liver cathepsin H used in this study was the corresponding form of cathepsin H from rat spleen.

**Assays:** The activity of cathepsin B was measured at pH 6.0 using N-α-benzoyl-DL-arginine 2-naphthylamide (BANA) and Z-Phe-Arg-MCA as substrates by the method of Barrett (18, 19), with a modification for the fluorometric assay as described (14). Cathepsin H was assayed at pH 6.5 spectrophotometrically using Leu-NA (10) and
fluorometrically using Arg-MCA as substrate (19). The incubation of each enzyme solution was carried out at 40°C for 10 min. One unit of each enzyme was defined as the amount of enzyme to release 1 μmol of each substrate per min at 40°C. Assays for testing the effects of SA and specific inhibitors on both enzymes were performed with the respective methylcoumaryl substrates. The molar concentration of solution of each enzyme was determined by inactivation with E-64 (20).

**Results**

**In vitro effect of SA on cathepsins B and H:** Figure 2 shows experiments in which the molar ratio of SA to each enzyme varied from zero to $10^5$. The reaction solution contained cysteine and EDTA at fixed concentrations. Cathepsin B was significantly activated by SA when the SA-to-enzyme molar ratio was greater than $6 \times 10^3$. The SA-stimulated reaction appears to attain a plateau at around the SA/enzyme ratio of $3 \times 10^4:1$. In contrast, little or no significant change in the cathepsin H activity was observed when similarly treated with SA. It has been known that cathepsin B requires the presence of a sulfhydryl compound plus EDTA for maximal activity (1).
cathepsin B was accomplished by the presence of 5 mM cysteine and 1 mM EDTA. No significant change in the cathepsin B activity was observed by increase in the concentrations of cysteine and EDTA or by use of other thiol reducing reagents such as dithiothreitol and 2-mercaptopetoethanol. The data given in Fig. 3 demonstrate the effect of these activators on the SA-stimulated reaction of cathepsin B. Upon the addition of SA to the cathepsin B solution, the enzyme activity increased linearly at the SA concentrations between $6.2 \times 10^{-6}$ M and $6.2 \times 10^{-3}$ M and then reached a plateau value which was not modified even at the higher concentrations. The extent of activation of cathepsin B by SA was not obtained by conventional activators such as cysteine and EDTA. SA alone and SA plus EDTA did not cause the activation of cathepsin B in accord with earlier reports (15). No significant change in the extent of cathepsin B activation by SA was observed by use of dithiothreitol, 2-mercaptopetoethanol and reduced glutathione in place of cysteine in the range of 2 mM to 10 mM (Table 1). The activation potency of SA for cathepsin B increased by decreasing pH values between 4.5 and 8.0 (Fig. 4). The activation at pH 4.5 was about twice that at pH 6.5 at the fixed molar ratio of SA to cathepsin B. Little or no SA-stimulated reaction for cathepsin B was observed at pH 7.0. The marked decrease in the activity

| Thiol compound          | Concentration (mM) | % of cathepsin B activity |
|-------------------------|--------------------|--------------------------|
| None                    |                    | 6                        |
| Cysteine                | 2                  | 223                      |
|                         | 5                  | 240                      |
|                         | 10                 | 260                      |
| Dithiothreitol          | 2                  | 210                      |
|                         | 5                  | 226                      |
|                         | 10                 | 230                      |
| 2-Mercaptopetoethanol   | 2                  | 193                      |
|                         | 5                  | 220                      |
|                         | 10                 | 248                      |
| Reduced glutathione     | 2                  | 230                      |
|                         | 5                  | 243                      |
|                         | 10                 | 251                      |

The purified cathepsin B (2.0 x 10^{-7} M) in 0.2 M sodium phosphate buffer, pH 6.0, containing 2 mM EDTA was preincubated at 25°C for 10 min under the following conditions: Control (○) was preincubated with 10 mM cysteine and 4 mM EDTA in the absence of SA. Others were treated with increased amounts of SA alone (●) or in combination with EDTA (▲), cysteine (△) and cysteine plus EDTA (■). After preincubation, each sample was subjected to the usual cathepsin B assay using Z-Phe-Arg-MCA as a substrate. To the controls was added SA after the reaction was terminated.
at pH values above 7.5 is ascribed to an inactivation of cathepsin B as described previously (10). The effect of preincubation time on the SA-stimulated reaction was also investigated. Cathepsin B was preincubated with SA up to 40 min at 25°C, and the remaining activity was measured by the usual assay. As shown in Fig. 5, the activation of cathepsin B by SA occurs immediately upon the addition of SA to the enzyme solution. At pH 5.0, no significant change in the activating potency of SA was observed up to 40 min. The SA-stimulated reaction was observed also at pH 7.0 within 5 min preincubation, but the longer preincubation times resulted in the loss of the SA-activating potency. The difference in the SA-activating potency between both pH values may be due to facilitation of the reaction of SA with cathepsin B at pH 5.0 rather than at pH 7.0.

Next, to investigate the nature and extent of the reaction of cathepsin B with SA, the effect of leupeptin and E-64 on the SA-stimulated reaction was examined. Leupeptin is known to be a tight-binding reversible inhibitor of cathepsin B (21). E-64 is also shown to inactivate cathepsin B by a stoichiometric reaction with the cysteine residue essential for catalytic activity (20, 22). Thus, both inhibitors can be used as the active-site-directed inhibitors of cathepsin B. The results shown in Fig. 6 indicate that the extent of inactivation of cathepsin B by leupeptin significantly decreased by the presence of SA prior to the addition of leupeptin. The concentrations of leupeptin necessary to achieve 50% inhibition of cathepsin B were $1.38 \times 10^{-8}$ M in the presence of SA and $5.24 \times 10^{-8}$ M in the absence of SA under the conditions employed. The decrease in the extent of inactivation by leupeptin in the presence of SA was about 50%. A similar result was also obtained with E-64 (data not shown). On the other hand, the extent of inhibition of cathepsin H by E-64, which is also an active-site-directed inhibitor for cathepsin H (20), was unaffected by SA.

Comparison of effects of several inhibitors on cathepsins B and H: Various cysteine
Fig. 6. Effect of SA on the rate of inactivation of cathepsins B and H by leupeptin and E-64. The purified cathepsins B (2.0×10^{-7} M) and H (1.6×10^{-6} M) in the respective assay buffers containing 5 mM cysteine and 2 mM EDTA were placed on ice for 5 min in the presence (○) or in the absence (●) of SA (6.2×10^{-3} M) and then incubated at 30°C for 5 min with leupeptin or E-64. The residual activities were measured by using the respective methylcoumarylamide substrates and are expressed as % of the controls which were determined in the absence of both SA and each inhibitor.

Fig. 7. Effects of specific proteinase inhibitors on the activities of cathepsins B and H purified from rat spleen. The activities of cathepsins B and H are shown by closed and open marks, respectively. The enzymes in the respective assay buffers containing 10 mM cysteine and 4 mM EDTA were preincubated at 37°C for 5 min with leupeptin (○, ●), antipain (▲, △) and chymostatin (□, ▼) at the concentrations indicated. Then each enzyme sample was subjected to the respective assays. Activities are expressed as % of those of the controls that were determined by omitting inhibitors.

Proteinase inhibitors were added to the assay mixture, and their effects on the activities of cathepsins B and H were examined. The results are presented in Figs. 7 and 8. The experiments were made at the fixed concentrations of enzyme and substrate by increasing concentrations of inhibitors. Cathepsins B and H were inhibited by the microbial peptide aldehydes leupeptin, antipain and chymostatin, but cathepsin H was much less reactive with these compounds than was cathepsin B (Fig. 7). Cathepsin B was more than 95% inhibited by 1 μM (0.43 μg/ml) of leupeptin, whereas cathepsin H was less than 20% inhibited by the same concentration of leupeptin. Antipain, an inhibitor of cysteine proteinases including papain and cathepsins (23), inhibited cathepsins B and H at extents comparable with those obtained with leupeptin. Chymostatin, which inhibits chymotrypsin, papain and cathepsins but not plasmin, trypsin and kallikrein (24), also inhibited cathepsin B strongly, although it was less reactive with cathepsin B than were
leupeptin and antipain.

The data given in Fig. 8 showed that inhibition profiles with iodoacetic acid, E-64 and mercuric chloride were apparently different between these two enzymes. Since rat spleen cathepsin H is shown to differ from the cathepsin H from other sources in the effect of mercuric compounds such as mercuric chloride and p-chloromercuribenzoate (10), rat liver cathepsin H was examined for sensitivity to these inhibitors. Iodoacetic acid and E-64 showed nearly the same inhibitory effect on both rat liver and spleen cathepsin H, but the effect of mercuric chloride on these enzymes were strikingly different. Rat spleen cathepsin H was not inhibited at all, even by a high concentration of mercuric chloride (1 mM) that caused more than 65% inhibition of rat liver cathepsin H. All of the inhibitors tested reacted much more slowly with cathepsin H than cathepsin B.

Discussion

The purification procedure reported in this paper seems to be very convenient if both cathepsins B and H are desired at the same time. Cathepsins B and H from rat spleen were easily separated by use of DEAE-Sephacel and chromatofocusing columns. Rat cathepsin H, in common with cathepsin H isolated from human liver (25), showed less affinity for DEAE-Sephacel at pH 6.0–6.5 than cathepsin B. On the chromatofocusing column, cathepsin H was eluted at pH values between 7.0 and 7.5, whereas cathepsin B revealed the peak of activity in the pH range of 5.0–5.4. Rat spleen cathepsin B had all the characteristics known for cathepsin B from other sources. Also, rat spleen cathepsin H (10) was similar in many enzymic properties to rat liver cathepsin H, despite the differences in susceptibility to mercuric compounds and stability to alkaline pH.

Cathepsins B and H have molecular weights in the range of 22,000–30,000 and optimum pH between 6.0 and 7.0. Both enzymes hydrolyzed blocked amino acid naphthylamides such as N-benzoyl-DL-arginine-2-naphthylamide. In addition, the homology of these two enzymes are shown by analysis of their amino acid sequences (9). Therefore, these two analogous enzymes seem to be difficult to distinguish from each other in biological functions. This may be ascribed to the lack of information on the catalytic site properties of cathepsin H as compared with those of cathepsin B. We thus thought it worthwhile to compare the characteristics of the catalytic sites of both enzymes in some detail. In previous works (14, 15), we found that cathepsin B could be distinguished from cathepsin H by differences in effects of flufenamic acid, indomethacin and sodium salicylate on their activities. The activating potency of SA for cathepsin B was also shown to be lost by the
substitution of SA for the meta and para isomers or aspirin, indicating that the SA-stimulated reaction is largely due to its high binding capacity as a hydrogen donor in the ortho position to the enzyme (15). The present study showed that the SA-stimulated reaction occurred at the molar ratio of SA to cathepsin B as high as 10^3: 1. With an increase in this ratio, the extent of activation for cathepsin B increased in a dose-dependent manner and attained a plateau. The SA-stimulated reaction of cathepsin B indiscreetly required the presence of thiol reducing reagents such as cysteine, 2-mercaptoethanol, dithiothreitol and reduced glutathione. Since the activation of the essential cysteine residue of cathepsin B by these thiol reducing compounds has been shown to be a slow process (26), the conformational change induced by SA-binding appears to activate the process. The extent of inactivation of cathepsin B by leupeptin and E-64 was apparently diminished by the presence of SA prior to the addition of the inhibitor. Since these inhibitors are known as active-site-directed titrants of cathepsin B, the data suggest that the SA-binding to the enzyme molecule occurs at least in part in the catalytic site region. The anti-inflammatory and analgesic activities of commonly used nonsteroidal anti-inflammatory agents including SA have been discussed mainly with regard to their ability to inhibit the biosynthesis of prostaglandins, and very little information is available on its subcellular effect on lysosomal proteinases. Nevertheless, the possibility that these agents may exert some of their effects by modifying enzymes other than prostaglandin-synthetase has been suggested. It may be of particular interest, therefore, that SA has the ability to activate cathepsin B. Whether or not SA is present outside the circulation in a sufficient amount to have the physiological efficiency for activating cathepsin B remains to be elucidated, but the present observation seems to be noted as the first known property of SA.

The activities of cathepsins B and H were also clearly distinguished by their susceptibilities to the specific cysteine proteinase inhibitors tested. In all cases, cathepsin H was much less effective than cathepsin B. All compounds tested except mercuric chloride showed nearly the same inhibitory effects on rat spleen cathepsins B and H as those obtained with the enzymes from other sources (1, 25, 27, 28). Mercuric chloride did not affect the activity of rat spleen cathepsin H, but this compound strongly inhibited rat liver cathepsin H as well as cathepsin B. The present results confirm the observations of our previous study that the efficiency of mercuric chloride for rat spleen cathepsin H was different from that of cathepsin H from other sources (10). Since the selectivity and potency of inhibitors are due to their affinity for the specificity sites of enzyme, the discrepancies in inhibitory effects of these compounds on cathepsins B and H indicate the structural difference of these enzymes. Especially, the discriminative effects of leupeptin and E-64 on cathepsins B and H indicate the difference in catalytic site properties between these two enzymes.

References
1 Barrett, A.J.: Cathepsin B and other thiol proteinases. In Proteases in Mammalian Cells and Tissues. Edited by Barrett, A.J., p. 181–208, North-Holland Publishing Co., Amsterdam (1977)
2 Dean, T.: The roles of cathepsins B1 and D in the digestion of cytoplasmic proteins in vitro by lysosomal extracts. Biochem. Biophys. Res. Commun. 68, 518–523 (1976)
3 Quinn, P.S. and Judah, J.D.: Calcium-dependent Golgi-vesicle fusion and cathepsin B in the conversion of proalbumin into albumin in rat liver. Biochem. J. 172, 301–309 (1978)
4 Pietras, R.J. and Roberts, J.A.: Cathepsin B-like enzymes. Subcellular distribution and properties in neoplastic and control cells from human ectocervix. J. Biol. Chem. 256, 5536–5544 (1981)
5 Recklies, A.D., Tiltman, K.J., Stoker, T.A.M., and Poole, A.R.: Secretion of proteinases from malignant and nonmalignant human breast tissue. Cancer Res. 40, 550–556 (1980)
6 Bayliss, M.T. and Ali, S.Y.: Studies on cathepsin B in human articular cartilage. Biochem. J. 171, 149–164 (1978)
7 Mason, R.W., Taylor, M.A.J. and Etherington, D.J.: Purification and characterization of collagenolytic cathepsins from rabbit liver. FEBS Lett. 146, 33–36 (1982)
8 Barrett, A.J. and Kirschke, H.: Cathepsin B, cathepsin H, and cathepsin L. Methods
9 Takio, K., Towatari, T., Katunuma, N., Teller, D.C. and Titani, K.: Homology of amino acid sequences of rat liver cathepsins B and H with that of papain. Proc. Natl. Acad. Sci. U.S.A. 80, 3666-3670 (1983).

10 Yamamoto, K., Kamata, O. and Kato, Y.: Separation and properties of three forms of cathepsin H-like cysteine proteinase from rat spleen. J. Biochem. 95, 477-484 (1984).

11 Yamamoto, K., Kamata, O., Takeda, M. and Kato, Y.: Characterization of rat spleen cathepsin B and differential effects of anti-inflammatory drugs on its activity. Japan. J. Oral Biol. 25, 834-838 (1983).

12 Kominami, E. and Katunuma, N.: Immunological studies on cathepsins B and H from rat liver. J. Biochem. 91, 67-71 (1982).

13 Ritonja, A., Popovic, T., Turk, V., Wiedenmann, K. and Machleidt, W.: Amino acid sequence of human liver cathepsin B. FEBS Lett. 181, 169-172 (1985).

14 Yamamoto, K., Kamata, O. and Kato, Y.: Differential effects of anti-inflammatory agents on lysosomal cysteine proteinases cathepsins B and H from rat spleen. Japan. J. Pharmacol. 35, 253-258 (1984).

15 Yamamoto K., Takeda, M. and Kato, Y.: Sodium salicylate activates cathepsin B but not cathepsin H from rat spleen. Japan. J. Pharmacol. 38, 215-218 (1985).

16 Yamamoto, K., Katsuda, N., Himeno, M. and Kato, K.: Cathepsin D of rat spleen. Affinity purification and properties of two types of cathepsin D. Eur. J. Biochem. 95, 459-467 (1979).

17 Yamamoto, K., Katsuda, N. and Kato, K.: Affinity purification and properties of cathepsin E-like cysteine proteinase from rat spleen. Eur. J. Biochem. 92, 499-508 (1978).

18 Barrett, A.J.: A new assay for cathepsin B1 and other thiol proteinases. Anal. Biochem. 47, 280-293 (1972).

19 Barrett, A.J.: Fluorimetric assays for cathepsin B and cathepsin H with methylcoumarylamide substrates. Biochem. J. 187, 909-912 (1980).

20 Barrett, A.J., Kembhavi, A.A., Brown, M.A., Kirschke, H., Knight, C.G., Tamai, M. and Hanada, K.: L-trans-Epoxy succinyl-leucylamido (4-quandio) butane (E-64) and its analogues as inhibitors of cysteine proteinases including cathepsins B, H and L. Biochem. J. 201, 189-198 (1982).

21 Knight, C.G.: Human cathepsin B. Application of the substrate N-benzylxycarbonyl-L-arginyl-L-arginine 2-naphthylamide to a study of the inhibition by leupeptin. Biochem. J. 189, 447-453 (1980).

22 Hashida, S., Towatari, T., Kominami, E. and Katunuma, N.: Inhibitions by E-64 derivatives of rat liver cathepsin B and cathepsin L in vitro and in vivo. J. Biochem. 88, 1805-1811 (1980).

23 Suda, H., Aoyagi, T., Hamada, M., Takeuchi, T. and Umezawa, H.: Antipain, a new protease inhibitor isolated from actinomycetes. J. Antibiot. 25, 263-266 (1972).

24 Tatsuta, K., Mikami, N., Fujimoto, K., Umezawa, S., Umezawa, H. and Aoyagi, T.: The structure of chymostatin, a chymotrypsin inhibitor. J. Antibiot. 26, 625-646 (1973).

25 Schwartz, W.N. and Barrett, A.J.: Human cathepsin B. Biochem. J. 191, 487-497 (1980).

26 Baici, A. and Gyger-Marazzi, M.: The slow, tight-binding inhibition of cathepsin B by leupeptin. A hysteric effect. Eur. J. Biochem. 129, 33-41 (1982).

27 Kirschke, H., Langner, J., Riemann, B., Wiederanders, S., Ansorge, S. and Bohley, P.: Lysosomal cysteine proteinases. In Protein Degradation in Health and Disease, p. 15-35, Ciba Foundation Symposium 75, Excerpta Medica, Amsterdam, Oxford and New York (1980).

28 Kirschke, H., Langner, J., Wiederanders, B., Ansorge, S., Bohley, P. and Hanson, H.: Cathepsin H. An endoaminopeptidase from rat liver lysosomes. Acta Biol. Med. Germ. 36, 185-199 (1977).