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Molecular Cloning of a cDNA Encoding a Cathepsin D Homologue from the Mulberry Longicorn Beetle, *Apriona germari*

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A cDNA encoding a cathepsin D homologue was cloned from a cDNA library of the mulberry longicorn beetle, *Apriona germari*. Sequence analysis of the cDNA encoding the cathepsin D homologue of *A. germari* revealed that the 1,158 bp cDNA has an open reading frame of 386 amino acid residues. The deduced protein sequence of the *A. germari* cathepsin D homologue shows high homology with cathepsin D in insects, *Aedes aegypti* (68.2% amino acid similarity) and *Drosophila melanogaster* (67.2% amino acid similarity). Two aspartic residues and six cystein residues in the *A. germari* cathepsin D homologue are present at identical locations in all of the other catepsins D. Unlike cathepsins D in two insect species, *A. germari* cathepsin D homologue appears to have two putative glycosylation sites, rather than one. Phylogenetic analysis revealed the *A. germari* cathepsin D homologue is more closely related to insect cathepsins D than to the other animal cathepsins D. Northern blot analysis suggests that *A. germari* cathepsin D homologue gene is expressed in most, if not all, body tissues.

Key words : Mulberry longicorn beetle, *Apriona germari*, cDNA cloning, Cathepsin D

Introduction

Aspartic proteases have been classified into several subgroups including the cathepsins, renins, pepsins and chymosins. Cathepsin D (EC 3.4.23) is a member of the aspartic proteases, proteolytic enzymes of the pepsin family (Barrett, 1977). The cathepsin D is present in most cells of many species and is probably one of the major factors contributing to lysosomal digestive activity (Metcalf and Fusek, 1993; Tsuji and Akasaki, 1994). The cathepsin D enzyme activity was elevated in a wide variety of tissues particularly during tissue remodeling or regression as well as in apoptotic cells (Yang and Schnellmann, 1996).

Cathepsins D have been widely investigated in mammals and fishes, but limited in insects. In insect, cathepsins D have been identified and characterized only in two insect species such as *Aedes aegypti* and *Drosophila melanogaster* (Cho and Raikhel, 1992; Page-McCaw *et al.*, 1999).

The Cerambycidae, commonly known as long-horned beetles is one of the largest groups in Coleoptera. The family has about 20,000 species throughout the world and most species of the family are wood-bores (Crowson, 1981; Daly *et al.*, 1998; Yoon *et al.*, 2001). The larvae generally bore into wood and last for several years. Although the long-horned beetles are known as the largest groups in Coleoptera, little is known at the molecular level of genetic information in the long-horned beetles.

Of the long-horned beetles, the mulberry longicorn beetle, *Apriona germari*, is a serious pest of mulberry tree. *A. germari* larvae hide in the trunk and feed on it. Also *A. germari* passes a long developmental period to complete its life cycle in the mulberry fields. Recently, the life history of *A. germari* has been mostly studied in the laboratory on an artificial diet rearing (Yoon and Mah, 1999).

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In order to obtain genetic information on the mulberry longicorn beetle, we have constructed cDNA library from the larvae whole body of *A. germari*. We have cloned and characterized a cDNA encoding the cathepsin D homologue from the cDNA library of *A. germari*. In this paper, the cloning, sequencing and characterization of the *A. germari* cathepsin D homologue gene are described.

Materials and Methods

Animals

The larvae of the mulberry longicorn beetle, *Apriona germari* were collected from the mulberry tree branch of the wild mulberry tree field in Korea. *A. germari* was reared on an artificial diet as described previously (Yoon and Mah, 1999).

cDNA library screening and nucleotide sequencing

A cDNA library was constructed from the poly(A)+ mRNA isolated from the whole body of *A. germari* larvae by Uni-ZAP XR vector and Gigapack III Gold Packing Extract (Stratagene). The cDNA was ligated into *Eco*RI-*Xho*I sites of Uni-ZAP XR vector. Ligated library was transformed into *E. coli* XL1-Blue MRF strain. *E. coli* XL1-Blue MRF strain was infected by the Uni-ZAP XR library harboring *A. germari* cDNA and cultured on the NZY agar medium. Each plaque was suspended in SM buffer [5.8 g/l NaCl, 2 g/l $MgSO_4 \cdot 7H_2O$, 0.05 M TrisCl (pH 7.5) and 0.01% gelatin solution] containing 0.02% (v/v) chloroform and stored at 4°C for 1 day. The phages were eluted into SM buffer. The pBluescript phagemids were *in vivo* excised from the Uni-ZAP XR vector using an ExAssist helper phage. *E. coli* strain, SOLR cell (Stratagene), was infected by the excised phagemids and plated on LB-Amp medium (50 μg/ml ampicillin). Plasmid DNA from the overnight culture was isolated. The size of inserted cDNA was estimated with a 1% agarose gel electrophoresis after treatment of restriction enzymes (*Eco*RI and *Xho*I). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program.

Phylogenetic analysis

With the ten GenBank-registered amino acid sequences of cathepsin D genes, phylogenetic analysis among the deduced amino acid sequences was performed with the PAUP (Phylogenetic Analysis using Parsimony) version 3.1 (Swofford, 1990). The accession numbers of the sequences in the GenBank are as follows: *A. germari* (AF454831, this study), *A. aegypti* (M95187), *D. melanogaster* (AF220040), rainbow trout *Oncorhynchus mykiss* (U90321), Atlantic herring *Clupea harengus* (AF312364), icefish *Chionodraco hamatus* (AJ007878), chicken *Gallus gallus* (S49650), human *Homo sapiens* (M63138), lizard *Podarcis sicula* (AJ009838), Norway rat *Rattus norvegicus* (X54467), and house mouse *Mus musculus* (X68378).

RNA isolation and Northern blot analysis

Total RNAs were isolated from the whole body, fat body, midgut and epidermis of the *A. germari* larvae by using the Total RNA Extraction Kit (Promega, Madison, WI). Total RNAs (10 μg/lane) from the larvae of *A. germari* were denatured by glyoxalation (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a buffer containing 2 × PIPES, 50% formamide, 1% sodium dodecyl sulphate (SDS) and blocking agent (Boehringer Mannheim, Mannheim, Germany). The probe used to detect the cathepsin D homologue gene transcripts was a 1,218 bp *A. germari* cathepsin D homologue gene cloned in this study and labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) at 65°C, and finally exposed to *X*-ray film. For rehybridization, the membrane was washed for 20 min at room temperature in sterile millipore water. Then the membrane was washed overnight at 65°C in 50 mM Tris-HCl (pH 8.0), 50% dimethylformamide and 1% SDS in order to remove the hybridized probe. The membrane was then rehybridized to [α-^{32}P] dCTP-labeled 16S rRNA probe. The 16S rRNA gene was used as an internal loading control.

Results and Discussion

Sequence of *A. germari* cathepsin D homologue gene and its deduced primary structure

Construction of cDNA library was prepared from the whole body of *A. germari* larvae. The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the *A. germari* ESTs (expressed sequence tags). Of these ESTs, one exhibited similarity to the reported cathepsins D. The complete DNA sequence

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-60  AAAATCATAGAACAGTAGTTCAGACTTTTTCGCTGATTGATATACAAAAGCAGCTATAAA
  1  ATGTCACGATTATTTTTAATGTCAGTTTTCTGCGTGTTTATTACAGTAAACTGCGATTTA
  1  M  S  R  L  F  F  M  S  V  F  C  V  F  I  T  V  N  C  D  L

 61  ATTAGGGTTCCTCTAGAACGCGGTAAATCCGCGCGAAGGACTTTACAAGAAGTCAATACC
 21  I  R  V  P  L  E  R  G  K  S  A  R  R  T  L  Q  E  V  N  T

121  CACGTACAACAAGTTAGGTTCAGGTACGGAGTCGGAGGTCCAGCTCCAGAACCTCTATCA
 41  H  V  Q  Q  V  R  F  R  Y  G  V  G  G  P  A  P  E  P  L  S

181  AATTATTTAGACGCTCAATACTTTGGACCGATATCAATTGGAAATCCCCCACAGAAATTT
 61  N  Y  L  D  A  Q  Y  F  G  P  I  S  I  G  N  P  P  Q  K  F

241  AAGGTGGTCTTCGACACTGGTTCGTCGAATTTGTGGGTACCCTCTAAAAAGTGCCACTAT
 81  K  V  V  F  D  T  G  S  S  N  L  W  V  P  S  K  K  C  H  Y

301  ACCAACATCGCCTGTCTTTTACATAATAAATATGACTCCTCGAAATCTTCCACCTATAAG
101  T  N  I  A  C  L  L  H  N  K  Y  D  S  T  K  S  S  T  Y  K

361  AAGAATGGAACTGACTTCTCCATCAAATATGGTTCTGGAAGTCTCAGCGGATTTTTATCT
121  K  N  G  T  D  F  S  I  K  Y  G  S  G  S  L  S  G  F  L  S

421  ACCGACGTTGTAACGGTTGGTTCACTTGCTGTTAAAGACCAAACCTTCGCAGAGGCCATG
141  T  D  V  V  T  V  G  S  L  A  V  K  D  Q  T  F  A  E  A  M

481  AGCGAACCGGGTCTCGCTTTTGTAGCCGCCAAATTCGACGAGTATCCTTGGCATGGCCTA
161  S  E  P  G  L  A  F  V  A  A  K  F  D  E  Y  P  W  H  G  L

541  CAGCAGGATCTCGGTAGTAGAGCGTCACTCCCGTTTTTTTATAACATGATCACTCAAGGA
181  Q  Q  D  L  G  S  R  A  S  L  P  F  F  Y  N  M  I  T  Q  G

601  CTTGTGTCACAACCGGTCTTTTCATTCTACTTAAATAGAGATCCAGATGCTGCCGAAGGT
201  L  V  S  Q  P  V  F  S  F  Y  L  N  R  D  P  D  A  A  E  G

661  GGCGAGCTGAGCCTTGGCGGATCCGATCCCAAATACTACAAAGGCAATTTCACTTACCTC
221  G  E  L  S  L  G  G  S  D  P  K  Y  Y  K  G  N  F  T  Y  L

721  TCGGTGGACCGACAGGCCTATTGGCAATTCAAGATGGATAAGATTCAACTAGGCAAAACT
241  S  V  D  R  Q  A  Y  W  Q  F  K  M  D  K  I  Q  L  G  K  T

781  GTCTTTTGCAAAAGCGGTTGCCAAGCTATCGCTGATACGGGCACCAGTTTGGTTGCTGGG
261  V  F  C  K  S  G  C  Q  A  I  A  D  T  G  T  S  L  V  A  G

841  CCTGTAGATGAAGTCACATCGATCAACAAACTAATTGGTGGCACTCCGATAATAGGTGGA
281  P  V  D  E  V  T  S  I  N  K  L  I  G  G  T  P  I  I  G  G

901  GAATATGTGGTAGACTGCTTAATTCCAAAGTTACCCGAAATTGACTTTATTTTGGGTGGG
301  E  Y  V  V  D  C  L  I  P  K  L  P  E  I  D  F  I  L  G  G

961  AAAACTTACACATTAGAAGGAAAAGACTATATTCTCAGAGTTTCCCAAGCCGGGAAAACA
321  K  T  Y  T  L  E  G  K  D  Y  I  L  R  V  S  Q  A  G  K  T

1021 ATATGTCTCTCCGGCTTCATGGGCATCGACATTCCCCCTCCAAACGGTCCTCTGTGGATC
341  I  C  L  S  G  F  M  G  I  D  I  P  P  P  N  G  P  L  W  I

1081 TTGGGAGACGTTTTCATAGGGAAGTTCTACACCGAATTCGATCTTGGCAATAATCGCATC
361  L  G  D  V  F  I  G  K  F  Y  T  E  F  D  L  G  N  N  R  I

1141 GGTTTCGCCGAAGCTGCTTAAACAGTCTCCTATTCAATCCAAAGATTAGCTTGTATTCAT
381  G  F  A  E  A  A  *
1201 TATAGTTTGCTCTCTTTTCACGTCTATTGAGAAGAAATGATAAATTCTTGTGTGATTTTA
1261 GAATAAACTAGGTTATTTTTAAGTAAAGTTCTACCAATTTATATCACTTTAATTTGTTTG
1321 ATTAGAATAAAATAATGCTGTACCAAATAAAAAAAAAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of *A. germari* cathepsin D homologue gene. The start codon of ATG is boxed and the termination codon is asterisk. The polyadenylation signal AATAAA is underlined. The two catalytic aspartyl residues are marked with solid squares and the two putative glycosylation sites with solid triangles. The six cystein residues are marked with open triangles. The putative signal peptide cleavage site is marked with arrow. The GenBank accession number is AF454831.

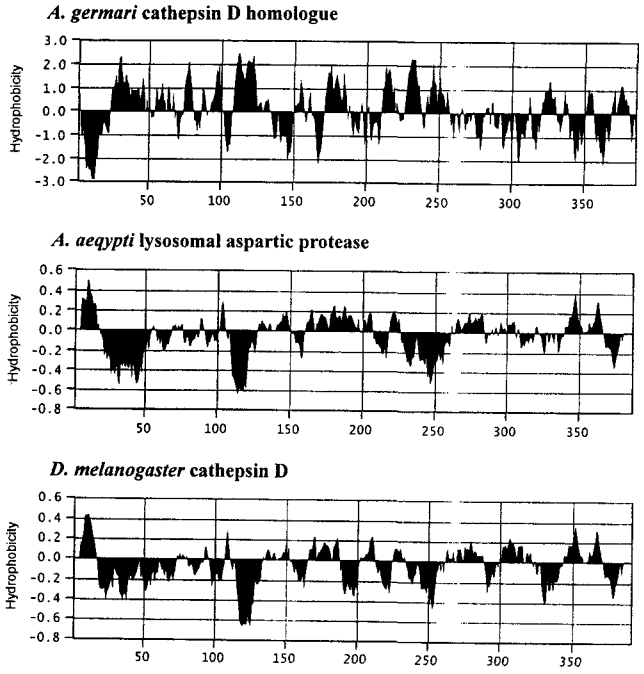

Fig. 2. The hydropathy profile of *A. germari* cathepsin D homologue and known insect cathepsins D. Hydropathic analysis was done as described by Kyte and Doolittle (1982). The known insect cathepsins D were: *A. aegypti* (Cho and Raikhel, 1992) and *D. melanogaster* (Page-McCaw *et al.*, 2000).

of a cDNA encoding a putative member of the insect cathepsin D gene family revealed that the 1,158 bp cDNA has an open reading frame of 386 amino acid residues, which we designated AgCatD (GenBank accession number AF454831) (Fig. 1). In the AgCatD gene sequence, a polyadenylation signal AATAAA was found at nucleotides 1,326, sixteen nucleotides upstream of the polyadenylation site. The AgCatD contains all the typical features of aspartic proteases, including the two aspartic residues that are involved in the catalytic mechanism. The two aspartic residues of the AgCatD are present at the same position in all catepsins D of the other organisms (Capasso *et al.*, 1999).

The hydropathy plot of AgCatD was analyzed by the Kyte and Doolittle method (Kyte and Doolittle, 1982), and compared with other insect cathepsins D with a high homology. As shown in Fig. 2, hydropathic analysis suggests the profile of AgCatD is similar to known insect cathepsins D such as *A. aegypti* and *D. melanogaster* (Cho and Raikhel, 1992; Page-McCaw *et al.*, 1999). Considering the amino acid sequence of other cathepsins D, AgCatD suggests an enzyme consisted of a putative signal peptide of 19 amino acids, a propeptide of 33 amino acids and a mature protein of 334 amino acids. The predicted molecular mass of the mature enzyme from the deduced amino acid sequence is approximately 36.3 kDa.

A multiple sequence alignment of the deduced protein sequence of AgCatD gene with other cathepsin D sequences is shown in Fig. 3. Alignment of the AgCatD sequence with those for cathepsin D from several other species indicates the extent of the identity that exists. All six cystein residues in the AgCatD were conserved at the same position among the species aligned (Capasso *et al.*, 1999; Cho and Raikhel, 1992; Page-McCaw *et al.*, 1999). The two putative glycosylation sites were identified in the sequence of the AgCatD enzyme. The first putative glycosylation

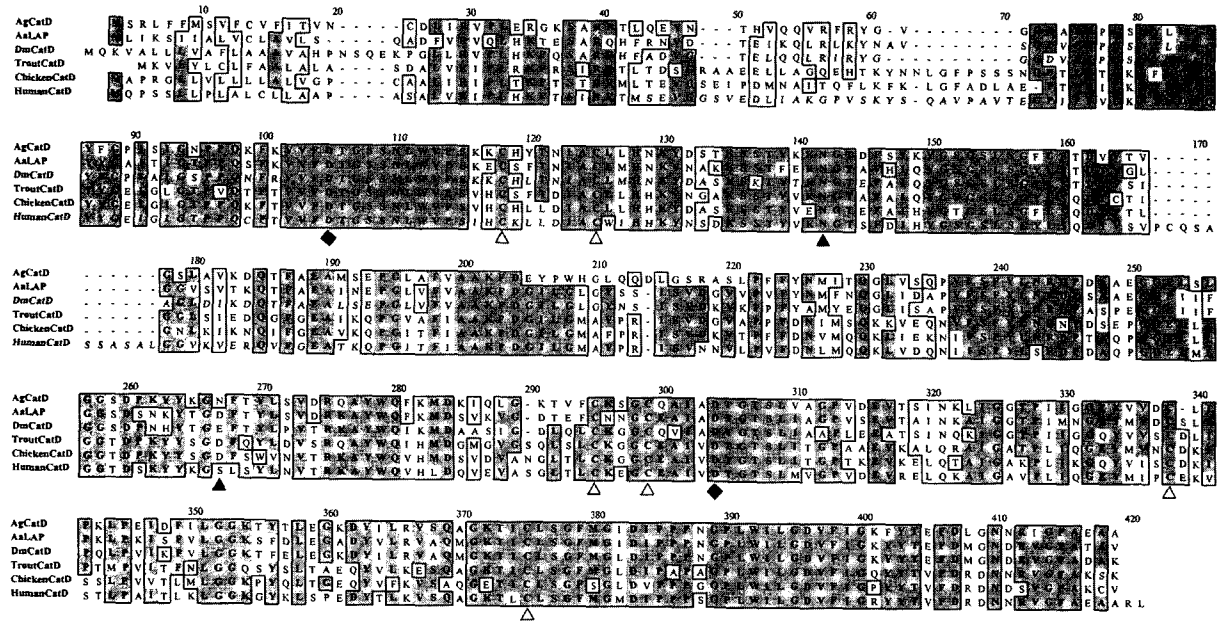

Fig. 3. Multiple sequence alignment of the deduced protein sequence of the *A. germari* cathepsin D homologue gene with other cathepsin D protein sequences. In solid box are the residues that are identical to those in *A. germari* cathepsin D homologue protein (AgCatD). The other cathepsin D protein sequences aligned were: *A. aegypti* (AaAspp; Cho and Raikhel, 1992), *D. melanogaster* (DmCatD; Page-McCaw *et al.*, 2000), *O. mykiss* (TroutCatD; Brooks *et al.*, 1997), *G. gallus* (ChickenCatD; Retzek *et al.*, 1992) and *H. sapiens* (HumanCatD; Redecker *et al.*, 1991). The two catalytic aspartyl residues are marked with solid squares and the two putative glycosylation sites with solid triangles. The six cystein residues are marked with open triangles.

Table 1. Pairwise comparison among amino acid sequences of the *A. germari* cathepsin D gene and the known cathepsin D genes

Species	GenBank No.	1	2	3	4	5	6	7	8	9	10	11
1. *A. germari*	AF454831	-	0.318	0.328	0.449	0.456	0.466	0.470	0.473	0.473	0.475	0.487
2. *A. aegyti*	M95187	134	-	0.280	0.416	0.430	0.435	0.451	0.458	0.447	0.454	0.451
3. *D. melanogaster*	AF220040	138	118	-	0.430	0.449	0.461	0.435	0.492	0.456	0.470	0.480
4. Trout	U90321	189	175	181	-	0.169	0.219	0.318	0.359	0.352	0.347	0.375
5. Herring	AF312364	192	181	189	71	-	0.233	0.352	0.356	0.352	0.352	0.368
6. Icefish	AJ007878	196	183	194	92	98	-	0.354	0.390	0.373	0.373	0.399
7. Chicken	S49650	198	190	183	134	148	149	-	0.325	0.247	0.325	0.333
8. Human	M63138	199	193	207	151	150	164	137	-	0.375	0.183	0.190
9. Lizard	AJ009838	199	188	192	148	148	157	104	158	-	0.361	0.373
10. Rat	X54467	200	191	198	146	148	157	137	77	152	-	0.088
11. Mouse	X68378	205	190	202	158	155	168	140	80	157	37	-

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

site was present at the identical location in other cathepsins D, but the second site of other cathepsin D was different from the AgCatD (Capasso *et al.*, 1999). The noticeably difference in the putative glycosylation site compared with two known insect cathepsins D with only single glycosylation site is that showed the second putative glycosylation site in the AgCatD (Cho and Raikhel, 1992; Page-McCaw *et al.*, 1999).

The deduced protein sequence of the AgCatD had a high homology with that of cathepsins D from other animals (Table 1). This alignment illustrates that AgCatD is 68.2% identical to the *A. aegypti* and 67.2% identical to the *D. melanogaster*. The AgCatD also had 51.3% to 55.1% amino acid identity with the other animals.

A phylogenetic tree was constructed using the protein sequences of cathepsins D (Fig. 4). The phylogenic anal-

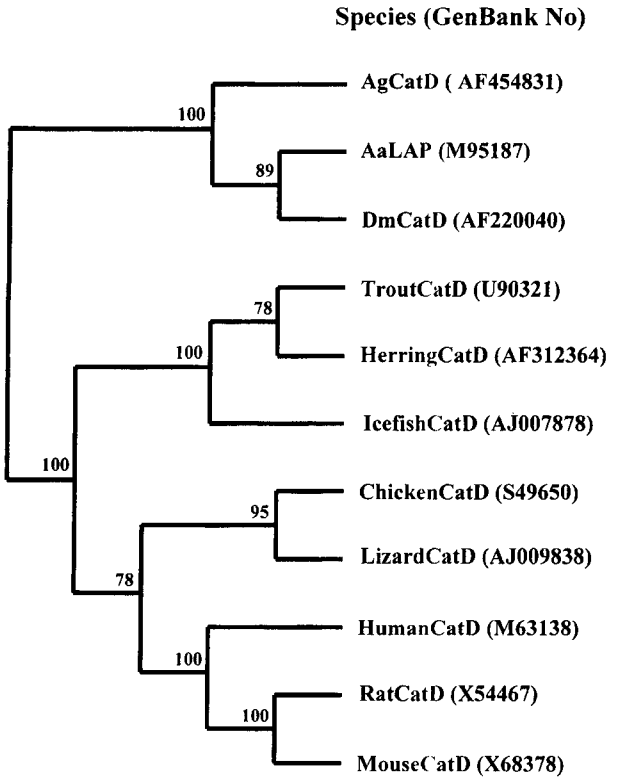

Fig. 4. Phylogenetic tree for aligned amino acid sequences of the *A. germari* cathepsin D homologue protein and the known cathepsin D proteins. The sequences were extracted from; *A. germari* (this study), *A. aegypti* (Cho and Raikhel, 1992), *D. melanogaster* (Page-McCaw *et al.*, 2000), *O. mykiss* (Brooks *et al.*, 1997), *C. harengus* (Nielsen *et al.*, 2000), *C. hamatus* (Capasso *et al.*, 1999), *G. gallus* (Retzek *et al.*, 1992), *P. sicula* (De Stasio *et al.*, 1999), *H. sapiens* (Redecker *et al.*, 1991), *R. norvegicus* (Birch and Loh, 1990), and *M. musculus* (Hetman *et al.*, 1994). The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates.

ysis revealed that AgCatD is closer to cathepsins D of insects, *A. aegypti* and *D. melanogaster* than other animal cathepsins D, forming insect cathepsin D one separate group.

Expression of *A. germari* cathepsin D homologue gene at the transcriptional level

To confirm the expression of the AgCatD gene at the transcriptional level, Northern blot analysis was carried out using the mRNA prepared from the three distinct tissues such as fat body, midgut and epidermis (Fig. 5). A hybridization signal was clearly detected as a single band in the mRNA from whole body as a positive control, fatbody and midgut. Weak but obvious hybridization signal of AgCatD gene was also detected in the epidermis. These

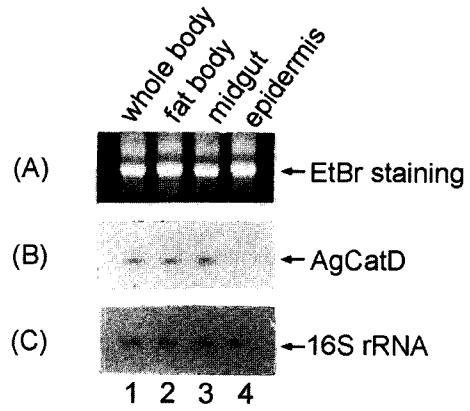

Fig. 5. Northern blot analysis of the *A. germari* cathepsin D homologue gene. Total RNAs were isolated from the whole body (lane 1), fat body (lane 2), midgut (lane 3) and epidermis (lane 4). The RNAs were separated by 1.0% formaldehyde agarose gel electrophoresis (A), transferred on to a nylon membrane, and hybridized with the radiolabelled 1,218 bp cathepsin D homologue gene (B). The 16S rRNA gene was used as an internal loading control (C). Transcripts of the cathepsin D homologue gene are indicated on the right of panel by an arrow.

results collectively suggests that the AgCatD gene is expressed in most, if not all, body tissues. The result is similar to the previous reports in that cathepsin D is a housekeeping gene common to most, if not all, body tissues (Brooks *et al.*, 1997; Hetman *et al.*, 1994).

In conclusion, we showed gene nucleotide sequence of the cDNA encoding a cathepsin D homologue from the mulberry longicorn beetle, *A. germari*, and expression of the gene in most body tissues for the first time.

References

Barret, A. J. (1977) Cathepsin D and other carboxyl proteinases; in *Proteinases in Mammalian Cells and Tissues*. Barrett, A. J. (ed.), pp. 209-248, Elsevier, Amsterdam.

Birch, N. P. and Y. P. Loh (1990) Cloning, sequence and expression of rat cathepsin D. *Nucleic Acids Res.* **18**, 6445-6446.

Brooks, S., C. R. Tyler, O. Carnevali, K. Coward and J. P. Sumpter (1997) Molecular characterization of ovarian cathepsin D in the rainbow trout, *Oncorhynchus mykiss*. *Gene* **201**, 45-54.

Capasso, C., W. E. Lees, A. Capasso, R. Scudiero, V. Carginale, P. Kille, J. Kay and E. Parisi (1999) Cathepsin D from the liver of the Antarctic icefish *Chionodraco hamatus* exhibits unusual activity and stability at high temperatures. *Biochem. Biophys. Acta* **1431**, 64-73.

Cho, W. L. and A. S. Raikhel (1992) Cloning of cDNA for mosquito lysosomal aspartic protease. *J. Biol. Chem.* **267**,

21823-21829.

Crowson, R. A. (1981) The biology of the Coleoptera. Academic Press. London.

Daly, H. V., J. T. Doyen and A. H. Purcell III (1998) Introduction to insect biology and diversity. 2nd edition, Oxford University Press, London.

De Stasio, R., L. Borrelli, P. Kille, E. Parisi and S. Filosa (1999) Isolation, characterization and molecular cloning of cathepsin D from lizard ovary: changes in enzyme activity and mRNA expression throughout ovarian cycle. *Mol. Reprod. Dev.* **52**, 126-134.

Hetman, M., A. Perschl, P. Saftig, K. Vonfigura and C. Peters (1994) Mouse cathepsin D gene-molecular organization, characterization of the promoter, and chromosome localization. *DNA Cell Biol.* **13**, 419-427.

Kyte, J. and R. F. Doolittle (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132.

McMaster, G. K. and G. G. Carmichael (1977) Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. USA* **74**, 4835-4838.

Metcalf, P. and M. Fusek (1993) Two crystal structures for cathepsin D: the lysosomal targeting signal and active site. *EMBO J.* **12**, 1293-1302.

Nielsen, L. B., P. Stougaard, P. S. Andersen and L. H. Pedersen (2000) Cloning and sequence determination of herring muscle cathepsin D. GenBank accession number AF312364.

Page-McCaw, A. W., G. Tsang and G. M. Rubin (2000) A Drosophila gene related to cathepsin D. GenBank accession number AF220040.

Redecker, B., B. Heckendorf, H. W. Grosch, G. Mersmann and A. Hasilik (1991) Molecular organization of the human cathepsin D gene. *DNA Cell Biol.* **10**, 423-431.

Retzek, H., E. Steyrer, E. J. Sanders, J. Nimpf and W. J. Schneider (1992) Molecular cloning and functional characterization of chicken cathepsin D, a key enzyme for yolk formation. *DNA Cell Biol.* **11**, 661-672.

Swofford, D. L. (1990) PAUP: phylogenetic analysis using parsimony, ver. 3.0. Illinois Natural History Survey, Champaign (on disk).

Tsuji, H. and K. Akasaki (1994) Identification and characterization of lysosomal enzymes involved in the proteolysis of phenobarbital-inducible cytochrome P450. *Biol. Pharm. Bull.* **17**, 568-571.

Yang, X. and R. G. Schnellmann (1996) Proteinases in renal cell death. *J. Toxicol. Environ. Health* **48**, 319-332.

Yoon, H. J. and Y. I. Mah (1999) Life cycle of the mulberry longicorn beetle, *Apriona germari* Hope on an artificial diet. *J. Asia-Pacific Entomol.* **2**, 169-173.

Yoon, H. J., J. S. Bae, I. Kim, B. R. Jin, Y. I. Mah, J. Y. Moon and H. D. Sohn (2001) A phylogenetic study in some long-horned beetle (Coleoptera: Cerambycidae) using mitochondrial COI gene and 16S rRNA sequences. *Int. J. Indust. Entomol.* **2**, 37-53.