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Full Length Research Paper

Molecular characterization and expression analysis of a hepcidin gene from rice field eel (*Monopterus albus*)

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Hepcidin is a cysteine-rich, dual-function peptide with antimicrobial activity that plays crucial roles in iron homeostasis. A few hepcidin-like genes have been isolated from teleost. Here, we have identified a hepcidin-like gene from rice field eel (RFE), *Monopterus albus*. Nucleotide sequences including cDNA and genomic DNA (GenBank accession numbers: FJ436808 and FJ594996, respectively) and deduced amino acid sequences were presented. In the 949 bp-long genomic sequence, two introns and three exons were identified. The full-length cDNA encodes a prepropeptide of 90 amino acid residues. RT-PCR analysis suggested that hepcidin transcripts are highly abundant in the liver and kidney, less abundant in the heart, skin, brain, blood cells, intestine, spleen and stomach and undetectable in muscle. After challenged with *Aeromonas hydrophila* infection or iron-dextran stimulation, the hepcidin transcript levels were analyzed by RT-PCR. The results revealed that the expression of hepcidin dramatically increased at 24 h post-infection of the pathogen injection. Moreover, hepcidin mRNAs in the liver, intestine and brain were 2.4, 1.5 and 2-fold increase, respectively, compared with the control animals after 5 days in iron-dextran injected RFEs.

Key words: Rice field eel, *Monopterus albus*, hepcidin, gene expression.

INTRODUCTION

Antimicrobial peptides (AMPs) are widely distributed from invertebrates to mammals and play an important role in host innate immune system against microbial invasion (Andreu and Rivas, 1998; Lehrer and Ganz, 1999). Hundreds of AMPs have been isolated from plants and mammals and display strong antimicrobial activity against a broad range of microbes. So these AMPs may serve as new potentially resources for the development of alternative therapeutants (Hancock and Lehrer, 1998; Patrzykat and Douglas, 2003). Cysteine-rich antimicrobial peptides are an important part of AMPs and have been identified in the hemolymph of crustaceans, fat bodies of insects and livers of teleost. Hepcidin originally isolated from human blood ultrafiltrate and urine, is one kind of cysteine-rich antimicrobial (Krause et al., 2000; Park et al., 2001). Subsequent study demonstrated that hepcidin is iron-regulatory hormone responsible for the regulation of body iron balance and recycling in mammals (Nicolas et al., 2001; Weinstein et al., 2002). To date, an increasing number of hepcidin has been identified and characterized from some fishes and amphibians (Shi and Alvin, 2006). However, little is known about its putative dual function in fish (Rodrigues et al., 2006), the functions of hepcidins in fishes and amphibians need to be further determined. Liver hepcidin expression was found to increase in both the iron-overloaded and infected sea bass, while in the iron-deficient fish no alteration in expression levels was detected when they were submitted either to iron status modulation or bacterial infection (Rodrigues et al., 2006). Hu et al. (2007) reported that

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Abbreviations: **RFE**, Rice field eel; **AMPs**, antimicrobial peptides

the hepcidin transcript levels was up-regulated in the liver by *Edwardsiella ictaluri* infection and moreover, hepatic hepcidin transcript levels correlated significantly with serum iron concen-trations. The amino acid organization of hepcidin is highly conserved across different species, sharing six to eight cysteine residues at conserved positions. This suggests that the disulfide bridges of hepcidin are evolutionarily conserved and may be necessary for the antimicrobial activity (Rodrigues et al., 2006). Fish hepcidin genes have been found in rockfish (Kim et al., 2008), turbot (Chen et al., 2007), gilhead seabream (Cuesta et al., 2007), red seabream (Chen et al., 2005), flounder (Kim et al., 2005; Hirono et al., 2005), catfish (Bao et al., 2005), zebrafish (Shike et al., 2004), white bass (Shike et al., 2002), winter flounder and Atlantic salmon (Douglas et al., 2003). Tissue-specific expression of hepcidins showed that they are expressed in a variety of tissues such as spleen, intestine, head kidney, muscle and brain (Douglas et al., 2003; Chen et al., 2007; Kim et al., 2008). Not only purified natural peptides (Shike et al., 2002), but also recombinant fusion hepcidin (Zhang et al., 2005) and synthesized peptides (Krause et al., 2000) exhibited an effective activity against several kinds of bacteria. Moreover, hepcidin expression in the liver can be induced dramatically by bacterial and lipopolysaccharide (LPS) infection (Nemeth et al., 2004; Shike et al., 2004). When challenged with pathogenic *Streptococcus iniae*, *Aeromonas salmonicida* and *Listonella anguillarum*, the expressional level of hepcidin was significantly up-regulated (Shike et al., 2002; Lauth et al., 2005; Chen et al., 2007; Kim et al., 2008). Therefore, it appears that hepcidin could be an effective component of the host innate immunity system in response to microbial invasion and infection.

Hepcidin has also been demonstrated to be an iron-regulatory hormone responsible for the regulation of body iron balance and recycling in mammals (Nicolas et al., 2001; Weinstein et al., 2002). However, the link between hepcidin and iron metabolism is not completely under-stood at present. The direct link between hepcidin and iron metabolism in murine was demonstrated almost immediately after it was shown to possess antimicrobial properties (Pigeon et al., 2001). Further studies showed that hepcidin gene expression was up-regulated under iron-overloaded conditions and the hepcidin gene knock-out of mouse led to hepatic iron accumulation, however, in humans, it leads to hereditary hemochromatosis (Nicolas et al., 2001; Roetto et al., 2003). Although, another report showed that the levels of zebrafish hepcidin were induced following acute iron-dextran injec-tion (Fraenkel et al., 2005). No other reports can be found about teleost hepcidin expression effected by iron-dextran stimulation. Further studies will be needed to demonstrate the dual functions of hepcidin as anti-microbial peptide and iron-regulatory protein.

Monopterus albus, commonly called the rice or swamp eel, was tentatively identified as belonging to the synbranchid genus *Monopterus* and was regarded as the unique representative of Synbranchidae (Collins et al., 2002; Li et al., 2007). It is one of the most economically important freshwater fishes found in aquatic habitats in China and other Southeast Asian countries (Zhou et al., 2002). However, the bacterial-resistance ability of farmed populations is very poor. And more seriously, the wide resources have declined in recent years due to over-fishing and environmental pollution (Yin et al., 2005). In the effort to determine how to conserve and sustainably exploit these resources, searching for new resistant-related genes and utilization of them in the molecular breeding of RFE is needed. Sequential hermaphroditism (sex change) of RFE attracted more attention (Cheng et al., 2003; Huang et al., 2005; Zhang et al., 2008). However, few reports can be found on study of RFE resistant-related genes. To date, no reports on hepcidin gene of RFE can be found.

In this study, we reported the cloning and structural analysis of the hepcidin genes from RFE (*M. albus*) and its expression in various tissues in response to infection with pathogenic bacteria and to iron-dextran stimulation.

MATERIALS AND METHODS

Experimental animals, DNA and total RNA isolation

Adult RFEs (weighing about 100 g) were purchased from Nan-men freshwater fish market (Jingzhou China). Total RNA was isolated from fish liver using Trizol reagent (Invitrogen) according to manufacture's instruction. Ten tissues were collected, frozen by liquid nitrogen and stored immediately at -80°C including liver, heart, skin, blood cells, kidney, intestine, muscle, stomach, spleen and brain. Total RNAs of these tissues were extracted and stored at -80°C until use.

Genomic DNA was extracted from RFE liver as described elsewhere (Strauss et al., 2000) and purified with phenol/chloroform twice.

Amplification of hepcidin cDNA

cDNA synthesis was carried out using a random primer as described (Chen et al., 2001). A pair of degenerate primer mahepN1 (5'-GATGRCHTTCAGBG-3') and mahepC1 (5'-AATSCT CAGAACCTGGA-3') was designed based on the sequence homology between known fish hepcidin cDNAs. The aligned sequences used in primer design were: Japanese flounder (C23298.1), red sea bream (AY452732), black porgy (AY669376), Nile tilapia (AY725227), Atlantic salmon (BI468191). The ampli-fication conditions were: an initial denaturation at 95°C for 4 min followed by 35 cycles of amplification followed by a 10 min extension at 72°C. Each cycle included denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension for 1 min. Five clones were sequenced to obtain the cDNA sequences.

To isolate full-length hepcidin cDNA, 5'-RACE and 3'-RACE were carried out. One pair of gene-specific primers (mahepGSP5 and mahepGSP3) was designed according to the earlier mentioned cDNA sequence. MahepGSP5 (5' –CCGGAGTGTCATTGCTCCC TGCTTCTTCC-3') was used to amplify the 5'end and mahepGSP3 (5'- ATGGAACCGTGGACGGTGCCGAGTCACATC-3') was used to

obtain the 3' end of hepcidin cDNA.

Introns amplification and sequencing

The specific primers hepN1 (5'-CTCGCCTTTATCTGCATTCTGG-3') and hepC1 (5'-CGCAGCCCTTGTAGTTCT-3') were designed based on the cDNA sequence obtained above and used to amplify the genomic DNA containing all introns. Genomic DNA (50 ng) was used as template. PCR was performed with the following conditions: denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 54°C for 30 s and 72°C for 1 min 20 s, with a final extension step of 72°C for 7 min.

Sequence analysis

The nucleotide sequences and deduced amino acid sequences were analyzed using DNASTAR (Dayhoff et al., 1978). Signal peptides were predicted using the Signal P program. Multiple alignments of the hepcidin proteins were constructed using the Clustal W program (Thompson et al., 1994). A phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) and analyzed with MEGA 3 (Kumar et al., 2004).

Bacteria challenge, iron treatment and sampling

Fifty (50) REFs were randomly assigned into four groups. Twenty (20) RFEs were intraperitoneally injected with 100 µl *Aeromonas hydrophila* ATCC35654 (2.6×10^6 cfu/ml). Ten REFs were intramuscularlly injected with iron-dextran (Sigma) at 10 mg iron/kg body weight. The last twenty (20) were injected with 100 µl NaCl (0.9%) as control. Two pathogen-infected fish and two NaCl-injected fish were sacrificed at 0, 24, 48 and 72 h after injection, respectively. Three of iron-dextran stimulated fish were sacrificed after 5 days after injection. Tissues were collected and stored at -80°C until use.

Expression analysis of hepcidin gene by RT-PCR

Total RNA from various tissues were extracted with Trizol Reagent (Invitrogen) according to the manufacturer's instructions. The reverse-transcription of mRNA was performed as previously reported (Chen et al., 2001). The pair of gene-specific primer hepN1 and hepC1 was used for amplifying RFE hepcidin cDNA fragments. Expression of b-actin was used as internal control. The primers maactinN1 (5'-GGCTACTCCTTCACCACCACAG-3') and mactinC1 (5'-GTCTCATGGATTCCGCAGGTCA-3') were used for amplifying b-actin fragment. PCR was run as follows: initial incubation at 94°C for 4 min, followed by 35 cycles of 94°C, 30 s 53°C, 30 s and 72°C, 30 s, with a final extension of 5 min at 72°C. 15 µl amplification products were analyzed on 1.5% agarose gel with a DL2000 DNA marker (TaKaRa).

RESULTS

Gene organization of rice field eel hepcidin

Five clones were sequenced and one cDNA fragment was obtained. The full-length cDNA is 700 bp in length, excluding the polyA tail and contained an open reading frame (ORF) of 273 bases encoding a protein of 90 amino acids. The signal peptide sequence was 24 amino acids in length; the predicted mature peptides were 26 amino acids long; the prodomian was 40 amino acids (Figure 1a). Intron 1 was 99 bp in length, whereas intron 2 was 160 bp in length. The first exon contains the 5' UTR, the signal peptide and a part of the prodomain. The prodomain extends from exon 1 through the exon 3. Exon 3 also encodes the mature peptide and the 3' UTR (Figure 1b).

Sequence alignment and phylogenetic analysis

The deduced amino acid sequence of RFE hepcidin has 71.1, 63.3, 57.8, 53.9, 52.4, 52.1, 51, 50, 37.8, 34.3, 27.8, 27.5, 26.7 and 25.6% identity with that of medaka (AU178966), winter flounder (AW013026), white bass (AF394246), Japanese flounder (C23298.1), red sea bream (AY452732), black porgy (AY669376), Nile tilapia (AY725227), Atlantic salmon (BI468191), rainbow trout (AF281354), zebrafish (NM_205583), mouse (BC021587), human (BC020612), dog (AY899807) and pig (NM_214117), respectively (Figure 2). It is obvious that the RFE hepcidin gene is more similar to those of winter flounder and medaka than those of other species (Figure 3).

Expression of RFE hepcidin genes by RT-PCR

Tissue-specific gene expression

Tissue specific expression of the hepcidin transcripts was assessed by RT-PCR. Expression of the hepcidin transcript was detected in a wide range of tissues. It was demonstrated that hepcidin transcripts were highly abundant in liver, abundant in kidney, less abundant in heart, skin, brain, blood cells, intestine, spleen and stomach and undetectable in muscle (Figure 4).

Effect of *A. hydrophila* and iron-dextran on hepcidin expression

The effect of *A. hydrophila* on hepcidin gene expression was assessed in the brain, heart, kidney, liver, skin and spleen. The result showed that: challenge with pathogenic bacteria, *A. hydrophila*, significantly up-regulated the expression of hepcidins in all the six tissues. The expression of hepcidin dramatically increased at 24 h post-infection of the pathogen bacteria (Figure 5).

After challenged with iron-dextran, the hepcidin mRNA levels of hepatic, brain and intestine were determined by a RT-PCR analysis. Data analysis was performed using one-way ANOVA of product and service solutions (SPSS 13.0). As shown in Figure 6, hepcidin expression of iron-treated group was 2.4, 1.5 and 2-fold increase in

A

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1      ATATGACAAGGGTCACCAAAAGATAGGAAGACATGGTCCTGCCCAATCAACCTCCATCCA
61     TCAAAGGACGTGAAGAATAAATCGAAGATATTGTGGTCCTGTCCGGTGCCCTGACTCACT
121    TGGGAAACAAGACATGAAGACATCAGTGTTTGCTGTTGCAGTGACACTCATGCTCGCCTT
                    M  K  T  S  V  F  A  V  A  V  T  L  M  L  A  F
181    TATCTGCATTCTGGAGAGCTCCGCCATCCCATTCAAAGAGGTGCAAGAGCTGGAAGAAGC
       I  C  I  L  E  S  S  A  I  P  F  K  E  V  Q  E  L  E  E  A
241    AGGGAGCAATGACACTCCGGTTGTGGCACATCAAGAGATGTCAATGGAATCGTGGATGAT
        G  S  N  D  T  P  V  V  A  H  Q  E  M  S  M  E  S  W  M  M
301    GCCTAATCACATCAGACAGAAGCGTTACAGCTATCTCTCCCTCTGCCGCTATTGCTGTGC
       P  N  H  I  R  Q  K  R  Y  S  Y  L  S  L  C  R  Y  C  C  A
361    CTGCTGCAAGAACTACAAGGGCTGCGGCATGTGCTGTAGGTTCTGACGGGTCCTGTGATG
         C  C  K  N  Y  K  G  C  G  M  C  C  R  F  *
421    ACCACTAAAATGTTAATTTATTACTCTTTTCCTCTTACCACAAGGGAAGACTTTTCTTGA
481    CCTCTTGAACTGTTTTGTTTCACTTTGAAGAGGCATGATTAATGGAAGAGCACTGCAGTA
541    AAAGGTATCTTTTGTATTTATTTTGTACATTTGTAACACTTTTAACCTAAAGTTCTTTTG
601    TAAGCTCAACAATGTCAAGTTGTTTCCAACAGTAATCCAGATGTGACTGTTTCTTTGTGT
661    AAAGTGTCTGTAATAAATAATGTATATTTGAAGCAACTGCAAAAAAAAAAAA
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B

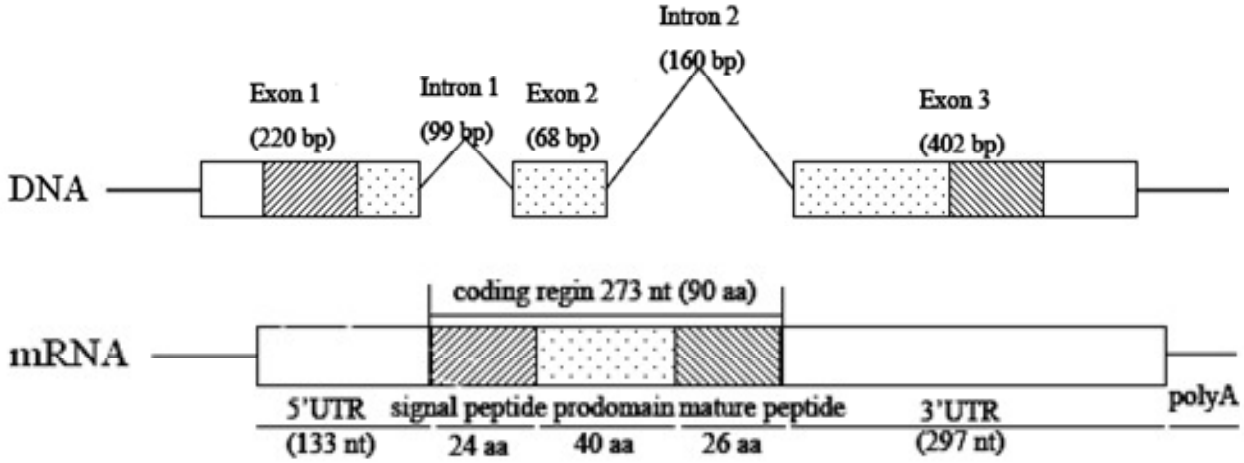

Figure 1. A: Nucleotide sequence of cDNA and deduced amino acid sequence of RFE hepcidin. The ploy $(A)^+$ signal sequence AATAAA is underlined; B: organization of RFE hepcidin genomic DNA and mRNA.

comparison to the control, respectively.

DISCUSSION

This study was designed to isolate and characterize hepcidin gene in rice field eel. Similar to some mammals and fishes, RFE hepcidin gene also consists of three exons and two introns. Moreover, like many reported fish hepcidins, the first intron of the RFE hepcidin gene (99 bp) is much smaller than the first intron of human and murine hepcidin genes (1.2 and 2.1 kb, respectively) and the second intron (160 bp) is larger than the corresponding intron of human (89 bp) and murine (83 bp) genes (Park et al., 2001; Pigeon et al., 2001).

The cDNA structure indicated that the RFE hepcidin is translated as a 90-amino acid prepropeptide that is cleaved to produce a mature peptide of 26-amino acids. Alignment indicated that both signal peptide sequences and mature peptide sequences of the hepcidins are highly conserved within the species examined. Similar to hepcidin from other fish and mammals, RFE hepcidin consists of 8 cysteine residues which is a feature of most hepcidins. All these result suggested that the gene belongs to hepcidin family. Hepcidins of Atlantic salmon, winter flounder and mouse are organized as gene clusters (Patrzykat and Douglas, 2003; Pigeon et al., 2001). So far, two or more hepcidins have been reported from winter flounder, Japanese flounder, Atlantic salmon, black rock fish, pig and mouse (Pigeon et al., 2001; Douglas et al., 2003; Fu et al., 2007; Kim et al., 2008). This result inferred the presence of polymorphism in

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rice field eel      MKTSVFAVAVTLMLAFICILESSAIPFK.EVQELEEAGSN  39
medaka              MKAFSIAVAVTLVLAFICILQSSAIPVN.GVKELEEAASN  39
winter flounder     MKAFSIAVAVTLVLAFVCIQCSSAVPFQ.GVQELEEAGGN  39
Japanese flounder   MKTFSVAVTVAVVLVFICIQQSSATSP..EVQELEEAVSS  38
Nile tilapia        MKTFSVAVAVAVVLTFICFQQSSAVPVTEQEQELEEPMSM  40
black porgy         MKTFSVAVAVAVVLTFICLQESSAGSFT.EVQEPEEPMNN  39
red sea bream       MKTFSVAVAVAVVLTFICLQESSAASFT.EVQELEEPMSN  39
white bass          MKTFSVAVAVAVVLAFICLQESSAVPVT.EVQELEEPMSN  39
Atlantic salmon     ....MKAFSVAVVLVIACMFILESTAVPFSEVRTEEVGSF  36
rainbow trout       ............................LQVLTEEVGSI  11
zebrafish           MKLSNVFLAAVVILTCVCVFQITAVPFIQQVQDEHHVESE  40
dog                 MALSTRIQAACLLLLLLAS.VASVSVLPHQTGQLTDLRAQ  39
pig                 MALSVQIRAACLLLLLLVS.LTAGSVLPSQTRQLTDLRTQ  39
human               MALSSQIWAACLLLLLLLASLTSGSVFPQQTGQLAELQPQ  40
mouse               MALSTRTQAACLLLLLLAS.LSSTTYLHQQMRQTTELQPL  39
Consensus

rice field eel      DTPVV.AHQEMSMESWMPNHIRQKRYSYLSLCRYCCACC  78
medaka              DTPVA.ARHEMSMQPWMLPNHIREKRQSHISMCTMCCNCC  78
winter flounder     DTPVA.EHQVMSMESWMENPTRQKRHISHISLCRWCCNCC  78
Japanese flounder   DNAAA.EHQEQSADSWMPQN.RQKRD...VKCGFCC...  70
Nile tilapia        DYPAA.AHEEASVDSWKMLYNSRHKRG...IKCRFCCGCC  76
black porgy         ESPVA.AHEEKSEESWKMPYNNRHKRS..PKDCQFCCGCC  76
red sea bream       GSPVA.ADEEMSEESWKMPYASRRWR.....CRFCCRCC  72
white bass          ......EYQEMPVESWKMPYNNRHKRHSSPGGCRFCCNCC  73
Atlantic salmon     DSPVG.EHQQPGGESMHLPEPFRFKRQIHLSLCGLCCNCC  75
rainbow trout       DSPVG.EHQQPGGESMRLPEHFRFKRXSHLSLCRWCCNCC  50
zebrafish           ELQENQHLTEAEHRLTDPLVLFRTKRQSHLSLCRFCCKCC  80
dog                 DT....AGAEAGLQPTLQLRR.LRRRDTHFPICIFCCGCC  74
pig                 DT....AGATAGLTPVAQR....LRRDTHFPICIFCCGCC  71
human               DR....AGARASWMPMFQRR...RRRDTHFPICIFCCGCC  73
mouse               HG....EESRADIAIPMQKR...RKRDTNFPICIFCCKCC  72
Consensus                                        c  cc

rice field eel      KNYKGCGMCCRF.                             90
medaka              KNYKGCGFCCR..                             89
winter flounder     KANKGCGFCCKF.                             90
Japanese flounder   .KDGGCGVCCNF.                             81
Nile tilapia        .TPGICGVCCRF.                             87
black porgy         PDMSGCGICCTY.                             88
red sea bream       PRMRGCGLCCQRR                             85
white bass          PNMSGCGVCCRF.                             85
Atlantic salmon     .HNIGCGFCCKF.                             86
rainbow trout       .HNKGXGFCCKF.                             61
zebrafish           .RNKGCGYCCKF.                             91
dog                 .KTPKCGFCCKT.                             85
pig                 .RKAICGMCCKT.                             82
human               .HRSKCGMCCKT.                             84
mouse               .NNSQCGICCKT.                             83
Consensus                 g  cc
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Figure 2. Alignment of deduced amino acids sequence of RFE hepcidin with those of human (BC020612), pig (NM_214117), dog (AY899807), mouse (BC021587), medaka (AU178966), winter flounder (AW013026), Japanese flounder (C23298.1), Nile tilapia (AY725227), black porgy (AY669376), red sea bream (AY452732), white bass (AF394246), Atlantic salmon (BI468191), rainbow trout (AF281354) and zebra fish (NM_205583).

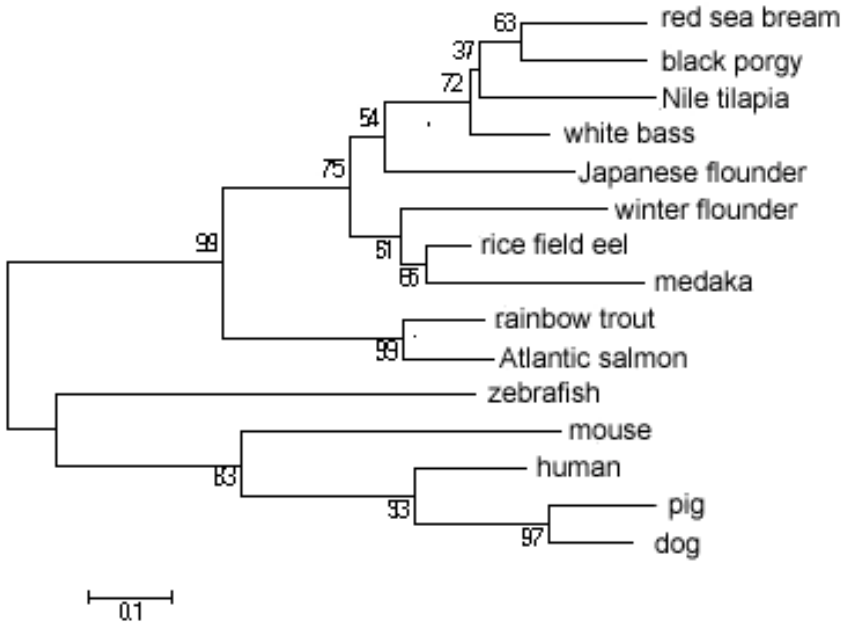

Figure 3. Phylogenetic analysis of RFE hepcidin and other vertebrates. Constructions are performed on the basis of the homology sequences calculated from the complete amino acid sequence of the hepcidins. The tree was constructed using a neighbour-joining method. Gaps are completely deleted. The scale bar is 0.1, which refers to percentage of divergence.

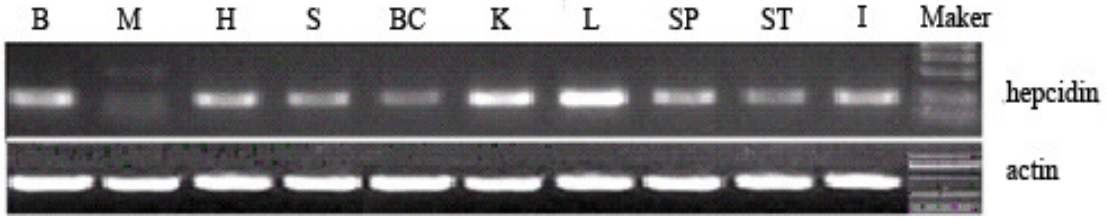

Figure 4. Tissue-specific expression analysis of RFE hepcidin. Tissues assayed were brain (B), muscle (M), heart (H), skin (S), blood cell (BC), kidney (K), liver (L), spleen (SP), stomach (ST) and intestine (I). Marker is the DL 2000.

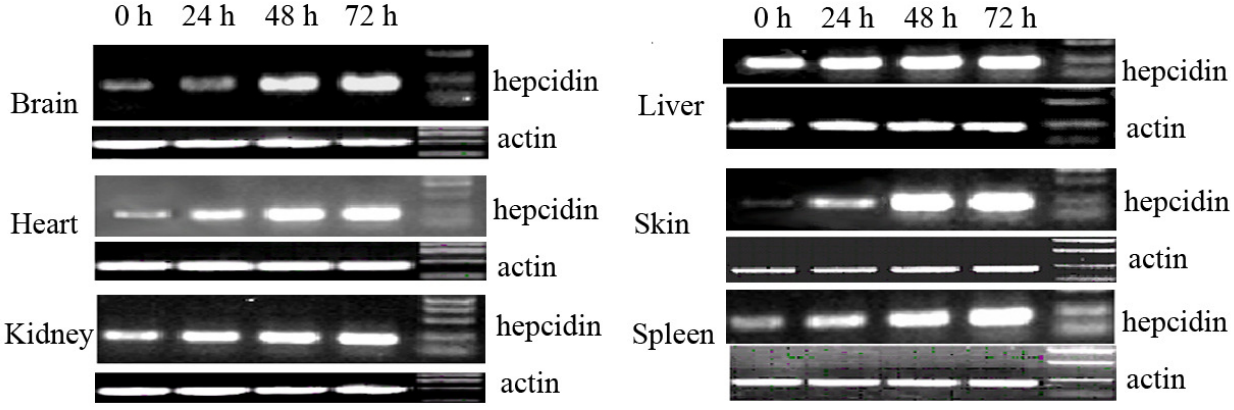

Figure 5. RT-PCR analysis of hepcidin expression after bacterial challenge with *A. hydrophila*. Samples of brain, heart, kidney, liver, skin and spleen were collected at 0, 24, 48 and 72 h after challenge. RT-PCR products were analyzed on agarose gels. b-actin was used as an internal control.

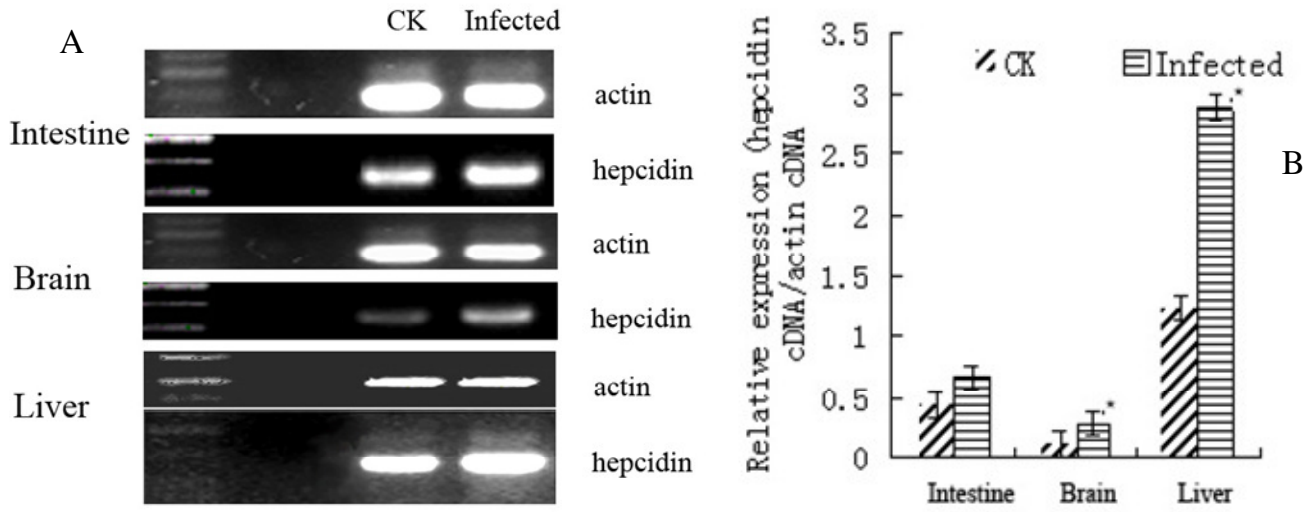

Figure 6. A: Hepcidin expression in iron-dextran stimulated animals. Five days after experimental treatment, hepcidin expression was assessed by comparative RT-PCR in control and iron-stimulation fish. b-Actin was used as control. **B:** Difference of liver and brain hepcidin were extremely significant at $P < 0.01$ and intestine hepcidin was not significant ($P > 0.05$). Data are the mean ± SE of three separate experiments, *$P < 0.05$.

hepcidin molecules in teleost. Whether there is molecular poly-morphism in RFE hepcidin gene or not needs further study.

Tissue-specific expression analysis of reported hepcidins revealed that hepcidin is predominantly expressed in the liver and less in muscle (Chen et al., 2007; Kim et al., 2008). So far, hepcidin transcripts were also detected in a variety of tissues such as skin, intestine, gill, stomach and brain in teleost. Previous studies showed that Sal1 hepcidin (Douglas et al., 2003), turbot hepcidin (Chen et al., 2007) and moronecidin of hybrid striped bass (Lauth et al., 2002) transcripts were found in blood cells. This study also found that the hepcidin transcripts were expressed in blood cells of RFE. Moreover, RFE hepcidin expression was too low, to be detectable in muscle which is similar to reported fish hepcidins (Chen et al., 2007; Kim et al., 2008).

Mammal hepcidins are defined as a type II acute-phase response protein (Nemth et al., 2003), which is characterized by a rapid expression following infection. The hepatic hepcidin expression level was upgraded a lot after LPS injection in mice (Pigeon et al., 2001), pig (Fu et al., 2007) and Japanese flounder (Hirono et al., 2005). Salmonella infection strongly increased porcine hepcidin transcripts in the liver at the early time of challenge Sanga et al., 2006). Challenge of turbot with pathogenic bacteria, *L. anguillarum*, significantly up-regulated the hepatic hepcidin expression (Chen et al., 2007). Challenged with the fish pathogen bacteria, *S. iniae*, two hepcidin genes of black rockfish were differentially expressed (Kim et al., 2008). The black rock fish hepcidin I and II dramatically increased at 24 h post-injection, then gradually declined at 3 days in hepcidin II, while hepcidin I expression continued at 3 days after challenge (Kim et al., 2008). In this study, we also demonstrated that the virulence bacteria *A. hydrophila* can dramatically up-regulate the expression of the hepcidin in liver, intestine, kidney, brain, heart and skin. These pathogen-induced hepcidin gene expression also inferred us that hepcidin plays a vital role in the immune defense system of RFE to inflammatory infection.

Hepcidin has also been demonstrated to be the long-sought hormone responsible for the regulation of iron balance and recycling in humans and mice (Nicolas et al., 2001; Weinstein et al., 2002). However, molecular mechanisms for hepcidin expression by iron are largely unknown at present. The first link between hepcidin and iron metabolism arose from the study of Pigeon et al. (2001), who were searching for new genes up-regulated during iron excess. Nicolas' observation (Nicolas et al., 2002) strongly supported the role of hepcidin as a putative iron-regulatory hormone. Expression of the hepcidin gene in mice is enhanced by iron overload (Xiao and Qian, 2000; Liu et al., 2006). In wild-type zebrafish, the levels of hepcidin were induced following acute iron-dextran injection (Fraenkel et al., 2005). In our study, the effects of the iron-dextran on the hepcidin expression were measured. Results showed that the mRNA transcripts of hepcidin were highly upgraded when challenged with iron-dextran in brain, intestine and liver.

In conclusion, we have firstly identified one hepcidin-like gene in rice field eel. The expression profile showed that the hepcidin-like gene is differently expressed in a tissue-specific manner. Hepcidin mRNA transcripts levels are closely influenced by pathogenic bacterial infection and iron stimulation to a significant extent. These inferred that hepcidins may have different functions in RFEs. Further studies will be needed to elucidate gene

regulation and peptide function of hepcidins in the innate immune response.

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