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Characterization of a cysteine proteinase from adult worms of *Paragonimus westermani*

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Abstract: *Paragonimus westermani*, the lung fluke, is known to migrate to the pulmonary tissue of mammalian hosts and causes pathological changes in the lungs. An acidic thiol-dependent proteinase with a molecular weight of approximately 20,000 daltons was purified to homogeneity using ion-exchange chromatography and gel filtration chromatography. On SDS-PAGE, the molecular weight of the enzyme was 17,500 daltons. Isoelectric point was 6.45. The enzyme was similar to the acidic cysteine proteinase of vertebrates in the properties of pH optimum, substrate specificity, and inhibitor sensitivity. Enzymatic activity was stable at pH 5.5 for at least two days when stored at 4°C. The cysteine proteinase was capable of degrading collagen and hemoglobin. Sera of patients with paragonimiasis and mice infected with *P. westermani* reacted in immunoblots with the partially purified proteinase. This result suggested that the cysteine proteinase of *P. westermani* may play a role in migration in tissues, and in acquisition of nutrients by parasites from the host. It is also potentially an antigen for the serodiagnosis of paragonimiasis.

Key words: *Paragonimus westermani*, cysteine proteinase, degradation, immunoblot analysis

INTRODUCTION

Paragonimus westermani is a pathogen that causes lung disease in carnivorous mammals and humans. After oral infection by metacercariae in the second intermediate host, the juvenile worms invade into intestinal wall, migrate into a variety of intraabdominal organs, and eventually arrive in the lung where they become adult worms. The lesions caused in the lung by the worms are resulted from mechanical damage through the migration of the worms into tissues and cyst formation. However, the biochemical mechanisms involved in the pathogenesis and the acquisition of nutrients of the parasite are not understood well.

The function of some proteolytic enzymes of the parasites is important not only to the physiology of parasites (Auriault *et al.*, 1982; McKerrow and Doenhoff, 1988) but also to the host-parasite relationship (McKerrow, 1989). A variety of proteolytic enzymes have been detected in many parasites and in different developmental stages of parasitic helminths. The proteolytic enzymes of the adult worms of the trematodes, *Schistosoma mansoni*, have been studied extensively (Dresden *et al.*, 1981; Chappell and Dresden, 1987). Similar proteolytic enzymes have also been identified in the liver fluke, *Fasciola hepatica* (Simpkin *et*

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al., 1980; Yamasaki *et al.*, 1989) and in the lung fluke (Hamajima and Yamakami, 1981; Yamakami, 1986; Yamakami and Hamajima, 1987). It has been suggested that the cysteine proteinases found in the adult worms of *S. mansoni* (Chappell and Dresden, 1987), *F. hepatica* (Rege *et al.*, 1989) and *Clonorchis sinensis* (Song and Rege, 1991) hydrolyze hemoglobin and collagen, and may play an important role in the acquisition of nutrients by parasites from the host tissues. A cysteine proteinase was also detected in various developmental stages of *P. westermani* (Song and Dresden, 1990), but not studied in the adult worms.

In order to obtain better understanding of the possible role of this enzyme in the host-parasite relationship, the present study was undertaken to isolate and characterize the cysteine proteinase from adult worms of *P. westermani*.

MATERIALS AND METHODS

Parasites

The metacercariae of *Paragonimus westermani* were obtained from naturally infected crayfish (*Cambaroides similis*) collected in Wando-Gun, Korea. Dogs were fed orally with 100 metacercariae and were dissected four months after the experimental infection. The worms were harvested from the cysts in the lung. The collected worms were washed five times with cold 0.85% saline and once with distilled water and lyophilized.

Extraction of proteinase from adult worms

All procedures from worm collection to enzyme purification were carried out at 4°C unless otherwise stated. For the preparation of crude extract enzyme, the frozen worms (6.79 g) were homogenized with a Teflon-pestle tissue homogenizer in 0.1 M sodium citrate buffer (pH 5.5) and centrifuged at 20,000 g for 30 min. The supernatants were tested for proteinase activity and protein concentration.

Quantitation of protein

Protein concentration was determined by the method of Lowry *et al.* (1951). Bovine serum albumin (0-20 mg, Sigma) was used as a standard protein. All assays were done in duplicate.

Assay of enzyme activity

Proteinase activity in the crude extract was estimated by synthetic dipeptide substrate with a fluorescent leaving group, carboxybenzoyl-phenylalanyl-arginyl-7-amino-4-trifluoromethylcoumarin (CBZ-phe-arg-AFC; Enzyme Systems Products, USA). The assay mixture contained 0.1 M sodium citrate (pH 5.5), 5 mM dithiothreitol (DTT) and 10 μg of CBZ-phe-arg-AFC dissolved in 10 μl of dimethyl sulfoxide in a total volume of 0.5 ml. After incubation for 3 hr at 37°C, the released 7-amino-4-trifluoromethylcoumarin (AFC) was measured in a Turner fluorometer (Model III, Sequoia-Turner Corporation, USA) at excitatory and emission wavelengths of 400 and 505 nm, respectively. Standard curve was constructed with various concentrations of AFC; a unit of the enzyme activity is expressed as nmol AFC min^{-1} ml^{-1}.

Purification of cysteine proteinase

All chromatography media were purchased from Pharmacia LKB Biotechnology, Sweden. The crude enzyme was applied to a CM-Trisacryl M column (2.6 × 15 cm) equilibrated with 0.1 M sodium citrate buffer (pH 5.5). The column was eluted 0.1 M sodium citrate (pH 5.5) containing 0.2 M NaCl. Fractions (3 ml each) were collected at a flow rate of 50 ml/hr, and assayed for enzyme activity. The active fractions were pooled, concentrated, dialyzed and applied to activated thiol Sepharose 4B affinity gel column (1 × 8 cm; bed volume 6 ml) pre-equilibrated with 0.1 M Tris-HCl buffer (pH 7.5), containing 0.3 M NaCl and 1 mM ethylenediaminetetraaceitc acid (EDTA). After loading the proteins, the gels were washed to free of non-binding proteins with equilibrating buffer. The bound protein was then eluted with the same buffer containing 25 mM cysteine (Sigma). Fractions (2 ml each) were collected at a flow rate of 20 ml/hr. The active fractions were pooled, concentrated and dialyzed with 0.1 M sodium citrate (pH 5.5). The final purification of proteinase was done with Sephacryl S-200 HR (1.6 × 40 cm) which was

pre-equilibrated with 0.1 M sodium citrate (pH 5.5). The proteolytic activity fractions (1.4 ml each) were assayed, collected, and stored at –70°C for further studies.

The column was calibrated with proteins of known molecular weight (Pharmacia LKB Biotechnology, Sewden), including bovine serum albumin (66 kDa), ovalbumin (43 kDa), chymotrypsinogen A (25 kDa), and ribonuclease (13.7 kDa).

Biochemical and immunological properties of the purified cysteine proteinase

Determination of optimal pH and molarity of the buffer: Enzyme activity was monitored in 0.1 M sodium citrate buffer (pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0), and in 0.1 M sodium phosphate buffer (pH 6.5 and 7.0). To determine the optimal molarity of the buffer, the reaction mixture was incubated in 0.01 M, 0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M sodium citrate buffer at pH 5.5 as described by Dresden *et al.* (1985). All assays were done in duplicate and were standardized using a blank each time.

Temperature stability: An effect of the temperature on the enzyme activity was estimated as following. The purified cysteine proteinase (20 μg of protein content) was preincubated at 4°C for 2, 4, and 8 day in the 0.1 M sodium citrate (pH 5.5).

Effects of proteinase inhibitors: Purified enzyme was preincubated at 37°C for 30 min in 0.1 M sodium citrate (pH 5.5), containing inhibitors. Substrate was then added. The reaction mixtures were incubated for 3 hr at 37°C and the released AFC was measured in a Turner III fluorometer.

The inhibitors employed in this study were L-trans-epoxy-succinyl-leucylamido (4-guanidino) butane (E-64, 10^{-5}M; Sigma), iodoacetic acid (1 mM; Sigma), N-ethylmaleimide (NEM, 10 mM; Sigma), leupeptin (10^{-4}M; Peninsula Laboratories, USA), N-tosyl-L-phenylalanine chloromethyl ketone (TPCK, 1mM; Sigma), N-α-p-tosyl-L-lysine chloromethyl ketone (TLCK, 1 mM; Sigma), diisopropyl fluorophosphate (DFP, 100 μM; Sigma), phenylmethyl sulfonyl fluoride (PMSF, 1 mM; Sigma), pepstatin (10^{-5}M; Peninsula Laboratories, USA), ethylenediaminetetraacetic acid (EDTA, 10mM; Sigma), 1, 10-phenanthroline (1 mM; Sigma).

Degradation of collagen and hemoglobin: Degradation of protein substrates was carried out as previously described by Rege *et al.* (1987) using calf skin, acidic soluble collagen (Type I; Boehringer Mannheim Biochemica, Germany) and bovine hemoglobin (Sigma). Briefly, 90 μg of collagen or 100 μg of hemoglobin were incubated with or without purified cysteine proteinase (8 μg) in 0.1 M sodium citrate (pH 5.5) containing 5 mM DTT. The reaction was stopped by the addition of as equal volume (100 μl) of reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis sample buffer containing β-mercaptoethanol and followed by boiling for 2.5 minutes. Degradation products were separated on a 7.5-15% gradient SDS-PAGE and visualized with Coomassie Blue R-250.

SDS-PAGE and immunoblot analysis: SDS-PAGE was carried out on 7.5-15% gradient gel under reducing conditions as the method described by Laemmli (1970). Molecular weight markers including the following proteins (Pharmacia LKB Biotechnology, Sweden) were employed phosphorylase B (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30.0 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa). The gel was stained with Coomassie Blue R-250.

After electrophoresis, the proteins were transferred to nitrocellulose membrane (pore size 0.2 μm; Pharmacia LKB, Sweden) according to the method of Tsang *et al.* (1983). The transferred proteins were immunoblotted by incubation for 2 hr at 37°C with 30 μl of antisera obtained from human paragonimiasis and/or experimentally infected mouse (1:100 dilution). Normal human serum served as control. The immunoreactive bands were visualized by an incubation for 2 hr at room temperature with horseradish peroxidase (HRP)-conjugated goat anti-human and anti-mouse IgG (1:1,000 dilution; Cappel, USA), and followed by a further incubation for 5-15 min at room temperature with 0.05% 3,3-diamino-benzidine (Sigma), and 0.001% H_2O_2 in PBS. The color development was stopped by rinsing the nitrocellulose membranes with

deionized water.

Isoelectric focusing (IEF): The isoelectric point (pI) of the aliquots from various stages of purification and finally purified enzyme was analyzed on pH 3.5-9.3 isoelectric focusing gel at 4°C (The manual of IEF; Pharmacia LKB Biotechonology, Sweden). In each lane, 1 μl (5-6 μg) of samples were loaded. After isoelectric focusing, the gel was fixed with fixing solution and stained with Coomassie blue R-250.

RESULTS

Assay of enzyme activity

To select proper substrate for proteolytic activity of *Paragonimus westermani* crude extract enzyme, preliminary experiments using two dipeptide substrates, CBZ-phe-arg-AFC and CBZ-arg-arg-AFC was carried out. It was observed that much higher activity (about 6-fold) was obtained with CBZ-phe-arg-AFC than with CBZ-arg-arg-AFC; hence, CBZ-phe-arg-AFC was used for subsequent experiments. The cysteine proteinase was thiol dependent (Table 1).

When the effect of pH activity of purified cysteine proteinase was tested with CBZ-phe-arg-AFC, maximum activity of purified cysteine proteinase was observed at pH 5.5. No activity was seen at below pH 3.0. Very little activity was seen above pH 7.0.

To optimize yields from extraction of the proteinase, the effect of ionic strength on solubilization of activity was tested with various extraction buffers containing 0.01-0.5 M sodium citrate (pH 5.5). Maximum activity of the enzyme (crude extract) was solubilized with 0.1 M sodium citrate (pH 5.5).

Purification, stability, SDS-PAGE and IEF of cysteine proteinase

Purification of a cysteine proteinase from adult worms was performed with a series of chromatographic steps, including CM-Trisacryl M cation-exchange, activated thiol Sepharose 4B affinity and Sephacryl S-200 HR gel filtration. CM-Trisacryl M column chromatography revealed the presence of three activity peaks at 0.2 M NaCl. The active fractions (Fig. 1A) were pooled, concentrated, and applied to activated thiol Sepharose 4B affinity column. One peak of the proteolytic activity was eluted with 0.1 M Tris-HCl buffer containing 0.3 M NaCl, 1 mM EDTA and 25 mM cysteine (pH 7.5). The peak of the active fraction (Fig. 1B) were pooled, concentrated, and applied to an Sephacryl S-200 HR gel filtration column. Two protein peaks were obtained according to their absorbance at 280nm. High proteolytic activity found in first peak (Fig. 2) was pooled, concentrated and used in further study. Molecular weight estimate of purified cysteine proteinase under native condition was done (Inset in Fig. 2). Native cysteine proteinase eluted from the gel filtration revealed that the enzyme had a molecular weight approximate 20,000 daltons.

Results of purified cyteine proteinase were summarized in Table 2. The proteinase activity was purified approximately 80-fold when compared to the crude extracts with the recovery of 2.45%. On 7.5-15% SDS-PAGE, the purified enzyme migrated as a single band at 17.5 kDa (Fig. 3).

When the purified proteinase was stored at 4°C for 2- or 8- day without any treatment, the purified cysteine proteinase lost about 20%

Table 1. Activity of cysteine proteinase from *Paragonimus westermani* against synthetic substrate and thiol dependence of enzyme[a]

Substrate	Activity[b]	
	With 5 mM DTT	Without 5 mM DTT
CBZ-phe-arg-AFC	10.0	3.6
CBZ-arg-arg-AFC	1.6	0.2

[a]Note: Equivalent amounts of frozen adult worms were extracted in 0.1 M sodium citrate buffer (pH 5.5), centrifuged, and assayed against dipeptide substrates linked to AFC (3 hr). Results were monitored at 505 nm and 400 nm.

[b]Activity is expressed as unit (nmoles of AFC/min).

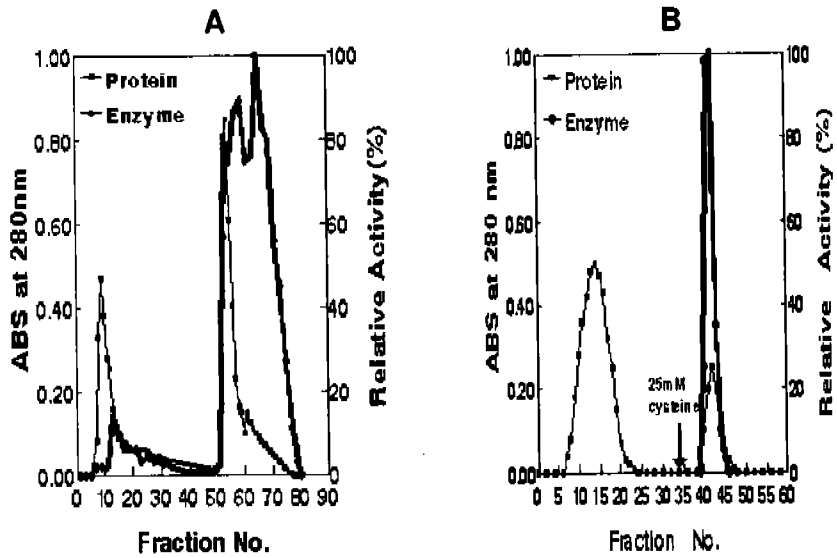

Fig. 1. Elution profile of cysteine proteinase of *P. westermani*. **(A)** The crude extracts were loaded on CM-trisacryl M cathion-exchange column. Fractions were assayed for activity on CBZ-phe-arg-AFC (●) and monitored for protein content (■) at 280 nm. **(B)** Affinity chromatography with thiol-activated Sepharose 4B. The enzyme purified from CM-trisacryl column chromatography was loaded. 2 ml fractions were assayed for activity as described previously. Markings are the same as in A.

Table 2. Summary for the purification of cysteine proteinase from *P. westermani* adult worms

Purification steps	Total protein (mg)	Total activity (Unit)[a]	Specific activity (Uint/mg)	Purification fold	Recovery (%)
Crude extract	1,045.00	36,290.00	34.70	1.00	100.00
CM-Trisacryl M[b]	205.80	25,123.00	122.10	3.52	69.20
Thiol-Sepharose 4B[c]	2.03	2,403.40	1,183.90	34.12	6.62
Sephacryl S-200HR[d]	0.32	890.40	2,782.50	80.19	2.45

[a]nmoles of AFC/min; [b]ion exchange chromatography; [c]affinity chromatography; [d]gel filtration chromatography

and 60% of the enzyme activity, respectively (Table 3); but no activity was lost even after 3 weeks at –20°C (data not shown).

To observe the pI of the purified cysteine proteinase, IEF analysis was carried out. As shown in Fig. 4, the purified cysteine proteinase revealed the pI of 6.45.

Inhibition profile

Cysteine proteinase purified from the crude extracts were tested with CBZ-phe-arg-AFC substrate for inhibition by various compounds known to affect endopeptidase activity. As shown in Table 4, E-64, iodoacetic acid, NEM, and leupeptin, which are known to cysteine proteinases inhibitors, showed significant inhibitory effect (87% or greater). No significant inhibitions were observed with pepstatin (inhibitor of aspartic proteinases), PMSF and DFP (inhibitors of serine proteinase), and 1,10-phenanthroline and EDTA (inhibitors of metallo proteinase).

Activity against collagen and hemoglobin

α- and β-chains of the reacted collagen were completely degraded to several distinct products by the purified cysteine proteinase.

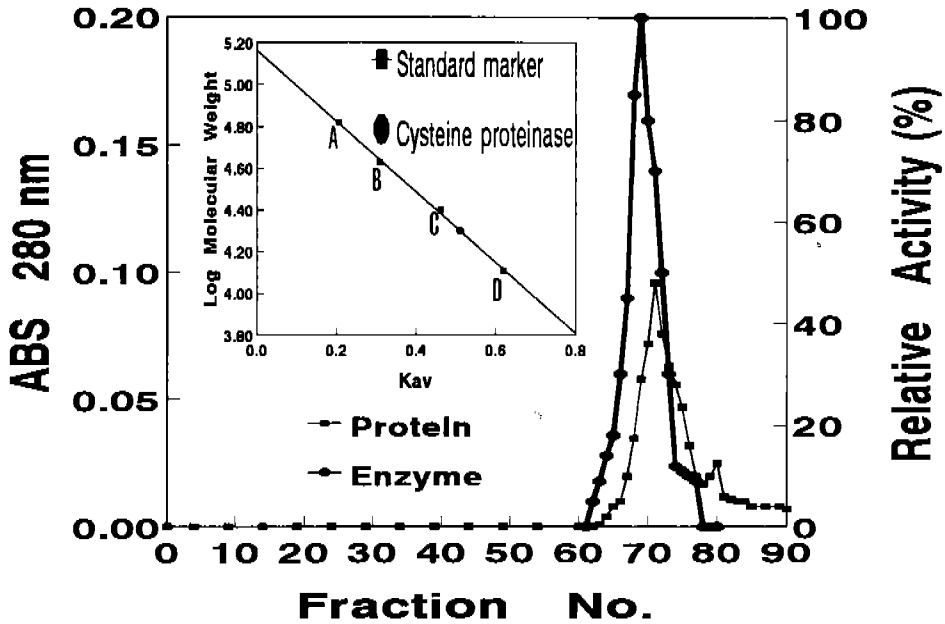

Fig. 2. Activity peaks purified from thiol-activated Sepharose 4B were loaded onto a column (1.6 × 40 cm) of sephacryl S-200 HR. 1.4 ml fractions were assayed for activity. Marking are the same as in A. Molecular weight determination of purified cysteine proteinase by Sephacryl S-200 HR gel filtration chromatography (inset). Purified enzyme was applied to Sephacryl S-200 column (1.6 × 40 cm) which had been precalibrated with marker proteinas. The markers were as follows. A: bovine serum albumin (66,000), B: ovalbumin (43,000), C: chymotrypsinogen (25,000), D: ribonuclease (13,700).

Table 3. Stability of cysteine proteinase purified from *P. westermani*

Periods (day)	Activity at 4°C	Remaining
	nmoles AFC/h/ml	Activity (%)[a]
0	1,500	100.0
2	1,200	80.0
4	700	46.7
8	600	40.0

[a]Activity is expressed as relative percent.

Long incubation times did not, however, result in further degradation of these products to peptides (Fig. 5). Degradation of hemoglobin was also noted. A marked decrease in the dimer band and a marginal decrease in the density of the monomer band were noted (Fig. 6).

Antigenicity of the purified cysteine proteinase

Antibodies in the sera of human (paragonimiasis) and mouse infected with *P. westermani* were tested by immunoblots with denatured proteinase samples. Purified preparations of the cysteine proteinase, previously shown by SDS-PAGE to contain a single molecular weight major band at 17,500 daltons, were used as a source of antigen. The results of this experiment are shown in Fig. 7. A specific immunoblotting pattern was observed. Antisera from both *P. westermani*-infected human and mouse showed a specific, positive reaction at molecular weight around 17,500 daltons. Normal human serum showed

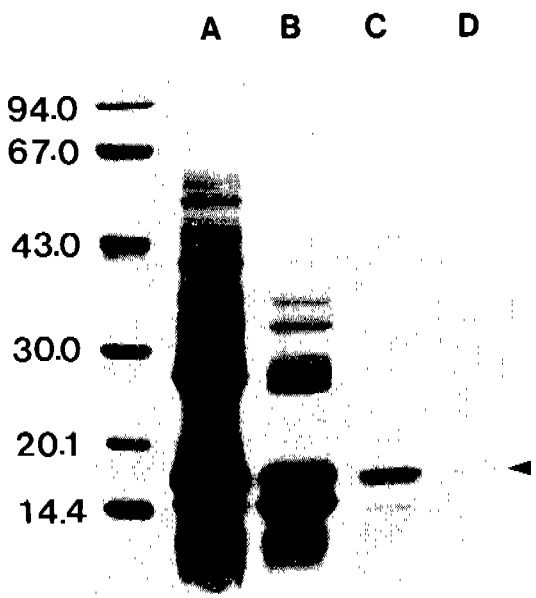

Fig. 3. 7.5-15.0% gradient SDS-PAGE analysis of cysteine proteinase purified from *P. westermani*. Lane A, homogenate supernatan; Lane B, active peak from CM-Trisacryl M; Lane C, active peak from thiol-activated Sepharose 4B affinity gel; Lane D, active peak from Sephacryl S-200 HR. Molecular weight markers included the following proteins (Pharmacia LKB, Sweden): phosphorylase B (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100) and α-lactalbumin (14,400).

no reaction.

DISCUSSION

In the present study, we have purified and characterized the cysteine proteinase from adult worms of *Paragonimus westermani* using a variety of biochemical techniques. Degradation and antigenicity of purified cysteine proteinase have also assayed with collagen and hemoglobin and anti-human sera (paragonimiasis), respectively.

Recently, many reports have been purified and characterized proteolytic enzymes in protozoan and helminths. These proteolytic enzymes existed in crude extracts and excretory-secretory products of parasites are related with migration, nutritional uptake and pathological symptoms in host, especially play major roles in depression of response immunological response (McKerrow, 1989).

Table 4. Effects of inhibitors on cysteine proteinase purified from *P. westermani*

Inhibitors	% Relative activity[a]
None	100.0
Cysteine class	
E-64 (10^{-5}M)	3.0
Iodoacetic acid (1 mM)	13.0
NEM (10 mM)	2.0
Leupeptin (10^{-4}M)	3.0
Serine class	
TPCK (1 mM)	17.5
TLCK (1 mM)	53.0
DFP (100 mM)	102.0
PMSF (1 mM)	100.0
Aspartic class	
Pepstatin A (10^{-5}M)	98.0
Metallo class	
EDTA (10 mM)	94.0
1,10-Phenanthroline (1 mM)	46.0

The concentration of inhibitors tested is shown in parenthese.

[a]The activity against CBZ-phe-arg-AFC substrate in the absence of inhibitors was taken as 100%.

E-64: L-trans-epoxyssuccinyl-leucylamide (4-guanidino) butane

NEM: N-ethylmaleimide

TPCK: N-tosyl-L-phenylalanine chloromethyl ketone

TLCK: N-α-p-tosyl-L-lysine-chloromethyl ketone

DFP: Di-isopropylfluoreophosphage

PMSF: Phenylmethyl sulfonyl fluoride

EDTA: Ethylenediaminetetraaceitc acid

The cysteine proteinase purified from adult worms of *P. westermani* also shared properties with mammalian cathepsins B, H, and L (Barrett, 1979) belonged to the cysteine proteinase class and showed similarities in their properties to cysteine proteinase obtained from other trematodes such as *Clonorchis sinensis* and sparganum (Song and Rege, 1991; Song *et al.*, 1992). Yamakami (1986) demonstrated an acidic cysteine proteinase in adult worms of the related parasite, *Paragonimus ohirai*. And Yamakami and Hamajima (1987) reported the presence of neutral thiol preoteinase activity in *P. westermani* metacercariae. Song and Dresden (1990) confirmed the presence of cysteine proteinase at various developmental stages of

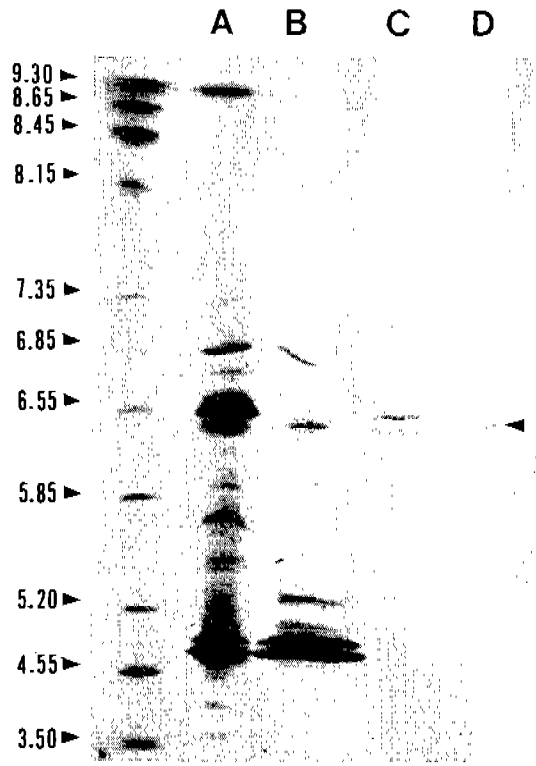

Fig. 4. Isoelectric focusing analysis (pH range 3.5-9.5) of the purified cysteine proteinase. Lane A, homogenate supernatant; Lane B, active peak from CM-Trisacryl M; Lane C, active peak from thiol-activated Sepharose 4B affinity gel; Lane D, active peak from Sephacryl S-200 HR. Isoelectric point markers included the following proteins (Pharmacia, Sweden): trypsinogen (pI 9.30), lentil lectin-basic band (pI 8.65), lentil lectin middles band (pI 8.45), lentil lectin-acidic band (pI 8.15), horse myoglobin-basic band (pI 7.35), horse myoglobin-acidic band (pI 6.85), human carbonic anhydrase B (pI 6.55), bovine carbonic anhydrase B (pI 5.85), β-lactoglobin (pI 5.20), soybean trypsin inhibitor (pI 4.55), and amyloglucosidase (pI 3.50).

P. westermani.

The cysteine proteinase purified from *P. westermani* adult worms hydrolyzed substrates normally used to assay cysteine proteinase, such as CBZ-phe-arg-AFC, and were thiol-activated and had an acidic pH optimum. These results were similar to those of the purified cysteine proteinase from various stages of *P. westermani* (Song and Dresden, 1990).

The mol. wt. of the enzyme, as determined

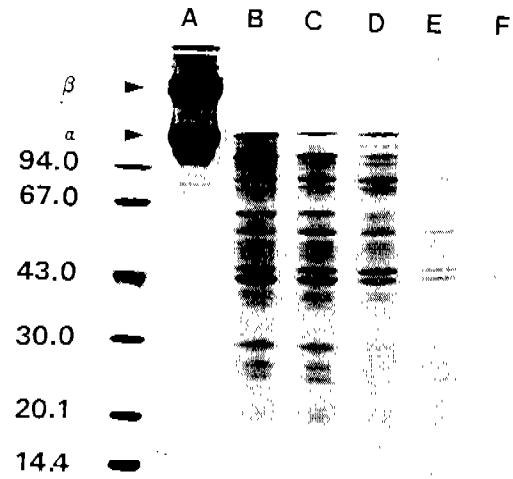

Fig. 5. Degradation of collagen substrates by *P. westermani* cysteine proteinase. Proteinase purified from gel filtration chromatography was assayed for collagenolysis in the presence of 5 mM DTT. Collagen degradation is indicated by the disappearance of α- and β-chains (upper two arrow heads) from a 7.5-15.0% SDS-PAGE. Lane A, control collagen (Type I); Lane B-F, incubated for 30 min, 1, 2, 4, and 8 hr, respectively.

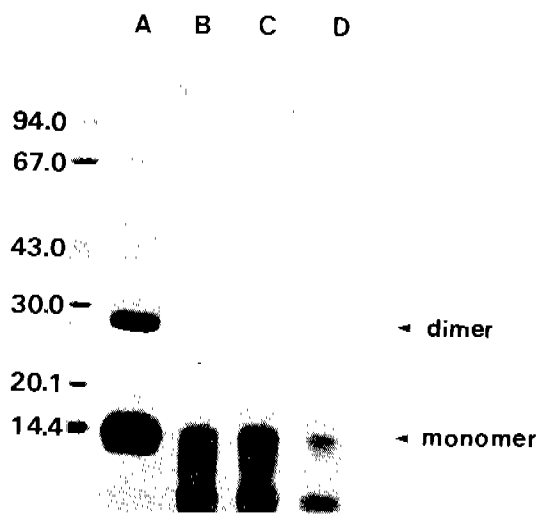

Fig. 6. Degradation of hemoglobin by *P. westermani* cysteine proteinase. Purified cysteine proteinase from gel filtration chromatography was assayed for hemoglobinolytic activity in the presence of 5 mM DTT (incubation time of lane B, C and D are 2, 4, and 8 hr, respectively). Disappearance of the monomeric and dimeric hemoglobin was analysed by a 7.5-15.0% SDS-PAGE. Lane A, control hemoglobin without enzyme.

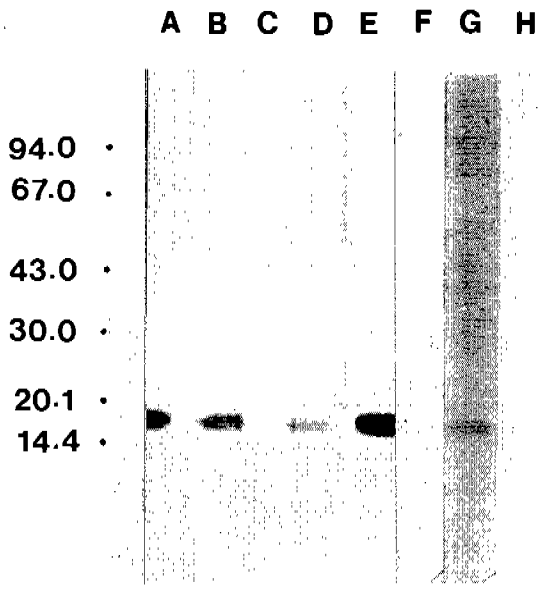

Fig. 7. Immunoblot analysis of *P. westermani* cysteine proteinase under denaturing conditions. 7.5-15.0% polyacrylamide gel electrophoresis and electrophoretic transfer of purified proteinase onto nitrocellulose membranes were performed as described in materials and methods. Lane A-F, human antisera (paragonimiasis); Lane G, mouse antiserum (3 months after experimental infection); Lane H, normal human serum. Molecular weight standards are indicated.

by Sephacryl S-200 HR gel filtration chromatography, was 20,000 daltons. These results were similar to those of the other lung parasite cysteine proteinases (Rege *et al.*, 1989), and of metacercaria (Yamakami and Hamajima, 1987), and the purified cysteine proteinase from various developmental stages of *P. westermani* (Song and Dresden, 1990).

The inhibitory effects of various proteinase inhibitors, particularly E-64, iodoacetic acid, NEM, and leupeptin indicate that the purified enzyme belong to a cysteine proteinase. In contrast, TPCK, TLCK and 1,10-phenanthroline also had a moderate inhibitory effect. It is considered that these compounds decreased proteinase activity of crude extract enzymes by 50% or more. It should be note, however, that minor amounts of other proteinases may be present in the purified enzyme. Other compounds known to inhibit serine or aspartic proteinases did not appreciably decrease activity. According these data, the purified enzyme from the crude extract enzyme contain cysteine proteinase activity.

The purified cysteine proteinase from adult worms of *P. westermani* was capable of digesting collagen and hemoglobin. This result was consistent with observations made by others using crude extracts (Halton, 1967; Aoki, 1980). Thus the cysteine proteinase of *P. westermani* could play a role either in migration of the worms into host tissue or in uptake of nutrients. Similar enzymes have been also demonstrated in closely related trematode parasites, *S. mansoni* (Dresden and Deelder, 1979; Chappell and Dresden, 1986) and *F. hepatica* (Rege *et al.*, 1989). The results of this experiment were similar when cysteine proteinase was purified from various developmental stages of *P. westermani* (data not shown).

By SDS-denaturing electrophoresis, the purified cysteine proteinase had a mol. wt. of 17,500 daltons, which correlates well with the mol. wt. estimated with the native enzyme. Antisera obtained both from pateints with paragonimiasis and mice experimentally infected with *P. westermani* reacted with the cysteine proteinase from adult worms.

This result suggests that the *P. westermani* proteinase may be useful as an antigen for the serodiagnosis of paragonimiasis. Similar antigenicity of proteinases from other parasites have been reported by others (Ruppell *et al.*, 1985; Yamasaki *et al.*, 1989; Song *et al.*, 1992). Further more study for clarifying the sites secreted cysteine proteinase of the worms will require.

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=국문초록=

폐흡충(*Paragonimus westermani*)성충에서 정제한 cysteine proteinase의 특성

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자연 감염된 가재에서 폐흡충의 피낭유충을 분리하고 개에 경구 감염시켜 성충을 얻었다. 폐흡충 성충의 조효소를 ion-exchange chromatography, affinity chromatography와 gel filtration chromatography를 실시하여 cysteine proteinase를 순수 정제하였다. 이들 효소의 생화학적 특성과 분해능을 관찰하였으며, 효소면역전기영동이적법을 이용하여 순수 정제한 효소의 항원성을 관찰하였다. 정제된 효소는 저분자 합성기질인 CBZ-arg-arg-AFC 보다 CBZ-phe-arg-AFC에서 높은 활성을 보였으며, 이들 효소는 thiol-dependent이었다. 정제된 효소 및 조효소의 최적 pH는 5.5이었고, 최적 mole 농도는 0.1 M(0.1 M sodium citrate, pH 5.5)이었고, 이들 효소는 4°C에서 48시간 동안 80%의 안정성을 보였다. 정제된 효소의 native 분자량은 20,000 dalton이었고, SDS-PAGE 상에 나타난 분자량은 17,500 dalton이었다. 정제된 효소는 cysteine proteinase 특이 억제인자인 E-64, Iodoacetic acid, NEM에 의해 활성이 완전히 억제되었으며, serine proteinase, aspartic proteinase 및 metallo proteinase 특이 억제인자에 의해 활성이 억제되지 않았다. 정제된 효소는 collagen(Type I)과 hemoglobin을 분해하였고, 효소면역전기영동이적법으로 정제된 효소의 항원성을 확인하였다.

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