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Characterization and Structural Analysis of Hepcidin Like Antimicrobial Peptide From *Schizothorax richardsonii* (Gray)

Preeti Chaturvedi · Meenakshi Dhanik · Amit Pande

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Abstract Innate immune system is a primary line of defense in fish that protects it from the invading pathogens. Antimicrobial peptides (AMPs) are widely distributed in nature and are essential components of innate immunity. These molecules enable the host's innate immune system to fight against a variety of infectious agents. One such AMP, hepcidin, is a cysteine rich amphipathic peptide. We have amplified, cloned and characterized hepcidin like AMP from *Schizothorax richardsonii* that inhabits one of the most difficult aquatic ecosystems in the Indian Himalayas. The cDNA encoding hepcidin like peptide was amplified as a 371 bp fragment with an open reading frame (ORF) of 279 nucleotides flanked by 5′ and 3′ UTRs of 70 and 22 bases respectively. This ORF encodes a peptide of 93 amino acids with a signal peptide of 24 amino acids and a mature peptide of 25 amino acids. The mature hepcidin like peptide of *S. richardsonii* has eight cystine residues that participate in the formation of four disulfide bonds, a unique feature of hepcidin like AMPs. A 3D model of hepcidin like mature peptide was generated using Modeller 9.10 which was validated using PROCHECK and ERRAT. Phylogenetic analysis of hepcidin like AMP from *S. richardsonii* revealed that it was closely related to hepcidin from olive barb (*Puntius sarana*).

Keywords Antimicrobial peptides · Hepcidin · Innate immunity · *Schizothorax richardsonii* · Infectious agents

P. Chaturvedi · M. Dhanik · A. Pande (✉)
Directorate of Coldwater Fisheries Research, Bhimtal, Nainital 263136, Uttarakhand, India
e-mail: compton.pande@yahoo.co.in

1 Introduction

Co-evolution of host and pathogens has developed a variety of versatile defense mechanisms that can be either acquired or germline-encoded [1]. Innate immune system, a key defense mechanism, provides first line of defense against pathogens [2]. Fish being poikilothermic, possess a limited antibody repertoire, affinity maturation and memory besides weak lymphocyte proliferation. This accounts for a poor acquired immune response compared with relatively temperature independent innate immune response [3]. Antimicrobial peptides (AMPs) one of the important mediators of innate immunity, are evolutionarily conserved elements and widely distributed in nature from invertebrates to mammals [4]. These molecules synthesized by host are involved in defense against a variety of infections [1]. A number of AMPs have been identified in fish for example hepcidin, defensins, misgurin, piscidin, parasin, moronecidin and daxin [1, 6, 7]. AMPs possess unique broad spectrum anti-microbial activity against a number of viruses, bacteria, fungi and protozoa. Besides being able to kill 99.9 % pathogens within 20 min [8] it has also been reported that AMPs can possess anticancer properties [9]. Synthetic tilapia hepcidin 2–3 [10] and TH-1-5 [11] have been demonstrated to possess antitumor activity in human cancer cells that may have an application in cancer therapy. Moreover AMPs are also effective against antibiotic resistant pathogens therefore, free from side effects associated with conventional antibiotics [12]. All these characteristics make AMPs attractive potential therapeutic agents that may find an application in both human and veterinary medicine [13].

Hepcidin is a 25 amino acid cysteine rich cationic peptide with hairpin structure in which two β-sheets are linked by four disulfide bonds. Initially this AMP was

termed as liver expressed antimicrobial peptide (LEAP) as it was primarily detected in liver [14]. Two types of LEAPs, LEAP1 and LEAP2 are widespread in fish species and humans. There are eight and four cysteine residues in LEAP1 and LEAP2 resulting in the formation of four and two disulfide bonds [15]. In humans, three isoforms of hepcidin are known to exhibit antibacterial activities that contain 20, 22 and 25 amino acids [16]. The amino acid sequences are highly conserved among species and share a characteristic feature of six to eight cysteine residues conserved at positions that signify the importance of disulfide bonds in antibacterial activity [17, 18]. Hepcidin also acts as an important component of host innate immune system upon pathogenic stimuli. It has been reported in various studies that natural and synthetic hepcidin possess antibacterial activity against Gram-positive and Gram-negative bacteria besides having strong activity against other fish pathogens [19]. Antimicrobial activity of purified hepcidin-P1 from large yellow croker [20], synthetic hepcidin peptide from Japanese flounder [21], tilapia hepcidin TH-1-5 and TH-2-3 [22], gilthead seabream hepcidin [4], large yellow croaker PC-hep [19], orange-spotted grouper EC-hepcidin 1 [23], black porgy AS-hepc2 and AS-hepc6 [24] and medaka Om-hep1 [25], recombinant Japanese flounder hepcidin fusion peptide JFL4 [26] and recombinant medaka hepcidin Pro-Omhep1[25] have been well documented. Besides these fish species, hepcidin has been reported in other fish species including striped bass, *Morone saxatilis* × *Morone chrysops* [27], winter flounder *Pseudopleuronectes americanus* [28], Atlantic salmon *Salmo salar* [28], rainbow trout *Oncorhynchus mykiss* [28], medaka *Oryzias latipes* [28], zebrafish *Danio rerio* [29], olive flounder *Paralichthys olivaceus* [21, 30], red sea bream *Chrysophrys major* [31], channel catfish *Ictalurus punctatus* [32], black porgy *Acanthopagrus schlegelii* [33], turbot *Scophthalmus maximus* [34], Atlantic cod *Gadus morhua* [35] and sea bass *Dicentrarchus labrax* [36]. In humans [14, 16] and mice [37] expression of hepcidin mRNA has primarily been reported in liver [22, 23, 32] while in fish, expression of this AMP has been demonstrated from liver, spleen, gill, intestine, brain, stomach and skin. The mRNA transcript of hepcidin in early stage blastula is maternally derived, implying a major role of hepcidin in innate defence of embryos in turbot. Bacterial challenge increases hepcidin transcript expression in different fish tissues such as head kidney, heart, spleen, skin and gills [34]. In hybrid striped bass antimicrobial activity and the induction of hepcidin has been observed upon infection [27]. It has been observed that expression of mRNA hepcidin transcript increases by 4,500 fold upon bacterial infection [38] advocating its role in innate immunity against bacterial pathogens [39]. Apart from antimicrobial activity, hepcidin has important role in iron regulation in fish [5, 36] for example, ferroportin, an iron transporter present on cells of the intestinal duodenum, macrophages, and cells of the placenta bind to ferroportin and induces its internalization as well as degradation [40]. Besides bactericidal and antifungal activity, AMPs have immune-modulatory functions and play a key role in iron metabolism, inflammation, hypoxia and anemia [41–46].

Emergence of antibiotic resistant microbes, a matter of grave concern, has resulted due to injudicious use of antibiotics. Therefore, a quest for development of new broad spectrum antimicrobial agents remains a challenge. A solution could be AMPs, which are active against broad spectrum of infectious agents that are resistant to conventional drugs [47, 48]. Looking into the tremendous applications that AMPs may find in agriculture, pharmaceutical and food industry [49], we attempted to unravel an AMP from Indian snow trout, *Schizothorax richardsonii*, a fish which inhabits a unique ecological niche in the foot hills of the Himalayas. In this report identification, cloning, structural and phylogenetic analysis of an AMP resembling hepcidin like AMP from *S. richardsonii* is discussed.

2 Materials and Methods

2.1 Fish and Tissue Collection

Eleven snow trout (*Schizothorax richardsonii)* weighing about 10–15 g were collected from Kalsa stream, Chafi 29.37°N, 79.57°E located at an altitude of 1,238 m above sea level. The fish were euthanized by immersion in 100 ppm of clove oil [50] before being dissected. Tissues including liver, spleen, kidney, brain, heart, gill, intestine, skin and muscle were collected in Ribozol (Ameresco) and homogenized using a dounce homogenizer.

2.2 Amplification of Hepcidin Gene

Total RNA was isolated from the tissues using Ribozol (Ameresco) following manufacturer's recommendations. The concentration of the purified RNA was estimated using Eon multimode spectrometer (BioTek) and integrity tested by agarose gel electrophoresis. Total RNA (2 µg) was used for the synthesis of cDNA using RevertAid Reverse transcriptase (Fermentas) and Oligo dT_{18} (Fermentas) primer. Clustal alignment of the mRNA encoding hepcidin from grass carp, common carp and zebra fish were carried out to design specific primers for the amplification of full length hepcidin cDNA. The cDNA was used as template to amplify hepcidin gene using gene specific primer sequence Hep1 for 5′ATCAGAGCCGAGCAGAAGA 3′ and Hep1 Rev 5′CAGCCTGCATTTATACCCG3′ corresponding to the conserved region. Amplification of hepcidin cDNA was

Table 1 Hepcidin sequences and their GeneBank accession number used for alignment and phylogenetic analysis

S. No.	Species name	GeneBank accession number
1	Atlantic salmon	AAO85553.1
2	Rainbow trout	ADU85830.1
3	Mandarin fish	ACO88905.1
4	Hong kong grouper	AEO44882.1
5	Olive flounder	AAZ43072.1
6	Swamp eel	ADK79123.1
7	Olive barb	CAZ68137.1
8	Common carp	AFY23859.1
9	Grass carp	AEZ51835.1
10	Wuchang bream	AFO84706.1
11	Zebra fish	AAI62314.1
12	Blue catfish	AAX39714.1
13	Channel catfish	ABA43709.1
14	Human	AAH20612.1
15	Sumatran orangutan	NP_001127676.1
16	Wild boar	NP_999282.1
17	House mouse	NP_115930.1
18	Rock dove	ABV00675.1
19	Moorish idol	AFQ32275.1

carried out by initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation, annealing and extension at 94 °C for 30 s, 51.6 °C for 30 s and 72 °C for 30 s, with final extension at 72 °C for 10 min. PCR products were resolved by agarose gel electrophoresis on 1.5 % agarose gel and visualized under UV using GelDoc XR (BioRad). The amplified products were gel purified using sure trap gel extraction kit (Nucleopore). The purified products were ligated into the InsTA cloning vector (Thermo-scientific) and transfected into *Escherichia coli* DH-5α cells. The nucleotide sequences of the inserts were obtained by outsourcing (Eurofin India).

2.3 Sequence Analysis of Hepcidin Like Peptide

Nucleotide sequence of hepcidin was analyzed for similarity with other known sequences using BLASTP [51] while the protein translation tool expert protein analysis system was used to predict amino acid sequence [52]. To predict the physiochemical properties of hepcidin such as molecular weight, theoretical pI, and grand average of hydropathy (Gravy) ProtParam tool [53] was used. The amino acid sequence of the predicted hepcidin like peptide of *S. richardsonii* was aligned to compare with hepcidin sequences of different species of fish and mammals (Table 1) using ClustalW [54].

2.4 Molecular Modelling of Hepcidin Mature Peptide

The 3D structural model of the hepcidin like mature peptide of *S. richardsonii* was constructed with the Modeller 9.10 [55]. The protein data bank (PDB) was searched for suitable template for active peptide using position specific iterated-basic local alignment search tool (PSI-BLAST). The alignment of the query- template was carried out using ClustalW program [56].

2.5 Model Optimization, Quality Assessment and Visualization

The model was viewed in YASARA view [56] and Swiss-Pdb Viewer 4.0.4 [57]. Hydrogen addition was done in Swiss-Pdb Viewer 4.0.4. Energy minimization was done *in vacuo* with GROMOS 96 implementation of Swiss-Pdb Viewer 4.0.4. The quality of the model was predicted by ERRAT [58]. PDBsum [59] was used for prediction of secondary structure. The stereo-chemical quality of the protein was analysed using PROCHECK. Superposition, alignment and RMSD of template and query were determined by YASARA View [56]. Structural alignment was done by MUSTANG implementation [60] of YASARA view.

2.6 Phylogenetic Analysis

MEGA 5 [61] was used to carry out phylogenetic and molecular evolutionary analyses [62]. Full length amino acid sequences of hepcidin from different species of fish and other mammals were retrieved from GenBank (Table 1) to construct phylogenetic tree by Neighbour-joining method.

3 Results

3.1 Characterization of *S. richardsonii* Hepcidin Like Antimicrobial Peptide

cDNA corresponding to hepcidin like AMP was amplified from the liver of *S. richardsonii* using the primers described earlier and a PCR product of 371 bp was obtained in all fish livers. Five PCR products were randomly sequenced and the deduced nucleotide sequence was submitted to Gen-Bank (Accession number KC894741). Amplified cDNA possesses an open reading frame (ORF) of 279 bp that encodes a predicted pre-prohepcidin of 93 amino acids. The cDNA of hepcidin like AMP from *S. richardsonii* has a 5′ UTR of 70 bp, an ORF followed by 3′ UTR of 19 bp. Using Signal P [63], a hydrophobic region rich in Val and Ala residues was determined in the signal

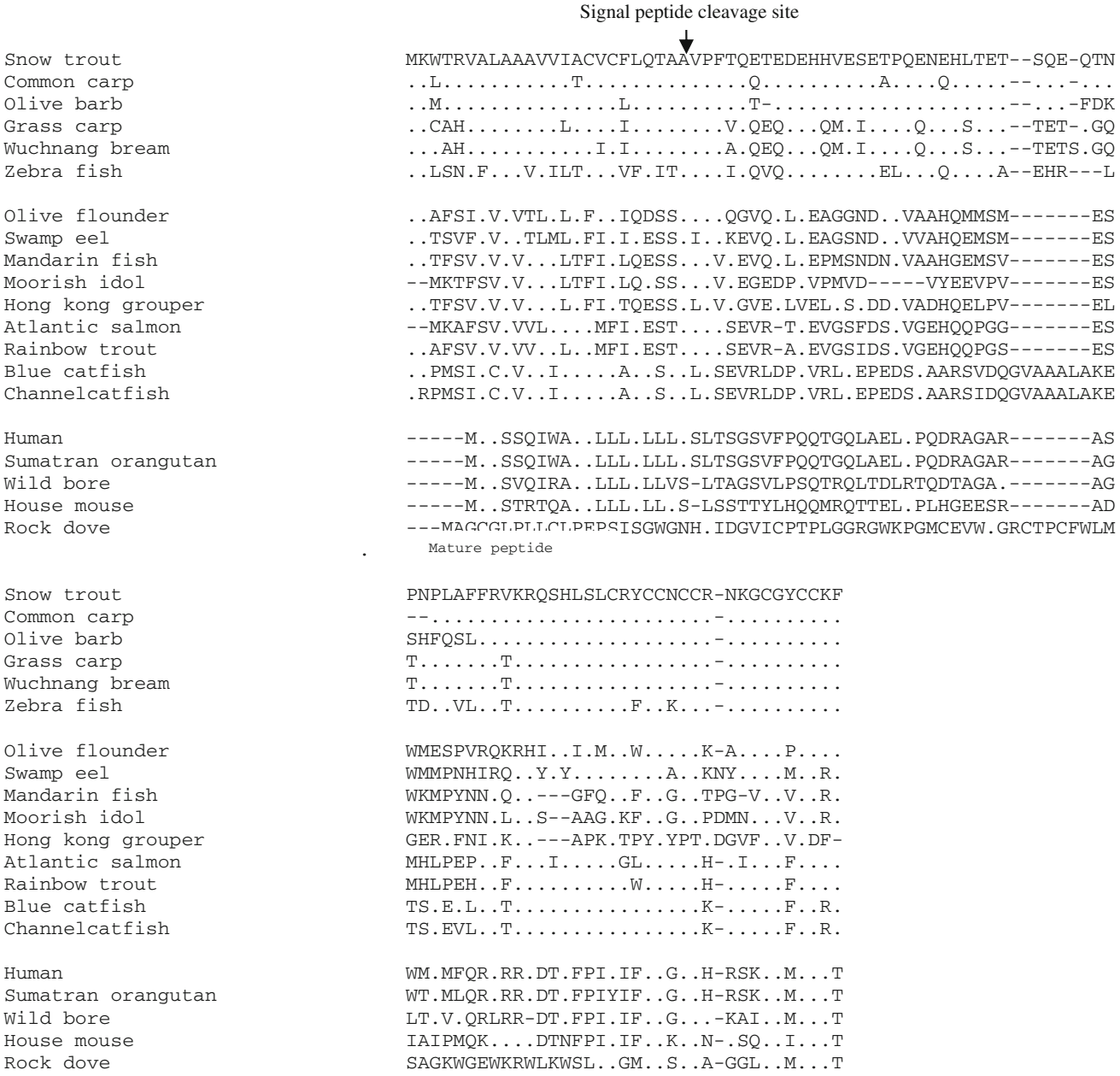

Fig. 1 ClustalW alignment and comparison of the amino acid sequence of hepcidin like AMP deduced from snow trout (*S. richardsonii)* and other vertebrate hepcidins. *Dots* indicate identical amino acids. Gaps are inserted to obtain maximum homology. The cleavage site in the signal peptide is shown by an *arrow*

peptide from *S. richardsonii* having a cleavage site between Ala 24 and Val 25. In *S. richardsonii* the mature peptide was predicted to possess 25 amino acid residues with a pro-domain of 44 amino acids. The molecular weight of hepcidin like mature peptide from *S. richardsonii* was predicted to be 2893D with theoretical pI 8.74. The mature hepcidin like AMP of *S. richardsonii* had a Gravy (grand average of hydropathicity) index of −0.204, exhibiting its hydrophilic nature. Intriguingly, we observed a substitution Gln^{30} in pro-domain, an unusual feature to be observed in cyprinids (Fig. 1).

3.2 Sequence Analysis of Hepcidin Like Peptide of *Schizothorax richardsonii*

BLASTP analysis revealed that the deduced amino acid sequence of *S. richardsonii* has 92, 87, 78, and 66 %, identity with *Cyprinus carpio, Systomus sarana, Megalobrama amblycephala* and *D. rerio* respectively. We observed eight cysteine residues in the mature peptide, motif [RX(K/R)R] for propeptide cleavage and (-CCR/KF) sequence at the C-termini of hepcidin like AMP of *S. richardsonii* (Fig. 1).

3.3 Molecular Modelling of Hepcidin Mature Peptide

3D model of the active peptide of hepcidin like AMP of *S. richardsonii* was generated by homology modelling that provides important information about its function. Upon a PSI-BLAST search against PDB 1S6 W from hybrid white striped bass hepcidin, best template with 70 % identity and 76 % query coverage was selected. The query and template were given as an input in Modeller 9.10 and the best model was selected based on the lowest discrete optimized protein energy. Upon predicting the 3D structure of the mature peptide using Modeller 9.10, it was revealed that hepcidin like mature peptide of *S. richardsonii* was a β-sheet structure with eight cysteine residues. All the eight cysteine residues were connected as Cys^{7}–Cys^{23}, Cys^{10}–Cys^{22}, Cys^{11}–Cys^{19}, $Cys1^{3}$–Cys^{14} to form four disulfide bonds (Fig. 2a) and stabilized hairpin shaped molecular structure.

3.4 Model Optimization, Quality Assessment and Visualization

The model generated from Modeller 9.10 was subject to energy minimization using Swiss-Pdb Viewer 4.0.4 to repair distorted geometries by moving atoms to release local constraints. The generated model showed a quality factor of 100 % in ERRAT. For evaluation of the said structure, 3D model was submitted to PDBsum and the position of secondary structure elements was generated. The model of the active peptide was found to contain one β-sheet with two anti-parallel β-strands, four disulfide bonds, a β-hairpin, a β-turn and a γ-turn (Fig. 2b). Ramachandran plot (Fig. 2c) and RMSD were used to validate the model. Ramachandran plot showed 85.7 % residues in most favored regions, 14.3 % residues in allowed regions and 0 % residues in disallowed region. Overall G factor, a measure of a protein's stereo-chemical property, was calculated to be −0.38 which is greater than the recommended value (−0.50). To further validate the model, it was compared with the respective crystal structure of the template, 1S6 W. The generated model and the template were superimposed for calculation of RMSD. RMSD of mature hepcidin like AMP from *S. richardsonii* was calculated by superimposition over the said template from hybrid white striped bass which was calculated to be 0.935 Å for 18 aligned out of 25 residues.

3.5 Phylogenetic Analysis

To reveal the phylogenetic relationship between hepcidin of *S. richardsonii* and other vertebrates, a phylogenetic tree was constructed using Nebigour-joining method [61]. In phylogenetic analysis, fish hepcidins separate in a different clade from the mammals, inferring that fish and mammalian hepcidins diversified from the same ortholog (Fig. 3). The fish clade could be further separated into three distinct clades. The hepcidin-like AMP of *S. richardsonii* reported herein closely resembles cyprinid hepcidin with olive barb (*Puntius sarana*) as its closest relative.

4 Discussion

AMPs are an important component of innate immune system in fish. In this study we amplified an ORF of 279 bp from *S. richardsonii* that encodes a protein of 93 amino acids and has a high similarity with fish and mammalian hepcidins. It is known that the number of amino acids residues in hepcidin ranges between 83–96 in fish and mammals. For example in gilthead seabream (*Sparus aurata*) an ORF of 255 bp has been reported that codes for pre-prohepcidin of 84 amino acid residues [4] while in blunt snout bream (*M. amblycephala*) an ORF of 285 bp codes for a polypeptide of 94 residues [15]. Mature hepcidin is known to possess 20, 22, 24, 25 and 26 residues in different fish species [4] while in humans three isoforms of 20, 22 and 25 amino acid residues have been reported [16]. It is known that eight cysteine residues constitute molecular signatures for most hepcidins [29] while some species have six. These eight cysteine residues are responsible for the formation of four disulfide bonds in mature peptide. The eight cysteine residues in the mature peptides are highly conserved among fish species including turbot, *S. maximus* [34], zebra fish, *D. rerio* [29], common carp, *C. carpio* [39], gilthead seabream, *Sparus aurata* [4], olive flounder, *P. olivaceus* [30], and humans [16] while in some fish species including Japanese flounder II and winter flounder II [30], six cysteine residues have been reported. The predicted mature peptide of hepcidin like AMP of *S. richardsonii* consists of 25 amino acid residues. We too have observed eight cysteine residues at conserved positions in hepcidin like AMP from *S. richardsonii* involved in formation of four disulfide bonds. The predicted molecular weight of *S. richardsonii* is comparable with blunt snout bream molecular weight 2,892.46 Da and pI 8.34. It has been well documented in several studies that positive charge is a prerequisite factor for interaction between the AMPs and bacterial lipid membrane that leads to pore formation. The hepcidin like mature peptide of *S. richardsonii* too is positively charged (pI 8.74) and may be involved in increasing the membrane permeability thus facilitating the destruction of microbes [64, 65]. We predicted Gravy (grand average of hydropathicity) index −0.204 value of mature hepcidin like peptide of *S. richardsonii* which revealed its hydrophobic nature. The hydrophilic amino acids participate in the formation of hydrogen bond with polar head groups in lipid bilayer and

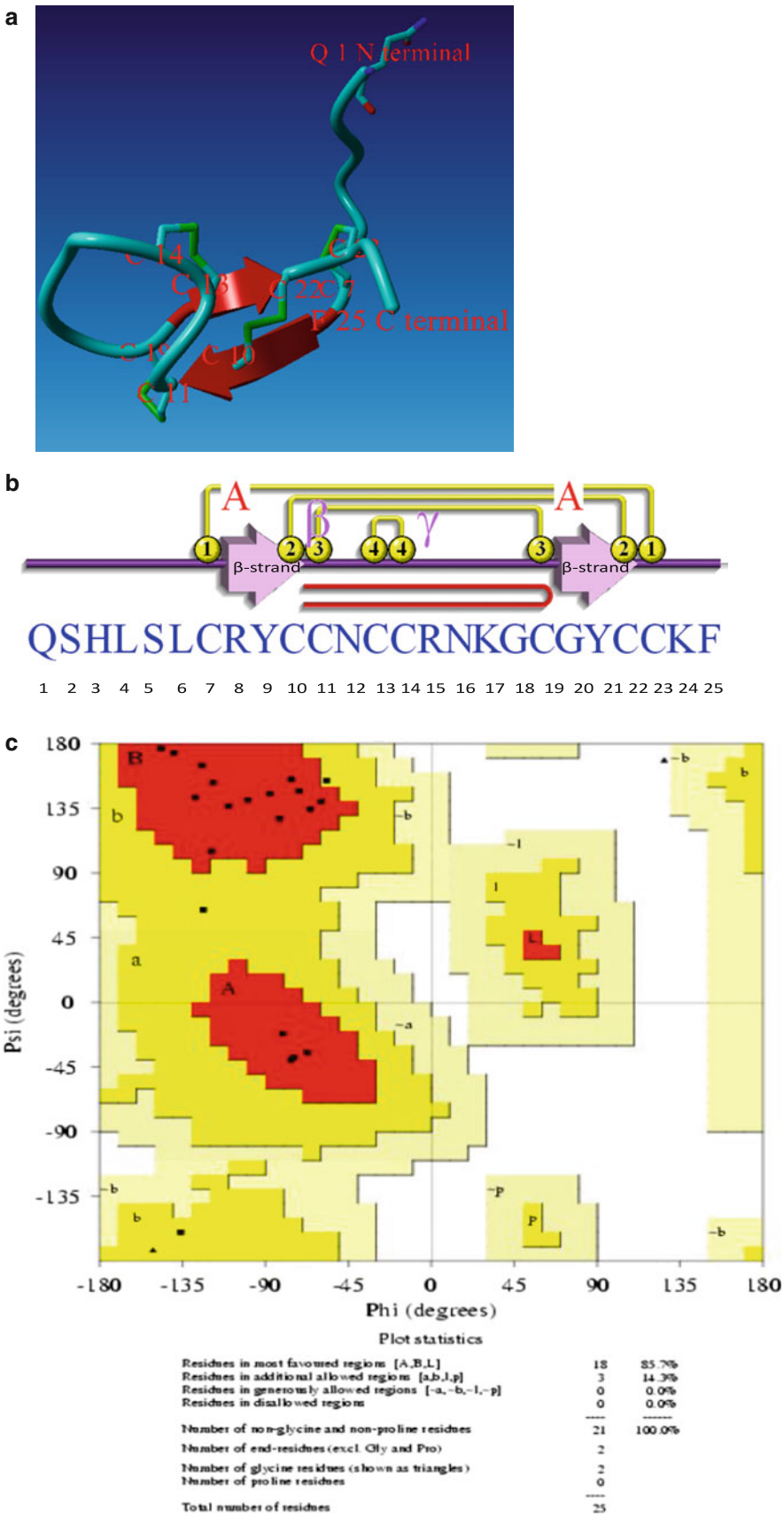

Fig. 2 Structural analysis of mature hepcidin like AMP from *S. richardsonii*. **a** 3D structure of mature peptide of hepcidin like AMP of *S. richardsonii*. *Green colour* in the model indicates the disulfide bonds. **b** Predicted secondary structure of mature peptide of hepcidin like AMP from *S. richardsonii*. *A* indicates β-sheet with two β-strands (one strand at Arg^{8}–Cys^{10} and second strand from Gly^{20} to Cys^{22}). , , β, γ represent disulfide bonds, β- hairpin, beta turn and gamma turns respectively. **c** Ramachandran plot showing plot statistics of the mature peptide comprising 25 amino acid residues of hepcidin like AMP from *S. richardsonii* (Color figure online)

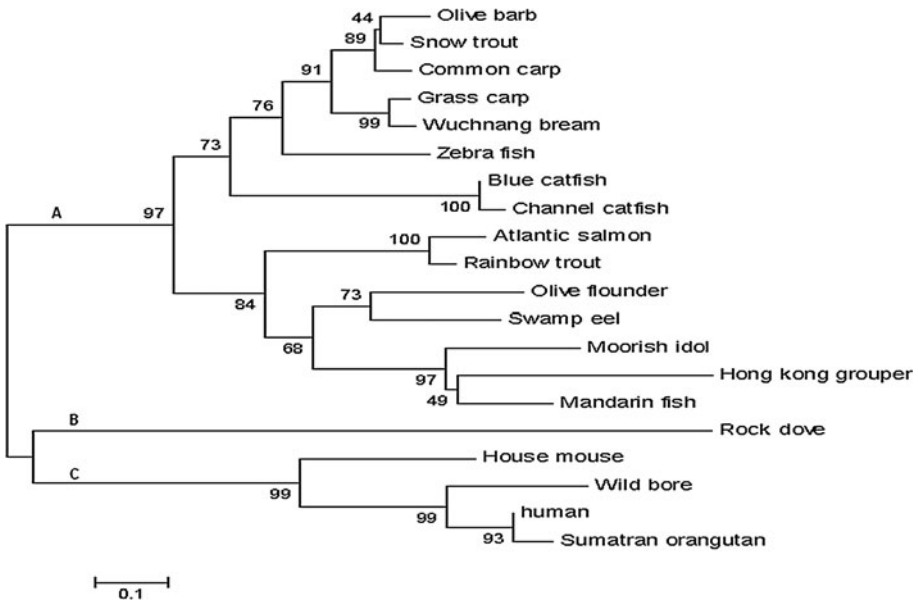

Fig. 3 Phylogenetic analysis of hepcidin like AMP from snow trout and other vertebrates. Phylogenetic tree was constructed using Mega 5. Neighbor-joining method was used to show the relationship between hepcidins of different fish families and other vertebrates. The GenBank accession numbers for these hepcidin sequences are shown in Table 1. The tree is divided into three clades A, B and C which represent hepcidin peptide from different fish families along with bird and mammals. The numbers at tree nodes indicate the percentage of 1,000 bootstrap samples. The scale bar refers to a phylogenetic distance of 0.1 amino acid substitutions per site

membrane proteins of bacterial cell membrane [66]. *In silico* analysis of the mature peptide suggests that the amino acid sequence of the predicted peptide is similar to most hepcidins and it may thus possess antimicrobial capability [4] something, that needs to be elucidated. We observed unique amino acid Gln^{30} in the prodomain of hepcidin like AMP of *S. richardsonii* which is absent in other cyprinids.

The amino acid sequences of hepcidin like AMP are highly conserved among fish species rather than between fish and other vertebrates [28]. Douglas et al. [28] reported that prepro-hepcidin like peptide of fish and mammals possess high similarity in the amino acids of their mature peptide while among fish, amino acids are highly conserved in signal peptide, pro-domain and mature peptide regions. Our findings are similar to the findings of Douglas et al. [28] as alignment of amino-acid sequence of *S. richardsonii* prepro-hepcidin like peptide were highly conserved in signal peptide, pro-peptide and mature peptide regions among the fish species (Fig. 1) while, the deduced amino acid sequence of the pre-propeptide was different from other mammals. Based on the amino acid sequence of human hepcidin [14, 16] and close proximity of [RX(K/R)R] motif, necessary for pro-peptide cleavage, the amino termini of hepcidin like peptides was allocated. C-termini (-CCR/KF) of mature peptide of prepro-hepcidin like peptide is conserved among fish species [5] and similar sequence at the C-terminus of *S. richardsonii* has been reported by us.

In 3D modeling, we predicted β-sheet structure of hepcidin like AMP of *S. richardsonii* in which eight cysteine residues are involved in formation of four disulfide bonds in a discrete pattern Cys^{7}–Cys^{23}, Cys^{10}–Cys^{22}, Cys^{11}–Cys^{19}, Cys^{13}–Cys^{14}. Same pattern of disulfide bonds have been reported in hepcidin from humans (25 or 20 amino acids) [17] and hybrid striped bass (*M. chrysops* × *M. saxatilis*) (21 amino acids) [67]. In the mature peptide of *S. richardsonii* the vicinal disulfide bonds between Cys^{13} and Cys^{14} located at the turn of the hairpin points may also be responsible for the activity of the molecule as reported earlier by Hunter et al. [17]. A quality factor of 100 % was observed for this model by ERRAT. Models having sequence similarity between template and query of more than 50 % are reliable, while similarity between 30 and 50 % and below 30 % results in errors such as mis-prediction of basic folds [68]. We observed 70 % sequence identity in the predicted 3D model of mature peptide of hepcidin like AMP of *S. richardsonii* therefore the predicted 3D model is reliable. To further evaluate the reliability of predicted 3D model, we used Ramachandran plot and RMSD structural assessment methods. In Ramachandran plot we observed 85.7 % residues in most favored region therefore the predicted 3D model of mature peptide of hepcidin like AMP of *S. richardsonii* is a good model. G factor provides a measure of unusualness in the stereo-chemical property of a molecule. The values below −0.5 shows the unusual property while the value below −1.0 high unusualness. The value of G

factor for predicted model is −0.38. Similarity between two 3D models is indicated by RMSD value. Lower the value of RMSD, higher the similarity between the two 3D models. The calculated RMSD value for the mature peptide of hepcidin like AMP from *S. richardsonii* is 0.935 Å. This indicates that the 3D models of the mature peptides derived from 1S6 W (hybrid white striped bass) and hepcidin like AMP of *S. richardsonii* are quite similar. Therefore, all the structure assessment methods Ramachandran plot, G-factor, RMSD value and quality factor confirm the quality of the predicted model.

Hepcidins from different fish species, bird and mammals are divided into three clades as evident from the phylogenetic analysis. All the fish hepcidins are clustered into clade A, while bird and mammalian hepcidins are clustered into clade B and C respectively. Therefore, phylogenetic analysis revealed distant evolutionary relationship between fish and mammalian hepcidins.

5 Conclusion

AMPs are the key mediators of innate immune system which play a vital role in host defense against microbial invasion. Hepcidin is multifunctional cysteine rich small cationic peptide. In this study, we identified and cloned hepcidin like AMP from *S. richardsonii* and a 3D model was predicted besides conducting the phylogenetic analysis. Hepcidin like AMP from *S. richardsonii* is 93 residues long with a signal peptide of 24 amino acid residues having alanine and valine rich hydrophobic region. Mature peptide consists of 25 amino acids with a stretch of eight cysteine residues at conserved positions that form the signature of hepcidins. 3D modeling predicted that the hepcidin like AMP from *S. richardsonii* has β-sheet structure in which all eight cysteine residues are involved in formation of disulfide bonds in a discrete pattern: Cys^{7}–Cys^{23}, Cys^{10}–Cys^{22}, Cys^{11}–Cys^{19}, Cys^{13}–Cys^{14}. The mature peptide of *S. richardsonii* has a vicinal disulfide bond (Cys^{13}–Cys^{14}) at the turn of the hairpin points that might be crucial domain in the active molecule. A unique amino acid Gln^{30} in propeptide of *S. richardsonii* was observed which is absent in other cyprinids. This amino acid might have an effect on function of pro-domain. A 3D model for hepcidin like mature peptide of *S. richardsonii* was generated, that may be employed to predict the functional behavior of the protein.

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