The in vitro activation of the recombinant purified human cathepsin K (EC 3.4.22.28) was examined by mutagenesis. Cathepsin K was expressed as a secreted proenzyme using baculovirus-infected SF21 insect cells. Spontaneous in vitro activation of procathepsin K occurred at pH 4 and was catalyzed by exogenous mature cathepsin K. Three intermediates were identified as recurring at pH 4 and was catalyzed by exogenous mature cathepsin K. Finally, procathepsin K has trace proteolytic activity, suggesting autocatalysis may occur in vivo.

In this report we provide the following evidence for an autocatalytic activation mechanism. First, in vitro self-activation of wild-type procathepsin K occurs spontaneously at 4°C, pH 4 and is catalyzed by mature cathepsin K. Second, unlike wild-type enzyme, the [Ser139,Ala162]procathepsin K mutant lacks the ability to self-process into a mature active enzyme, but can be processed by addition of wild-type mature cathepsin K. Significantly, the intermediates observed in this trans-processing of [Ser139,Ala162]procathepsin K are identical to those observed in the spontaneous activation of wild-type procathepsin K. Finally, procathepsin K has trace proteolytic activity, suggesting autocatalysis may occur in vivo.

EXPERIMENTAL PROCEDURES

Materials—Z-Phe-Arg-AMC was obtained from Bachem; L-cysteine-HCl was from Amresco; MES was from Calbiochem Corp.; EDTA and E64 were from Sigma; precast SDS-PAGE gels were purchased from Bio-Rad. Protein concentrations were estimated by the Bradford method using the Bio-Rad protein assay except where otherwise specified.

Cells and Virus—SF21 insect cells and wild-type baculovirus were purchased from Invitrogen. The cells were cultured in Grace’s medium (Invitrogen) supplemented with 10% bovine fetal serum (Life Technologies, Inc.), penicillin-streptomycin (Life Technologies, Inc.), L-cysteine-HCl, and amphotericin B (Boehringer Mannheim).

Construction of Wild-type and Mutant Procathepsin K Expression Vectors and Recombinant Virus—The wild-type procathepsin K expression vector and recombinant virus were constructed according to published procedures (14). The mutant expression vector was constructed by DNA site-directed mutagenesis as follows. A 1.36-kilobase NotI/HindIII fragment containing the coding region of the human cathepsin K was inserted into the unique NotI and HindIII sites of pVL1393, yielding pVL1393pK. The mutagenic linker was synthesized following a published procedure (14) and the linker was inserted into pVL1393pK by digestion with NotI and HindIII and religation. The resulting plasmid was used to transform E. coli strain SURE. The resulting mutant plasmid was designated pVL1393pKmut.

This paper is available on line at http://www-jbc.stanford.edu/jbc/13955
Autoactivation of Human Cathepsin K

SpeI fragment containing the coding region of pre-procathepsin K was subcloned into pSelect301, a modified version of pSelect (Promega) designed to contain additional restriction sites within the multiple cloning region. Single-stranded phagemid DNA was generated in Köt- infected Escherichia coli strain JM101. The following oligonucleotides were used for mutagenesis:

- 5'-ACAGCTTAAAAACCGaggggggggggAGCTGTTTCTGACGAGTTTAAAGGAGTTCCGAGG-3'.
- 5'-CATTAGTTCTTGCGGaggggggggggAGCTGTTTCTGACGAGTTTAAAGGAGTTCCGAGG-3'.

This changed Cys193 to Ser and Ser163 to Ala, respectively, creating a double mutant. Both the C193S and S163A mutations were confirmed by automated DNA sequencing (Applied Biosystems, Inc.). The mutagenized 1.36-kilobase fragment was then subcloned into the baculovirus transfer vector pVL1393, which had been digested with BamHI and XhoI, generating the plasmid pBacMut1CatK.

For construction of recombinant viruses, SF21 cells were co-transfected with purified AcNPV linear DNA (Pharbiogen) and pBacMut1CatK using the method described to generate recombinant virus (vBacCatK) for native cathepsin K (14).

Expression of Wild-type or Mutant Cathepsin K in Recombinant Virus-infected Cells—SF21 cells were infected with four plaque-forming units of either wild-type vBacCatK or mutant vBacMut1CatK recombinant virus/cell at 27 °C in serum-containing medium. Twenty-four hours after infection, the cells were pelleted, resuspended in a serum-free medium, and incubated for an additional 72 h. Western blot analyses were performed on aliquots of the medium as described previously elsewhere (14).

Purification of Wild-type and Mutant Procathepsin K—Baculovirus conditioned medium (10 liters) containing wild-type or [Ser139,Ala163]procathepsin K at pH 6.5 was loaded onto a 100-mL Sepharose Fast Flow column (Pharmacia XK50, 5 × 5 cm) pre-equilibrated with 20 mM sodium phosphate, pH 6.9 (buffer A). The column was washed to base line with 10 column volumes of buffer A. Bound material was eluted with a series of NaCl steps (0.35 M, 0.5 M, and 1 M) in buffer A. Fractions were analyzed by SDS-PAGE and Western blot. Procathepsin K eluted in the 0.5 M NaCl fraction and was analyzed by N-terminal sequencing and MALDI-MS (see below). Purified wild-type and mutant procathepsin K were concentrated to between 1 and 2.5 mg/ml using an Omegacell (Filtron Technology Corp.) with a 10-kDa molecular mass cut-off membrane.

E64 Treatment of Procathepsin K—E64 (70 mM) was added to 0.2 ml of procathepsin K (2.5 mg/ml, 14 mM) in 20 mM sodium phosphate, 0.5 M NaCl at pH 6.8. After 15 min at 25 °C, the mixture was dialyzed against a series of 20 mM sodium phosphate, 0.5 M NaCl at pH 6.8. Purified mature procathepsin K was obtained as described above (4), dialyzed against 20 mM sodium phosphate, 0.5 M NaCl at pH 4 until the enzyme was diluted to 1 mg/ml with dialysis buffer prior to activation.

Activation of Wild-type Procathepsin K—Concentrated procathepsin K was incubated in activation buffer consisting of 0.2 mM sodium acetate, 20 mM l-cysteine adjusted to pH 4.0 at 4 °C on a rotary mixer. Where indicated, 1% (mass/mass) mature cathepsin K was added as seed after pH adjustment. The mature cathepsin K that was used as seed was initially obtained from 60 °C induced activation of procathepsin K, a gift from M. Bossard (SmithKline Beecham Pharmaceuticals) (14). Subsequently, "seed" was made by the 4 °C activation procedure described here. The extent of activation and processing were assessed hourly by measuring hydrolytic activity as described below and by SDS-PAGE, respectively. SDS-PAGE analysis was carried out using 15% Tris-glycine gel-type acrylamide gels (Bio-Rad). When the specific activity stopped increasing (~15–25 μmol/min/mg), the reaction was stopped by the addition of E64 or by snap-freezing in a bath of dry ice in acetone.

Cathepsin K Activity Assay—Cathepsin K activity was determined using a fluorogenic substrate in a microtiter plate format. The reactions consisted of 0.04–1 μM of mature cathepsin K, 20 μM Z-Phe-Arg-AMC in 100 mM sodium acetate, 20 mM l-cysteine, 5 mM EDTA at pH 5.5. Reactions were initiated by the addition of substrate-containing assay buffer to the enzyme solution. The assay followed Z-Phe-Arg-AMC hydrolysis, which was measured using a Dynatech microtiter plate reader with excitation at 365 nm and fluorescence emission at 530 nm. Under these conditions, the sensitivity of the assay was typically 4.7 × 10⁵ μmol of AMC/fluorescence unit. Procathepsin K was assayed under the same conditions in the same buffer, but also included 100 mM MES and 100 mM HEPES so that the pH could be varied between 4 and 7.

Activation of [Ser139,Ala163]procathepsin K by Wild-type Cathepsin K or Pepsin—[Ser139,Ala163]procathepsin K was concentrated to 1 mg/ml and was used in three 0.1 ml reactions. All reactions contained activation buffer (0.2 mM sodium acetate, 20 mM l-cysteine at pH 4) and were run on a rotary mixer at 4 °C. No mature cathepsin K was added to the first reaction, whereas the second reaction included 1% wild-type mature cathepsin K and 1 mM activation buffer (0.2 M sodium acetate, 20 mM l-cysteine, and 1 mM E64). The third reaction consisted of a mixture of 50 μl of [Ser139,Ala163]procathepsin K (1 mg/ml), 50 μl of wild-type cathepsin K (1 mg/ml) and 1 mM mature cathepsin K. One-microliter and 10-μl samples were taken at 1-h intervals for activity and SDS-PAGE analysis, respectively. Proteinolytic cleavage of the propeptide from [Ser139,Ala163]procathepsin K was also accomplished using a modification of the published procedure utilizing pepsin (13). Briefly, [Ser139,Ala163]procathepsin K (1 mg/ml) was incubated with pepsin (20 μg/ml) in 0.2 mM sodium acetate, 5 mM EDTA, 1 mM l-cysteine, and 5 mM dithiothreitol at 40 °C for 45 min.

N-terminal Sequence Analysis—Sequence analysis was performed on a Beckman LC-3490 TriCrt gas-phase protein sequencer equipped with a Beckman 126/166 system for on-line phenylthiodyantoin analysis (Beckman Instruments, Inc., Fullerton, CA). Data was acquired using System Gold chromatography software. Samples were electroblotted onto polyvinylidene difluoride type supports (Problott), and standard Beckman optimized polyvinylidene difluoride sequencing cycles were used.

MALDI-Mass Spectrometry—MALDI-MS data were obtained on a Voyager RP laser desorption time-of-flight mass spectrometer (PerSeptive Biosystems, Framingham, MA). Protein samples were prepared for analysis by diluting analyte 1:5 with 3,5-dimethoxy-4-hydroxycinnamic acid (10 mg/ml in 2:1 0.1% trifluoroacetic acid/acetonitrile) for a final concentration of 1–10 pmol/μl. Bovine β-lactoglobulin A (Sigma) was included as an internal calibrant (MH+ 18364 Da). Desorption/ionization was accomplished using photon irradiation from a 357-nm pulsed nitrogen laser and 25-kV accelerating energy. Spectra were averaged over ~100 laser scans. Calibrations were carried out using a customized version of ITOG Pro (WaveMetrics, Inc., Lake Oswego, OR) on a Macintosh personal computer.

Electrospray Mass Spectrometry—Both the apo-cathepsin K (32 μM) and the E64-cathepsin K product (39 μM) were supplied in 4 mM MES buffer also containing 10 mM NaCl and 0.4 mM l-cysteine at a pH of 6.06. These solutions were diluted with 10 μl of 88:8:4 MeOH:water:formic acid, followed by 80 μl of a 1:10:0.2% solution of MeOH:water:formic acid, resulting in a final cathepsin K concentration of ~1.5 μM and a final volume of 100 μl. Two microliters of this solution was loaded into a pulled glass capillary for ultravalow volume electrospray (nano-spray) mass spectrometry. Mass spectra were recorded on a PE-Sciex API III (PE-Sciex Instruments, Concord, Ontario, Canada) by repetitively scanning the m/z range of 1050–1650 with a step size of 0.05 and a dwell of 1 ms, and averaging 5–10 min of accumulated data. Results for all charge states corresponding to [M + nH]+ and [M + (n−1)H + Na]+ between 15+ and 22+ were averaged together, producing a typical 95% confidence interval of ± 1.5 Da.

RESULTS

Expression of Wild-type and Mutant Cathepsin K—Infection of SF21 cells with either recombinant wild-type cathepsin K (vBacCatK) (14) or mutant (vBacMut1CatK) virus resulted in the production of identically sized proenzyme protein bands of approximately 35,000 Da as determined by Western blot analysis using a previously generated antiserum (14). As was determined for the wild-type proenzyme (14), most of the mutant protein was also secreted into the medium with a smaller percentage of the expressed protein retained in the cell pellets (data not shown).

Purification of Wild-type Procathepsin K—The secreted proenzyme was purified to greater than 85% homogeneity, as described under "Experimental Procedures" (Fig. 1, lane f). The N-terminal sequence of this purified proenzyme was LYPPEELLD, which corresponded exactly with the N-terminal signal sequence occurred after Ala25. No secondary sequence was observed. Analysis the proenzyme by MALDI-MS yielded a mass of 36366 Da, which exceeded by 3% the theoretical mass, 35300.5 Da, calculated from the amino acid sequence. Edman sequencing data gave a very low yield for Asn103, the potential N-linked glycosylation site in the propeptide domain, consist-
ent with glycosylation of this residue. Absence of glycosylation at Asn\(^{161}\) in the mature form of the protein was indicated by MS (see below). Several attempts to further purify the enzyme by a variety of ion exchange and size exclusion chromatography resulted in poor recovery of procathepsin K.

**Activation of Wild-type Procathepsin K: Catalysis by Mature Cathepsin K**—The time courses of activation reactions containing zero (open circles) and 1% mature cathepsin K (filled circles) were determined (Fig. 2). Procathepsin K activated without mature cathepsin K at 4 °C, pH 4. The reaction containing 1% mature cathepsin K had no apparent lag and required a shorter time to obtain full enzymatic activity. SDS-PAGE analysis of the proteolytic conversion to mature enzyme in the absence of seed (Fig. 1) indicated the accumulation of intermediates (25–35 kDa) and propeptide fragments (6.5–12 kDa). Full activity, typically 15–25 \(\mu\)mol/min/mg, was determined at the end of the activation when an accurate protein concentration was determined. The reaction of 1% mature cathepsin K with procathepsin K that had been pretreated with E64 did not produce any activated mature cathepsin K (Fig. 2, open triangles), and <5% propeptide degradation was observed by SDS-PAGE (data not shown).

Early cleavage sites were determined by N-terminal sequencing of the protein bands isolated from a blot of a SDS-PAGE gel from a subsequent activation reaction using identical conditions to the one described above. The two largest intermediates resulted from cleavages which occurred immediately after Glu19 and Ser98, respectively. The third and smallest intermediate was derived from cleavage after Glu110.

**Characterization of Mature Cathepsin K—N-terminal sequence analysis** indicated that mature cathepsin K was composed of a mixture of enzymes with N termini of Gly\(^{113}\)RAP-, Arg\(^{114}\)APD- and Ala\(^{115}\)PD-, with varying ratios depending on the preparation. These results were consistently observed in four individual activation reactions. MALDI-MS of a pool of activated enzyme that exhibited a 1:1 ratio of Gly\(^{113}\) and Arg\(^{114}\) N termini yielded a \(M_0\), of 22,896, for the unresolved protein components. Electrospray mass spectrometry (ESMS) for a preparation in which Arg\(^{114}\) and Ala\(^{115}\) dominated as the N termini yielded molecular masses of 23,646.7 ± 1.5 Da and 23,590.1 ± 1.5 Da for the two protein species (calculated = 23,645.7 and 23,489.5, respectively, for the protein ending with Met\(^{199}\)). The electrospray data are consistent with six of the eight Cys residues being in disulfide linkage. Both the electrospray and MALDI-MS data indicate that the mature protein is not glycosylated.

ESMS of the complex formed by reaction of a preparation of activated, mature cathepsin K (Arg\(^{114}\) major and Ala\(^{115}\), minor N termini) with E64 yielded a determined \(M_0\) for the major component of 24,001.8 (calculated = 24,003.0). This mass is consistent with addition of the entire inhibitor into the protein via ring opening of the epoxide by the thiol of the active-site Cys residue.

** Demonstration of Autoprocessing: Activation of [Ser\(^{139}\),Ala\(^{163}\)]Procathepsin K—[Ser\(^{139}\),Ala\(^{163}\)]Procathepsin K was purified to greater than 85% homogeneity as described under “Experimental Procedures” (Fig. 3A, lane 1). The N-terminal sequence of proenzyme indicated that cleavage of the secretion signal sequence occurred after the Ala\(^{15}\) residue, consistent with that observed for wild-type protein. MALDI-MS analysis indicated the mutant procathepsin K contained approximately 2% glycosylation by mass.

The studies designed to distinguish between autocatalytic activation and activation catalyzed by a protease other than cathepsin K consisted of three different experiments. The first two experiments contained mutant procathepsin K, which was incubated in pH 4 buffer either in the absence or presence of one percent wild-type mature cathepsin K. The third experiment was done to observe full processing of the mutant procathepsin K and consisted of a 1:1 mixture of wild-type procathepsin K and mutant procathepsin K with 1% wild-type mature cathepsin K.

The result of the first experiment is shown in Fig. 3A. Mutant procathepsin K did not process to mature cathepsin K in the absence of wild-type mature cathepsin K seed, but was partially processed in the presence of a catalytic amount of wild-type mature cathepsin K as shown in Fig. 3B. There was no increase in activity (Fig. 4, open circles), but rather the activity of the seed decreased 15-fold during the first 2 h of the reaction. Under the reaction conditions the initially formed propeptide fragments were stable, which allowed for their characterization (see below). The third experiment consisting of a 1:1 mixture of mutant and wild-type cathepsin K, and one percent mature cathepsin K seed resulted in complete conversion to mature cathepsin K as shown in Fig. 3C. The specific activity of the resultant mature enzyme was 11 \(\mu\)mol/min/mg, which was about one half that observed for the wild-type enzyme used in this experiment (25 \(\mu\)mol/min/mg). The mutant enzyme was also processed to mature enzyme by pepsin (data not shown).

The intermediates and propeptide fragments (Fig. 3B) were

![Fig. 1. SDS-PAGE analysis of procathepsin K activation at 4 °C, catalyzed by 0% mature cathepsin K. Lanes M, molecular size markers; lane 1, purified procathepsin K; lane 2, procathepsin K incubated at pH 4 in buffer at time = 1 h. Lanes 3–5 are procathepsin K incubated at 4 °C in buffer at times = 3, 5.75, and 8 h, respectively. Samples from the time course correspond to the open circles in Fig. 2.](Image 93x632 to 263x729)

**Fig. 1.** SDS-PAGE analysis of procathepsin K activation at 4 °C, catalyzed by 0% mature cathepsin K. Lanes M, molecular size markers; lane 1, purified procathepsin K; lane 2, procathepsin K incubated at pH 4 in buffer at time = 1 h. Lanes 3–5 are procathepsin K incubated at 4 °C in buffer at times = 3, 5.75, and 8 h, respectively. Samples from the time course correspond to the open circles in Fig. 2.

![Fig. 2. Activity of wild-type mature cathepsin K during activation.](Image 323x546 to 549x729)

**Fig. 2.** Activity of wild-type mature cathepsin K during activation. Samples of purified procathepsin K were incubated at 4 °C, in the presence of 0.2 m sodium acetate, 20 m M \(\epsilon\)-cysteine at pH 4.0 with no added mature cathepsin K (○), 1% mature cathepsin K (●), or procathepsin K that was preincubated with E64, dialyzed, and activated in the presence of 1% mature cathepsin K (□). The proteolytic activity was evaluated using the Z-Phe-Arg-AMC assay as described under “Experimental Procedures” and is expressed here in fluorescence units/minute (FU/min). S.A., specific activity.
Autoactivation of Human Cathepsin K

Activity of Procathepsin K—Activation of procathepsin K in the absence of mature cathepsin K suggests that the proenzyme has proteolytic activity. Procathepsin K isolated from baculovirus medium, as described under “Experimental Procedures,” was assayed for activity at pH 3.5–7.0 and was found to be able to hydrolyze Z-Phe-Arg-AMC having a maximal activity at pH 4. The specific activity, based upon estimates from initial velocity measurements, was 0.007–0.014 μmol/min/mg, approximately 2000-fold lower than mature cathepsin K activity.

**DISCUSSION**

Bossard et al. (14) reported a successful small scale (80 μg/ml) activation of semi-pure procathepsin K under two conditions: (a) brief exposure to elevated temperatures or (b) incubation at 4 °C in the presence of a catalytic amount of preactivated mature cathepsin K. Their results suggested an autocatalytic mechanism for cathepsin K activation. Evidence against an autocatalysis mechanism was provided by a study by Brömmel et al. (13), in which no autoprocessing was observed and the activation could only be accomplished using pepsin (13). To examine the mechanism of cathepsin K activation, we examined the putative catalytic effect of preactivated mature cathepsin K.

In agreement with Bossard’s observation, procathepsin K could be activated at 4 °C and pH 4 in the absence of preactivated mature cathepsin K, and the activation was catalyzed by...
the addition of 1% mature cathepsin K. These results differ from those of Brömme et al.; however, there were several differences in the procedures employed. We activated enzyme that was greater than 85% pure and was at a much higher concentration in the activation reaction. Brömme et al. attempted to activate procathepsin K without purification in the baculovirus crude cell lysate. Additionally, we observed that the spontaneous activation in the absence of 1% mature cathepsin K could be inhibited by pretreating the procathepsin K with the cysteine protease inhibitor, E64, thereby demonstrating that activation was due to a cysteine protease.

Mature cathepsin K produced under the conditions described under “Experimental Procedures” contained a ragged N terminus, composed of a mixture of NH2-G11RAPHD-, NH2-R114APD- and NH2-A115PD-. The site for N-terminal cleavage for mature cathepsin K was predicted to be after NH2-A115 based upon a sequence alignment of cathepsin S and L (14). The one or two extra N-terminal amino acids observed in the activation of cathepsin K were consistent with the N-terminal extension of several amino acids of the propeptide, which were observed in the autoprocessing of cathepsin B (16) and cathepsin S (17).

The MALDI-MS of wild-type procathepsin K indicated that approximately 3% of the mass was due to glycosylation at one or both of two potential glycosylation sites in the procathepsin K sequence, one of which was located in the propeptide domain (14). To determine if the mature enzyme was glycosylated, we analyzed mature cathepsin K and E64-inhibited mature cathepsin K by MALDI-MS and ESM, which indicated the absence of glycosylation on the mature cathepsin K. These data, together with the Edman sequencing results, indicate that all of the carbohydrate is located on Asn103 in the propeptide domain, although our results do not rule out the possibility of O-glycosylation elsewhere in the propeptide domain. Our results contrast with those of Brömme et al. (13), who observed no glycosylation on procathepsin K. The discrepancy may be a result of different expression conditions.

In vitro activation of procathepsin K in the absence of procathepsin K is autoactivated mature cathepsin K could be the result of limited proteolytic activity of procathepsin K under the reaction conditions, i.e. autoactivation, or by the activity of a contaminating baculovirus protease as suggested by Brömme. Autocatalytic processing mechanisms have been verified for propapain (15) and procathepsin B (16) through experiments that showed that an active site cysteine to serine mutation eliminated the proenzyme’s ability to autoactivate. Analogously, we constructed a mutant in which the active site cysteine of procathepsin K, Cys139, was mutated to serine. We reasoned that such a mutant should lack the ability to autoprocess, and any processing observed under the activation conditions in the absence of mature cathepsin K would have to be due to a contaminating protease. The additional mutation at residue 163, serine to alanine, was incorporated to remove the potential glycosylation site in the mature enzyme.

The [Ser139-Ala163]procathepsin K did not process to the mature form at pH 4, but did undergo limited proteolytic cleavage when 1% wild-type mature cathepsin K was included in the reaction mixture. The peptide intermediates generated in this reaction match exactly the peptide intermediates observed in the activation of wild-type procathepsin K (Fig. 5). These results clearly demonstrated that mature cathepsin K could proteolytically process mutant procathepsin K and gives strong evidence in support of a trans-autoactivation process.

Additionally, the intermediates formed during the reaction had the ability to inhibit the processing of the mutant enzyme by decreasing the activity of the wild-type seed 15-fold during the first 2 h of the reaction (data not shown). Inhibition of cysteine proteases by their respective propeptides has been previously observed for cathepsin L (18), papain and papaya protease IV (19), and cathepsin B (20). Evidently, the inhibitory intermediates formed during activation of cathepsin K are proteolytically degraded during the activation of wild-type enzyme due to the concomitant generation of molar equivalents of mature, active enzyme. An active enzyme is not generated in the processing of [Ser139-Ala163]procathepsin K; hence, the reaction stops.

To see if the mutant could be completely processed to mature cathepsin K, we activated the mutant in the presence of one equivalent of wild-type pro-cathepsin K. Therefore, active enzyme would be formed in concert with the inhibitory intermediates. This reaction successfully converted the mixture of procathepsin K to mature cathepsin in a nearly quantitative conversion (Fig. 3C). Since mature cathepsin K is a relatively nonspecific protease, which would be expected to proteolyze an unfolded mutant protein, and extensive proteolysis of mutant procathepsin K was not observed, it seems likely that the mutant procathepsin K is folded properly.

The activation of wild-type procathepsin K consists of initial cleavage at three preferred sites, namely residues Glu110, Ser139, and Glu110 as illustrated in Fig. 5. An additional cleavage site is observed in the activation of [Ser139,Ala163]procathepsin K after Ala74. Inspection of the preferred cleavage sites revealed few trends. Cleavage occurred one or two amino acids after each and every proline residue in the propeptide, and at an additional cleavage site, after Ala74, where proline was not present. This led us to postulate that even though cathepsin K has a somewhat broad substrate specificity (13, 14), it may prefer sites that possess secondary structural features imparted by a nearby proline residue. A preference for sites containing proline would be conducive for activity with one of its natural substrates, type I collagen, which is proline-rich.

The unique site not containing proline lies within a putative consensus motif, Gly-X-Asn-X-Phe-X-Asp, (Fig. 5) found in papain, caracain, and papaya protease IV, which was postulated to be the site of initial cleavage in the pH-dependent autoactivation of papain (21). The protonation of the aspartic acid residue was postulated to be a trigger for activation. Although the authors could not identify the site of cleavage they speculated it to be between X and Asp, since this would place the preferred phenylalanine residue in the S2 pocket. In the case of cathepsin K, leucine is a preferred residue for P2 (12, 14), which predicts cleavage after Ala74, which is consistent with the observed cleavage within this motif in cathepsin K. More information about the structures around the proline residues and the putative consensus motif and their mechanistic implications in cathepsin K autoactivation await the determination of the three dimensional x-ray structure of procathepsin K.

If autoprocessing were to occur in vivo, procathepsin K would have to be able to initiate its activation without the aid of mature cathepsin K under the conditions in the bone resorption pit. The spontaneous activation of procathepsin K (Figs. 1 and 2), and the proteolytic cleavage of Z-Phe-Arg-AMC by procathepsin K demonstrates that such a process is possible.

In summary, the in vitro activation of cathepsin K is autocatalytic and does not require a different protease. We postulate a similar mechanism is occurring in vivo. Once procathepsin K is secreted into the resorption pit, it undergoes a conformational change induced by the lower pH, which unmask the active site and makes the propeptide more vulnerable to endoproteolysis. The ensuing activation of procathepsin K in the resorption pit would be accelerated by the action of catalytic amounts of the newly formed mature cathepsin K.
Once the propeptide is fragmented at the preferred Pro-X-X sites, it is degraded further by endoproteolysis at less preferred sites, resulting in fully active mature cathepsin K.

Acknowledgments—We thank Dr. George I. Glover, Dr. Allan Shatzman and Dr. George P. Livis for support of this work; Stephannie Van Horn for sequencing of the mutant cDNA; Jim Forwald for the pselect 301 vector; and Dr. Mary Bossard for heat-activated mature cathepsin K.

REFERENCES
1. Baron, R. (1989) Anat. Rec. 224, 317–324
2. Delaisse, J. M., Eeckhout, Y., and Vaes, G. (1984) Biochem. Biophys. Res. Commun. 125, 441–447
3. Delaisse, J. M., Eeckhout, Y., and Vaes, G. (1989) Biochem. J. 192, 365–368
4. Lerner, U. H., and Grubb, A. (1992) J. Bone Miner. Res. 7, 433–439
5. Delaisse, J. M., Boyde, A., Macconnachie, E., Ali, N. N., Sear, C. H. J., Eeckhout, Y., Vaes, G., and Jones, S. J. (1987) Bone 8, 305–313
6. Hill, P. A., Buttle, D. J., Jones, S. J., Boyde, A., Murata, M., Reynolds, J. J., and Metke, M. C. (1994) J. Cell. Biochem. 56, 118–130
7. Li, Y. P., Alexander, M. B., Wucherpfennig, A. L., Chen, W., Yelick, P., and Stashenko, P. (1994) Mol. Biol. Cell 5, 335a
8. Inaoka, T., Bilbe, G., Ishibashi, O., Teruza, K., Kumegawa, M., and Kokubo, T. (1995) Biochem. Biophys. Res. Commun. 206, 89–96
9. Teruza, K., Teruza, Y., Maejima, A., Sato, T., Nemoto, K., Kamioka, H., Hakeda, Y., and Kumegawa, M. (1994) J. Biol. Chem. 269, 1106–1109
10. Brümme, D., and Okamoto, K. (1995) Biol. Chem. Hoppe-Seyler 376, 379–384
11. Drake, F. H., Dodds, R. A., James, I. E., Connor, J. R., Debouck, C., Richardson, S., Lee-Ryłaczewski, E., Coleman, L., Rieman, D., Barthlow, R., Hastings, G., and Gowen, M. (1996) J. Biol. Chem. 271, 12511–12516
12. Shi, G.-P., Chapman, H. A., Bhaig, S. M., Deleeuw, C., Reddy, V. Y., and Weiss, S. J. (1995) FEBS Lett. 357, 129–134
13. Brümme, D., Okamoto, K., Wang, B. B., and Biroc, S. (1996) J. Biol. Chem. 271, 2126–2132
14. Bossard, M. J., Tomaszek, T. A., Thompson, S. K., Amegadzie, B. Y., Hanning, C. R., Jones, C., Kurylja, J. T., McNulty, D. E., Drake, F. H., Gowen, M., and Levy, M. A. (1996) J. Biol. Chem. 271, 12517–12524
15. Vernet, T., Khouri, H. E., Lafhamme, P., Tessier, D. C., Musil, R., Gour-Salin, B. J., Storer, A. C., and Thomas, D. Y. (1991) J. Biol. Chem. 266, 21451–21457
16. Rowan, A. D., Mason, P., Mach, L., and Mott, J. S. (1992) J. Biol. Chem. 267, 15993–15999
17. Brümme, D., Bonneau, P. R., Lachance, P., Wiederanders, B., Kirschke, H., Peters, C., Thomas, D. Y., Storer, A. C., and Vernet, T. (1993) J. Biol. Chem. 268, 4832–4838
18. Carmona, E., Dufour, E., Plouffe, C., Takebe, S., Mason, P., Mott, J. S., and Menard, R. (1996) Biochemistry 35, 8149–8157
19. Taylor, M. A. J., Baker, K. C., Briggs, G. S., Connerton, I. F., Cummings, N. J., Pratt, K. A., Revell, D. F., Freedman, R. G., and Goodenough, P. W. (1995) Protein Eng. 8, 59–62
20. Fox, T., de Miguel, E., Mott, J. S., and Storer, A. C. (1992) Biochemistry 31, 12571–12576
21. Vernet, T., Berti, P. J., de Montigny, C., Musil, R., Tessier, D. C., Ménard, R., Magny, M.-C., Storer, A. C., and Thomas, D. Y. (1995) J. Biol. Chem. 270, 10838–10846
Autocatalytic Activation of Human Cathepsin K
Michael S. McQueney, Bernard Y. Amegadzie, Karla D'Alessio, Charles R. Hanning, Megan M. McLaughlin, Dean McNulty, Steven A. Carr, Carl Ijames, Jeff Kurdyla and Christopher S. Jones

J. Biol. Chem. 1997, 272:13955-13960.
doi: 10.1074/jbc.272.21.13955

Access the most updated version of this article at http://www.jbc.org/content/272/21/13955

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 21 references, 8 of which can be accessed free at http://www.jbc.org/content/272/21/13955.full.html#ref-list-1