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Chicken cathelicidins as potent intrinsically disordered biocides with antimicrobial activity against infectious pathogens

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ABSTRACT

This study was performed to identify the expression patterns of the cathelicidin genes in a local chicken breed and to evaluate the antimicrobial activities of the cathelicidin peptides against pathogenic bacteria. This analysis revealed that the coding regions of *CATH-1*, *-2*, and *-3* genes contain 447 bp, 465 bp, and 456 bp, respectively, and encode proteins of 148, 154, 151 amino acids, respectively. The complete amino acid sequences of the cathelicidin peptides are similar to those found in *Meleagris gallopavo*, *Phasianus colchicus*, and *Coturnix coturnix*, and show high sequence identity to their *Columba livia* and *Anas platyrhynchos* counterparts. In contrast, these avian peptides shared a very low sequence identity with the mammalian cathelicidins. The analysis further revealed that the cathelicidin genes are expressed in various organ and tissues. We also show that the CATH peptides 1, 2, 3 and their amide-modified structures possess potent antimicrobial activities against both Gram-positive and Gram-negative pathogens, with these bacteria being affected to different extents. The antimicrobial activities of the peptides are slightly lower than those of their amide analogs. Computational analysis revealed that pre-pro-cathelicidins are hybrid proteins that contain ordered domains and functional intrinsically disordered regions. Furthermore, high structural and sequence variability of mature cathelicidins is a strong indication of their rather disordered nature. It is likely that intrinsic disorder is needed for the multifarious functionality of these antimicrobial peptides. Our analyses indicated that cathelicidin peptides require further study to better understand their full potentials in the treatment of diseases in both humans and animals. The data obtained for synthetic avian peptides will help elucidating of their potential applications in the pharmaceutical industry.

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1. Introduction

Antimicrobial peptides (AMPs) are classified in several families and are widely found in living organisms, including mammalian, plants, insects and avian species. These peptides display antimicrobial potential against a broad spectrum of microorganisms, including bacteria, fungi, parasites and viruses. Such peptides are called host defense peptides (HDPs). In addition to functions in control and inactivation of microbial growth, their involvement into the immunomodulatory mechanisms are being discovered (Cuperus et al., 2013; Sugiarto and Yu, 2004). These peptides have short amino acid chains of less than 100 amino acids and are cationic molecules rich in arginine, lysine and histidine residues. They are amphipathic, containing both a hydrophobic and hydrophilic region, and serve as important players of an ancient defense mechanism against various types of pathogenic agents. Currently, the full sequences and phylogenies of the defensin and cathelicidin genes have been established for the avian class. In addition, several reports have focused on determining the function of these peptides as templates for novel anti-microbial agents (Cuperus et al., 2013; Yacoub et al., 2015a).

Cathelicidins are one of the major HDP groups and have been found in mammals (Zanetti, 2005), fish (Chang et al., 2006; Uzzell et al., 2003), reptiles (Zhao et al., 2008), and birds (Lynn et al., 2004; van Dijk et al., 2008; Xiao et al., 2006a). The name cathelicidin is derived from the similarity of the cathelicidin large middle domain to cathelin, a cathepsin L inhibitor that was originally isolated from porcine leukocytes (Ritonja et al., 1989). The cathelicidin genes vary considerably between species, with humans, rhesus monkeys, mice, rats, and guinea pigs possessing only one gene, whereas eight or more catheliciden genes have been found in pig, sheep and cattle (Frohm Nilsson et al., 1999; Nagaoka et al., 1998; Scocchi et al., 2009; Termen et al., 2003; Zhao et al., 2008). Currently, four avian cathelicidin peptides have been identified (Lynn et al., 2004), including fowlicidin-2 (Goitsuka et al., 2007; van Dijk et al., 2008; Xiao et al., 2006a). Three cathelicidens genes, *Pc-CATH-1*, *-2*, and *-3*, have been described in pheasant (*Phasianus colchicus*). These three genes share a high level of similarity with chicken cathelicidins-1, -2, and -3 (Wang et al., 2011).

Most cathelicidins, particularly avian cathelicidins, exhibit antimicrobial activity toward a broad spectrum of pathogens, including bacteria and fungi. The underlying mechanism by which AMPs exert their effect is through their interaction with the negatively charged phospholipid bilayer of the cell, which leads to the disorganization of the cell membrane, disruption of membrane integrity, and eventually results in killing of the infectious agents (Brogden, 2005; Ganz, 2004; Powers and Hancock, 2003). The cathelicidin peptides possess high positive net charge and contain conserved cysteine residues responsible for the formation of intramolecular disulfide bonds. These structural characteristics are believed to be important for the antimicrobial potential of cathelicidins (Zasloff, 2002). Importantly, these peptides have bactericidal activity with little probability of consolidated bacterial resistance (Zasloff, 2002).

Several other mechanisms by which cathelicidins are able to kill pathogens have been revealed, including involvement in the intracellular processes of blocking nucleic acid and protein synthesis (Brogden, 2005; Nicolas, 2009). Furthermore, some other roles have been described for several AMPs, especially those involved in the immune response consolidation and regulation (Finlay and Hancock, 2004). However, the primary biocidal mechanism of AMPs is killing microbes via disruption of their membrane integrity, the action that depends on two main characteristics, their cationic nature and amphipathicity (Evans and Harmon, 1995; Powers and Hancock, 2003). In this study, we aimed to identify the expression patterns of the cathelicidin genes in a local Saudi chicken, to evaluate the antimicrobial activity of the corresponding peptides against pathogenic bacteria, and to monitor the changes in their antimicrobial activity by using amidated analogs of these peptides.

Many biologically active proteins and peptides are known to be intrinsically disordered (IDPs); i.e., they either contain intrinsically disordered protein regions (IDPRs), which are functionally important regions without unique 3D structure, or are disordered as a whole (Dunker et al., 1998, 2001, 2000; Dyson and Wright, 2005; Habchi et al., 2014; Iakoucheva et al., 2002; Radivojac et al., 2007; Tompa, 2002, 2012; Turoverov et al., 2010; Uversky, 2002a, 2015; Uversky and Dunker, 2010; Uversky et al., 2000, 2005; van der Lee et al., 2014; Ward et al., 2004; Wright and Dyson, 1999). The disorder contents and flexibility vary between different proteins, and this variability is determined by the peculiarities of their amino acid sequences (Dunker et al., 2001; Habchi et al., 2014; Uversky, 2003, 2013; Uversky and Dunker, 2010; Uversky et al., 2000). IDPs/IDPRs are commonly found in all proteomes analyzed so far (Cheng et al., 2007; Dunker et al., 2000; Mohan et al., 2008; Oldfield et al., 2005; Pancsa and Tompa, 2012; Peng et al., 2015; Schad et al., 2011; Uversky, 2010; Uversky and Dunker, 2010; Uversky et al., 2006; Ward et al., 2004; Xue et al., 2012; Xue et al., 2010), where they have a multitude of crucial functions (Dunker et al., 2002a; Dunker et al., 2001, 2002b, 2005, 2008; Dunker and Obradovic, 2001; Dunker and Uversky, 2008; Oldfield et al., 2008; Radivojac et al., 2007; Tompa, 2002, 2003, 2005; Tompa et al., 2009, 2015, 2005; Uversky, 2002a, b, 2003; 2011; Uversky and Dunker, 2010; Uversky et al., 2000, 2005; Wright and Dyson, 1999). Earlier we showed that intrinsic disorder is important for functionality of two HDPs, defensins (Mattar et al., 2016) and NK-lysins (Yacoub et al., 2016). Keeping this in mind, we analyzed intrinsic disorder predisposition in cathelicidins.

2. Materials and methods

2.1. Animals

Fifty healthy, Baladi (local) chickens (*Gallus gallus* domesticus) obtained from King Abdulaziz University farm, Jeddah, Saudi Arabia, were used. The birds were kept in cages and provided with water and food ad libitum. This study was approved by the ethical committee of King Abdulaziz University and followed the health guidelines for completing the animal experiments.

2.2. Tissue collection

Tissue specimens were collected from the organs of nineteen chickens, including the bone marrow, spleen, liver, oviduct, ovum, large and small intestines, pancreas, skin, egg yolk, muscles, heart, testis, duodenum, gizzard, uterus, and kidney. These tissues were dissected and frozen in liquid nitrogen until use.

2.3. RNA isolation and cDNA synthesis

The total RNA was isolated from 30 to 60 mg of the bone marrow, spleen, liver, oviduct, ovum, large and small intestines, pancreas, skin, egg yolk, muscles, heart, testis, duodenum, gizzard, uterus, and kidney tissues using the EZ RNA Clean Up Plus DNase Kit (EZ BioResearch, St Louis, MO, USA). The RNA concentrations were measured using a NanoDrop Spectrophotometer (Jenway, UK). Reverse transcriptions (RT) was performed using the oligo-dT primers (Bioneer, Inc., Daejeon, Republic of Korea) in a 20 μL reaction mixture containing 5 μL of the RNA. The cDNAs obtained