the extent that a comparison of the lysed and intact cell systems is valid, there are several possible explanations for this discrepancy. It is possible that the level of 5-HPETE generated is sufficient to deplete glutathione, the principal reducing agent for peroxides. This is unlikely, however, because the intracellular concentration of glutathione is about 5 mM (Egan & Gale, 1985) and the total intracellular concentration of 5-lipoxygenase products from endogenous arachidonic acid reaches only about 0.08 mM (Sun & McGuire, 1984; calculated assuming 5×10^8 cells/g wet weight). It is possible that the 5-HPETE that is formed in intact cells is segregated from the peroxidases so that the rapid rate of reduction of 5-HPETE in vitro is not indicative of the in vivo rate. It is also possible that the intrinsic partitioning of 5-HPETE on the enzyme between dissociation and conversion to LTA_4 is different for 5-lipoxygenase in its native intracellular state and the relative inefficiency observed in vitro is an artifact. We cannot at present provide evidence for either of these possibilities.

Registry No. LTA_4, 72059-45-1; 5-HPETE, 70608-72-9; 5-LO, 80619-02-9; arachidonic acid, 506-32-1.

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Purification and Amino Acid Sequence of Chicken Liver Cathepsin L

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ABSTRACT: Cathepsin L was purified from chicken liver lysosomes by a two-step procedure. Cathepsin L exhibited a single band of M_r 27 000 by sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions, presented a high affinity for the substrate Z-Phe-Arg-NMec, was very unstable at neutral pH, and was inhibited by Z-Phe-Phe-CHN_2. The complete amino acid sequence of cathepsin L has been determined and consists of 215 residues. The sequence was deduced from analysis of peptides generated by enzymatic digestions and by chemical cleavage at methionyl bonds. Comparison of the amino acid sequence of cathepsin L with those of rat liver cathepsins B and H and papain demonstrates a striking homology among their primary structures.

Lysosomal proteinases play an important role in intracellular protein catabolism (Barrett & Kirschke, 1981). Among lysosomal proteinases, cathepsin L (EC 3.4.22.15), because of its high proteolytic activity, probably makes a major contribution to intralysosomal proteolysis. Cathepsin L has been purified from rat (Kirschke, 1977), rabbit (Okitani et al., 1980; Mason et al., 1984), chicken (Wada & Tanabe, 1986), and human (Pagano & Engler, 1982; Mason et al., 1985) liver and exhibits general characteristics including high affinity for the substrate Z-Phe-Arg-NMec,[1] inhibition by Z-Phe-Phe-CHN_2,

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instability at neutral pH, and a molecular weight in the range 25 000–29 000.

Although primary structures of cathepsins B and H are known (Takio et al., 1983; Ritonja et al., 1985), only N-terminal amino acid sequences have been reported for cathepsin L (Turk et al., 1985; Mason et al., 1986; Wada & Tanabe, 1986). Rat, human, and chicken cathepsin L, when purified from tissue homogenates, were also shown to consist of both a heavy chain (M_r 24 000–25 000) and a light chain (M_r 4000–5000) (Mason et al., 1985; Kirschke & Barrett, 1985; Wada & Tanabe, 1986). However, it is not clear whether this results from limited proteolysis of cathepsin L during the purification procedure or whether cathepsin L does exist as a double-chain form within lysosomes.

This paper describes a two-step procedure for the purification of cathepsin L from chicken liver lysosomes. We also present for the first time the complete amino acid sequence of the cathepsin L, which is compared with those of other cysteine proteinases, and we show that cathepsin L exists as a single-chain form when directly purified from lysosomes.

Experimental Procedures

Materials. TPCK-trypsin was from Boeringher (Mannheim, FRG); staphylococcal V-8 protease was from Sigma (St Louis, MO); yeast carboxypeptidase Y was from Carlbiotech (Coepenhagen, Denmark); sequanal-grade TFA and PITC were from Pierce (Rockford, IL); Z-Phe-Arg-NMec, Z-Arg-Arg-NMec, and Arg-NMec were purchased from Bachem AG (Budendorf, Switzerland); Z-Phe-Phe-CHN_2 was kindly provided by Dr. E. Shaw (Basel, Switzerland). All other reagents were of the purest grade commercially available. The Waters (Milford, MA) HPLC equipment consisted of a 720 system controller, two 6000 A pumps, and a 440 absorbance detector. ODS-2 columns (SFCC, Gagny, France) with particle diameters of 10 μm (3.9 × 300 mm), 5 μm (3.9 × 150 mm) and 3 μm (3.9 × 150 mm) were used. Purification of cathepsin L was achieved by FPLC with a Mono-S column (Pharmacia, Uppsala, Sweden).

Purification of Cathepsin L from Chicken Liver. Chicken livers were obtained from a local slaughterhouse, and lysosomes were prepared at 0–4 °C according to Lardeux et al. (1983). Livers were homogenized in 10 volumes of 10 mM sodium phosphate buffer, pH 7.4, containing 0.25 M sucrose and 1 mM EDTA. The liver homogenate was centrifuged 10 min at 1000*g* and then at 4000*g* for another 10 min. The resulting supernatant was centrifuged at 20000*g* for 15 min and the crude lysosomal pellet resuspended in 0.6 volume of 30 mM sodium phosphate buffer, pH 5.8, and frozen (−20 °C). The frozen homogenate was thawed and the lysosomal extract recovered after 20-min centrifugation at 100000*g*. Only two further steps were then sufficient to purify cathepsin L from the extract.

The lysosomal extract was adjusted to pH 4.2 with 1 M sodium acetate buffer, pH 4.2, and centrifuged at 15000*g* for 5 min. The pellet containing cathepsin L was solubilized in 1 volume of 20 mM sodium pyrophosphate buffer, pH 6, cleared by centrifugation at 15000*g* for 5 min, and directly applied to a Mono-S column preequilibrated in the same buffer. The column was eluted at a flow rate of 0.8 mL·min^{-1} with a linear gradient of NaCl (0–0.5 M). Fractions (0.8 mL) were collected and tested for enzymatic activities and absorbance at 280 nm. Cathepsin L was eluted at 0.45 M NaCl. We purified 4 mg of cathepsin L from 6 kg of chicken liver. The purified enzyme was dialyzed against water for 48 h at 4 °C and lyophilized.

Reduction and Alkylation. Cathepsin L (3.5 mg) was dissolved in 0.6 mL of 0.36 M Tris, pH 8.6, containing 5 mM EDTA and 3 M guanidine hydrochloride, reduced with 10 mM 2-mercaptoethanol, and then alkylated with 10 mM iodoacetic acid. Excess reagents were removed by dialysis against water, and alkylated cathepsin L (SCM-cathepsin L) was lyophilized.

Amino Acid Analysis. Samples (200 pmol) of SCM-cathepsin L were hydrolyzed with 5.7 N HCl in evacuated sealed tubes for 24, 48, and 96 h at 110 °C. Peptides were hydrolyzed for only 24 h. Amino acids were derivatized with PITC, and PTC amino acids analyses were performed by RP-HPLC on a 3-μm ODS-2 column (Bidlingmeyer et al., 1984). Carboxypeptidase Y digests were also analyzed as described above after vacuum drying.

CNBr Treatment. SCM-cathepsin L (2 nmol) was dissolved at room temperature in 200 μL of 70% formic acid, and 0.6 mg of CNBr was added. Twenty-four hours later, the reaction mixture was dried under vacuum. Homoserine was then transformed into homoserine lactone with 200 μL of anhydrous TFA at 20 °C for 1 h.

Enzymatic Hydrolysis. SCM-cathepsin L (3 nmol) was digested in 0.2 mL of 0.1 M ammonium bicarbonate, pH 7.8, and 3 μL of trypsin (2 mg/mL in 1 mM HCl) at 37 °C for 10 h. Digestion of SCM-cathepsin L (3 nmol) with *Staphylococcus aureus* V-8 protease was performed at glytamyl (and occasionally aspartyl) residues in 0.2 mL of 50 mM NH_4HCO_3, pH 7.8, and 20 μL of staphylococcal protease (1 mg/mL in H_2O) for 15 h at 37 °C. Digestion at glutamyl and aspartyl residues was carried out in 0.2 mL of 50 mM KH_2PO_4, pH 7.8, and with 20 μL of staphylococcal protease (1 mg/mL in H_2O), also for 15 h at 37 °C. SCM-cathepsin L (0.1 nmol) and peptides from the CNBr digest (0.1 nmol) were also digested in 30 μL of 50 mM pyridine–acetate, pH 5.5, and 2 μL of carboxypeptidase Y (0.2 mg/mL in 50 mM pyridine–acetate, pH 5.5) for 2 h at 25 °C.

Fractionation of Peptide Mixtures. All separations were achieved by RP-HPLC on a 10-μm ODS-2 column. Most fractionations were carried out with system I: (A) 0.115% TFA; (B) 0.1% TFA in 60% CH_3CN. Some purifications were achieved with system II: (A) 25 mM $NH_4CH_3CO_2$, pH 6.0; (B) 40% 50 mM $NH_4CH_3CO_2$, pH 6.0, in 60% CH_3CN. All separations were carried out at 40 °C with a flow rate of 1 mL·min^{-1}. The absorbance was recorded at 214 nm. The manually collected fractions were dried under vacuum.

Edman Degradations. Large peptides were analyzed with Applied Biosystems gas-phase sequencer 470A. The manual "partitioning method for small peptides" described by Tarr (1982) was used. For the identification of PTH amino acids, the procedure of S. Cohen (personal communication) was employed with the following system: (A) 83% sodium acetate (35 mM), pH 5, and 17% acetonitrile; (B) 60% 2-propanol. A 5-μm ODS-2 column was used at 41 °C. Elution was carried out successively for 1 min with 100% A, for 3.5 min with a hyperbolic gradient (Waters System, curve 2) to 66% A and 34% B, and followed by 4 min at 66% A and 34% B, and finally, the column was reequilibrated in 100% A. The

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; RP-HPLC, reverse-phase high-performance liquid chromatography; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PITC, phenyl isothiocyanate; TFA, trifluoroacetic acid; SCM, *S*-carboxymethyl; TPCK-trypsin, trypsin treated with L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; Z, benzyloxycarbonyl; NMec, 4-methyl-7-coumarylamide; FPLC, fast protein liquid chromatography; PTC, phenylthiocarbamyl; PTH, phenylthiohydantoin; E-64, L-1-[(*trans*-epoxysuccinyl)leucylamido]-4-guanidinobutane.

Table I: Purification of Chicken Liver Cathepsin L[a]

purification step	total protein (mg)	activity (units)	sp act. (units mg of protein)	purification (x-fold)	yield (%)
lysosomal extract	405	1860	4.6	1	100
after precipitation, pH 4.2	22	1330	60.4	13	71
Mono-S	0.08	200	2500	545	11

[a] One unit of activity releases 1 μmol of (aminomethyl)coumarin/min.

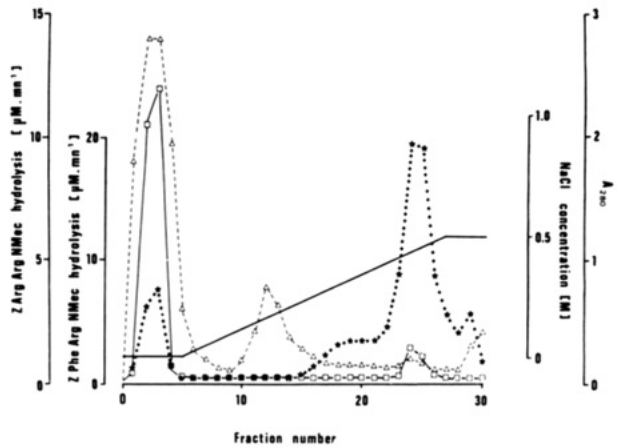

FIGURE 1: FPLC on Mono-S column of lysosomal extract after precipitation at pH 4.2. Activities against Z-Phe-Arg-NMec (Δ), Z-Arg-Arg-NMec (□), and A_{280} (▲) were measured.

absorbance was recorded at 254 nm with 0.05 AUFS full scale.

Enzyme Assays. The assay for Z-Phe-Arg-NMec, Z-Arg-Arg-NMec, and Arg-NMec hydrolysis was carried out according to Barrett (1980) with the cathepsin L fraction eluted from Mono-S column. Activation by DTT was tested by preincubating enzyme fractions at 37 °C for 5 min at optimum pH of activity prior to assay. Inhibitors were tested by preincubating enzyme with different concentrations of E-64 and Z-Phe-Phe-CHN_2 for 5 min at 37 °C and at optimum pH. The residual Z-Phe-Arg-NMec activity was then assayed as described above. The concentration of cathepsin L used for activation and inhibition studies was 1.3 nM as determined by E-64 titration (Barrett et al., 1982). Inhibition studies of cathepsin L by leupeptin were according to Green et al. (1984).

Other Methods. SDS–PAGE was performed as described by Laemmli (1970) under reducing conditions. A separating gel of 12% acrylamide and 0.4% methylenebis(acrylamide) was used. Silver staining was used to reveal proteins (Merril et al., 1983). Protein concentration was determined by the method of Bradford (1976) with bovine serum albumin (Sigma) as standard.

RESULTS

Purification. Our purification procedure (Table I) was based on two observations. First, chicken cathepsin L precipitated by reducing the pH to 4.2 and was easily resolubilized in pyrophosphate buffer at pH 6. This stage preserved 71% of the initial hydrolyzing activity. Second, in contrast with cathepsins B, H, and D, cathepsin L bound very tightly to the Mono-S column at pH 6 and was eluted as a single peak with 0.45 M NaCl (Figure 1). On the basis of the total activity against Z-Phe-Arg-NMec in lysosomal extract, a final purification of 545-fold was achieved in only two steps. Purified chicken liver cathepsin L exhibited a single band of M_r 27 000 by SDS–PAGE under reducing conditions (Figure 2).

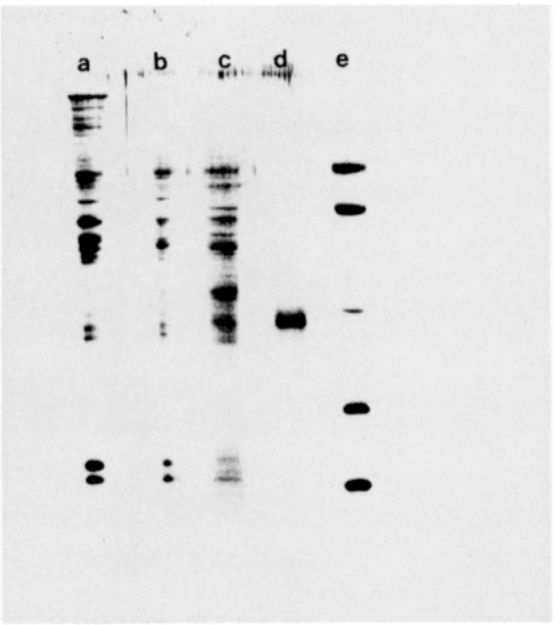

FIGURE 2: SDS–polyacrylamide gel electrophoresis under reducing conditions, as well as silver staining, were as described under Experimental Procedures. (a) Supernatant of liver homogenate after centrifugation at 4000*g* (5 μg of protein); (b) lysosomal extract (6 μg of protein); (c) lysosomal extract after treatment at pH 4.2 (5 μg of protein); (d) 5 μg of cathepsin L; (e) standard proteins—phosphorylase B (M_r 94 000), bovine serum albumin (M_r 67 000), ovalbumin (M_r 43 000), carbonic anhydrase (M_r 30 000), soybean trypsin inhibitor (M_r 20 000), and α-lactalbumin (M_r 14 400).

Table II: Amino Acid Composition of Chicken Liver Cathepsin L

	from amino acid analysis	from sequence		from amino acid analysis	from sequence
Ala	15.7	17[a]	Lys	15.7	15
Asp		16	Met	0.6	3
	24.8	24	Phe	7.9	8
Asn		8	Pro	10.5	8
Arg	8.2	6	Ser	14.4	16
Cys	4.9	7	Thr	9.5	7
Gly	23.4	25	Tyr	12.6	14
Glu		12	Trp	ND[b]	5
	20.2	23	Val	14.4	16
Gln		11			
His	5.9	5	total		215
Ile	7.0	7	M_r		23 668
Leu	10.2	9			

[a] Expressed in mol/mol of protein. [b] ND, not determined.

Properties. The activity toward Z-Phe-Arg-NMec required a thiol activator. Maximum hydrolysis was achieved with 2 mM DTT and was optimum in a pH range of 5.0–5.5. The enzyme was very unstable at neutral pH, and 97% of its activity was lost after only 2 min of incubation at pH 7.4. Michaelis constant (K_m) and k_{cat} for Z-Phe-Arg-NMec were determined to be 6 μM and 30 s^{-1}, respectively. Neither cathepsin B (Z-Arg-Arg-NMec) nor cathepsin H (Arg-NMec) substrates were hydrolyzed by chicken cathepsin L. Cathepsin L activity was also totally inhibited by 10 μM E-64 and by 10 μM of the specific inhibitor Z-Phe-Phe-CHN_2. Leupeptin was also a tight binding inhibitor of cathepsin L since a K_i value of 0.4 nM was estimated.

Analysis of Amino Acid Sequence. The amino acid composition of cathepsin L is presented in Table II, and the overall strategy for analysis of the amino acid sequence is outlined in Figure 3. The sequence of the first 31 N-terminal amino acids was obtained by automated Edman degradation of

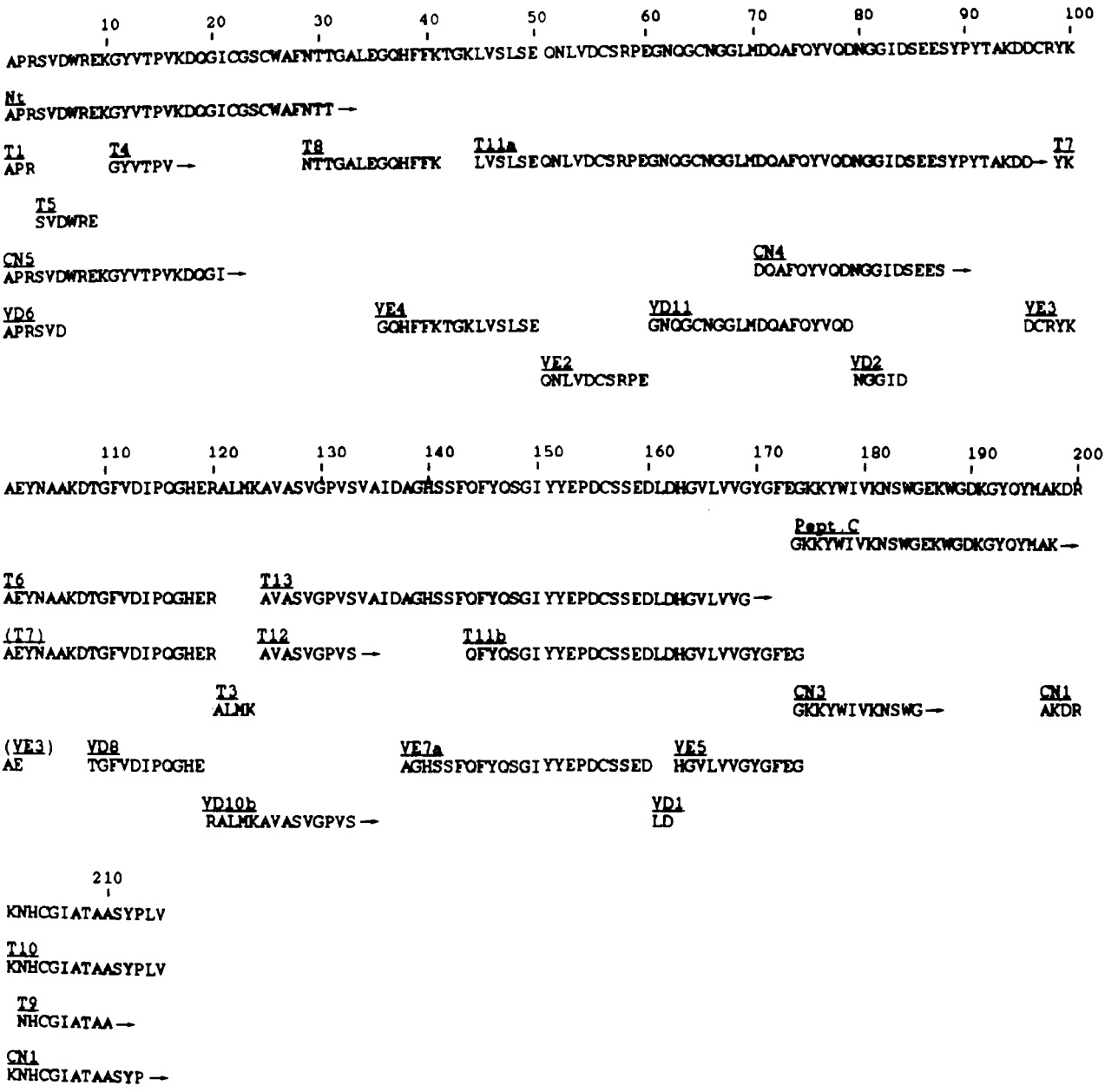

FIGURE 3: Alignment of peptides used for sequence determination. (T) Tryptic peptides; (VE) peptides obtained by cleavage at Glu with protease V8; (VD) peptides generated by digestion at Glu and Asp by protease V8; (CN) cyanogen bromide peptides; (Pept. C), peptide C; (Nt) automated Edman degradation on SCM-cathepsin L.

SCM-cathepsin L (500 pmol). Two sequences were obtained from the whole protein: a major one attributed to the N-terminal part of cathepsin L and a minor one, peptide C (minor sequence/major sequence ca. 1/2 in moles of PTH amino acids). Peptide C was isolated taking advantage of its solubility in TFA 1%. The insoluble SCM-cathepsin L was removed by centrifugation, and peptide C was sequenced (Figure 3). Because SDS–PAGE under reducing conditions of purified cathepsin L showed only one band, peptide C probably resulted from limited autolysis of cathepsin L during the 48-h dialysis step.

From a tryptic digest of SCM-cathepsin L, 13 main fractions were obtained by RP-HPLC (Figure 4). Automated sequence determinations of peptides T11a and T13 were carried out through 52 and 45 cycles, respectively. Fraction T11 was a mixture of two peptides (T11b/T11a, ca. 1/4 in moles of PTH amino acid), which were sequenced together.

The HPLC elution profile of the CNBr digest of SCM-cathepsin L is shown in Figure 5. Only five fractions, CN1 to CN5, of the six ones expected were collected, and four

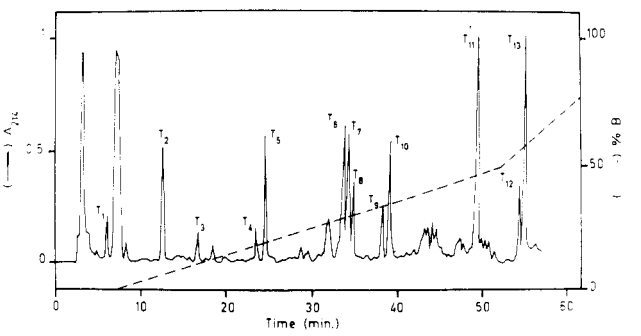

FIGURE 4: Separation of tryptic peptides of SCM-cathepsin L. Elution was in system I, as described under Experimental Procedures.

(CN1, -3, -4, and -5) were submitted to automated sequence analysis. Peptide CN2 was lost. Digestion of SCM-cathepsin L and peptide CN1 by carboxypeptidase Y released valine, suggesting that peptide CN1 was the C-terminal part of cathepsin L. The sequence of peptide CN5 agreed with that of the N-terminal region of the enzyme. Peptide CN3 corre-

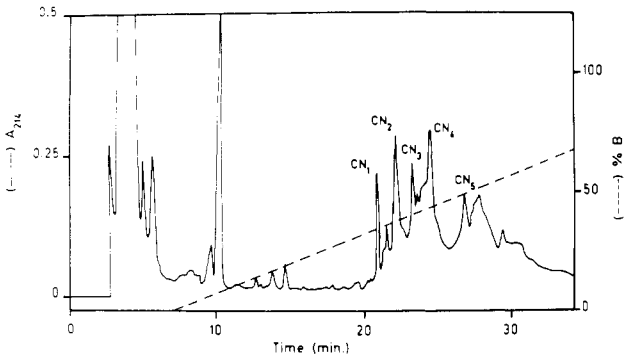

FIGURE 5: Separation of cyanogen bromide peptides of SCM-cathepsin L. Elution was in system I as described under Experimental Procedures.

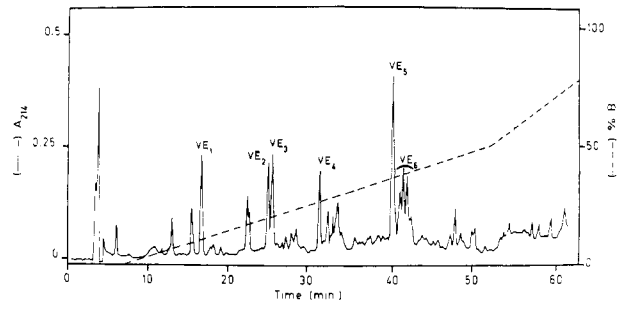

FIGURE 6: Separation of protease V8 peptides generated by digestion at Glu. Elution was in system I as described under Experimental Procedures.

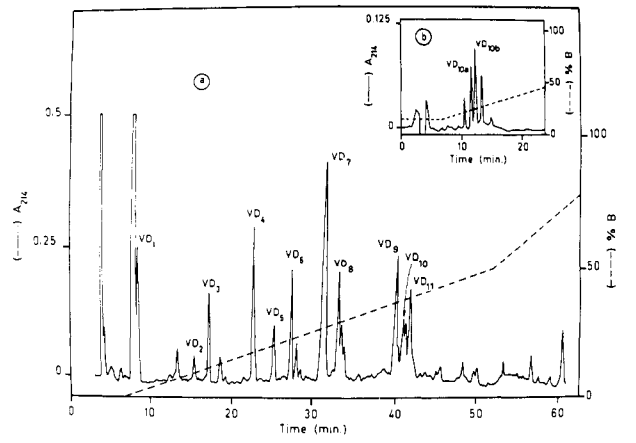

FIGURE 7: Separation of peptides generated by digestion with protease V8 at Glu and Asp residues: (a) separation of fractions VD1–VD11 with system I; (b) resolution of fraction VD10 with system II as reported under Experimental Procedures.

sponded to the N-terminal part of peptide C.

Digestion at glutamyl residues was performed in ammonium bicarbonate, pH 7.8, because SCM-cathepsin L was insoluble in ammonium acetate, pH 4.0. From digestion of alkylated cathepsin L at glutamyl (Figure 6) and at glutamyl and aspartyl (Figure 7a) residues, we manually collected 6 and 11 fractions, respectively. Fractions VD10 (Figure 7b) and VE6 were repurified in system II. Two peptides (VD10a, VD10b) and three peptides (VE6a, -b, and -c) were resolved from fractions VD10 and VE6, respectively.

Alignment of the Peptides. The amino-terminal sequence obtained for SCM-cathepsin L was linked to that of peptide VE4 by the sequence of peptide T8 obtained by an atypical cleavage between Phe-28 and Asn-29 (Figure 3). Peptide VE4 overlapped with peptide T11a and the sequence of peptide T11a was confirmed by those of peptides VE2 (residues 51–60), VD11 (61–79), VD2 (80–84), and CN4 (71–88).

Table III: Amino Acid Compositions of Peptides CN4 and T13

fragment residues	CN4 (71–123)	T13 (125–176)	fragment residues	CN4 (71–123)	T13 (125–176)
Ala	5.3 (6)[a]	3.7 (4)	Leu	1.1 (1)	2.7 (2)
Asx	7.4 (8)	4.4 (4)	Lys	3.5 (3)	2.5 (2)
Arg	1.8 (2)	0 (0)	Met (HSe)	0.5 (1)	0 (0)
Cys	0.5 (1)	0.4 (1)	Phe	1.8 (2)	2.5 (3)
Gly	4.2 (4)	6.8 (7)	Pro	2.5 (2)	1.2 (1)
Glx	8 (8)	5.3 (5)	Ser	2 (2)	6.3 (7)
His	1 (1)	2.6 (2)	Thr	2.6 (2)	0.2 (0)
Ile	2.4 (2)	2 (2)	Val	2.3 (2)	6.3 (7)

[a] Amino acid residues from the sequence.

Peptides T11a and VE3 overlap by only one amino acid. However the sequence between residues 71 and 123 agrees with the amino acid composition of peptide CN4 (Table III). Fragments T6 and T7 generated by cleavage at Lys-100 and Arg-98, respectively, were linked to peptide T11a by the sequence of peptide VE3. Peptide T13 was linked to fragment T6 by the peptide VD10b with an overlap of only one amino acid between T6 and VD10b. However, the sequence between residues 109 and 130 was completely determined twice: first with tryptic peptides T6, T3, and T12 and then with peptides VD8 and VD10b. Fragment T13 overlapped with peptide VE5 whose C-terminal amino acid was Gly-174 and not Glu-173 as might be expected. Fragment T11b included residues 144–174 and agreed with the previous sequence. Peptides C and CN3 overlapped by one residue (Gly-174) with peptide T11b. However, amino acid composition of peptide T13 (Table III) agrees with the sequence between residues 125 and 176. The sequence of peptide T10 contains the C-terminal valine and overlapped peptides T9 and CN1. This last one overlapped also peptide C. The complete primary structure of chicken liver cathepsin L, as shown in Figure 3, contained 215 residues and had a calculated M_r of 23 668.

Discussion

According to enzymatic criteria, the cysteine proteinase we have purified from chicken liver lysosomes can be identified with cathepsin L: it presents a high affinity for the substrate Z-Phe-Arg-NMec with a K_m of 6 μM and no activity toward cathepsin B and cathepsin H substrates, Z-Arg-Arg-NMec and Arg-NMec, respectively. Avian cathepsin L is inhibited by Z-Phe-Phe-CHN_2, E-64, and leupeptin and appears to be strikingly unstable at neutral pH: after 10-min incubation at pH 7.0, 97% of the activity toward Z-Phe-Arg-NMec was lost.

We show that cathepsin L purified from chicken liver lysosomes exhibits a single band on SDS–PAGE under reducing conditions, and therefore, cathepsin L probably exists as an uncleaved single chain within lysosomes. Pagano and Engler (1982) obtained also a single band of M_r 30 000 for human cathepsin L on SDS–PAGE under reducing conditions. In our case, limited autolysis and apparition of the "light chain" (peptide C) could be detected only after extensive dialysis of cathepsin L. When purified after overnight autolysis of tissue extracts, human and chicken cathepsins L were also shown to consist of both a heavy chain (M_r 24 000–25 000) and a light chain (M_r 4000–5000) (Mason et al., 1985; Wada & Tanabe, 1986). The light chains of human (Mason et al., 1986) and chicken (Wada & Tanabe, 1986) cathepsins L were shown to contain 42 and 40 amino acids, respectively, and correspond to our peptide C. The site of cleavage that produces the "light" and "heavy" chains is the bond Glu-173–Gly-174 for the avian enzyme.

The tryptic peptides T8 and T11b were generated by atypical cleavage at Phe residues. These chymotryptic-like

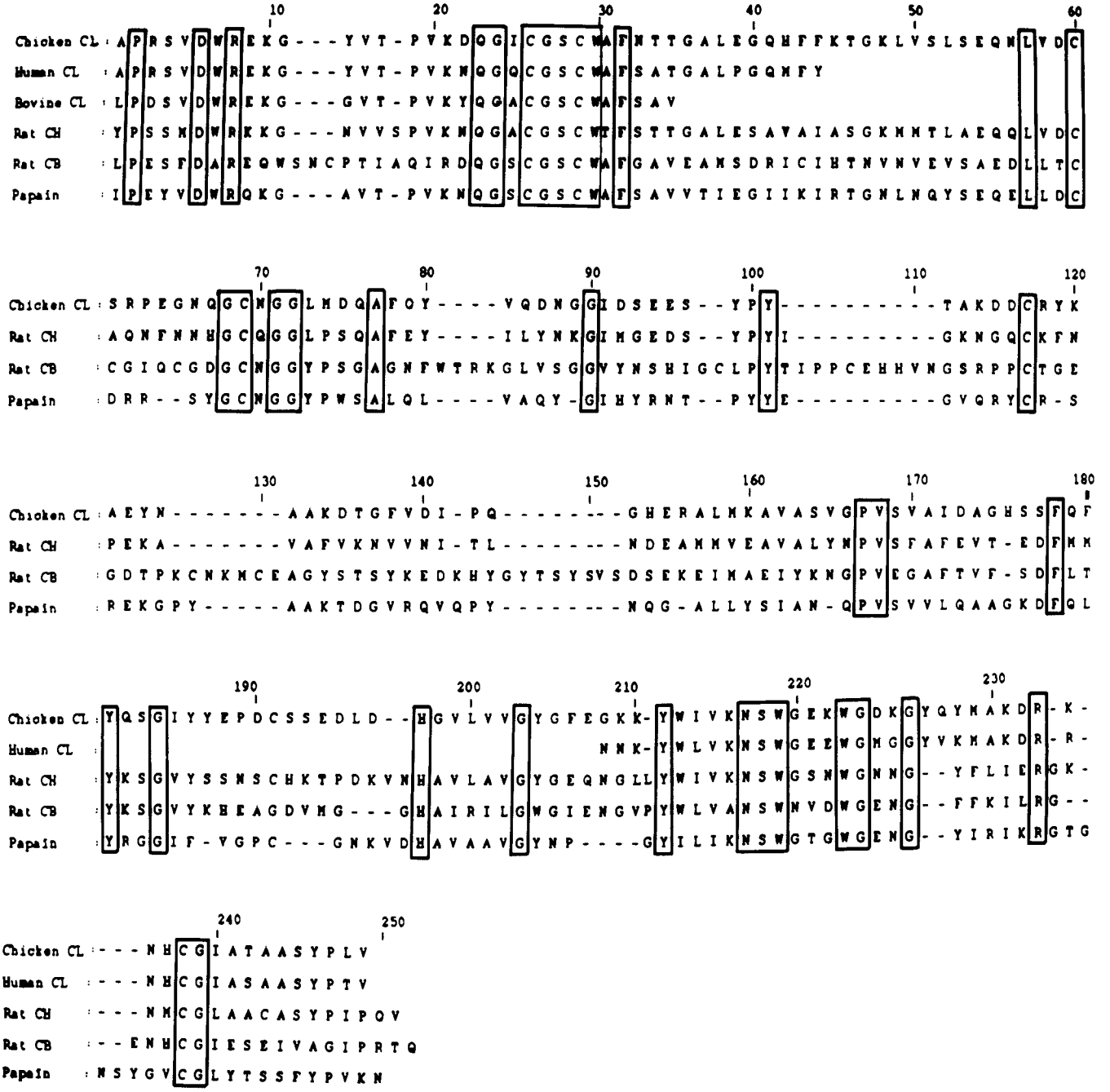

FIGURE 8: Alignment of the sequences of chicken liver cathepsin L (CL), human liver cathepsin L (N-terminal region and light chain), bovine liver cathepsin L (N-terminal region), rat liver cathepsins B (CB) and H (CH), and papain. The residue numbers are those of cathepsin B. Identical residues are enclosed in boxes.

cleavages occur during prolonged tryptic digestion (Keil-Dlouha et al., 1971). This long incubation period was employed because of the incomplete solubility of SCM-cathepsin L. Nevertheless, sequencing of fragments T8 and T11b provided important data for the construction of the complete sequence. The digestion at Glu residues by *S. aureus* V8 protease in ammonium bicarbonate, pH 7.8, generated some fragments by cleavage at Asp residues as previously reported (Walsh et al., 1986). Yet atypical cleavage at Gly-174 is not explained. Digestion of the whole protein by carboxypeptidase Y released only the C-terminal valine and not the heavy chain C-terminal residue (Glu-173). This can be explained by a low enzyme/substrate ratio and by the fact that carboxypeptidase Y releases glutamic acid more slowly than valine (Tsugita et al., 1980).

In Figure 8, the sequence of avian cathepsin L is compared with partial sequences of bovine (Turk et al., 1985) and human (Mason et al., 1986) cathepsins L as well as with the complete amino acid sequence of papain (Mitchel et al., 1970) and rat cathepsins B and H (Takio et al., 1983). The sequences are aligned so as to achieve maximal homology. For comparison, chicken cathepsin L, rat cathepsins B and H, and papain sequences are arbitrarily divided into three regions (Takio et al., 1983): an N-terminal (or active site cysteinyl) region, a central one, and a C-terminal (or active site histidyl) region. The N-terminal region (77 residues in cathepsin B) contains the cystein-rich site (C-G-S-C-W), and Cys-29 is probably the active site cysteine for chicken cathepsin L. The three cathepsins L show 77–82% identity (Table IV) in this region. In the same region, homology between chicken cathepsin L–rat cathepsin H and chicken cathepsin L–papain is the highest (57% and 55% identity, respectively) whereas it is the lowest when avian cathepsin L is compared with rat cathepsin B (37% identity). The homology in the central region (residues 78–152

Table IV: Homologies of Amino Acid Sequences of Thiol Endopeptidases[a]

residues in cathepsin B	region of comparison: amino terminal (1–77)	central (78–152)	carboxyl terminal (153–252)	whole protein (1–252)
chicken CL/rat CH	57.5	35.7	51.7	50.5
chicken CL/rat CB	37.0	16.6	35.2	32.0
chicken CL/papain	54.9	30.0	44.3	45.2
chicken CL/human CL	82.5[b]		73.8[c]	
chicken CL/bovin CL	77.4[d]			

[a] Identity (%) was calculated from the sequence alignment shown in Figure 8: (CL) cathepsin L; (CB) cathepsin B; (CH) cathepsin H. [b] Comparison of the 40 amino-terminal residues. [c] Comparison of the light chains (residues 208–250). [d] Comparison of the 35 amino-terminal residues.

in cathepsin B) is low, especially between chicken cathepsin L and rat cathepsin B. However, with only one deletion, 36% identity is observed between avian cathepsin L and rat cathepsin H.

The degree of identity between chicken and human cathepsins L is lower in the light chain than in the N-terminal region. In the C-terminal region (residues 153–252 in cathepsin B), homology between chicken cathepsin L and rat cathepsin H is higher than between chicken cathepsin L and papain or chicken cathepsin L and rat cathepsin B (Table IV). Asn-32 (Figure 8) is a possible residue for glycosylation that is found in a typical Asn-X-Thr (Ser) sequence. Histidine-197 (active site histidine in cathepsin B) is retained in all sequences (Figure 8), and it is likely to be the active site histidine in chicken cathepsin L. Residues 217–219 are retained in the five thiolproteinases (Figure 8). In papain, Asn-217 orients the His-197 imidazole ring by forming a hydrogen bond, and Trp-219 shields this bond from the solvent (Drenth et al., 1985). Among the four sequences, 20% of the residues are identical. Of the 38 invariants residues, 6 are Cys that are probably involved in active site and disulfide bonds. Overall, the sequence of chicken liver cathepsin L is more closely related to those of rat liver cathepsin H and papain than to that of rat liver cathepsin B.

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