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Antimicrobial activity of trout hepcidin

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ABSTRACT

Hepcidin is an antimicrobial peptide and a hormone produced mostly the liver. It is a cysteine-rich peptide with a highly conserved β-sheet structure. Recently, we described the hepcidin expression in liver of rainbow trout and its inducibility by iron overloading and lipopolysaccharide (LPS). Thus, in this work, we focused in analyzing the importance of the peptide conformation associated to its oxidative state in the antimicrobial activity. This peptide showed a α-helix conformation in reduced state and the characteristic β-sheet conformation in the oxidized state. Antimicrobial activity assays showed that the oxidized peptide is more effective than the reduced peptide against *Escherichia coli* and the important salmon fish pathogen *Piscirickettsia salmonis.* In addition, confocal analysis of *P. salmonis* culture exposed to trout hepcidin coupled with rhodamine revealed the intracellular location of this peptide and Sytox permeation assay showed that membrane disruption is not the mechanism of its antimicrobial action. Moreover, a conserved ATCUN motif was detected in the N-terminus of this peptide. This sequence has been described as a small metal-binding site that has been implicated in DNA cleavage. In this work we proved that this peptide is able to induce DNA hydrolysis in the presence of ascorbate and $CuCl_2$. When the same experiments were carried out using a variant with truncated N-terminus no DNA hydrolysis was observed. Our results suggest that correct folding of hepcidin is required for its antimicrobial activity and most likely the metal-binding site (ATCUN motif) present in its N-terminus is involved in the oxidative damage to macromolecules.

1. Introduction

Hepcidin is an antimicrobial peptide (AMP) identified in mammals, fishes, birds, reptiles, and amphibians [1,2]. Moreover, it is considered an important hormone involved in the iron homeostasis [3]. Fish hepcidin genes encodes a preproprotein with a signal peptide, a prodomain and the mature peptide [4,5]. The mature hepcidin is a cysteine-rich peptide of 20–25 amino acids in length. Interestingly, the cysteines residues are conserved from mammals to teleost fishes such as *Oreochromis niloticus, Gadus morhua, Oryzias melastigma, Epinephelus coioides, Salmo salar* and *Oncorhynchus mykiss* [4,6–8]. It has been described different variant of teleost hepcidin with eight, six or four cysteines [9]. In human, this residues are involved in the conformation of the characteristic antiparallel β-sheet [10].Thus, it is possible that this folding is conserved in teleost hepcidin. In addition, NMR spectroscopy analyses indicated that hybrid striped bass and human hepcidin peptides adopt a similar three dimensional structure and have the same disulfide-bonding pattern [11].

The antimicrobial activity of the teleost hepcidin has been demonstrated against different bacterial and viral pathogens, and also against certain fungi. Medaka hepcidin showed growth inhibition against the Gram-positive bacteria *Corynebacterium glutamicum*, *Staphylococcus aureus* and against the Gram-negative bacteria *Escherichia coli*, *Aeromonas hydrophila*, and *Pseudomonas stutzeri* at lowest concentration [12]. Two synthetic hepcidin variants from orange-spotted grouper caused growth delay in the Gram-negative bacterium *Vibrio vulnificus* and the Gram-positive

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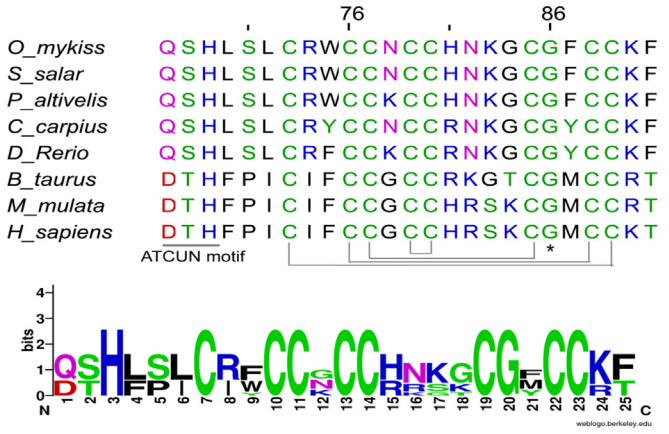

Fig. 1. Conserved ATCUN motif in the mature peptide hepcidin sequence. Conserved ATCUN motif in the mature peptide hepcidin sequence. Sequence alignment of the rainbow trout mature hepcidin with other species. The eight cysteines forming the disulfide bridge pattern are indicated (gray lines) and the conserved N-terminal ATCUN motif (XXH) is shown. At the bottom the logo of the sequence indicating the conservation.

bacterium *S. aureus* [7]. Interestingly, the replication of Singapore grouper iridovirus (SGIV) was inhibited by these synthetic peptides [7]. Moreover, antifungal activity of marine fish hepcidin has been described against the fungi *Aspergillus niger*, *Fusarium graminearum* and *Fusarium solan* [4].

Recently, we described the hepcidin expression in liver of rainbow trout and its inducibility by iron overloading and pathogen-associated molecular patterns, such as bacterial lipopolysaccharides [13]. Moreover, hepcidin level (quantified by sandwich ELISA) in rainbow trout challenged with *Aeromonas salmonicida* was increased twofold with respect to the untreated fish in head kidney samples, in parallel to the increase in the observed transcriptional level in the head kidney cells [14]. These results suggest the involvement of hepcidin in response to pathogens as an innate component of teleost fishes.

However, few studies have described the importance of the hepcidin conformation and the possible mechanism for the antimicrobial activity of this peptide. Like human hepcidin, trout hepcidin possesses eight cysteines involved in four disulfide bonds. It is has been reported that the absence of disulfide bridges in peptides results in a decrease in their antimicrobial activity [15]. Human and mouse defensins in its reduced forms were shown to possess fewer antimicrobial activity than in their oxidized forms [16,17]. Therefore, the oxidized state of peptides with several cysteines, like hepcidin, is very important for its optimal conformation and antimicrobial activity [18].

Interestingly, a histidine residue in the N-terminus of teleost and mammals hepcidin is highly conserved. This residue has been described to form a putative metal binding motif [19]. This region is known as "amine terminal Cu^{2+} and Ni^{2+}binding" or ATCUN motif [20]. The ATCUN motif is present in the N-terminus of serum albumins and has been shown to possess nucleolytic activity [21].

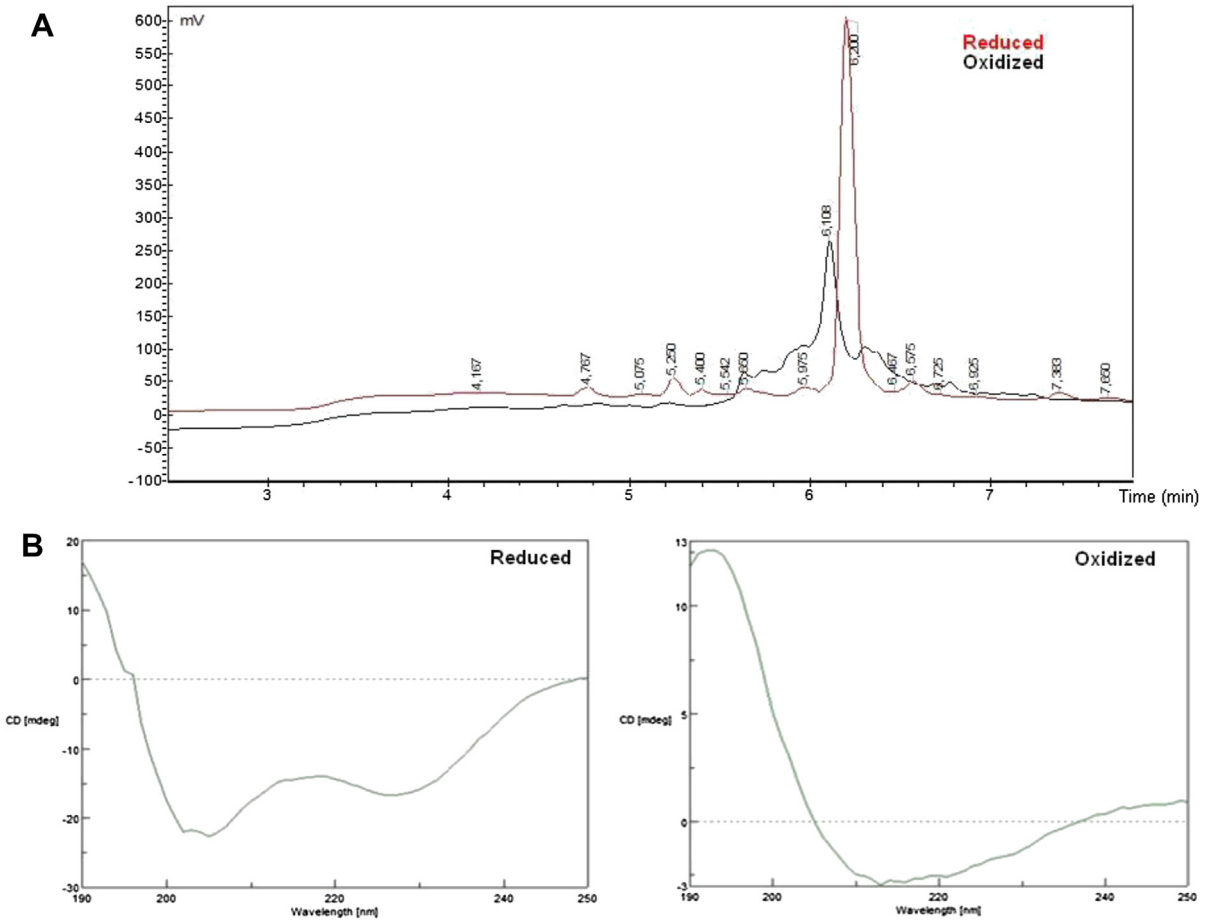

Fig. 2. Conformational change of trout hepcidin by cys-oxidation. A) RP-HPLC analysis of the Hep25 peptide in oxidized and reduced state. B) Circular dichroism of the Hep25 in the far UV in 30% TFE in its oxidized (right) and reduced state (left).