Gallinacin and Fowlicidin: Two Promising Antimicrobial Peptides in Chickens—A Review

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Abstract

Antimicrobial peptides (AMP) which have been identified in almost all groups of organisms, are the small cationic molecules that recognize the pathogen associated molecular patterns of the microbes. In chicken two main AMPs that play significant roles in bolstering the innate immunity are gallinacins and fowlicidins, which are the functional analogues of the mammalian beta-defensins and cathelicidins. Gallinacin identifies the Gram negative bacteria while fowlicidin exerts broad spectral activity. The basic mechanism of action is by far similar in both groups of AMPs. The 'docking sites' of these antimicrobial peptides includes the "lipid A" moiety of lipo polysaccharides, lipo-teichoic acids, anionic membrane phospholipids on bacterial surfaces. These AMPs block the DNA replication and protein synthesis in bacteria causing death of the microbe. Researchers have identified reproducible molecular markers of those peptides for selection of disease resistant stock of chickens.

Key words: Gallinacin, Fowlicidin, Antimicrobial peptides, Chicken, Innate immunity.

Introduction

The innate immunity which is the major system of host defense against pathogens in nearly all living organisms, has originated prior to the specific or adaptive immunity. The later is supposed to have arisen first in the gnathostomata on the way through the evolution of the vertebrates, to provide the immunological memory for specific pathogens and to mount stronger attacks during further encounter. Genetically transcribed antimicrobial peptides have been recognized as key players in non-adaptive defense systems. Starting with the discovery of small cationic antimicrobial proteins in rabbit and guinea pig granulocytes (Zeya and Spitznagel, 1966) and thionin (Fernandez de Caleya et al., 1972) in plants, scores of antimicrobial peptides have been discovered in almost all groups of animals. Antimicrobial peptides (AMPs) are cationic molecules, less than 100 amino acids long and are rich in histidine, lysine, and arginine. They are amphipathic, containing hydrophobic and hydrophilic regions with a broad spectral antimicrobial activity (Sugiarto and Yu, 2004).

The basic mechanism of action of these antimicrobial peptides is the electrostatic interaction between the cationic peptides and the anionic membrane component of the pathogen, with exception to some strongly anionic antibiotic peptides (Andreu and Rivas, 1998). Pathogen-associated molecular patterns (PAMPs), small molecular motifs consistently found on pathogens, are recognized by pattern recognition receptors (PRRs) in plants and animals to activate the host innate immune responses. Bacterial lipopolysaccharides (LPS), anionic molecules that contribute to the structural integrity of the bacterial membrane, are considered to be the prototypical PAMPs. Antimicrobial peptides play an integral role in the innate immune response to microbial infections in poultry because avian heterophils lack the superoxide ion and myelo-peroxidase (Xiao et al., 2006); therefore, they rely more on the non-oxidative mechanisms involving lysozyme and cationic proteins (Ma et al., 2008). In the present context we will restrict our discussion to Gallinacin and Fowlicidin AMPs of chickens.

Gallinacin: the functional Equivalent of ß-Defensin in Chicken:

Defensins are ß-sheet proteins of 3.5 to 4.5 kDa size (Bals et al., 1998), characterized by the presence of a conserved cysteine-rich defensin motif (Ma et al., 2008) that contribute to the antimicrobial properties of mammalian granulocytes, epithelial cells, and certain secretions. Although defensins are very prominent in

granulocytes, they are not ubiquitous, since defensin expression is absent in the neutrophils of horses (Couto et al., 1992), mice (Eisenhauer and Lehrer, 1992) and pigs (Kokryakov et al., 1993). Among the mammals, the primary structures of defensin molecules are considerably variable except for the conserved cysteines (Lehrer and Ganz, 2002). Three types of mammalian defensin have been identified, based on the ordered array of three disulfide bonds formed between six cysteine molecules. The a-defensins are abundant in granulocytes (Ganz and Lehrer, 1995) and small intestinal Paneth cells (Mallow et al., 1996), while ß-defensins occur in leukocytes of many species (Selsted et al., 1993; Harwig et al., 1994). The third one of defensin genes, theta (?)-defensins, has been identified only in leukocytes of the rhesus monkey (Tang et al., 1999). In chicken, only the ß-defensin gene families exist, which is termed as gallinacin (Xiao et al., 2004), where as in turkey, the same gene family is termed as 'Gallopavin' (Zhao et al., 2001). Evolutionary studies indicate that a-, ß- and ?-defensins originated from a common ancestral ß-defensin gene (Liu et al., 1997; Harwig et al., 1994).

The Gallinacins have been extensively studied in chicken as compared to other avids. Gallinacins pass through the bacterial membrane and hinder transcription and translation (Ganz, 2003) after being activated by the pathogens. A total of 13 different gallinacin genes, designated as Gal-1 to -13, have been identified (Xiao et al., 2004) and mapped within an 86-kb region of chromosome 3q3.5-q3.7 in chickens. Their expressions of gallinacins are abundant in cells that are involved in the innate immune response against microbial infections. This illustrates the importance of the gallinacin peptides as a bridge between the innate and adaptive immune responses in chickens (Menendez and Finlay, 2007.). Detailed study has revealed the expression of Gals in different organs as, Gal1 to 7 in bone marrow, tongue, trachea, and bursa of Fabricius (Zhao et al. 2001), Gal 8-13 in liver, kidney, testicle, ovary, and male and female reproductive tract tissues (Xiao et al. 2004). The expression of Gal-1, 2 and 3 is higher in infundibulum and surface epithelium of vagina since the vaginal immunity is indispensable to protect the oviduct which, otherwise, may be perturbed by the microbial load being harbored in the cloaca (Ohashi et al., 2005).

Gallinacin as a potential marker against Gram Negative Bacterial Infection:

The range of antimocrobial activity of avian ß–defensins have been studied to unveil their action against bacteria and fungi (Higgs et al., 2005) vis-à-vis their correlations with TLR4 genes involved in Salmonella enterica serovar Enteritidis (SE) resistance (Akter et al., 2007). The heritability of SE counts in the spleen (0.10–0.32) and the cecum (0.06–0.20) of chicken demonstrates the genetic control of the trait (Berthelot et al., 1998). Keeping in view the possibility to develop genetically immune chicken lines, research endeavors have mainly been emphasized on the tissue specific expression pattern and marker assisted selection studies. Hasenstein et al. (2006), Hasenstein and Lamont (2007) identified a mean of 13.2 to 17 single nucleotide polymorphisms (SNP) per kilobase in five candidate genes (Gal-2, 3, 4, 5, and 7) in White Leghorn chickens. They proposed to utilize the SNPs as molecular markers for the response to S. enterica serovar Enteritidis to enhance the immune response in poultry. The Gallinacin genes (Gal-8 and -9) have also been cloned to produce recombinant Gallinacin (Gal) proteins (Ma et al., 2008) while the global gene expression profile in chicken heterophils has been studied to establish the association between Gallinacin expression with in vitro Salmonella infection (Chiang et al., 2008). The up-regulation of the Gal expression has been ardently studied to establish some application in nutri-genomics. Expression of ß-defensin genes is induced by inflammation or bacterial infection (Akbari et al., 2008, Menendez and Finlay, 2007) or lipopolysaccharide (LPS) (Subedi et al., 2007).

Fowlicidin: A broad spectral Antimicrobial peptide:

Cathelicidins, identified in mammalian species, contain a conserved cathelin precursor domain. To date, cathelicidins have been described in various mammalian species (Bals et al., 2001; Agerberth et al., 2000). Extensive research work has been undertaken on the sequence and structural biology of cathelicidin (Ganz, 1997; Bals et al., 2001). Inactive pro-peptides of cathelicidins are stored in the secretory granules of neutrophils and the active counterparts are released by proteolytic processing, into the phagosomes or into the extracellular environment of the cell. Cathelicidins evoke innate immunity against Gram-positive and negative bacteria, enveloped viruses and fungi. Cathelicidins are chemo-attractant and they activate a variety of immune cells, interact with lipopolysaccharide (LPS) as well as promote angiogenesis, cytolysis and wound healing. Cathelicidins, like defensins, bind negatively charged microbial LPS membranes by physical interaction and induce bacterial cell death by membrane permeabilization and disruption. Xiao et al. (2006) identified 3 novel cathelicidins in chicken (fowlicidin-1, fowlicidin-2, and fowlicidin-3) densely clustered within 7.5kb on the p-arm of chromosome 2. The genes coding for the three fowlicidins consists of the 4 exon- 3 intron structure, typical of mammalian cathelicidin. The three peptides are positively charged at the C-terminus due to an excess of arginine and lysine.

Lynn et al. (2004) reported the bioinformatic properties and tissue expression pattern of nine host

defense peptide genes in chicken, including those of fowlicidin. Xiao et al. (2006) studied the broad spectral and salt-insensitive antibacterial activities of fowlicidins and also certified these host defense peptides as excellent candidates for novel antimicrobial and antisepsis agents due to their potent LPS-neutralizing activity. Goitsuka et al. (2007) described a previously unrecognized cathelicidin gene in chickens, chCATH-B1 that is expressed exclusively in the epithelium of the bursa of Fabricius. Bhuina et al. (2009) proposed that the helical structures of peptide fragments derived from fowlicidin-1 in LPS could be utilized to develop non-toxic anti-endotoxic compounds. They elucidated the structural and functional relationship of the amino acid sequence of fowlicidin genes to their bactericidal properties. The optimized truncated peptides of fowlicidins has been patented (Patent Number: US 2008/0119405 A1) which could retain bactericidal properties yet with reduced toxicity to host cells. To the best of our knowledge, no report has been furnished on polymorphism study or SNP detection of the fowlicidin genes. It indicates a vast scope of research work that may be carried out to explore new areas of anti microbial peptides and their application in host defense enhancement.

Conclusion

To date several host defense petides (namely, defensins, alamethicin, bactenecins, magainins, cecropins, melittins etc) have been screened and identified in different vertebrate species. Protection against the pathogenic contamination in animal food has become the mammoth challenge in the food-animal industry. Poultry or poultry products account for a significant proportion of human diseases. Application of antibiotics to the chicken has raised serious concerns about the drug residues in the processed foods, which has detrimental effect on the flora and fauna of the digestive tract. Further, the extensive use of antibiotics has evolved antibiotic resistant pathogens. Hence, mining novel antimicrobial agents as natural alternatives for antibiotics has become a feasible and promising approach. It would be visionary to expect some constructive roles of these antimicrobial peptides to enhance the host immunity. This will be helpful by far to reduce the application of antibiotics which have certain unsought consequences. In depth research work may be designed to unveil the mechanism of action of the novel antimicrobial peptides in different species of animals. It would be advantageous to select the livestock for general rather than specific disease resistance, as specific immunity would not act on wide range of pathogens, but innate immunity does. Nutrigenomics study can expound possible ways to up regulate the expression pattern of the desired host defense peptides. In long run, if certain antimicrobial peptides with spectacular disease resistance capacity are discovered, that may be used for transgenesis in susceptible species. Thus the research area of antimicrobial peptides is still left untouched up to a great extent that would entice many interested researchers to explore the field.

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