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Review

The human beta-defensin-3, an antibacterial peptide with multiple biological functions

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Abstract

A group of interesting molecules called defensins exhibit multiple functions but have been primarily recognized to possess a broad spectrum of antimicrobial activities. Studies have reported two different types of defensins (α and β) from human and animals, a cyclic θ defensin from rhesus, and several defensin-like peptides from plants. There is no amino acid sequence homology between these peptides, but they all contain three Cys–Cys disulfide linkages while the connectivities are different. Human β-defensin-3 (HβD-3) is the most recently discovered member of the host-defense peptide family that has attracted much attention. This molecule is expressed either constitutively or induced upon a challenge, and a growing evidence indicates the involvement of such molecules in adaptive immunity as well. It has been shown to exhibit antibacterial activities towards Gram-negative and Gram-positive bacteria as well as an ability to act as a chemo-attractant. Analysis of NMR structural data suggested a symmetrical dimeric form of this peptide in solution, which consists of three β strands and a short helix in the N-terminal region. While the disulfide linkages are known to provide the structural stability and stability against proteases, the biological relevance of this dimeric form was contradicted by another biological study. Since there is considerable current interest in developing HβD-3 for possible pharmaceutical applications, studies to further our understanding on the determinants of antibacterial activities and immunomodulatory function of HβD-3 are considered to be highly significant. The knowledge of its biosynthetic regulation will also help in understanding the role of HβD-3 in immunity. This article presents an overview of the expression and regulation of HβD-3 in humans, and the structure–function correlations among HβD-3 and its modified peptides are discussed emphasizing the functional importance. The future scope for studies on HβD-3 and design of short potent antimicrobial peptides, based on the native HβD-3 molecule, that do not interfere in the immunomodulatory function is also outlined.

Keywords: Antibacterial peptide; Defensin; HβD-3; Structure; Innate and adaptive immunity; Membrane-disruption

Contents

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1. Introduction

Defensins are a family of antimicrobial peptides and vital contributors to host immune response. Being constitutively or inducibly expressed, they have been shown to contribute to innate host defense via direct bacteriocidal activity, as well as to adaptive immunity through effector and regulatory functions. Defensins are an efficient part of the first line of host defense because of their ability to recognize and neutralize invading microorganisms quickly and specifically. Defensins show antimicrobial activities against Gram-negative and Gram-positive bacterial strains and fungi, as well as some parasites and enveloped viruses. Their mechanism of activity is known to involve membrane permeabilization, although different peptides act in different ways and exact mechanisms are only beginning to be elucidated [1–3]. This relatively non-specific membrane permeating mechanism makes incidence of resistant bacteria rare. Defensins also exhibit chemotactic behavior for certain cells and function to induce the adaptive immune system. Certain defensins play different roles in recruitment and exhibit receptor-specific chemotactic activity. Overall, defensins have a great potential for pharmaceutical applications as antibiotics as well as modulators of inflammation [1]. As a family, the defensins deserve a special attention due to their particular prominence in humans, constituting a number of genes and being extensively present in human tissues.

While there are three distinct classes of defensins (α, β, and θ-defensins), only α- and β-defensins are expressed in humans (for a regularly updated list of plant and animal antimicrobial peptides, see the website: http://www.bbcm.univ.trieste.it/~tossi/antimic.html, and also references [4] and [5]). Both α- and β-defensins are short cationic peptides (29–45 residues) containing six conserved cysteine residues involved in disulfide linkages. The tertiary structure of these peptides consists of three antiparallel β-sheets, which are constrained by cysteine residues, making up the characteristic "defensin-like" fold and spatially separated hydrophobic and hydrophilic regions. α- and β-defensins are products of distinct gene families that are thought to have evolved from an ancestral β-defensin gene; α-defensins show an evidence of being newer because they are more homologous as a group and exist only in mammals. This divergence resulted in adjacent clusters on chromosomal maps for α- and β-defensin genes; in humans on chromosome 8p23, although some newly identified β-defensin genes map to different chromosomes [6,7].

The disulfide connectivities in α-defensins are Cys1–Cys6, Cys2–Cys4 and Cys3–Cys5 (the number indicates the location of the Cys residue in the amino acid sequence from the N-terminus). They are expressed in human neutrophil cells, Paneth cells of the small intestine, and a very few epithelial cells. The four human α-defensins originally isolated from neutrophil cells are named as HNP1–4 (human neutrophil peptides); HD-5 and HD-6 (where HD stands for the human defensin) are products of Paneth cells.

The disulfide connectivities in β-defensin are Cys1–Cys5, Cys2–Cys4 and Cys3–Cys6. β-defensins are found in epithelial cells. Human β-defensins are named as HβD-1–4 and were originally isolated from human plasma (HβD-1) and psoriatic scales (HβD-2, HβD-3); HβD-4 has not yet been isolated, but identified solely by genomics. The human genome suggests that there are at least 25 β-defensins that are yet to be discovered [8]. HβD-1 is constitutively expressed in some tissues (but can also be upregulated), while HβD-2–4 are inducible, usually in response to pro-inflammatory stimuli. β-defensins have been shown to be ligands for chemokine receptor CCR6 on dendritic cells (DCs) and T cells; this is the basis of their activity as effector molecules of adaptive immunity.

Studies continue to show specific activities of certain defensins and their activity against specific microbial agents. Difficulties arise in correlating *in vitro* and *in vivo* activities of defensins, as well as differentiating the activities of antimicrobial peptides from that of other components of the immune system due to their overlap in function. Another problem is that the *in vitro* antimicrobial activities of most defensins are dulled by physiological salts, divalent cations and serum proteins; the magnitude of inhibition depends on the defensin and its target bacteria. These sensitivities suggest that most of the defensin activities take place in membrane sequestered environments where salt and serum concentrations are low and defensin concentrations are high, such as phagocytic vacuoles or the external surface of skin and mucus membranes.

2. β-Defensins and their roles in diseases

As defensins are a part of the host immune system, they are implicated in a wide variety of conditions and diseases. In many cases, a disease state is accompanied by a change in the amount of defensin expression in the diseased tissue. Patients with vascular diseases have shown high levels of defensins in atherosclerotic plaques in humans. In this way, defensins may be mediators of vascular diseases. It was found that defensins interfere with LDL (low density lipoprotein, known as "bad cholesterol") and Lp(*a*) (lipoprotein *a*) degradation and therefore contribute to the accumulation of these lipoproteins. Defensins also appear to inhibit angiogenesis, a defect associated with traumatic aortic dissection and coronary artery disease [9].

Crohn's disease is an inflammatory disease of the intestinal tract that until recently had no identifiable cause. It has recently been shown that the relationship between the host and commensal gastrointestinal bacteria in Crohn's patients has been disturbed. In healthy patients, defensins help keep up the beneficial relationship with these commensal bacteria; disturbance of defensin levels can therefore cause commensal bacteria to become pathogenic, leading to gastrointestinal infections and disease [10]. Defensin levels have also been shown to be low in patients suffering from irritable bowel syndrome [11].

In bronchoalveolar inflammation and skin diseases (such as psoriasis and mastitis) expression and peptide concentrations of HβD-2 and HβD-3 are increased; as a result of these high defensin levels psoriatic lesions rarely become infected [3,12]. In contrast, the skin condition atopic dermatitis shows decreased HβD-2 and HβD-3 levels; this condition is often accompanied by bacterial, fungal, or viral infection [3].