Primary Structure and mRNA Expression of Carp (*Cyprinus carpio*) Cathepsin B

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We cloned a cathepsin B cDNA from the muscle of carp. The nucleotide sequence of carp cathepsin B cDNA consisted of 1470 bp including a 993 bp open reading frame, encoding a deduced protein of 330 amino acids. The deduced amino acid sequence of carp cathepsin B has similarity of 80 % to that of rainbow trout cathepsin B and of 76-79% to mammalian cathepsins B. Based on a multiple sequence alignment, carp cathepsin B was considered to work in two-chain form consisting of a light chain and a heavy chain. Tissue distribution of the carp cathepsin B mRNA has been studied by northern blot analysis. The result revealed that cathepsin B was constitutively expressed in every tissue, and the relative amount of cathepsin B mRNA expression showed decreased in the order of ovary, gill, intestine, testis, hepatopancreas, spleen, kidney, heart, and brain. A high expression of cathepsin B transcripts in ovary might be necessary for yolk formation.

Key Words: cathepsin B, carp, primary structure, two-chain form, northern blot analysis

Introduction

Cathepsin B [EC 3. 4. 22. 1] is a lysosomal cysteine protease with dual activities of endopeptidase and dipeptidylcarboxypeptidase, and it is usually considered to be an essential enzyme, which functions throughout the process of protein degradation in lysosome. It is also believed that cathepsin B is concerned with skeletal myoblast differentiation,[1] antigen presentation in the immune response,[2-5] hormone activation[6-9] and tissue remodeling, such as bone matrix resorption.[10,11] Many studies have shown that under the special conditions the increased expression or secretion outside lysosomes of cathepsin B cause pathological conditions such as arthritis and pancreatitis.[10,12] Also, because cathepsin B is a tumour-associated cysteine peptidase, which plays an important role in the proteolytic cascade involved in cancer invasion,[10,11] many researchers are interested in the application of cathepsin B determination to the diagnosis of cancer.[13] In food science, possibility of using cathepsin B as a candidate gene for meat quality in dry-cured ham production was investigated.[14] As an important enzyme both in medicine and in food science, mammalian cathepsin B is well studied in structure, tissue distribution, gene structure and altered expression in pathological conditions, *etc.* In fish, it is known to be an enzyme related to soften muscle,[15] however, there is few reports about the structure and tissue distribution of fish cathepsin B. Here, to clarify the primary structure of carp cathepsin B, we determined the complete nucleotide sequence of carp cathepsin B based on the genetic information of conserved regions from other cathepsins B and the N-terminal amino acid sequence. Also we studied the tissue distribution of carp cathepsin B mRNA by northern blot analysis and discussed its physiological functions based on our data and previous reports.

Materials and Methods

Materials

Cultured carp *Cyprinus carpio* (body weight 800-900 g) were obtained from Koda Suisan Co., Nagasaki, Japan. Ten kinds of tissues (hepatopancreas, spleen, kidney, intestine, muscle, heart, gill, brain, ovary and testis) of the carp were collected and immediately frozen in liquid nitrogen.

Molecular cloning of carp cathepsin B cDNA

1) *RNA isolation and cDNA synthesis*

Total RNA was extracted from fresh carp muscle (1 mg) with an ISOGEN kit (Nippon Gene, Toyama, Japan). The RNA was first denatured and then reverse transcribed with SuperScript RT (Gibco BRL, Paisley, UK) using the oligo (dT)-adaptor primer (5'-GGCCACGCGTCGACTAG TAC$(T)_{17}$-3') for first-stranded cDNA synthesis.

2) *Reverse transcription-polymerase chain reaction (RT-PCR)*

DNA sequences of five other cathepsins B (rainbow trout, chicken, mouse, bovine, and human) were got from international DNA database (DDBJ /EMBL /GenBank). For initial RT-PCR, primers were designed based on conserved sequences of other cathepsins B. The degenerate

oligonucleotide primer sequences were: 5'-AT(A/T/C)(A/C)G(A/T/C/G)GA(C/T)CA(A/G)GG(A/T/C/G)(A/T)(C/G)(A/T/C/G)TG(C/T)GG-3' for the sense primer and 5'-CC(A/C/T/G)C(T/G)(A/C/T/G)A(A/G)(A/T/G)AT(C/T)TT(A/G)AA(A/G)AA(A/T/C/G)CC-3' for the antisense primer. PCR was carried out in the reaction mixture to a final volume of 50 μL, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 1 μM of each primer, 5 μL of cDNA, and 5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems Inc., Forster city, CA, USA). Parameters used for PCR amplification were 9 min at 94 ℃ for 1 cycle, and 1 min at 94 ℃, 1 min at 50 ℃, and 2 min at 72 ℃ for 40 cycles, followed by a final extension period of 7 min at 72 ℃. The PCR reaction was performed using Gene Amp PCR System 2400 (Applied Biosystems Inc., Forster city, CA, USA). The PCR products were purified from an agarose gel and cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA). Plasmids were prepared for double-stranded DNA sequencing, using an alkaline-SDS method.

3) *5' and 3'-rapid amplification of cDNA ends (RACE)*

The first-stranded cDNA for 5'RACE was synthesized using a specific primer (5'-CGCAAGAACCCTGGTCTCTG-3') and added poly (A) into the 3'-terminus by terminal deoxynucleotidyl transferase (GIBCO BRL, Paisley, UK). The PCR reaction used both the sense primers (5'-GGCCACGCGTCGACTAGTAC $(T)_{17}$-3') and the antisense primers (5'-TCTGATCTCTTTAAGAGTGGG-3'). Parameters used for PCR amplification were 9 min at 94 ℃ for 1 cycle, and 1 min at 94 ℃, 1 min at 45 ℃, and 2 min at 72 ℃ for 40 cycles, followed by a final extension period of 7 min at 72 ℃. 3'RACE was amplified using both the sense (5'-CAGCATGTGAGTGGACCTGC-3') and the antisense primers (5'-GGCCACGCGTCGACTAGTAC-3'). Parameters used for PCR amplification were 9 min at 94 ℃ for 1 cycle, and 1 min at 94 ℃, 1 min at 55 ℃, and 2 min at 72 ℃ for 40 cycles, followed by a final extension period of 7 min at 72 ℃. The PCR reactions were performed using GeneAmp PCR System 2400 (Applied Biosystems Inc., Forster city, CA, USA). Both the 5'-RACE (450 bp) and the 3'-RACE (860 bp) products were cloned into pGEM-T Easy vector and the sequences of three independent clones were verified.

4) *DNA sequencing and analysis*

DNA sequencing was performed by the dideoxy chain termination method using BigDye™ Terminator Cycle Sequencing FS Ready Reaction Kits (Applied Biosystems Inc., Forster city, CA, USA) with a DNA sequencer (model 377, Applied Biosystems Inc., Forster city, CA, USA). Sequence analysis and comparisons were performed with DNASIS-MAC Ver 3.6 (Hitachi Software, Kanagawa, Japan). Predictions of signal peptides were made using SignalP (v.3.0) software, provided interactively on the center of Technical University Denmark server (www.cbs.dtu.dk/services/SignalP/).

The nucleotide sequence data reported in the present paper have been deposited in DDBJ /EMBL /GenBank databases under accession number AB215097.

Northern blot analysis

Total RNA was extracted from 0.2 g tissue with Trizol (Invitrogen life science Co., MD, USA). DIG-labeled cathepsin B probe was prepared by PCR using sense primer NTCBS1 (5'-CCACTAACTTTGATGCCAGAG-3'), antisense primer CAB-AP (5'-TTACTGTGGGATTCCAGCCAC -3') and PCR DIG Probe Synthesis Kit (Roche, Barsel, Switzerland). Parameters used for amplification were 2 min at 94 ℃ for 1 cycle, and 20 sec at 94 ℃, 30 sec at 53 ℃, and 2 min at 72 ℃ for 30 cycles, followed by a final extension period of 7 min at 72 ℃. The mRNA was purified with GeneElute mRNA mini Prep Kit (SIGMA-ALDRICH Co., MO, USA), and 2 μg mRNA was loaded in duplicate and run on 1% denatured formaldehyde agarose gel in gel-running buffer (0.04 M MOPS acid, 10 mM sodium acetate, 1 mM EDTA). Then the mRNA was transferred from the gel onto a nylon membrane (Pall Biosupport Co., NY, USA) for 2 h using a vacuum pump. After transfer, the mRNA was immobilized on the membrane by incubation at 80 ℃ for 2 h. Another membrane was prepared in the same method. The membranes were hybridized with the cathepsin B probe or β-actin probe overnight at 42 ℃ in 10 mL buffer (20×SSPE, 2.5 mL; formamide, 5 mL; distilled water, 1.5 mL; 10 % SDS, 0.5 mL; blocking reagent, 500 μL; probe, 1 μL). The membranes were washed three times in 1×SSPE / 0.1% SDS for 15 min and three times in 0.1×SSPE / 0.1% SDS for 15 min at 68 ℃, and then the membrane were blocked in 10 mL buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5) with 1 mL blocking reagent at 37 ℃ for 30 min and hybridized with 1 μL anti-DIG-AP Fab fragments at 37 ℃ for 1 h. The hybridization signals were detected by adding 30 μL CSPD (Roche Diagnostic, IN.,USA) in 3 mL detection buffer (0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5) at 37 ℃ for 15 min. The intensity of the bands were measured and quantified using image-analysis software CS analyzer (Ver. 1.02 a, ATTO Co., Tokyo, Japan).

Results

Primary structure of carp cathepsin B

The nucleotide and deduced amino acid sequences of

carp cathepsin B cDNA (1470 nucleotides) are shown in Fig. 1. The coding region for cathepsin B includes 993 nucleotides and corresponded to 330 amino acids (precursor protein). Based on a comparison of amino acid sequences from other sources, carp cathepsin B is predicted to have a processing site at Ala^{18}-Arg^{19} between pre-region and pro-region, and a processing site at Lys^{78}-Leu^{79} between pro-region and mature form. It is conceivable that the three

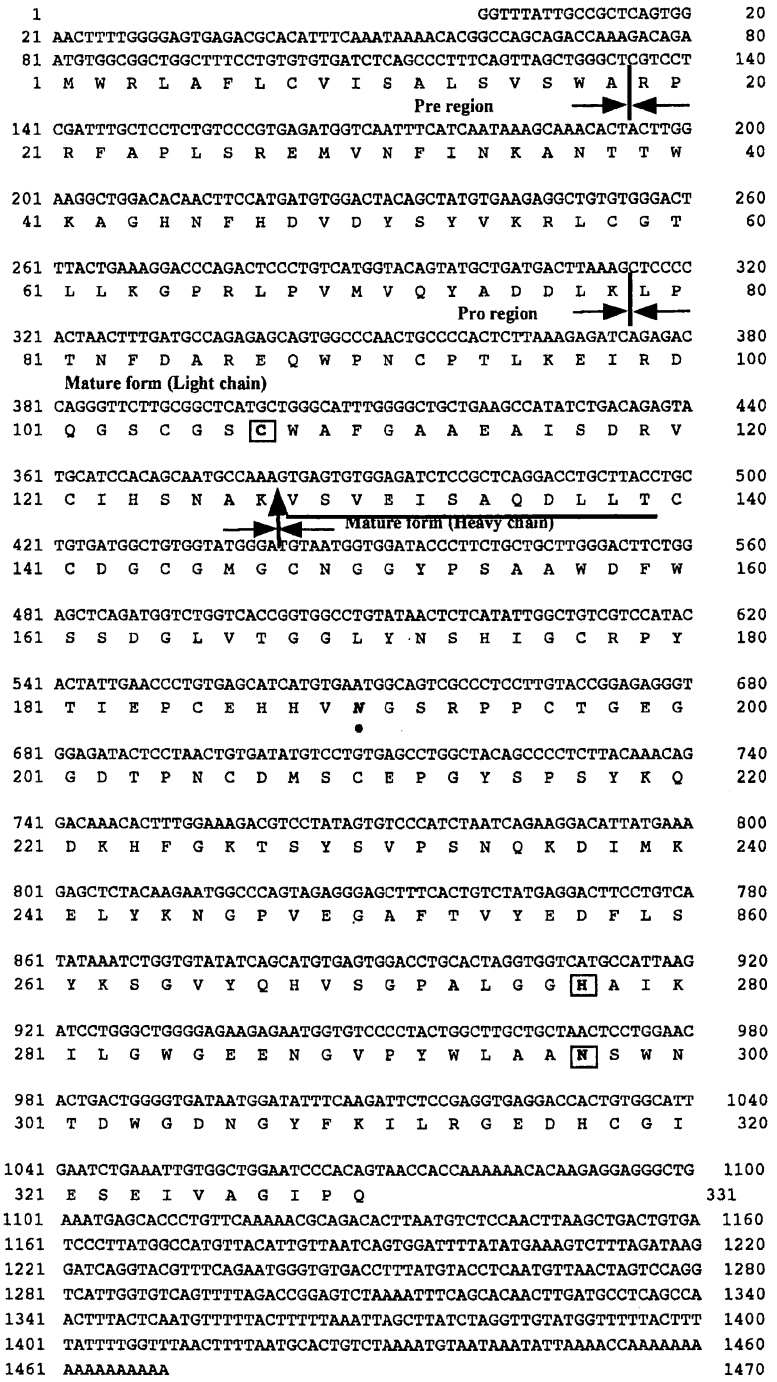

Fig. 1 Nucleotide and deduced amino acid sequences of carp cathepsin B cDNA.
The cleavage site between pre-region and pro-region, and the cleavage site between pro-region and mature enzyme, are marked by the line and arrows. The cleavage site between light chain and heavy chain is marked by the arrows. The N-terminal amino acid sequence of the purified cathepsin B heavy chain is underlined. The putative active site residues are indicated by the boxes and bold letters, the potential *N*-glycosylation site is indicated by the point and Italic letter.

active sites are Cys[107], His[277] and Asn[297]. The potential *N*-glycosylation site was Asn[190]. Therefore, the deduced amino acid sequence of cathepsin B cDNA consisted of a pre-region (18 residues), a pro-region (60 residues), and the full sequence of the mature form (252 residues).

Comparison of amino acid sequences of cathepsins B is shown in Fig. 2. Carp cathepsin B exhibited 80 % amino acid identity with rainbow trout cathepsin B,[16] 77 % with chicken cathepsin B,[17] and 76-78 % with mammalian cathepsins B.

mRNA expression of carp cathepsin B

Northern blot analysis revealed that cathepsin B is constitutively expressed in all the tissues studied (Fig. 3A). Fig. 3B shows the expression level of cathepsin B mRNA being normalized against β-actin expression. The relative amount of the expression decreased in the order of ovary, gill, intestine, testis, hepatopancreas, spleen, kidney, heart, and brain. It appears that there are significant differences of the relative amount in these tissues. Especially, the carp cathepsin B mRNA in ovary was expressed 28-fold as

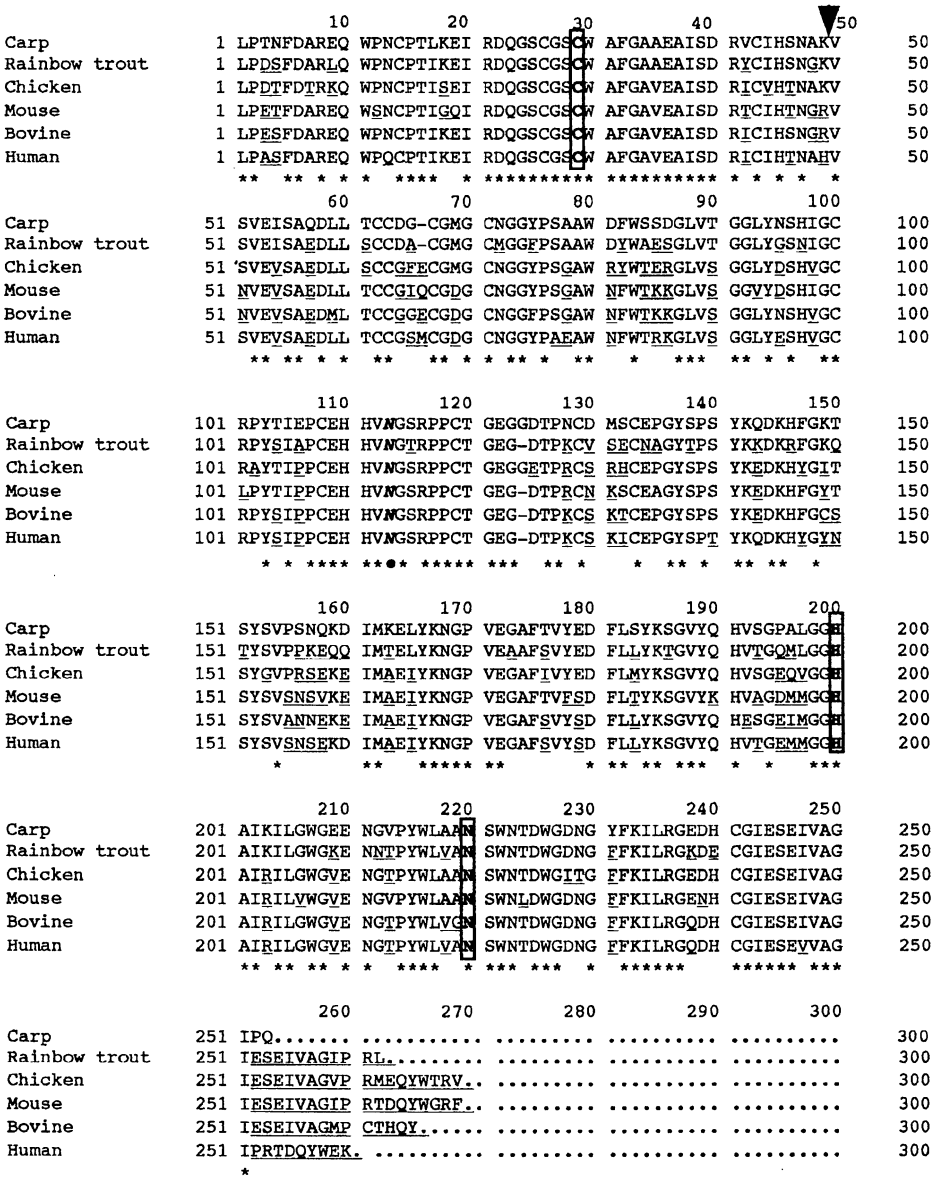

Fig. 2 Amino acid sequence alignment of carp muscle cathepsin B (mature form) and other cathepsins B. Rainbow trout,[16] chicken (GenBank accession number U18083), mouse (NM_007798), bovine (L06075), human (XM_035662). Identical residues and different residues with carp cathepsin B are marked with the asterisks and underlined, respectively. Active site residues of Cys, His, and Asn are indicated by the boxes and bold letters; *N*-glycosylation site are indicated by the point and Italic letter. The cleavage site between the light chain and heavy chain of mature enzyme is marked by the vertical arrow.

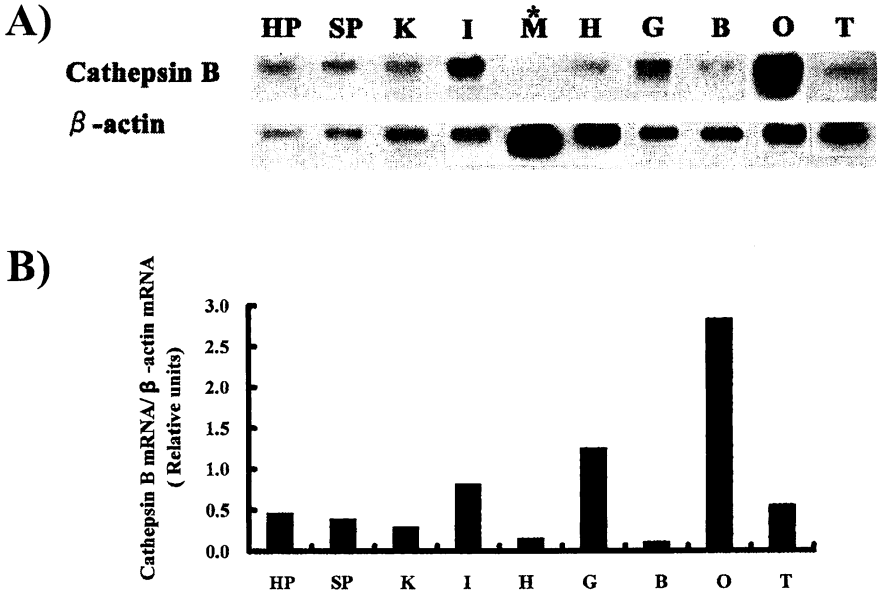

Fig. 3 A) Northern blot analysis of carp cathepsin B mRNA in various tissues. HP, hepatopancreas; SP, spleen; K, kidney; I, intestine; M, muscle; H, heart; G, gill; B, brain; O, ovary; T, testis. The mRNAs from each carp tissue were blotted onto a nylon membrane and hybridized with carp cathepsin B cDNA probe. The amounts of mRNAs were 5 μg for hepatopancreas, 1 μg for gill, 3 μg for other tissues. The amounts of mRNA hybridized with β-actin cDNA probe were 2.5 μg for hepatopancreas, 1 μg for gill, 3 μg for heart and testis, 1.5 μg for other tissues.
B) Quantitative analysis of cathepsin B mRNA in various tissues. Values are expressed as the relative ratio of cathepsin B mRNA against β-actin mRNA amount. HP, hepatopancreas; SP, spleen; K, kidney; I, intestine; H, heart; G, gill; B, brain; O, ovary; T, testis.
*In the case of muscle, we could not get the correct relative amount of cathepsin B mRNA against β-actin mRNA because the β-actin cDNA probe hybridized with not only β-actin but also the skeletal muscle actin at almost the same position, therefore the relative amount in muscle was not counted.

much as that in brain (calculated from value of band intensity measured using software CS analyzer).

Discussion

Mammalian cathepsin B is considered to function in two-chain form consisting of a light chain and a heavy chain. But for fish cathepsin B, the structure of active enzyme is still unknown. Comparing the amino acid sequence of cathepsins B at cleavage site between light chain and heavy chain, carp cathepsin B (Lys^{49}-Val^{50}) are similar to mammalian cathepsins B (Arg^{49}-Val^{50} of mouse and bovine cathepsins B), which are composed of a basic residue and a Val (Fig. 2). Therefore it is reasonable that carp cathepsin B acts in two-chain form, similar to mammalian cathepsin B.[18,19] This was supported by the result of SDS-PAGE of purified carp cathepsins B (data not shown). As indicated in Fig. 2, the N-terminal amino acid sequence of the 29 kDa band in SDS-PAGE under reducing conditions showed high homology with heavy chain of mammalian cathepsins B. Therefore, carp cathepsin B may work in two-chain form consisting of a light chain and a heavy chain bound by disulfide bond, like the mammalian cathepsin B.

To study the function of cathepsin B in carp, the mRNA expression of carp cathepsin B was analyzed by northern blot analysis. Though carp cathepsin B cDNA was cloned from muscle since cathepsin B was studied as an enzyme related to muscle softening, the expression level of cathepsin B mRNA was very low by northern blot analysis (Fig. 3A). On the other hand, it has been demonstrated that human cathepsin B exists in various tissues, such as hepatocytes, renal tubules, gastrointestinal epithelium, fibroblasts squamous epithelium, salivary glands and pancreas.[20] Rat cathepsin B mRNA is also expressed in a wide range of variation in the tissues and the highest levels is found in spleen and kidney.[21] Here the northern blot analysis indicates that cathepsin B is widely distributed in carp tissues as well (Fig. 3A). However, the relative amounts of the mRNA is different in tissues (Fig. 3B). Especially in ovary, gill, and intestine, the mRNA transcripts of cathepsin B appeared to be higher than those in other tissues. As cathepsin B has been proved to function in activation of proenzymes such as trypsinogen and prorenin,[12,22-25]

it is reasonable that cathepsin B mRNAs are abundantly transcribed in intestine which requires the activation of digestive enzymes. It is notable that the relative amounts of cathepsin B mRNA in ovary and gill are much higher than those in other tissues, for example, they are 28-fold and 12-fold higher than that in brain, respectively. This suggests that fish cathepsin B may participate in special cellular function. Zoltán *et al*, reported that the activity of cathepsin-B like enzyme localized in the endodermal cells which directly contact to the yolk when studying the yolk sac membrane of quail eggs.[26] The result indicated that cathepsin-B like enzyme has a significant correlation between growth of the yolk sac membrane and provide the amount of available free amino acids to support embryonic growth. In the study of the thiol protease of the yellow fever mosquito, cathepsin -B like enzyme was found to locate in the matrix surrounding the crystalline yolk protein, vitellin, and was involved in embryonic degradation of vitellin.[27] Moreover, the researches on yolk formation and degradation during oocyte maturation in sea bream[28] and on enzymes during oogenesis and embryogenesis in rainbow trout[29] have shown that the activity of cathepsin B or the amount of cathepsin B mRNA is significantly increased during oogenesis. This suggested that the activity of cathepsin B might be necessary for yolk formation. Cathepsin B in carp ovary may also have multiple functions, in yolk formation and embryonic growth. As for gill, the high level of cathepsin B mRNA may be relative with degradation of pathogen. The gill is constantly in contact with water which may contain pathogens. Fish gill is covered with only a thin layer of fragile cells to separate the vascular system from the external environment and proved to be capable of active taking up particles[30,31] or viral fish pathogens[32]. Mammalian cathepsin B is considered to act in antigen presentation.[2-5] Though further experiment is need to clarify the role of carp cathepsin B in gill, carp cathepsin B could have the similar function in the accessible site of pathogen. The wide variations in tissues of cathepsin B mRNA may be due to regulation by tissue specific controls. It suggests that cathepsin B has the special functions besides protein degradation. In rat the highest trancription level of cathepsin B was found in spleen and kidney compared with lung, brain, heart, liver, thymus, insulinoma, islets and pancreas.[21] On the other hand, their contents in carp was relativley low content although the reason is unclear.

To clarify the function of carp cathepsin B in tissues, it is necessary to investigate the protein amount and its enzymatic activity of cathepsin B.

References

1) D.T. Jane, L. DaSilva, J. Kobliński, M. Horwitz, B.F. Sloane, and M.J. Dufresne: Evidence for the involvement of cathepsin B in skeletal myoblast differentiation. J. Cell Biochem., **84**, 520-531 (2002).

2) C. Barrera, G. Ye, R. Espejo, S. Gunasena, R. Almanza, J. Leary, S. Crowe, P. Ernst, and E.V. Reyes: Expression of cathepsins B, L, S, and D by gastric epithelial cells implicates them as antigen presenting cells in local immune responses. Human Immunol., **62**, 1081-1091 (2001).

3) Y. Matsunaga, T. Saibara, H. Kido, and N. Katunuma: Participation of cathepsin B in processing of antigen presentation to MHC class II. FEBS Lett., **324**, 325-330 (1998).

4) V. Turk, B. Turk, G.. Guncar, D. Turk, and J. Kos: Lysosomal cathepsins: structure, role in antigen processing and presentation, and cancer. Adv. Enzyme. Regul., **42**, 285-303 (2002).

5) C. Driessen, A.M. Lennon-Dumenil, and H.L. Ploegh: Individual Cathepsins degrade immune complexes internalized by antigen-presenting cells via Fc-gamma receptors. Eur. J. Immunol., **31**, 1592-1601 (2001).

6) M. Linke, S. Jordans, L. Mach, V. Herzog, and K. Brix.: Thyroid stimulating hormone upregulates secretion of cathepsin B from thyroid epithelial cells. Biol. Chem. **383**, 773-784 (2002).

7) A.D. Dunn and J.T. Dunn: Cysteine proteinases from human thyroids and their actions on thyroglobulin. Endocrinology., **123**, 1089-1097 (1988).

8) R.R. MacGregor, J.W. Hamilton, G..N. Kent, R.E. Shofstal, and D. V. Cohn: The degradation of proparathormone and parathormone by parathyroid and liver cathepsin B. J. Biol. Chem., **254**, 4428-4433 (1979).

9) F. Bianca, T. Carmen, R. Thomas, D. Jan, V. F. Kurt, H. Volker, P. Christoph, S. Paul, and B. Klaudia: Thyroid functions of mouse cathepsins B, K, and L. J. Clin. Invest., **111**, 1733-1745 (2003).

10) B. Turk, D. Turk, and V. Turk: Lysosomal cysteine proteases: more than scavengers. Biochim. Biophys. Acta., **1447**, 98-111 (2000).

11) J.S. Mort and Buttle D. J.: Cathepsin B. Int. J. Biochem. Cell Biol., **29**, 715-720 (1997).

12) W. Halangk, M.M. Lerch, and B. Brandt-Nedelev: Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. J. Clin. Invest., **106**, 773-781 (2000).

13) H. Haczynska and M. Warwas: Cathepsin B--role in cancer invasion and diagnosis. Postepy. Hig. Med.,

Dosw., **48**, 729-743 (1994).

14) V. Russo, L. Fontanesi, R. Davoli, C. L. Nanni, M. Cagnazzo, L. Buttazzoni, R. Virgili, and M. Yerle: Investigation of candidate genes for meat quality in dry-cured ham production: the porcine cathepsin B (CTSB) and cystatin B (CSTB) genes. Anim. Genet., **33**, 123-131 (2002).

15) M. Yamashita: Study on cathepsins in the muscle of chum salmon. Bull. Natl . Res. Ins. Fish. Sci., **5**, 9-116 (1993).

16) J.Y. Kwon, F. Prat, C. Randall, and C. R. Tyler: Molecular characterization of putative yolk processing enzymes and their expression during oogenesis and embryogenesis in rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod., **65**, 1701-1709 (2001).

17) S.S. Dong, G.I. Stransky, C.H. Whitaker, S.E. Jordan, P.H. Schlesinger, J.C. Edwards, and H.C. Blair: Avian cathepsin B cDNA: sequence and demonstration that mRNAs of two sizes are produced in cell types producing large quantities of the enzyme. Biochim. Biophys. Acta., **1251**, 69-73 (1995).

18) B. Wiederanders, D. Broemme, H. Kirsche, N. Kakkinen, A Rinne, T. Pquette, and P. Toothman: Primary structure of bovine spleen cathepsin S, a comparison with rat liver cahepsin L, H, B and papain. FEBS Lett., **286**, 189-192 (1991).

19) E. Kominami, T. Tsukahara, K. Hara, and N. Katunuma: Biosyntheses and processing of lysosomal cysteine proteinases in rat macrophages. FEBS Lett., **231**, 225-228 (1988).

20) A.J. Howie, D. Burnett, and J. Crocker: The distribution of cathepsin B in human tissues. J. Pathol., **145**, 307-314 (1985).

21) S.B. San, S.J. Chan, and D.F. Steiner: Differences in cathepsin B mRNA levels in rat tissues suggest specialized functions. FEBS Lett., **201**, 251-256 (1986).

22) A.R. Francisco, G.D. Keith, and D.B. John: Cathepsin B is a prorenin processing enzyme. Hypertension., **27**, 514-517 (1996).

23) P.H. Wang, Y.S. Doi, L. Macaulay, T. Shinagawa, P.W. Anderson, J.D. Baxter, and W.A. Hsueh: Identification of renal cathepsin B as a human prorenin-processing enzyme. J. Biol. Chem., **266**, 12633-12638 (1991).

24) T. Shinagawa, K. Nakayama, Y. Uchiyama, E. Kominami, Y.S. Doi, K. Hashiba, K. Yano, W.A. Hsueh, and K. Murakami: Role of cathepsin B as prorenin processing enzyme in human kidney. Hypertens. Res., **18**, 131-136 (1995).

25) I. Jutras and T.L. Reudelhuber: Prorenin processing by cathepsin B in vitro and in transfected cells. FEBS Lett., **443**, 48-52 (1999).

26) K. Zoltan, M. Julia, K. Burkhard, T. Miklos, M. S. Paul, H. Walter, M. L. Markus, and S.T. Miklos: Proteolytic activity in the yolk sac membrane of quail eggs. Comp. Biochem. Physiol. A, **123**, 1-8 (1999).

27) W.L. Cho, S.M. Tsao, R.H. Alan, R. Walter, J.S. Chen, S.S. Ekaterina, and S.R. Alexander: Mosquito cathepsin B-like protease involved in embryonic degradation of vitellin is produced as a latent extraovarian precursor. J. Biol. Chem., **274**, 13311-13321 (1999).

28) O. Carnevali, R. Carletta, A. Cambi, A. Vita, and N. Bromage: Yolk formation and degradation during oocyte maturation in seabream Sparus aurata: involvement of two lysosomal proteinases. Biol. Reprod., **60**, 140-146 (1999).

29) J.Y. Kwon, F. Prat, C. Randall, and C.R. Tyler: Molecular characterization of putative yolk processing enzymes and their expression during oogenesis and embryogenesis in rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod., **65**, 1701-1709 (2001).

30) S.A. Goldes, H.W. Ferguson, P.Y. Daoust, and R.D. Moccia: Phagocytosis of the inert suspended clay kaolin by the gill of the rainbow trout salmo gairdneri. J. Fish Dis. **9**, 147-151 (1986).

31) P.D. Smith: Analysis of the hyperosmotic and bath methods for fish vaccination-comparison of uptake of particulate and nonparticulate antigens. Dev. Comp. Immunol. Suppl., **2**, 181-186 (1982).

32) W. Ahne: Uptake and multiplication of spring viremia of carp virus in carp, *Cyprinus carpio*. J. Fish Dis., **1**, 265-268 (1978).

コイカテプシンBの一次構造とmRNAの発現

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コイ筋肉からのカテプシンB遺伝子のクローニングを行った結果，コイカテプシンB cDNA（1,470 bp）は330アミノ酸をコードするORF（993 bp）で構成されていた。ORFはプレ部（18アミノ酸残基），プロ部（60アミノ酸残基）及び成熟型酵素（252アミノ酸残基）をコードしていた。コイカテプシンB（成熟型酵素）の一次構造を他生物種（ヒト，マウス，ウシ，ニワトリ及びニジマス）と比較すると，76-79%の相同性を示した。他生物由来カテプシンBのプロセシング部位と相同なアミノ酸配列を持つことから，コイカテプシンBは重鎖と軽鎖からなる二本鎖構造で機能していると考えられた。コイカテプシンB mRNAの組織分布をノーザンブロット法により調べた。その結果，カテプシンB mRNAの発現レベルは卵巣＞鰓＞腸＞精巣＞肝膵臓＞脾臓＞心臓＞脳の順であった。卵巣においてmRNAの発現レベルが特に高かったが，これはカテプシンBが卵黄形成に深く関与しているためと考えられる。