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Bovine Spleen Cathepsin A: Characterization and Comparison with the Protective Protein

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Cathepsin A was purified approximately 550-fold with an overall yield of 4% from bovine spleen crude extracts by successive chromatographies on DEAE-Sephadex A-50, phenyl-Toyopearl 650C, and Con A-agarose. PAGE of the purified enzyme without 2-mercaptoethanol revealed an apparent molecular size of 110 kDa, and SDS-PAGE with 2-mercaptoethanol gave two polypeptide bands corresponding to 32 and 25 kDa and without 2-mercaptoethanol a single polypeptide 52 kDa band. These results indicate that the enzyme has an $(\alpha\beta)_2$ tetrameric structure in which the α (32 kDa) and β (25 kDa) subunits are linked by disulfide bond(s). The enzyme exhibited peptidase activities, hydrolyzing various Z-dipeptides with optimum pHs between 5.0 and 5.8. The hydrolytic rate for Z-Phe-Ala was 15 times higher than that for Z-Glu-Tyr, the traditional cathepsin A substrate. The enzyme also catalyzed the hydrolysis of the C-terminal amino acids of RCM-RNase A and showed esterase activity toward BTEE at pH around 7.5. DFP and TPCK completely inhibited both peptidase and esterase activities, and [1,3-^{3}H]DFP was bound to the α subunit. All these results support the fact that the enzyme is a serine carboxypeptidase. The N-terminal amino acid sequences of the α and β subunits are highly homologous to those of the human protective protein in galactosialidosis, strongly supporting the identity between cathepsin A and the protective protein.

Key words: cathepsin A, serine carboxypeptidase, protective protein.

Cathepsin A [EC 3.4.16.1] was found in bovine spleen in the early stage of intracellular protease studies and was defined as an enzyme hydrolyzing Z-Glu-Tyr at acidic pH by Fruton *et al.* in 1941 (*1*). Nowadays, the enzyme is regarded as a carboxypeptidase located in lysosomes based on studies of cathepsin A found in pig kidney (*2-4*), rat liver (*5, 6*) and chicken breast muscle (*7*). However, cathepsin A in bovine spleen actually has never been purified to homogeneity for full characterization and thus, its enzymatic properties remained to be investigated, as indicated in the last review (*8*).

There is a group of serine carboxypeptidases (*9*), including cathepsin A, which are commonly found in animal lysosomes or plant vacuoles. This type of enzyme has been isolated from a variety of species, such as carboxypeptidase Y from *Saccharomyces cerevisiae* (*10*), carboxypeptidase S_1 and S_2 or P from *Penicillum janthinellum* (*11, 12*), carboxypeptidase C from citrus peel (*13*), and carboxypeptidase WII from wheat (*14, 15*). All these serine carboxypeptidases have a catalytic triad composed of serine (*16*), histidine (*17*), and aspartic acid (*18, 19*) in their active sites. Serine carboxypeptidases are characterized by having carboxypeptidase activity toward peptides and proteins at acidic pH, and esterase activity toward amino acid esters at alkaline pH. These properties are different from those of serine endopeptidase, such as trypsin and chymotrypsin, that are inactive below pH 7. Therefore, it would be of interest to determine the detailed mechanism and structure-function relationship of a typical serine carboxypeptidase, such as cathepsin A, in order to understand the intracellular role(s) of the enzyme in eukaryotes.

Additionally, the physiological role of cathepsin A is of great importance. This enzyme may be related to a human lysosomal disorder, galactosialidosis (*20*), the disease caused by a deficiency of the protective protein, which forms a complex with β-galactosidase and neuraminidase and stabilizes these two enzymes (*21, 22*). Interestingly, the protective protein exhibits serine carboxypeptidase activity similar to that of carboxypeptidase Y, a well characterized yeast serine carboxypeptidase, with respect to amino acid sequence homology (*23*), and hydrolytic activity toward Z-dipeptides (*24*). Since cathepsin A is a serine carboxypeptidase as well, the human protective protein was assumed to be cathepsin A without detailed characterization of cathepsin A itself. To clarify this

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Abbreviations: BTEE, benzoyl-tyrosine ethylester; Con A, concanavalin; DFP, diisopropyl fluorophosphate; EDTA, ethylenediaminetetraacetic acid; IAA, iodoacetamide; PAGE, polyacrylamide gel electrophoresis; PCMB, *p*-chloromercuribenzoic acid; PMSF, phenylmethane sulfonylfluoride; RCM-RNase A, reduced and carboxymethylated bovine pancreatic ribonuclease A; SDS, sodium dodecylsulfate; TPCK, chloromethyl ketone derivative of tosyl-L-Phe; Z, carbobenzoxy; ZPCK, chloromethyl ketone derivative of Z-Phe.

uncertainty, cathepsin A in bovine spleen, which was the first enzyme defined as cathepsin A, was therefore purified and characterized, as reported herein.

MATERIALS AND METHODS

Materials—Fresh bovine spleens were obtained from a local slaughterhouse. Z-Dipeptides were obtained from the Peptide Institute (Osaka); DEAE-Sephadex A-50 from Pharmacia (Uppsala, Sweden); ConA-agarose from Sigma (St. Louis, USA); phenyl-Toyopearl 650 C from Tosoh (Tokyo); and [1,3-^{3}H]DFP and autoradiography enhancer (En^{3}Hance) from Du Pont NEN Research Laboratories (Boston, USA). RCM-RNase A was prepared by the method of Crestfield *et al.* (*25*). All other reagents were of analytical grade.

Enzyme Assay—Peptidase activity toward Z-dipeptides was measured by determining the amount of amino acid released. The reaction mixture, comprising 3 mM substrate, 0.5 M sucrose, 0.1 M KCl, and the enzyme in 0.05 M sodium acetate buffer, pH 5.4, in 1 ml, was incubated at 37 °C for 5 to 15 min. The reaction was terminated by the addition of 2 ml of the ninhydrin reagent (*26*). The mixture was heated at 100°C for 15 min, and then the absorbance at 570 nm was measured after cooling in an ice bath.

Esterase activity toward BTEE was measured by monitoring the increase in absorbance at 256 nm at 37°C (*27*). The reaction mixture comprised 0.5 mM BTEE, 10 to 15 μl of enzyme solution, and 100 mM sodium phosphate, pH 7.0, in a final volume of 1 ml. One unit (U) of activity is defined as 1 mmol of product formed per minute. Specific activity is expressed as U per mg of protein.

Enzyme Purification—All procedures were performed at 4°C, and the activity toward Z-Glu-Tyr was measured for the enzymatic activity, unless otherwise indicated. Protein concentrations were determined by the method of Bradford (*28*) using ovalbumin as a standard.

Sliced bovine spleen tissue soaked in 3 volumes of 0.1 M sodium acetate, pH 5.4, containing 0.4 M KCl and 1 mM EDTA was ground with a meat grinder for 3 min and then homogenized with a Warning blender. The homogenate was centrifuged at 5,000 × g for 30 min and the supernatant was taken as the first extract. The precipitate was mixed with 2 volumes of the same buffer and centrifuged again in the same manner, and the supernatant was taken as the second extract. The two extracts were combined and filtered through three layers of cheesecloth, and then the pH was adjusted to 5.4.

Sucrose was added to the filtrate to 1.0 M. The solution was then heated at 50°C for 30 min and the insoluble materials formed were removed by centrifugation. Solid ammonium sulfate was added to the supernatant to obtain 70% saturation. The resulting precipitate was collected by centrifugation, redissolved in a small volume of 0.01 M sodium acetate, pH 5.4, containing 0.1 M sucrose and 0.02 M KCl, and then dialyzed overnight against the same buffer. This buffer is referred to as ASP.

DEAE-Sephadex A-50 chromatography: A column of DEAE-Sephadex A-50 (2.5 × 25 cm) was equilibrated with ASP. After application of the supernatant obtained on the 70% ammonium sulfate fraction, the column was washed with 3 column volumes of ASP, and then linear gradient elution was performed with 0.02 to 0.4 M KCl in ASP. The fractions containing cathepsin A activity were collected, and the protein was precipitated with 80% ammonium sulfate. The precipitate was collected by centrifugation and then dissolved in a small volume of ASP.

Phenyl-Toyopearl 650C chromatography: A column of phenyl-Toyopearl 650 C (2 × 8 cm) was equilibrated with ASP. The enzyme solution obtained on DEAE-Sephadex A-50 was applied to the column and then the column was washed with ASP. Cathepsin A was eluted with a linear gradient of 0 to 50% ethylene glycol in ASP. The fractions containing cathepsin A activity were collected, concentrated by ultrafiltration using an Amicon XM-50 membrane, and then dialyzed against ASP.

Con A-agarose affinity chromatography: A column of Con A-agarose (1.1 × 5 cm) was equilibrated with ASP. The enzyme solution obtained on phenyl-Toyopearl 650C was applied to the column, and then the column was washed with ASP containing 0.5 M KCl. The enzyme was eluted with 0.1 M sodium acetate, pH 5.4, containing 0.5 M KCl, 0.1 M sucrose, and 0.5 M α-methylmannoside. The fractions containing cathepsin A activity were collected and concentrated by ultrafiltration as described above.

Electrophoresis—Non-denaturing PAGE was performed on an 8% polyacrylamide gel. SDS-PAGE was performed according to the method of Laemmli (*29*). A sample for electrophoresis was prepared by heating at 100°C for 5 min with or without 2-mercaptoethanol.

Labeling of the Active Site Serine Residue—Purified cathepsin A (8 μg) was mixed with [1,3-^{3}H]DFP (10 μM, 22 mCi) in ASP and then incubated at 37°C for 30 min. The reaction was terminated by the addition of the SDS-PAGE sample buffer with or without 2-mercaptoethanol, followed by heating at 100°C for 5 min. The gel was impregnated with the autoradiography enhancer and then dried under vacuum. The radiolabeled protein was detected by exposing the dried gel to an X-ray (X-Omat) film in a cassette containing a Lightning plus intensifying screen at −80°C for 10 days.

RCM-RNase A Digestion with Cathepsin A—A mixture of RCM-RNase A (0.5 mg) and cathepsin A (0.05 mg) in ASP was incubated at 37°C for 30 min. The reaction was stopped by the addition of 10% trifluoroacetic acid. After brief centrifugation, the supernatant was dried on a Speed-vac AES 1000 (Savant, Farmingdale, NY). The dried material was redissolved in 0.2 M sodium citrate, pH 2.2, for amino acid analysis. Amino acid analyses were performed with a JEOL JLC-300 amino acid analyzer.

N-Terminal Sequence Analysis—The SDS-PAGE gels were electroblotted onto PVDF membranes according to the instructions of the manufacture (BioRad, Richmond, USA), and the protein bands were stained with amido black, followed by background removal with 10% methanol in 10% acetic acid. The protein bands at the 32 and 25 kDa positions were cut into small pieces and applied to a protein sequencer (Applied Biosystem 476A).

RESULTS

Purification of Cathepsin A—The purification steps for one blade of bovine spleen tissue (700 g) are summarized in Table I. The enzyme was purified 541-fold with an overall yield of 4%. When the extract was heat-treated,

there was a 3-fold increase in specific activity with only 26% activity lost. In a test, the activity was completely lost on incubation at 70°C for 30 min. The solubilized enzyme was rather unstable. Thus, sucrose and KCl were added as stabilizing reagents in the remaining steps of purification.

On DEAE-Sephadex A-50 chromatography, two peaks, peaks I and II, of cathepsin A activity were detected (Fig. 1). Only peak II, with higher specific activity was used for the subsequent steps of purification. The enzyme bound tightly to a hydrophobic chromatography column, phenyl-Toyopearl 650C, and was eluted with 40% ethylene glycol (Fig. 2). The enzyme was also strongly adsorbed to a Con A-agarose column and eluted with 0.5 M α-methylmannoside, indicating that the enzyme is a glycoprotein. At this stage, the enzyme preparation was homogeneous, as revealed on PAGE (Fig. 3).

Molecular Properties of Cathepsin A—Non-denaturing PAGE without 2-mercaptoethanol gave a single band

TABLE I. **Purification of bovine spleen cathepsin A.**

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)
Crude extract	9,850	312	0.03	100
Heat treatment at 50°C	2,120	230	0.09	74
70% $(NH_4)_2SO_4$ precipitates	310	158	0.51	51
DEAE-Sephadex A-50 chromatography[a]	28	102	3.54	33
Phenyl-Toyopearl 650C chromatography	2.6	28	10.7	9
Con A-agarose chromatography	0.8	13	16.3	4

[a]Only peak II was collected for the subsequent steps of purification.

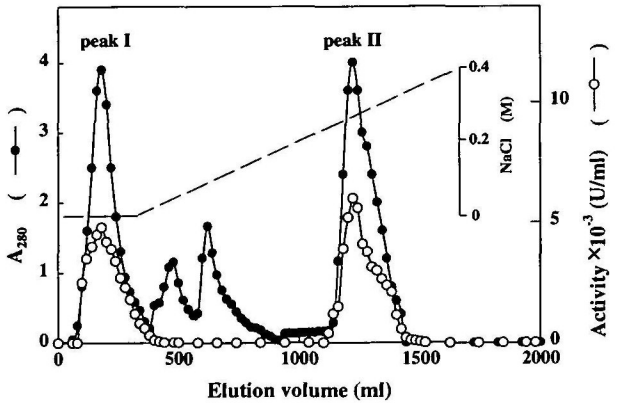

Fig. 1. **DEAE-Sephadex A-50 chromatography of bovine spleen cathepsin A.** The 70% ammonium sulfate fraction sample was applied to a column at the flow rate of 60 ml per h, fractions of 20 ml being collected.

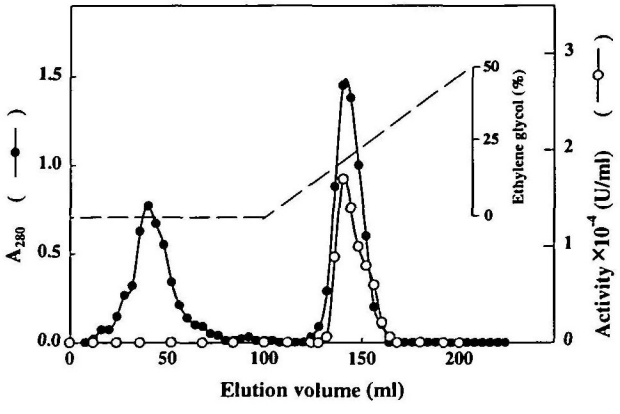

Fig. 2. **Phenyl-Toyopearl 650 C chromatography of bovine spleen cathepsin A.** The sample obtained on DEAE-Sephadex A-50 chromatography was applied to a column at the flow rate of 12 ml per h, fractions of 5 ml being collected.

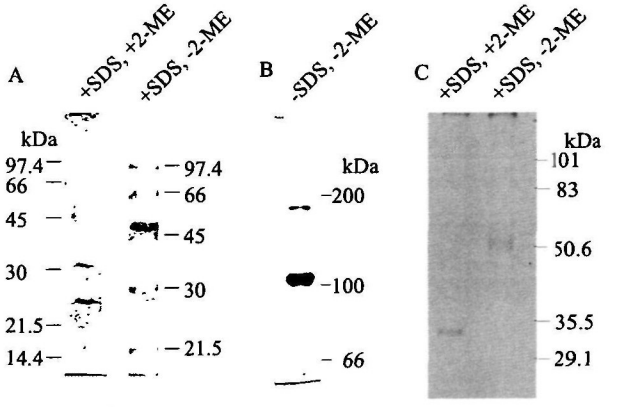

Fig. 3. **PAGE analysis of bovine spleen cathepsin A.** (A) SDS-PAGE. A 12% gel was used. (B) Non-denaturing PAGE. An 8% gel was used. Both (A) and (B) gels were stained with Coomassie Brilliant Blue R-250. (C) SDS-PAGE labeled with [1,3-^{3}H]DFP, a 12% gel was then exposed to X-ray film. The molecular size markers are as indicated. The dried gels (A and B) and X-ray film (C) were digitized on a flat bed scanner. With and without SDS or 2-mercaptoethanol are indicated as +SDS, −SDS, +2-ME, and −2-ME, respectively.

TABLE II. **Comparison of the amino acid compositions of bovine spleen cathepsin A, and the human, mouse, and chicken protective proteins.**

Amino acid	Cathepsin A[a] (mol%)	Protective protein		
		Human (*23*) (mol%)	Mouse (*35*) (mol%)	Chicken (*24*) (mol%)
Aspartic acid	12.3	12.6	13.3	11.2
Threonine	3.9[b]	3.7	3.8	4.2
Serine	7.5[b]	7.5	6.4	5.9
Glutamic acid	12.8	11.0	9.1	9.0
Proline	4.4	5.5	6.2	6.2
Glycine	8.4	6.8	6.6	8.1
Alanine	7.2	5.3	5.3	5.5
Half-cystine	2.1[c]	2.0	2.4	2.4
Valine	6.1	6.1	6.2	6.2
Methionine	2.1[c]	2.4	2.4	3.7
Isoleucine	2.7	2.6	2.9	3.1
Leucine	11.2	11.7	11.1	10.1
Tyrosine	3.9	6.4	6.4	5.9
Phenylalanine	4.5	5.1	4.9	5.3
Histidine	3.0	2.0	2.0	2.4
Lysine	4.7	4.6	4.4	4.6
Tryptophan	n.d.[d]	1.8	1.6	2.0
Arginine	4.1	3.7	4.4	4.0

[a]The values are the averages for two hydrolyzates obtained after 22 h hydrolysis with 6 N HCl *in vacuo* at 110°C, and are expressed as mol% of the protein. [b]The values for threonine and serine were corrected for 5 and 10% destruction, respectively, during the hydrolysis. [c]Half-cystine and methionine were determined as cysteic acid and methionine sulfone, respectively, after performic acid oxidation (*30*). [d]Tryptophan was not determined (n.d.).

corresponding to a molecular size of 110 kDa. However, SDS-PAGE with 2-mercaptoethanol gave two protein bands corresponding to molecular sizes of 32 and 25 kDa and without 2-mercaptoethanol, a single polypeptide of 52 kDa (Fig. 3). Based on these results, the enzyme can be regarded as an $(\alpha\beta)_2$ tetramer in which the α (molecular size, 32 kDa) and β (molecular size, 25 kDa) subunits are linked by disulfide bond(s).

N-terminal amino acid sequencing on the 32 and 25 kDa subunits gave the following results for the first 10 amino acid residues as Ala-Pro-Asp-Gln-Asp-Glu-Val-Gln-Arg-Leu- and Leu-Asp-Pro-Pro-Cys-Thr-Asn-Thr-Thr-Ala-, respectively. The amino acid compositions of the enzyme are shown in Table II. The amino acids in greatest abundance were aspartic acid, glutamic acid, and leucine.

Enzymatic Properties—Bovine spleen cathepsin A exhibited a pH optimum between 5.2 and 5.7 for peptidase activity, and at pH around 7.5 for esterase activity (Fig. 4). The activity at pH 8 or above was not measured because of the enzyme's instability.

Hydrolysis of Z-dipeptides revealed that cathepsin A was able to release hydrophobic amino acids rapidly, and glycine and proline slowly (Table III). The enzyme released C-terminal amino acids from RCM-RNase A sequentially (Fig. 5).

DFP, PMSF, TPCK, and ZPCK strongly inhibited both

TABLE III. **Synthetic Z-dipeptide hydrolyzing activity of bovine spleen cathepsin A.** The K_m and V_{max} values were determined at substrate concentrations ranging from 0.01 to 10 mM. The reaction was performed in 0.01 M sodium acetate, pH 5.4, containing 0.02 M KCl and 0.5 M sucrose at 37°C for 5 to 15 min.

Substrate Z-R_1-R_2	K_m (mM)	V_{max} (units/mg)
Z-Gly-Gly	3.8	0.15
Z-Gly-Phe	1.4	3.2
Z-Gly-Pro	1.8	0.05
Z-Gly-Glu	4.5	0.19
Z-Ala-Ala	2.8	34
Z-Ala-Leu	1.6	28
Z-Ala-Phe	1.2	35
Z-Ala-Glu	3.1	4.5
Z-Phe-Ala	2.1	125
Z-Phe-Leu	1.2	43
Z-Phe-Phe	1.6	40
Z-Phe-Glu	1.8	5.4
Z-Glu-Tyr	1.6	8.3
Z-Glu-Phe	0.8	2.1

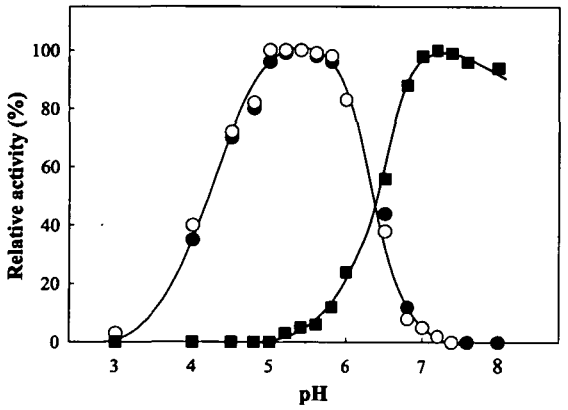

Fig. 4. **Effect of pH on the activity of bovine spleen cathepsin A.** The peptidase and esterase activities of the enzyme were determined at various pHs using the following buffers; sodium acetate (pH 3.0-6.5), Tris-Cl (pH 6.5-7.5), and HEPES (pH 7.5-8.0) buffer. Each buffer contained 0.02 M KCl and 0.1 M sucrose. The reaction was carried out at 37°C for 20 min. The peptidase activities toward Z-Glu-Tyr and Z-Ala-Phe, and the esterase activity toward Bz-Tyr-OEt are indicated as ●, ○, and ■, respectively.

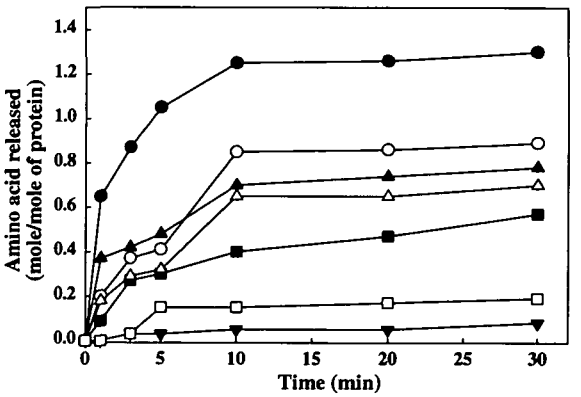

Fig. 5. **Rates of release of amino acids from RCM-RNase A on digestion with cathepsin A.** The reaction was carried out at 37°C with 0 to 30 min incubation (●, Val; ○, Ser; ▲, Ala; △, Asp; ■, Phe; □, His; ▼, Pro). The results are consistent with the C-terminus sequence of RCM-RNase A (-Val-Pro-His-Phe-Asp-Ala-Ser-Val-OH).

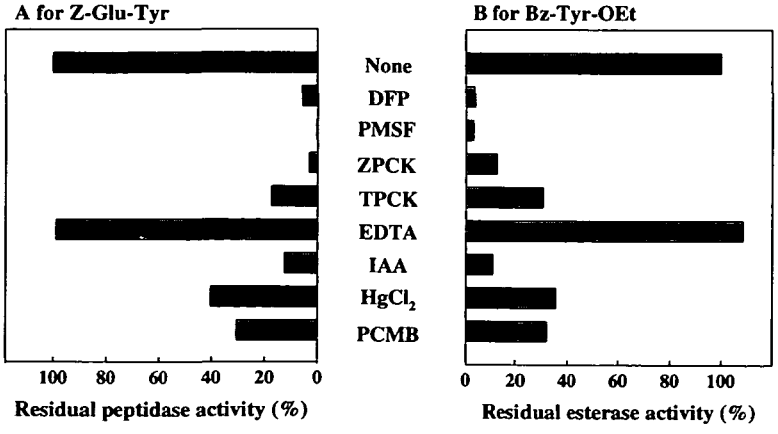

Fig. 6. **Effects of inhibitors on the activity of bovine spleen cathepsin A.** The enzyme was preincubated in 0.01 M acetate buffer, pH 5.4, or Tris-Cl buffer, pH 7.5, containing 0.02 M KCl and 0.5 M sucrose with 1 mM of an inhibitor at 37°C for 24 h. The enzyme activity was assayed by adding 0.01 M Z-Glu-Tyr or Bz-Tyr-OEt to the preincubation mixture.

the peptidase and esterase activities (Fig. 6). Sulfhydryl reagents, *i.e.*, PCMB, IAA, and $HgCl_2$, also inactivated both activities. EDTA had no effect on either activity. After the enzyme had been incubated with [1,3-^{3}H]DFP, autoradiography of the gels after SDS-PAGE with and without 2-mercaptoethanol showed a single radioactive band corresponding to molecular sizes of 32 and 52 kDa, respectively, indicating that the active serine residue is located in the α subunit (Fig. 3).

DISCUSSION

Cathepsin A, purified from pig kidney (*2*) and chicken breast muscle (*7*), showed specific activity of only 9.7 and 0.052 U/mg toward Z-Glu-Tyr, respectively. The cathepsin A purified in the present study exhibited a much higher specific activity of 16.3 U/mg, which is also 300-fold higher than that of the enzyme partially purified by Fruton *et al.* in the very first study of this enzyme (*1*). Thus, the enzyme preparation used in the present study is pertinent to discussion of the enzymatic and molecular properties.

Bovine spleen cathepsin A, as a carboxypeptidase, favors bulky and hydrophobic amino acid residues at the penultimate position as well as at the C-terminus position (Table III). Generally, when the penultimate amino acid is bulky and that at the C-terminus is non-bulky, the catalytic rate is high, but when glycine is at the penultimate position, the release of the C-terminal amino acid is slow. It is extremely low when the amino acid, proline, is linked to glycine. The V_{max} values for Z-Phe-Ala and Z-Glu-Tyr are 2,500 and 160 times, respectively, higher than that for Z-Gly-Pro. The broad substrate specificity toward peptides and proteins may make cathepsin A applicable to peptide sequencing like carboxypeptidase Y (*31, 32*).

The fact that both the peptidase and esterase activities of bovine spleen cathepsin A are inhibited proportionally by DFP, PMSF, TPCK, and ZPCK substantiates the idea that a single enzyme with a single charge relay system of a serine, a histidine and an aspartic acid residue might be responsible for the two activities. The parallel inhibition of both the peptidase and esterase activities by the SH reagents such as PCMB and IAA indicates that a SH group, located near the active site, interferes with the catalytic function or the binding of the substrate, similar to the SH group implicated for carboxypeptidase Y (*33*).

Although, in terms of the specificity and the active site structure, cathepsin A of bovine spleen, rat liver, pig kidney, and chicken breast muscle, as well as serine carboxypeptidases from yeast, mold and plants are very similar, there are some subtle differences. For example, the SH group of bovine spleen cathepsin A can react with IAA in the native state but not that of carboxypeptidase Y (*33*). The pH activity dependencies of bovine spleen cathepsin A toward Z-Phe-Ala and Z-Glu-Tyr were very similar; but they are quite different in carboxypeptidase Y. In carboxypeptidase Y, the K_m values toward Z-Glu-Tyr increased markedly with a slight change in k_{cat} when the pH of the assay mixture was changed from 5.5 to 6.5 (*32*).

Bovine spleen cathepsin A showed a V_{max} value for Z-Phe-Ala of 15 times higher than that for Z-Glu-Tyr. Thus, Z-Glu-Tyr is not the best substrate for cathepsin A or other serine carboxypeptidases such as carboxypeptidase P (*34*) and carboxypeptidase WII (*14, 15*). Z-Phe-Ala is therefore recommended as a better substrate for bovine spleen cathepsin A.

The molecular architecture of cathepsin A varies between species and organs. Cathepsin A from bovine spleen is an $(\alpha\beta)_2$ tetrameric protein of which the molecular sizes of the α and β subunits are 32 and 25 kDa, respectively. The two heteromeric subunits are linked by intersubunit disulfide bond(s) to make the 52 kDa subunit and two of the 52 kDa subunits are then complexed to form the mature 110 kDa protein. On the other hand, pig kidney cathepsin A (*3*) has two isoenzymes with molecular sizes of approximately 650 and 100 kDa, respectively, and the subunit compositions of these two enzymes are not clear. The molecular sizes reported for rat liver cathepsin A (*6*) are 420, 200, and 100 kDa sharing a common 25 kDa subunit.

A human lysosomal disorder, galactosialidosis, which is characterized by severely reduced activities of β-galactosidase and neuraminidase, is caused by a deficiency of the protective protein (*21*). The human protective protein has characteristics similar to those of a serine carboxypeptidases, carboxypeptidase Y, in the amino acid sequence (*23, 35, 36*) and enzymatic properties (*24*). Cathepsin A and the protective protein also exhibit catalytic similarities. For example, both cathepsin A and the protective protein hydrolyzes the N-blocked dipeptides, Z-Phe-Ala and Z-Phe-Leu, at acidic pH, and is inhibited by serine protease inhibitors. The protective protein is composed of two subunits of 32 and 20 kDa, similar to cathepsin A, and the larger subunit has been labeled with [1,3-^{3}H]DFP (*24*). The N-terminal sequence of the α subunit of bovine spleen cathepsin A (Ala-Pro-Asp-Gln-Asp-Glu-Val-Gln-Arg-Leu-) is highly homologous to that of the human protective protein (Ala-Pro-Asp-Gln-Asp-Glu-Ile-Gln-Arg-Leu-). The N-terminal sequence of the β subunit of bovine spleen cathepsin A (Leu-Asp-Pro-Pro-Cys-Thr-Asn-Thr-Thr-Ala-) is also very similar to that of the human protective protein (Met-Asp-Pro-Pro-Cys-Thr-Asn-Thr-Thr-Ala-). These results indicate that both bovine spleen cathepsin A and the human protective protein undergo similar processing to yield the α,β two-chain structure. The amino acid composition of cathepsin A, however, differs from that of the protective protein, especially in the glutamic acid, glycine, alanine, and tyrosine contents (Table II).

Human platelet deamidase is reported to be very similar to the human protective protein (*37*), and is also composed of 33 and 21 kDa subunits, whose N-terminal amino acid sequences are the same as those of the human protective protein (*23*). The similarities among the three proteins (the protective protein, placenta deamidase, and cathepsin A) raise an interesting question as to whether *in vivo* cathepsin A is multifunctional. It remains unknown whether or not cathepsin A forms a complex with β-galactosidase and neuraminidase as does the protective protein. The intracellular roles of serine carboxypeptidases, including cathepsin A, remain to be clarified in relation to the function of the human protective protein. Further studies including primary structure determination, cDNA sequencing and X-ray crystallography for the purified bovine spleen cathepsin A would be helpful for answering the above questions.

In conclusion, bovine spleen cathepsin A should be

classified as a serine carboxypeptidase with respect to its amino acid sequence, subunit structure and enzymatic properties.

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