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Bass hepcidin is a novel antimicrobial peptide induced by bacterial challenge

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We report the isolation of a novel antimicrobial peptide, bass hepcidin, from the gill of hybrid striped bass, white bass (*Morone chrysops*) × striped bass (*M. saxatilis*). After the intraperitoneal injection of *Micrococcus luteus* and *Escherichia coli*, the peptide was purified from HPLC fractions with antimicrobial activity against *Escherichia coli*. Sequencing by Edman degradation revealed a 21-residue peptide (GCRFCCNCCPNMSGCGVCCRF) with eight putative cysteines. Molecular mass measurements of the native peptide and the reduced and alkylated peptide confirmed the sequence with four intramolecular disulfide bridges. Peptide sequence homology to human hepcidin and other predicted hepcidins, indicated that the peptide is a new member of the hepcidin family. Nucleotide sequences for cDNA and genomic DNA were determined for white bass. A predicted prepropeptide (85 amino acids) consists of three domains: a signal peptide (24 amino acids), prodomain (40 amino acids) and a mature peptide (21 amino acids). The gene has two introns and three exons. A TATA box and several consensus-binding motifs for transcription factors including C/EBP, nuclear factor-κB, and hepatocyte nuclear factor were found in the region upstream of the transcriptional start site. In white bass liver, hepcidin gene expression was induced 4500-fold following challenge with the fish pathogen, *Streptococcus iniae*, while expression levels remained low in all other tissues tested. A novel antimicrobial peptide from the gill, bass hepcidin, is predominantly expressed in the liver and highly inducible by bacterial exposure.

Keywords: antimicrobial peptide; fish; hepcidin; innate immunity; *Streptococcus iniae*.

Antimicrobial peptides (AMPs) are a broadly distributed group of molecules that are important in host defense against microbial invasion. A growing number of peptides involved in innate immunity have been isolated from plants, invertebrates, and higher vertebrates. Human hepcidin and liver-expressed antimicrobial peptide (LEAP-1) are identical AMPs, which were isolated independently from urine and human blood ultrafiltrate, respectively [1,2]. Peptide sequences of additional hepcidins have been predicted from expressed sequence tag databases from the liver of mouse [3], rat, various fish species including medaka, rainbow trout, Japanese flounder [4], winter flounder [5], long-jawed mudsucker [6], and Atlantic salmon. To date, only human hepcidins have been isolated as mature peptides, which are 20, 22 or 25 residues and exhibit antimicrobial activity. Human hepcidins and the other predicted hepcidins share eight cysteines at conserved positions.

Fish have evolved to thrive in an aqueous environment with a rich microbial flora, and several AMPs have been isolated from fish [7]. During our search for AMPs from gills of hybrid striped bass, three RP-HPLC fractions with antimicrobial acitivity were found [8]. One contained moronecidin, a 22-residue AMP with an amphipathic α-helical structure. From two other adjacent fractions, we isolated another novel AMP, bass hepcidin, a 21-residue, cysteine-rich peptide, which is a homologue of human hepcidin. We report here the first hepcidin to be isolated from a nonhuman vertebrate, the first cysteine-rich AMP isolated from fish, and the first demonstration of hepcidin gene expression induced by live bacterial challenge.

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Abbreviations: AMP, antimicrobial peptide; Ct, threshold cycle; HNF, hepatocyte nuclear factor; IL, interleukin; NF, nuclear factor.

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MATERIALS AND METHODS

Tissue collection and purification of bass hepcidin

Three fractions with antimicrobial activity were obtained from the RP-HPLC fractions from gill extracts of adult hybrid striped bass, as described previously [8]. Briefly, fish were harvested at 12 h following intraperitoneal injection with *Micrococcus luteus* and *Escherichia coli* D22. The acidified extract from gills was prepurified by solid-phase extraction and subjected to RP-HPLC. Three fractions demonstrated antimicrobial activity against *E. coli* by the liquid-growth inhibition assay. One fraction contained two isoforms of a novel AMP, moronecidin [8]. The two other adjacent fractions were further purified to homogeneity with two additional RP-HPLC steps using appropriate linear biphasic gradients of acidified acetonitrile. After each purification step, fractions were lyophilized, resuspended in

water, and tested for antimicrobial activity against *E. coli* by the liquid-growth inhibition assay as described previously [8].

Peptide structure

The purity of the peptides was confirmed by capillary zone electrophoresis and MALDI-TOF MS as described [8]. Peptide microsequencing was performed by Edman degradation (PE Applied Biosystems, model 473A) on native and on reduced and pyridylethylated peptides.

Bacterial challenge of white bass and RNA sampling

The challenge experiment for molecular studies and for assessing induction of gene expression was designed to mimic the natural route of infection with *Streptococcus iniae*, a pathogenic bacterial isolate for this fish species. Eight white bass fingerlings (20–30 g) were immersed for 2 min in a suspension of *S. iniae* or sterile solution, as described previously [8]. Three challenged and three mock-challenged fingerlings were randomly selected, anesthetized, and sacrificed 27 h postchallenge. Tissue samples (approximately 100 mg for intestine, liver, spleen, and anterior kidney; 10–50 mg for skin, gill, and whole blood) were homogenized in TRIzol (GibcoBRL) and total RNA was extracted.

Nucleotide sequence of white bass hepcidin cDNA

Aliquots of total RNA were subjected to reverse transcription, using Moloney murine leukemia virus reverse transcriptase (GibcoBRL) and a primer poly T [8] (Fig. 1). A degenerate, sense primer 1F (5′-GGNTGYNGNTTYT GYTGYAAYTGYTG-3′) was deduced from the amino acid consensus sequence, GCRFCCNCC, corresponding to residues 1–9 in the hepcidin mature peptide (Fig. 1). The 3′ region of the hepcidin mRNA was determined by direct sequencing of the RT/PCR product amplified from cDNA generated with the poly T primer with the primer pair 1F and poly T. The 5′ region of the mRNA was determined by 5′ RACE [9]. Briefly, cDNA was synthesized with primer 219R, and a 'poly A head' was created following incubation with dATP and terminal deoxynucleotide transferase (Stratagene). The cDNA with the polyA head was amplified with the primer pair, 219R and poly T.

PCR was performed using r*Tth* DNA polymerase XL (PE Applied Biosystems) in a GeneAmp 9600 thermocycler (PE Applied Biosystems). The PCR products were purified from an agarose gel using a QiaQuick gel purification kit (Qiagen) and directly sequenced by the Applied Biosystems BigDye terminators™.

Nucleotide sequence determination of white bass hepcidin genomic DNA

DNA was extracted from the skin of white bass using DNAzol (Molecular Research Center, Inc.). A PCR product was generated by amplifying DNA with a primer pair 1F and 219R (Fig. 1) and sequenced. The 3′ and 5′ flanking sequences were determined by inverse PCR [10]. Briefly, DNA was double-digested with *Dra*I and *Hpa*I, incubated with T4 ligase (Promega) to create intramolecular ligations, and amplified with a primer pair 158F and 86R. Amplification and sequence determination of the PCR products were performed as described above.

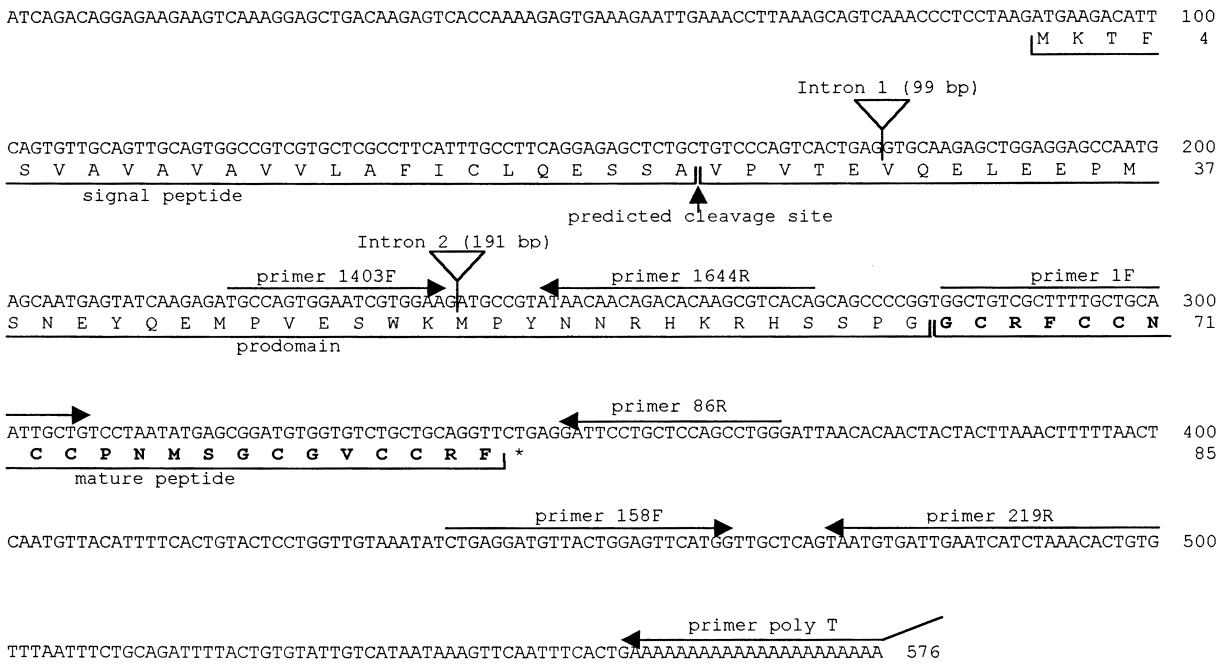

Fig. 1. cDNA and predicted amino-acid sequence of white bass hepcidin. Primer binding sites are shown with arrows (5′ to 3′). The organization of the peptide domains (signal peptide, prodomain, and mature peptide) is shown by amino-acid sequence enclosed by a underlined bar. The stop codon is indicated by an asterisk. Location of introns and the predicted peptide cleavage site are also shown.

Quantitative evaluation of white bass hepcidin mRNA by kinetic RT-PCR

To determine the sites and inducibility of gene expression, hepcidin mRNA and 18S rRNAs were quantitated in the RNA samples from the *S. iniae*- and mock-challenged fish by kinetic RT-PCR using a GeneAmp 5700 thermocycler (PE Applied Biosystems) [11]. A primer pair, 1403F and 1644R, was designed to span an intron in the hepcidin gene to preferentially amplify cDNA (52 bp) over genomic DNA (243 bp). A primer pair, 18S-F and 18S-R, which amplifies the conserved region of 18S rRNA cDNA, was used to evaluate each sample for cDNA yield and quality [8]. The cDNA was prepared with primers 1644R and 18S-R in a single reaction tube and the cDNA equivalent to 2×10^{-3} % of the harvested tissue was used for each PCR reaction. The quantity of hepcidin and 18S mRNA in each sample was expressed as relative units determined by standard curves created by the threshold cycle (Ct) values of the serially diluted cDNA from the liver of a challenged fish. The level of hepcidin gene expression was determined by the formula: units of hepcidin cDNA/units of 18S cDNA $\times$ 100 = % expression relative to the liver of a challenged fish. As an alternative way of expressing the quantity of hepcidin cDNA in the liver, absolute copy number of hepcidin cDNA templates per μg liver tissue was also determined. The copy number of hepcidin cDNA was determined using a kinetic PCR standard curve prepared from the Ct values of the serially diluted 5′ RACE product of known size and concentration (531 bp, 0.58 attogram per copy). The melting temperature (T_m) of the PCR products was used to distinguish amplification of cDNA vs. genomic DNA.

Computer analysis

Homology search was performed using BLASTP 2.1.2 and TBLASTN 2.1.3 by Genome Net WWW Server (http://www.genome.ad.jp) [12]. Putative transcription factor binding sites were predicted by TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) [13]. The cleavage sites for the signal peptide were predicted using SIGNALP (http://www.cbs.dtu.dk/services/SignalP) [14].

RESULTS

Purification and primary structure of bass hepcidin

Two fractions with antimicrobial activity from the gill of hybrid striped bass were purified to homogeneity by two additional analytical RP/HPLC purification steps as confirmed by capillary zone electrophoresis (data not shown). MALDI-TOF MS analysis of both fractions revealed the presence of an identical molecule with a molecular mass of 2255.97 MH^+.

Edman degradation of this molecule resulted in eight unidentified amino acids in a peptide of 21 residues. The peptide was reduced, alkylated, then re-analyzed by MALDI-TOF MS and Edman degradation. The eight blanks were determined to be cysteine residues and the amino acid sequence was completed as GCRFCCNCCP NMSGCGVCCRF. The mass of the peptide after reduction and S-pyridylethylation was measured as 3107.40 MH^+, which is 851.43 Da bigger than the mass of the native peptide, indicating the presence of eight cysteine residues (8 $\times$ 106 Da for the pyridylethyl group) engaged in the formation of four internal disulfide bridges in the native peptide. The measured mass of the native peptide agreed with the calculated mass of the 21-residue peptide with four disulfide bridges (2256.74 MH^+), with only a 0.8-Da difference. Computer analysis indicated that this peptide is a new member of the hepcidin family, bass hepcidin (SwissProt number P82951) (Fig. 2).

Because only a single peptide was isolated from a hybrid striped bass, we inferred that identical peptides were encoded by genes from the two parental species, striped bass and white bass. We chose white bass for characterization of the gene and expression studies because striped bass fingerlings were not available.

White bass hepcidin cDNA sequence

RT/PCR with a primer pair, 1F and poly T, yielded a positive signal (305 bp) from an RNA sample from the liver of an *S. iniae*-challenged white bass, but not from other tissues (data not shown). Thus, this liver RNA was used for 5′ RACE and the complete sequence of hepcidin cDNA was determined (GenBank accession number AF394246, Fig. 1). The complete cDNA is 554 bases exclusive of the polyA tail and contains an ORF of 347 bases with a coding capacity of 85 amino acids. The amino acid sequence of the 21-residue peptide was found at the C terminus of the ORF. Four methionine codons (nucleotides 90, 198, 219, and 240) were identified upstream of the mature peptide sequence. The first methionine codon (nucleotides 90) is probably the translational start site because it is followed by a typical signal peptide motif with a basic residue (lysine) and a hydrophobic region (rich in valine and alanine) and matches four of the seven nucleotides of the Kozac consensus sequence (A/G

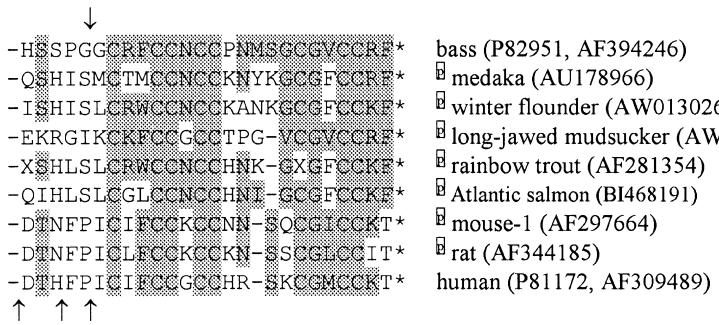

Fig. 2. Amino-acid sequence similarity of known and predicted hepcidins. Identical or similar amino acid residues are shaded. The cleavage sites for mature peptides of bass (↓) and humans (↑) are shown. Boxed p indicates a predicted hepcidin sequence. For the mouse hepcidins, the predicted product of only one of the duplicated hepcidin genes (*Hepc1*) is shown [3]. SwissProt and GenBank accession numbers are shown in parentheses.

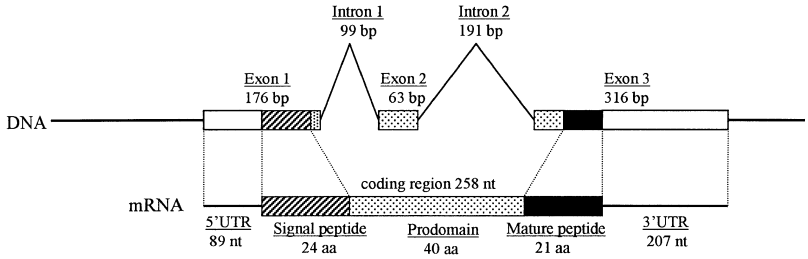

Fig. 3. Genetic organization of white bass hepcidin genomic DNA and mRNA.

CCAUGGG) for initiation of eukaryotic protein translation. Thus, the prepropeptide was predicted to be an 85-residue peptide.

A potential cleavage site for the signal peptide was predicted between Ala24 and Val25 in the 85-residue precursor. Thus, three domains are proposed for bass preprohepcidin: (a) a hydrophobic signal peptide (24 amino acids); (b) a prodomain (40 amino acids); and (c) a mature peptide (21 amino acids) (Fig. 3). A canonical polyadenylation signal was found in the 3′ UTR.

White bass hepcidin genomic DNA sequence and gene organization

The nucleotide sequence for the hepcidin gene and upstream region was determined for white bass (GenBank accession number AF394245, Fig. 4). The white bass hepcidin gene consists of two introns and three exons (Fig. 3). The first exon contains the 5′ UTR, the signal peptide, and 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.

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-1085                tttaa atctatatta aaaaagactg ataactggtt tagcagggtt aatggctaat tttacgcaga aactaaccaa ccatttgggg
-1000 taaagaattc tgtttgactt ctcttctatc agcgtttcca tccaaacgca cacaatgtaa caaataaacc acacaggaag aagaatgatt aagaaaagtt
-900  tcaaaagatt aaggcatgtg actcaagagg tagagcaggt cgtctgctaa ttggaaggtt ggcggttcga tccccggctc ttttcgctgc atgttgaagt
-800  atccttgagc aagatactga acccccccatt gcacttgatg ctgtgcataa gtgtatgaat gtgtttgaat aaatgacttt ctgtttgtga tggcctttgt
-700  catcactgta tgaataggtg agtgtggtat atagtgtaca gcgctttgag aggtctggag actagatgat cttcaataga tgctgatggt tgtgctctct
-600  gtcacattaa tgaaataatt attataaaac ctgggtaatc agatatatcc aacagctgta gagctgtttg ttgacagcag gtgtcaaagt caggcatttt
-500  agttattgca catcataggc ctctttgtga aagactgact tgcccctgac ttgtatttta tatgacactg atctattaac cattggtaac atattccagg
                                  CEBPβ

-400  acattagtgc caatatactg cagttaaggt gtaagaaaac aaaagcattt acaaaatctg ctgttatttt aatattgtat gatatgcatc ttctttattg
                                                 CEBPβ                                GATA

-300  tatataactg aaagatgcta gaagtgcaaa ataagcactg tataaccata cactctgtga tagaactgca tttgcaataa aagatacaaa tgttaatgat
                                                       GATA                                          NHF1

-200  tgaaacgtgg aacagacaaa caaagataaa aatttcccaat tcccactgct gagaatttcc cccattctcc ttcccagcat ccctcagaga gtgatgcaag
                                  GATA                                                                CEBPβ

-100  cccatccctg acagcagcta ctgtactgta ggtgctgtca gtgatgaggc aacagtggcc ttagtgagta taaataccag caggttttac acattcaacc
                                                                                TATA
+1 (Exon 1)
 ATCAGACAGG AGAAGAAGTC AAAGGAGCTG ACAAGAGTCA CCAAAAGAGT GAAAGAATTG AAACCTTAAA GCAGTCAAAC CCTCCTAAGA TGAAGACATT 100
                                                                                          M   K   T   F   4

 CAGTGTTGCA GTTGCAGTGG CCGTCGTGCT CGCCTTCATT TGCCTTCAGG AGAGCTCTGC TGTCCCAGTC ACTGAGgtaa caacttgact tcaatcattt 200
 S   V   A   V   A   V   A     V   V   L     A   F   I     C   L   Q     E   S   S   A     V   P   V     T   E                                  29
                                                                                                  (Exon 2)
 catttgctta ttggctgtga atgttttgtc caaatactga gacgttgttc ctaaaagtac aaaattcatt accagGTGCA AGAGCTGGAG GAGCCAATGA 300
                                                                                          V   Q     E   L   E     E   P   M   S   38

 GCAATGAGTA TCAAGAGATG CCAGTGGAAT CGTGGAAGgt acgttgagtt aaatgaatga agaaagtcag ataccatgag ccagttctaa tggaagtgga 400
   N   E   Y     Q   E   M     P   V   E   S     W   K                                                          50

 tgcatttcca ctgtaaagtg ctctgatgct ctctccagga gaaatgatca gagcaggaga gagaattaac agagtgagag atgatgctgg agatgaacac 500

                                  (Exon 3)
 cttgctcatg tctcttgtct ttcacacagA TGCCGTATAA CAACAGACAC AAGCGTCACA GCAGCCCCGG TGGCTGTCGC TTTTGCTGCA ATTGCTGTCC 600
                                  M   P   Y   N     N   R   H     K   R   H   S     S   P   G   G   C   R     F   C   C   N     C   C   P   74

 TAATATGAGC GGATGTGGTG TCTGCTGCAG GTTCTGAGGA TTCCTGCTCC AGCCTGGGAT TAACACAACT ACTACTTAAA CTTTTTAACT CAATGTTACA 700
 N   M   S     G   C   G   V     C   C   R     F   *                                                            85

 TTTTCACTGT ACTCCTGGTT GTAAATATCT GAGGATGTTA CTGGAGTTCA TGGTTGCTCA GTAATGTGAT TGAATCATCT AAACACTGTG TTTAATTTCT 800

 GCAGATTTTA CTGTGTATTG TCATAATAAA GTTCAATTTC ACTGAtatat aatgtgtaaa catgttcttt taccatttta ttttaccatt gttttagtct 900
                                  poly A
 tataataatc tggtgtgact tgtgagagtt aacttttctt tcaactgtgt gtcaaatgaa tccaa  965
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Fig. 4. Genomic sequence of white bass hepcidin. Numbering of the genomic sequence is relative to the transcription start site. Location of putative transcription factor binding sites are indicated by an arrow. The TATA box and polyadenylation signal are underlined. Exons are shown in upper case letters. The predicted peptide sequences are translated below the coding sequence and the mature peptide sequence is bold and underlined. The stop codon is indicated by an asterisk. (GenBank accession number AF394245).

Table 1. Expression of bass hepcidin gene in white bass tissues normalized for 18S gene expression and shown as a percentage of the expression level of the liver of a challenged fish. A 4500-fold increase in hepcidin expression was seen in the liver of challenged fish.

Tissues	Mock-challenged fish ($n = 3$) mean percentage expression (range)	*S. iniae*-challenged fish ($n = 3$) mean percentage expression (range)
Liver	0.02 (0.004–0.03)	88.87 (36.6–131.4)
Skin	0.001 (0–0.003)	0.29 (0.005–0.85)
Gill	0.0003 (0–0.0008)	0.04 (0.005–0.11)
Intestine	0.0001 (0–0.0003)	0.16 (0.04–0.12)
Spleen	0 (0–0)	0.01 (0–0.03)
Anterior kidney	0 (0–0)	0.28 (0.04–0.76)
Blood	0.04 (0–0.13)	0.15 (0–0.4)

The 1085 bp-upstream sequence of the white bass hepcidin gene contains regulatory elements and several binding motifs for transcription factors. Sequence analysis revealed a TATA box 32 nucleotides upstream from the transcriptional start site (nucleotide −32), four putative binding sites for CAAT enhancer-binding protein β (C/EBPβ) (nucleotides −111, −354, −798 and −914), one putative binding sites for nuclear factor (NF)-κB (nucleotide −150), three putative binding sites for hepatocyte nuclear factor (HNF) 1 (nucleotide −210) and HNF-3 β (nucleotide −184, −367).

White bass hepcidin gene expression

Levels of hepcidin gene expression were assessed by kinetic RT-PCR in three *S. iniae*-challenged and three mock-challenged fish. *S. iniae* was cultured from the brain in two out of three challenged fingerlings, thus confirming systemic infection. In all samples with detectable hepcidin amplification, the T_m of the PCR product was 80.0 °C (T_m for the PCR product from hepcidin cDNA), as opposed to 83.9 °C (T_m for the PCR product from hepcidin genomic DNA). This means there was no detectable amplification from genomic DNA. Thus, genomic DNA contamination did not affect the results of the kinetic RT-PCR. The average hepcidin expression in the liver of challenged and mock-challenged fish was 89% and 0.02%, respectively (% relative to the liver of a challenged fish). Accordingly, the hepcidin gene was induced approximately 4500-fold following bacterial challenge (Table 1). The level of expression remained low in other tissues, although induction was also demonstrated in every tissue tested. As an alternative approach to normalizing these data, we used a hepcidin PCR product of known quantity as the template for the standard curve. The average hepcidin copy number per μg liver was determined as 5.7×10^6 and 1.2×10^3 copies for the bacteria- and mock-challenged groups, respectively (Table 2). Thus, the hepcidin cDNA copy number per μg liver is low in the unchallenged state, but increases to extremely high levels following bacterial challenge. This is in contrast to another AMP, moronecidin, found in the bass gill and skin that was analyzed in these same fish and found not to be induced in any tissue [8].

Table 2. Estimated copy number of bass hepcidin cDNA molecules per μg liver in mock- and *S. iniae*-challenged white bass.

Experimental fish	cDNA copy number·μg^{-1} liver
Mock-challenged	
Fish 1	2.32×10^3
Fish 2	1.12×10^3
Fish 3	0.14×10^3
S. iniae-challenged	
Fish 1	1.8×10^6
Fish 2	10.8×10^6
Fish 3	4.4×10^6

DISCUSSION

We report here the discovery of a novel AMP, bass hepcidin, isolated from the gills of hybrid striped bass. This is the first member of the hepcidin family isolated and characterized from fish. Bass hepcidin was strongly induced in the liver of white bass following bacterial challenge.

Hepcidins are predicted to be a conserved peptide family with eight cysteine residues at identical positions (Fig. 2). Although the peptide sequence had previously been confirmed only for human hepcidin, similar peptides have been predicted from mRNA analysis in rat, mouse, and six species of fish (medaka, winter flounder, Japanese flounder, Atlantic salmon, rainbow trout, and long-jawed mudsucker). The predicted organization of the signal peptides, propeptides, and mature peptides is identical for bass and human hepcidins. Only a single 21-residue hepcidin was isolated from bass, whereas three processed hepcidins differing by N-terminal truncation, with 25, 22 or 20 residues, were found in humans [1]. The cleavage site for mature bass hepcidin is identical to the cleavage site for human hepcidin-20 (Fig. 2). The genes for bass, murine, and human hepcidin share a similar genetic organization with three exons and two introns [1,3]. Although the first intron of the bass hepcidin gene (99 bp) is much shorter than the corresponding introns of human and murine hepcidin genes (2.1 and 1.2 kb, respectively), the overall organization demonstrates remarkable conservation.

The white bass hepcidin gene was strongly induced in liver following bacterial challenge. The analysis of the upstream region of the gene revealed a TATA box and putative binding sites for transcription factors C/EBPβ, NF-κB, and HNF. The transcription factor C/EBPβ is regulated by complex interactions of cytokines and protein kinases, and mediates transcription of acute phase response genes by binding to the interleukin (IL)-6-responsive element in the promoters of genes, such as tumor necrosis factor α, IL-8, and granulocyte-colony stimulating factor [15]. Both C/EBPα and β are known to be important transcription

factors for hepatic gene expression [16]. The Rel/NF-κB, transcription factors are conserved from *Drosophila* to humans and play an important role in the Toll signaling pathway and hosts defense [17]. In *Drosophila*, κB motifs are found in the upstream region of all AMP genes [18]. HNFs are transcription factors expressed in liver and gut. HNF-1 and -4 have been reported to be essential for liver-specific gene expression and HNF-3β has been linked to differentiation of hepatocytes [16]. Interestingly, binding motifs for HNF, C/EBPβ, and NF-κB have also been described in the upstream region of the human and mouse hepcidin genes [3]. The mouse hepcidin gene was induced twofold to 10-fold following iron-overload or lipopolysaccharide challenge. However, the magnitude of the induction for the bass hepcidin gene was much greater following bacterial challenge (4500-fold). This is comparable to the AMPs of *Drosophila* and other insects, for which rapid, transient gene transcription follows septic injury [19,20]. Another similarity, highlighted by Park and colleagues [1], is that hepcidins and insect AMPs are synthesized in the liver and fat body (insect liver equivalent), respectively. However, hepcidins do not share structural characteristics with any of the cysteine-rich insect AMPs, insect defensins, or *Drosophila* drosomycin [21]. The different cysteine positions and disulfide arrays predict completely different three-dimensional structures.

Although bass hepcidin was isolated from the gills, gene expression was detected predominately in the liver. Discordance between the site of peptide isolation and the site of maximal gene expression was also noted in the case of human hepcidin [1,3]. Human hepcidin was isolated from urine and plasma ultrafiltrate [1,2]. Expression levels for both human and mouse hepcidins were high in the liver, and lower in the heart and brain [2,3]. These observations suggest that AMPs synthesized in the liver travel to distant sites through the circulation. Similarly, bass hepcidin is probably transported to the gill from the liver via blood stream. The peptide may enter the hepatic vein or portal system directly, or may be secreted into bile and enter the portal system by re-absorption in the intestine. As bass hepcidin was found in the gills but not in the skin, despite use of the same purification procedures for both tissues [8], gills may have a mechanism to bind or concentrate bass hepcidin.

In summary, bass hepcidin, a homologue of human hepcidin, was isolated from the gills, demonstrates antibacterial activity against *E. coli*, and was dramatically induced in the liver following the challenge with fish pathogen, *S. iniae*.

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