Interrelationship of active and latent secreted human cathepsin B precursors

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Two high- M_r forms of cathepsin B have been described previously, both of which are stable at alkaline pH, in contrast with the lysosomal proteinase. One form is latent and activated by pepsin treatment; the other form is active as measured with synthetic substrates. In the present study it was shown that the two forms are indistinguishable on the basis of molecular size as determined by gel-filtration chromatography or sodium dodecyl sulphate/polyacrylamide-gel electrophoresis followed by immunoblotting. Both forms lose their alkali-stability upon exposure to Hg^{2+} , and after Hg^{2+} treatment the latent form becomes immunoprecipitable by an antiserum that reacts only with denatured cathepsin B. Lysosomal cathepsin B is bound by the plasma proteinase inhibitor α_2 -macroglobulin, a process that requires proteolytic cleavage of the inhibitor. In contrast, the stable active form of cathepsin B is not bound by this inhibitor unless this enzyme is first destabilized by Hg^{2+} treatment. These results indicate that cathepsin B exists in three different states of activity, completely latent, partially active and fully proteolytically active. To exhibit true endopeptidase activity it seems that the enzyme must be in an alkali-unstable form.

INTRODUCTION

Cathepsin B is a lysosomal cysteine proteinase (Barrett & Kirschke, 1981) that is also thought to play an extracellular role in neoplastic and inflammatory diseases (Poole *et al.*, 1980; Mort *et al.*, 1984). Two extracellular high- M_r forms of human cathepsin B have been described previously. In ascitic fluid and culture medium of ascites cells from cancer patients, a latent form of cathepsin B was characterized that could be rendered active by prior proteinase treatment (Mort *et al.*, 1981a, 1983). In addition, human malignant breast tumours in organ culture release a high- M_r form of cathepsin B that was detected by its ability to hydrolyse synthetic substrates for which lysosomal cathepsin B has a high affinity (Mort *et al.*, 1980). A similar activity has been characterized in culture media of spontaneous mammary tumours (Recklies *et al.*, 1982a) and lactating mouse mammary gland (Recklies *et al.*, 1985; Recklies & Mort, 1985). Unlike the lysosomal proteinase, this active enzyme is stable at neutral pH but loses this property on treatment with Hg^{2+} ions, and by radioimmunoassay it has been shown that both of these secreted enzymes are high- M_r forms of cathepsin B (Recklies *et al.*, 1982b; Mort *et al.*, 1983).

It is now known that many lysosomal enzymes are synthesized as higher- M_r proenzyme forms (Hasilik & von Figura, 1984), which in the case of proteinases would be expected to be inactive. Since lysosomal cathepsin B is irreversibly inactivated at neutral to basic pH (Barrett, 1973), the cathepsin B precursor would be expected to be stable under normal physiological conditions to allow it to pass from its site of synthesis to the acid environment of the lysosome. These are exactly the properties that we have described for the latent form of cathepsin B, and so

it seems likely that this is a precursor of the lysosomal enzyme. The detection of a stable active high- M_r form is therefore somewhat unexpected; however, activity has only been demonstrated with synthetic substrates for which cathepsin B has a high affinity. In order to evaluate the endopeptidase activity of this species in partially purified culture media, binding of the enzyme to α_2 -macroglobulin was investigated. α_2 -Macroglobulin is a general high- M_r (760 000) proteinase inhibitor present in plasma. In order for a proteinase to be bound by this molecule it must make a proteolytic cleavage in the 'bait' region of the inhibitor (Barrett & Starkey, 1973; Barrett *et al.*, 1979); after such a cleavage a conformational change occurs and the inhibitor engulfs the proteinase, rendering it inactive against protein substrates. Activity against low- M_r substrates however, remains. Binding of cathepsin B forms to α_2 -macroglobulin can easily be assessed by the large increase in M_r of the entity with cathepsin B activity, measured with synthetic substrates, after complex-formation. In the present paper we describe a comparison of the two high- M_r cathepsin B forms that shows that they are in fact very similar and that the active species may not have complete proteolytic function.

EXPERIMENTAL

Materials

Human liver cathepsin B was purified and sheep anti-(cathepsin B) serum prepared as previously described (Mort *et al.*, 1981b). Anti-(cathepsin B) antibody was affinity-purified and ^{125}I -labelled by using the chloramine-T method (Greenwood *et al.*, 1963). Rabbit anti-[sheep F_c fragments (the non-antigen-binding fragments

produced by papain digestion of IgG)] were prepared as previously described (Recklies & Mort, 1982). Human α_2 -macroglobulin was purified by using the initial steps of the method of Barrett *et al.* (1979) followed by zinc-chelate chromatography (Kurecki *et al.*, 1979).

Medium from human breast-tumour explants, cultured in serum-free medium as previously described (Recklies *et al.*, 1980), was used as a source of high- M_r active cathepsin B. Media were dialysed into 50mM-Hepes/NaOH buffer, pH 7.0, and concentrated by ultrafiltration on PSAC membranes from Millipore (Mississauga, Ont., Canada). Gene Screen transfer membranes and Na¹²⁵I were from New England Nuclear (Montreal, Que., Canada), Protein A-Sepharose 4B was from Pharmacia (Montreal, Que., Canada), Toyo Soda TSK columns were from Bio-Rad (Mississauga, Ont., Canada) and Ultrogel AcA resins were from Fisher Scientific (Montreal, Que., Canada).

Assay of cathepsin B activity

Cathepsin B activity was measured by using the method of Barrett (1976), with Cbz-Arg-Arg- β -naphthylamide (prepared as described by Knight, 1980) as substrate. As shown previously, the various cathepsin B forms have different pH optima (Recklies *et al.*, 1982a; Mort *et al.*, 1981a). Assay pH values were therefore modified to give maximal activity of the form under study. The active high- M_r form was assayed at pH 5.5 (Recklies *et al.*, 1982a), and the latent form, after pepsin activation at pH 3.0, was assayed at pH 6.5 (Mort *et al.*, 1981a).

Partial purification of latent cathepsin B

Enzyme from ascitic fluid of cancer patients was purified by Affi-Gel Blue chromatography as described previously (Mort *et al.*, 1983). This was followed by gel filtration through an Ultrogel AcA-44 column followed by h.p.l.c. through a TSK-2000 SW-TSK-3000 SW column combination (each column 0.75 cm $\times$ 30 cm) in 0.1 M-sodium phosphate buffer, pH 7.0. Active enzyme was prepared from this partially purified preparation by pepsin digestion as described previously (Mort *et al.*, 1983).

When prepared by this procedure the latent enzyme showed a major band of M_r 40000, as was seen previously (Mort *et al.*, 1983), and a small amount of a faster-migrating component, when analysed by SDS/polyacrylamide-gel electrophoresis followed by electroblotting and localization by the use of anti-(cathepsin B) antibody (Fig. 1, lane A). This may be the result of limited proteolysis occurring during the purification procedure.

Gel-filtration chromatography

Serum-free medium from human breast carcinoma explants was concentrated to 2 ml and chromatographed through a Ultrogel AcA-54 column (1.6 cm $\times$ 90 cm) in 50 mM-Hepes/NaOH buffer, pH 7.0, containing 0.2 M-NaCl at 4 °C as described previously (Recklies & Mort, 1985). Ovalbumin (20 μ g) was included as an internal standard and its position in the resulting fractions determined by rocket immunoelectrophoresis. Calibration runs were carried out to determine the void volume (Blue Dextran), total column volume [K_3 Fe(CN)₆] and the elution position of liver cathepsin B.

For assessing the binding of cathepsin B forms to

α_2 -macroglobulin, samples (100 μ l) were analysed by high-speed gel filtration through a TSK-3000 SW column (7.5 mm $\times$ 30 cm with a 7.5 mm $\times$ 7 cm precolumn) in 0.1 M-sodium phosphate buffer, pH 6.5, at 1 ml/min.

Electrophoresis and electroblotting

Slab gels (10%) were made and run according to the method of Laemmli (1970). Transfer was carried out as described by Towbin *et al.* (1979). Initially nitrocellulose membranes were used, but there was significant non-specific binding of radiolabelled antibody. Much less non-specific binding was seen with the use of Gene Screen membranes. After the electroblotting, the membrane was blocked in 3% (w/v) bovine serum albumin in phosphate-buffered saline (10 mM-sodium/potassium phosphate buffer, pH 7.2, containing 0.145 M-NaCl and 0.05% NaN₃), incubated in ¹²⁵I-labelled affinity-purified anti-(cathepsin B) antibody in phosphate-buffered saline and then washed with phosphate-buffered saline containing 0.1% Tween 20. Radiolabelled standard proteins were used to provide reference M_r positions on the membranes. Cathepsin B-related bands were detected by autoradiography with Kodak XAR film.

Immune precipitation of latent cathepsin B

Latent enzyme was immune-precipitated by using affinity-purified sheep anti-(human cathepsin B) IgG bound to Protein A-Sepharose through an intermediate rabbit anti-(sheep Fc fragment) antibody (Recklies & Mort, 1982). The incubation and washing procedures of Gordon *et al.* (1983) were followed. For Hg²⁺ treatment, latent cathepsin B, partially purified from ascitic fluid, was incubated with 0.5 mM-HgCl₂ at room temperature for 15 min, and then EDTA was added to a final concentration of 2mM. Anti-(cathepsin B)-IgG bearing Protein A-Sepharose was added, and the mixture was left shaking for 16 h at room temperature. The Protein A-Sepharose was then washed, and the immune-precipitated material removed from the gel by boiling in the presence of 2 vol. of 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol and 0.001% Bromophenol Blue in 0.125 M-Tris/HCl buffer, pH 6.8, was analysed by SDS/polyacrylamide-gel electrophoresis followed by electroblotting and identification of cathepsin B-reactive material by use of the ¹²⁵I-labelled anti-(cathepsin B) antibody.

RESULTS

Secretion of both active and latent high- M_r cathepsin B forms from human mammary tumours

The two high- M_r cathepsin B forms previously characterized originated from different sources. Latent enzyme was obtained from ascitic fluid or from culture media of ascites cells and was detected after pepsin activation. Active enzyme was only found in culture media of tumour explants or lactating mammary gland. We have since found that medium from all breast tumours tested showed cathepsin B activity after pepsin digestion. Active enzyme was secreted to a greater degree by malignant rather than non-malignant tumours. When activity after pepsin digestion (measured at pH 6.5) was compared with endogenous activity (measured at pH 5.5) a wide range of ratios (0.5–60) was found for media from different specimens. This suggests that the latent and the

Fig. 1. Molecular sizes of cathepsin B-cross-reactive material before and after Hg^{2+} treatment

Samples were run on SDS/polyacrylamide gels under reducing conditions, and cathepsin B-like material was localized after electroblotting with the use of ^{125}I -labelled anti-(cathepsin B) IgG. Lanes A–D, latent cathepsin B partially purified from ascitic fluid: A, untreated; B, preincubated with HgCl_2 ; C, preincubated with HgCl_2 followed by pepsin treatment; D, pepsin-treated without prior HgCl_2 preincubation (details of these treatments are in Table 1). Lanes E and F, concentrated medium from human breast-tumour explants: E, no treatment; F, preincubated with HgCl_2 (0.5 mM, 15 min at 20 °C).

active high- M_r forms of cathepsin B are both present in most media but in different concentrations. When culture medium containing both active and latent enzyme was analysed by gel-filtration chromatography it was found that the activity, revealed by pepsin treatment of the column fractions, was co-eluted with the high- M_r active enzyme (Fig. 2) at the elution position previously found for the latent enzyme (Mort *et al.*, 1981a). In addition, when media containing both activities were subjected to polyacrylamide-gel electrophoresis followed by electroblotting, in every case only one band (M_r 40000) reactive with anti-(cathepsin B) antibody was observed (Fig. 1, lane E). The physical characteristics of the two secreted cathepsin B forms are thus indistinguishable on the basis of molecular size.

Similarity of the latent and active stable forms to Hg^{2+} treatment

The active and the latent secreted cathepsin B forms are also characterized by their stability at neutral and alkaline pH. Since the stability of the active form is

Fig. 2. Gel-filtration chromatography (Ultragel AcA-54) of tumour-explant conditioned medium

The elution profile of the high- M_r active form was determined by assay at pH 5.5 with Cbz-Arg-Arg- β -naphthylamide as substrate (○). Fractions were then treated at pH 3.0 with or without pepsin, and cathepsin B activity was assayed at pH 6.5, with the same substrate. Pepsin-generated activity (●) was calculated from difference between the activities resulting from these treatments. (Values in the absence of pepsin were 10–15% of the activity measured after pepsin treatment). Arrows show the elution positions of standard proteins obtained from separate runs, except for ovalbumin, which was used as an internal standard.

Table 1. Effect of Hg^{2+} treatment on pepsin activatability of latent cathepsin B

Samples of latent cathepsin B from ascitic fluid were incubated with or without 0.5 mM- HgCl_2 for 15 min (22 °C) at neutral pH in a total volume of 10 μl , and then 140 μl of 0.1 M-sodium formate buffer, pH 3.0, with or without pepsin was added to give a final pepsin concentration of 0.1 mg/ml. Incubation was then continued for 1 h at 40 °C. Duplicate 50 μl samples were then taken for cathepsin B assay at pH 6.5, with Cbz-Arg-Arg- β -naphthylamide as substrate as described previously (Mort *et al.*, 1983). The concentration of HgCl_2 used had no observable effect on pepsin activity and was neutralized by the excess EDTA and cysteine used in the cathepsin B assay. Values expressed are based on that obtained with pepsin digestion in the absence of HgCl_2 being taken as 100%. Letters in parentheses refer to corresponding lanes of similarly treated material analysed by SDS/polyacrylamide-gel electrophoresis (Fig. 1).

Preincubation conditions		
Hg^{2+}	Pepsin	Activity (%)
—	—	2.4 (A)
+	—	2.7 (B)
+	+	3.7 (C)
—	+	100.0 (D)

Fig. 3. Immune-precipitation of latent cathepsin B

Partially purified latent enzyme from ascitic fluid was immune-precipitated as described in the Experimental section after pretreatment with or without HgCl₂ as indicated. Antibody-bound material was analysed by SDS-polyacrylamide-gel electrophoresis and immunoblotting with ¹²⁵I-labelled anti-(cathepsin B) IgG. Lanes A and B, immune IgG; lanes C and D, non-immune IgG. Non-specific binding at M_r around 50 000 visible in all lanes is due to the presence of excess IgG heavy chain used in the immune-precipitation step.

irreversibly lost on treatment with low concentrations of Hg²⁺ (Recklies *et al.*, 1982b), it was decided to determine the effect of Hg²⁺ on the latent enzyme. Samples of latent enzyme from ascitic fluid were first treated with Hg²⁺ and then subjected to pepsin activation (Table 1). Whereas pepsin digestion without Hg²⁺ treatment generated cathepsin B activity, prior incubation with Hg²⁺ completely prevented activation. Since a vast excess of cysteine and EDTA is present when enzyme activity is measured, the amount of Hg²⁺ used in prior incubations to destabilize the enzyme had no effect on the enzyme activity, nor does Hg²⁺ inhibit pepsin activity. Treatment of the latent enzyme with Hg²⁺ but without pepsin treatment had no activation affect. Since exposure to Hg²⁺ resulted in the loss of pepsin-activatable enzyme, the products of these treatments were analysed by SDS/polyacrylamide-gel electrophoresis and cathepsin-B-cross-reactive material was localized by immunoblotting. Hg²⁺ treatment by itself had no effect on the M_r of the latent species (Fig. 1, lanes A and B). Similarly no effect on the size of the high- M_r active enzyme from tumour medium was observed after exposure to Hg²⁺ (Fig. 1, lanes E and F). When latent enzyme was treated with Hg²⁺ followed

Fig. 4. Binding of liver cathepsin B to α_2 -macroglobulin

Samples were chromatographed through a TSK-3000 column and fractions were assayed for cathepsin B activity: (a) liver cathepsin B alone [84 ng diluted in bovine serum albumin (0.1 mg/ml)]; (b) liver cathepsin B (84 ng) preincubated with 46 μ g of α_2 -macroglobulin in 0.1 M-sodium phosphate buffer, pH 6.5, containing bovine serum albumin (0.1 mg/ml), 2 mM-cysteine and 1 mM-EDTA for 30 min at 22 °C. The arrow indicates the elution position of α_2 -macroglobulin.

by pepsin digestion, no immune-reactive material could be detected (Fig. 1, lane C), whereas pepsin digestion of untreated enzyme yielded mainly one immune-reactive band in the 34 000- M_r region (Fig. 1, lane D). These findings suggest that Hg²⁺ induces a conformational change in the latent enzyme that renders it very sensitive to pepsin digestion, resulting in degradation to small peptides.

Immunological evidence that Hg²⁺ destabilizes the latent proteinase

Antisera raised against purified cathepsin B from liver by the present investigators (Mort *et al.*, 1981b; Recklies & Mort, 1985) and other groups (Barrett, 1973; Graf *et al.*, 1981) only react with the enzyme in a denatured state. All forms therefore react on immunoblots of SDS/polyacrylamide gels, but the lysosomal enzyme also reacts

Fig. 5. Binding of stable cathepsin B to α_2 -macroglobulin

Samples were chromatographed through a TSK-3000 column and fractions were assayed for cathepsin B activity: (a) concentrated medium from organ culture of breast tumours in 50 mM-sodium phosphate buffer, pH 6.5, containing 2 mM-cysteine; (b) tumour medium as in (a) preincubated with α_2 -macroglobulin (0.46 mg/ml) for 30 min at 22 °C; (c) tumour medium in 50 mM-sodium phosphate buffer, pH 6.5, incubated with 0.5 mM- HgCl_2 for 15 min at 22 °C and then dialysed overnight against 50 mM-sodium phosphate buffer, pH 6.5; destabilized enzyme was then incubated with α_2 -macroglobulin (0.46 mg/ml) in the presence of 2 mM-cysteine for 30 min at 22 °C; cathepsin B activity in medium preincubated with HgCl_2 was eluted as in (a) in the absence of α_2 -macroglobulin. The arrow indicates the elution position of α_2 -macroglobulin.

after exposure to alkaline pH, whereas the secreted forms do not. Once activated by pepsin treatment the latent enzyme becomes alkali-labile and is thus immune-reactive at elevated pH (Mort *et al.*, 1983). In addition, the active secreted enzyme from human tumours was shown to be immune-reactive at alkaline pH after destabilization with

Fig. 6. Binding of pepsin-activated latent cathepsin B to α_2 -macroglobulin

Samples were chromatographed through a TSK-3000 column and fractions were assayed for cathepsin B activity: (a) pepsin-activated latent enzyme in 50 mM-sodium phosphate buffer, pH 6.5, containing 2 mM-cysteine; (b) enzyme as in (a) preincubated with α_2 -macroglobulin (0.46 mg/ml) for 30 min at 22 °C. Note the difference in scales of the ordinates. Arrow indicates the elution position of α_2 -macroglobulin.

HgCl_2 (Recklies *et al.*, 1982b). Untreated latent enzyme does not react with the anti-(cathepsin B) antibody at alkaline pH, as the lack of immune-precipitability with this antibody shows (Fig. 3, lane A). However, if it was first exposed to Hg^{2+} , immune precipitation was observed (Fig. 3, lane B). This indicates that Hg^{2+} destabilizes the latent enzyme in such a way as to allow denaturation of alkaline pH conditions, and again demonstrates the similarity between the latent and the active secreted cathepsin B forms.

Effect of destabilization of high- M_r forms of cathepsin B on their ability to bind to α_2 -macroglobulin

When purified human liver cathepsin B was incubated with α_2 -macroglobulin most of the cathepsin B activity, measured with Cbz-Arg-Arg- β -naphthylamide as substrate, was eluted at the void volume (Fig. 4), indicating almost complete capture of the proteinase by the inhibitor. Although activity against the small synthetic substrate is retained on binding of the proteinase to α_2 -macroglobulin, it is clear from Fig. 4 that the complexed enzyme is not as active as it is in the free state. In contrast, when stable cathepsin B from tumour

Scheme 1. Schematic representation of the inter-relationship between stable and unstable forms of cathepsin B

(i) Latent cathepsin B; (ii) stable form active against synthetic substrates (high- M_r active form); (iii) unstable form, proteolytically active, generated from (ii) by Hg^{2+} treatment; (iv) unstable inactive form generated from (i) by Hg^{2+} treatment; (v) unstable proteolytically active form generated from (i) by pepsin treatment; (vi) lysosomal cathepsin B produced from probable precursor (i) by proteolytic processing *in vivo*. The active site is indicated by -SH. Regions contributing to maintenance of alkaline stability and that are affected by Hg^{2+} are indicated by A. Latency of form (i) is thought to be due to an interaction B that causes the blockage of the active site. This interaction is unaffected by Hg^{2+} treatment. Interactions A that stabilize form (ii) are thought to restrict the active site, limiting enzymic activity to action on synthetic substrates. Pepsin cleavage of form (i) at C decreases the M_r from 40 000 to 33 000. The mechanism of conversion of (i) into (ii) and the effect of pepsin treatment on (ii) are unknown at present.

medium was incubated with α_2 -macroglobulin, on gel-filtration analysis all the activity remained in the low- M_r region, indicating that this form does not bind to the inhibitor (Fig. 5b). If the enzyme preparation was first treated with Hg^{2+} and the Hg^{2+} then dialysed away, binding to α_2 -macroglobulin was observed (Fig. 5c).

Latent cathepsin B also did not bind to α_2 -macroglobulin; in fact, in ascitic fluid it coexists with large amounts of this inhibitor. When cathepsin B activity was generated by pepsin digestion, the active form was shown to bind to α_2 -macroglobulin (Fig. 6b).

DISCUSSION

Two high- M_r cathepsin B forms have been studied previously. Active cathepsin B stable to culture conditions has been characterized from organ cultures of human (Mort *et al.*, 1980) and mouse (Recklies *et al.*, 1982a) mammary tumours and lactating mammary gland (Recklies & Mort, 1985). In our early report the M_r of the stable enzyme from human tumours was estimated to be about 35 000 and the preparation was bound by α_2 -macroglobulin (Mort *et al.*, 1980). In those studies the enzyme was handled under conditions designed to prevent denaturation of lysosomal cathepsin B (pH 5.5 in the presence of EDTA) (Barrett, 1973). Under these conditions we now know that the secreted enzyme is destabilized, and we believe that limited proteolysis could have occurred, trimming the enzyme to a smaller molecular size. Similar cleavages occur when the latent enzyme is treated with various proteinases, and have also been observed when it is taken through several purification steps. If the active enzyme is kept under conditions where stability is maintained an M_r of 40 000 is seen by gel-filtration chromatography.

The latent form of cathepsin B was characterized from ascitic fluid of cancer patients (Mort *et al.*, 1981a, 1983), but we now realize that latent cathepsin B is present to some extent in all cultures of cancer cells and tumour explants. When media containing both forms are analysed by gel-filtration chromatography or by polyacrylamide-gel electrophoresis, it has not been possible to separate active from latent enzyme, suggesting that they are in fact very similar species. This similarity is further manifested in the stability of both secreted forms at elevated pH and the loss of their stability on treatment with Hg^{2+} , as demonstrated by the generation of immune-precipitable forms with the use of an antibody to lysosomal cathepsin B that only recognizes denatured enzyme. Although Hg^{2+} treatment converts latent cathepsin B into an unstable form, it does not, however, induce enzymic activity. The difference between the two high- M_r cathepsin B forms seems therefore to reside in access to their respective active sites.

Binding studies of cathepsin B forms to the plasma proteinase inhibitor α_2 -macroglobulin provide further insight into this area. Full proteolytic activity is required for capture of proteinases by this inhibitor (Barrett *et al.*, 1979). The finding that active high- M_r cathepsin B is not bound by α_2 -macroglobulin unless converted into an unstable form by Hg^{2+} pretreatment could be due to either of two reasons. Firstly, it is possible that the stable form has restricted substrate specificity when compared with the lysosomal enzyme and that the 'bait' region of the inhibitor does not contain an appropriate cleavage site. This seems unlikely, since previous studies have shown that secreted cathepsin B cleaves a range of synthetic substrate in a similar manner to that of the lysosomal enzyme (Mort *et al.*, 1980). In addition, the amino acid sequence of the 'bait' region of α_2 -

macroglobulin (Mortensen *et al.*, 1981) indicates that it contains several potential cleavage sites. Alternatively, the active site of both secreted cathepsin B forms may be restricted, the latent form being completely closed, the active form being open enough to allow entry of small peptides, such as synthetic substrate, while not allowing entry of protein substrates. A scheme representing this explanation is illustrated in Scheme 1. Treatment with Hg^{2+} is thought to loosen the whole molecule, thus opening up the active site, whereas in the latent form the active site remains blocked.

The instability of lysosomal cathepsin B at alkaline pH is intriguing, since significant sequence homology has been demonstrated between this enzyme and the cysteine proteinases cathepsin H from rat liver and papain. On the basis of sequence data similar tertiary structures have been suggested for the three enzymes (Takio *et al.*, 1983). A significant difference between cathepsin B on the one hand and cathepsin H and papain on the other is that the last-mentioned two enzymes are relatively stable at alkaline pH. A study of the amino acid sequence of cathepsin B reveals that, in addition to the histidine residue involved in the active site, there are six other histidine residues in this molecule, none of which is conserved in cathepsin H or papain. As the ionization status of histidine residues (pK 6) changes in the same pH range as that at which cathepsin B becomes unstable, histidine residues could be involved in maintaining the tertiary structure of cathepsin B. It is possible that in the precursor form of cathepsin B some of the histidine residues are complexed to a metal ion, possibly Zn^{2+} . Since they are both group IIB elements, Hg^{2+} can replace Zn^{2+} in many proteins, and such substitutions can induce conformational changes in the protein (see, e.g., Nelbach *et al.*, 1972). This would account both for the Hg^{2+} effects on the stability of the higher- M_r forms as well as for the finding that dialysis at acid pH, but not at neutral pH (Recklies & Mort, 1985), destabilizes the active secreted form.

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REFERENCES

- Barrett, A. J. (1973) *Biochem. J.* **131**, 809–822
 Barrett, A. J. (1976) *Anal. Biochem.* **76**, 374–376
 Barrett, A. J. & Kirschke, H. (1981) *Methods Enzymol.* **80**, 535–561
 Barrett, A. J. & Starkey, P. M. (1973) *Biochem. J.* **133**, 709–724
 Barrett, A. J., Brown, M. A. & Sayers, C. A. (1979) *Biochem. J.* **181**, 401–418
 Gordon, J. I., Sims, H. F., Lentz, S. R., Edelstein, C., Scanu, A. M. & Strauss, A. W. (1983) *J. Biol. Chem.* **258**, 4037–4044
 Graf, M., Baici, A. & Straüli, P. (1981) *Lab. Invest.* **45**, 587–596
 Greenwood, F. C., Hunter, W. H. & Glover, J. S. (1963) *Biochem. J.* **89**, 114–123
 Hasilik, A. & von Figura, K. (1984) in *Lysosomes in Biology and Pathology* (Dingle, J. T., Dean, R. T. & Sly, W., eds.), vol. 7, pp. 3–16, Elsevier, Amsterdam
 Knight, C. G. (1980) *Biochem. J.* **189**, 447–453
 Kurecki, T., Kress, L. F. & Laskowski, M. (1979) *Anal. Biochem.* **99**, 415–420
 Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
 Mort, J. S., Recklies, A. D. & Poole, A. R. (1980) *Biochim. Biophys. Acta* **614**, 134–143
 Mort, J. S., Leduc, M. & Recklies, A. D. (1981a) *Biochim. Biophys. Acta* **662**, 173–180
 Mort, J. S., Poole, A. R. & Decker, R. S. (1981b) *J. Histochem. Cytochem.* **29**, 494–500
 Mort, J. S., Leduc, M. S. & Recklies, A. D. (1983) *Biochim. Biophys. Acta* **755**, 369–375
 Mort, J. S., Recklies, A. D. & Poole, A. R. (1984) *Arthritis Rheum.* **27**, 509–515
 Mortensen, S. B., Sottrup-Jensen, L., Hansen, H. F., Petersen, T. E. & Magnusson, S. (1981) *FEBS Lett.* **135**, 295–300
 Nelbach, M. E., Pigiet, V. P., Gerhart, J. C. & Schachman, H. K. (1972) *Biochemistry* **11**, 315–327
 Poole, A. R., Recklies, A. D. & Mort, J. S. (1980) in *Proteinases and Tumor Invasion* (Straüli, P., Barrett, A. J. & Baici, A., eds.), pp. 81–95, Raven Press, New York
 Recklies, A. D. & Mort, J. S. (1982) *Clin. Chim. Acta* **123**, 127–138
 Recklies, A. D. & Mort, J. S. (1985) *Cancer Res.* **45**, 2302–2307
 Recklies, A. D., Tiltman, K. J., Stoker, T. A. M. & Poole, A. R. (1980) *Cancer Res.* **40**, 550–556
 Recklies, A. D., Mort, J. S. & Poole, A. R. (1982a) *Cancer Res.* **42**, 1026–1032
 Recklies, A. D., Poole, A. R. & Mort, J. S. (1982b) *Biochem. J.* **207**, 633–636
 Recklies, A. D., White, C., Mitchell, J. & Poole, A. R. (1985) *Cancer Res.* **45**, 2294–2301
 Takio, K., Towatari, T., Katunuma, N., Teller, D. C. & Titani, K. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3666–3670
 Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354

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