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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].

Cystic fibrosis (CF) is a recessive genetic disease caused by mutations in the CF transmembrane regulator gene, which encodes regulated chloride ion channels. The main cause of death in patients with CF is respiratory failure, perhaps due to progressive damage to lungs and airways due to recurring infections and inflammation. Infection with *Pseudomonas aeruginosa* marks the onset of progressive lung disease. The infections are almost always localized only in the lung, indicating a defect in local epithelial host defense. It has therefore been suggested that this defect is caused by the inhibition of defensin activity, which is due to the abnormal ionic state of CF airway fluid. A normal fluid is low in salt, which favors the activity of defensins, but CF fluid has been reported to be very high in salt due to lack of the function of CFTR protein that forms chloride ion channels. This high salt environment may inhibit defensin activity and compromise host respiratory defense [6]. On the other hand, the pro-inflammatory activity of antimicrobial peptides is likely to have negative consequences. For example, in CF patients, lung washings have cytotoxic levels of α-defensins [1].

Among the identified human β-defensins, HβD-3 is of special interest for structural and functional studies and also for possible pharmaceutical applications. It is also one among the identified human defensins that has the ability to undergo oligomerization [13–15]. Its ability to exhibit antibacterial activity at physiological salt concentrations towards Gram-positive bacteria and its involvement in adaptive immunity are of biological significance as compared to other human defensins. The ability to form a dimer that leads to the formation of higher ordered oligomeric structures is possibly responsible for unique characteristics of HβD-3. These properties of HβD-3 are of current interest and warrant further studies, though a modified analog of HβD-3 has been shown to form only monomeric structures and retain antibacterial activity [16]. It is therefore expected that future studies on this peptide will be directed towards understanding the discrete structural elements that may be responsible for its biological activity and the steps involved in the functional regulation of HβD-3.

3. Expression and regulation of HβD-3

The molecular evolution of gene coding for β-defensin 3 deduced 17 amino acid sequences in primates including human [16]. The primates analyzed were from Great Apes, Hylobatidae, Cercopithecidae, and Platyrrhine species. The alignment of nucleotide sequences from the coding region of the β-defensin 3 gene showed a greater identity implying conservation and evolutionary significance [16,17].

In humans, β-defensins are found mainly at epithelial surfaces, for example in gut and lung [18,19]. It has been reported that there are 28 defensin-like sequences in the human genome [20] but currently only HβD-1–6 have been characterized as part of the β-defensin family [21–26]. The β-defensins are further subdivided on the basis of their expression: those that are constitutively expressed (e.g. HβD-1) and those that are induced upon challenge with inflammatory or pathogen-derived stimuli [24,25,27–29]. In addition to their role as antimicrobials, β-defensins are chemoattractants promoting interactions between innate and acquired immune systems [23,24,30].

HβD-3 was first isolated from human lesional psoriatic scales. It has also been isolated from primary keratinocytes and lung epithelial cells pretreated with *P. aeruginosa* [25]. Table 1 shows some of the reports on the expression of HβD-3 in different tissues of the human system. Recently, the presence of HβD-3 peptide has been confirmed in homogenates of human lung, serum and gingival epithelia using RP-HPLC, radioimmunoassay, immunohistochemistry and *in situ* hybridization indicating the pathophysiological significance of this molecule in respiratory infections and in maintenance of periodontal homeostasis [31,32]. The expression of HβD-3 in engineered epidermis shown to provide protection against bacterial infection and thereby indicating potential therapeutic applications of this molecule by gene therapy to combat infectious diseases [33].

Analysis of HβD-3 gene expression in various body organs by real-time reverse-transcription polymerase chain reaction (RT-PCR) indicated strong expression of HβD-3 mRNA in skin, trachea, tongue and tonsils, whereas lower levels of expression were shown in organs like salivary glands, uterus, kidney, bone marrow, thymus, colon, stomach, adenoid, pharynx, and larynx. The RT-PCR also detected expression of HβD-3 in both inflamed and non-inflamed oral tissues and in salivary glands [34]. The HβD-3 expression is inducible on ocular surface epithelial cells and was observed to a greater extent in corneal and conjunctival infected samples than that of the noninfected samples [35]. A modified quantitative RT-PCR method is a fast and reliable tool for the screening of copy numbers of polymorphisms in β-defensin genes and is also useful for expression and epidemiologic studies [36].

The expression, regulation, and roles of β-defensins at non-reproductive sites have been reviewed in detail [37–39]. In human endometrial epithelium, the expression profiles of each of the beta-defensins (defensins 1–4) are related to the stage of the menstrual cycle. HβD-3 has been shown to express at the highest level compared to other defensins during early and late secretory phases [40,41].

The expression of HβD-3 has been found to be induced by external stimuli including interleukin-1, tumor necrosis factor-

Table 1
Expression of HβD-3 in different parts of the human body

Site of expression	Reference
Skin, trachea, tongue, uterus, pharynx, kidney, thymus, colon, stomach, adenoid	[25]
Placenta	[42]
Endometrium	[41]
Non-inflamed oral tissue samples:	
Gingiva, tongue, buccal mucosa, labial mucosa, floor of the mouth—mucosa, dental follicle	[34]
Inflamed oral tissue samples:	
Gingivitis, marginal periodontitis, apical periodontitis, candidiasis	[34]
In salivary glands:	
Submandibular gland, small labial gland	[34]

α, interferon-γ, as well as Gram-negative and Gram-positive bacteria [23,25,42,43]. cDNA isolation from primary keratinocytes encoded a 67-residue precursor preprotein that is processed by proteolytic activation to a mature 45-residues peptide having structural similarity to vertebrate epithelial defensins. A 3–8 fold increase in mRNA levels of HβD-3 was observed when normal human oral epithelial cells were challenged with *C. albicans*, while challenging with HIV-1 strains of X4 and R5 viral bio-phenotypes resulted in a 78 fold increase in mRNA levels of HβD-3 [44–46]. The induced expression of HβD-3 in keratinocytes by microbial stimuli was found to be mediated by transactivation of epidermal growth factor receptor (EFGR), which is distinct and also suggests differential regulation of expression among human β-defensins 1, 2 and 3 [47].

Jia et al. used a genomics based approach to identify the HβD-3 genes [42]. The human defensin genes are clustered to a <8 Mb region of the chromosome 8p22–p23 [48–50]. This kind of gene clustering pattern has been observed for defensin genes in other species as well [51–54]. The HβD-3 gene contains two exons located 13 kb upstream of HβD-2 gene. The first exon includes the 5′ untranslated region of the gene that encodes a domain of the preprotein. The second exon encodes the mature peptide, which contains 6 cysteine residues that form three intramolecular disulfide bonds. A genomics approach employing a computational search strategy for the discovery of β-defensin genes (using Hidden Markov Models for the six-cysteine conserved structural motifs) identified 28 new genes clustered on several different chromosomes [20].

In vitro studies indicated that the expression of HβD-3 mRNA in cultured primary bronchial epithelial cells is inhibited by dexamethasone, a corticosteroid, but not by mRNA levels of HβD-1 and HβD-2 [55]. Corticosteroids are known to inhibit the synthesis of many pro-inflammatory cytokines and cell surface molecules [56]. The *in vitro* bactericidal activity of HβD-3 has been found to be inhibited by saliva and serum against *S. mutans* and *A. actinomycetemcomitans* but the inhibition of the activity can be overcome with an increase in the peptide concentration [57]. It has been shown that cysteine proteases like cathepsins B, L, and S that are present and active in CF bronchoalveolar lavage have the ability to degrade and inactivate HβD-3. Hence, HβD-3 is susceptible to cathepsins (e.g. host proteases) and therefore, cathepsins play an important role in the regulation of HβD-3 activity. The over expression of cathepsins may lead to degradation and thereby favor bacterial infection [58]. It has been reported that antibacterial activities of HβD-3 and other host-defense peptides are inhibited by a 31 kDa protein, streptococcal inhibitor of complement (SIC) secreted predominately by M1 strains of group A *streptococci* (GAS). Another protein that is distantly related to SIC also inhibited the antibacterial activity of HβD-3, but to a lesser extent compared to SIC, indicating a role for virulence factors of bacteria that protect them from antibacterial action [59,60]. Hence, the identification of several defensin genes and their homologs in diverse biological systems indicates either the common need for these molecules throughout evolution or that they have been tuned to a specific sequence by mutation, resulting in their common tertiary structure without a significant sequence homology.

There are only a few studies on the isolation, cloning and expression of HβD-3 but the chemical synthetic protocols are well documented [3,16,23,25,61–66]. Further studies involving a synthetic approach for the generation of HβD-3 or its derivatives would help in exploring the involvement of this molecule in unrecognized biological activities.

4. Structure of HβD-3

HβD-3 is a 45-residues, cationic peptide with an asymmetrical distribution of charged residues, mostly clustered at the carboxyl-terminal region. It has a low sequence similarity among β-defensin class of peptides [16,20,23,25,42]. HβD-3 has been primarily isolated, characterized from psoriatic scales, and classified as a member of the β-defensin family of peptides [25,67]. The isolated and characterized human β-defensins are defined by six-cysteine motif spacing C–X6–C–X4–C–X9–C–X6–CC (where X*n* indicates *n* non-cysteine residues) and have the same cysteine connectivity as found in bovine neutrophil beta-defensin-12 (BNBD-12), bovine tracheal antimicrobial peptide (TAP), and bovine lingual antimicrobial peptide (LAP) [68]. However, the spacings are some what different in other defensins like HβD-4, HβD-27 and HβD-28. The sequence alignments and disulfide connectivities of defensins are shown in Fig. 1, including the recently synthesized and characterized HβD-27 and HβD-28 peptides that are found in chromosome 20 [66]. The three disulfide bonds found in HβD differ from α-defensins with respect to first and third Cys–Cys connectivities whereas the disulfide connectivity between the second and fourth cysteine residues is conserved among these two classes of peptides. Despite the differences in disulfide connectivities, the tertiary structure of these two classes of peptides is similar [13,15,61,62,69].

Studies on the secondary structure of HβD-3 using circular dichroism (CD) experiments in aqueous buffer, trifluoroethanol (TFE) and sodium dodecyl sulfate (SDS) micelles have been reported [16]. The results in aqueous medium showed approximately 25% of residues in a β-sheet conformation and <10% of the residues in an α-helical conformation. However, in the presence of 50% TFE, the peptide undergoes a marked conformational transition with an increase in the helicity of the peptide to 25% while its β-sheet conformation is unchanged (16). Similar structural transition was also observed in SDS micelle. These studies attributed the increase in helical structure of the peptide to the flexible N-terminus region of the peptide.

The solution nuclear magnetic resonance (NMR) spectroscopy studies in water at a low pH showed the formation of three anti-parallel beta-sheets in the stabilization of the tertiary structure and a short helical loop at the amino-terminal region of the peptide [62]. The primary sequence and the regions spanning the secondary structures are shown in Fig. 2A. The assigned nuclear Overhouser effect (NOE) connectivities indicate that the β3-sheet serves as a template for the β2 and β1 sheets in the stabilization of the tertiary structure among the

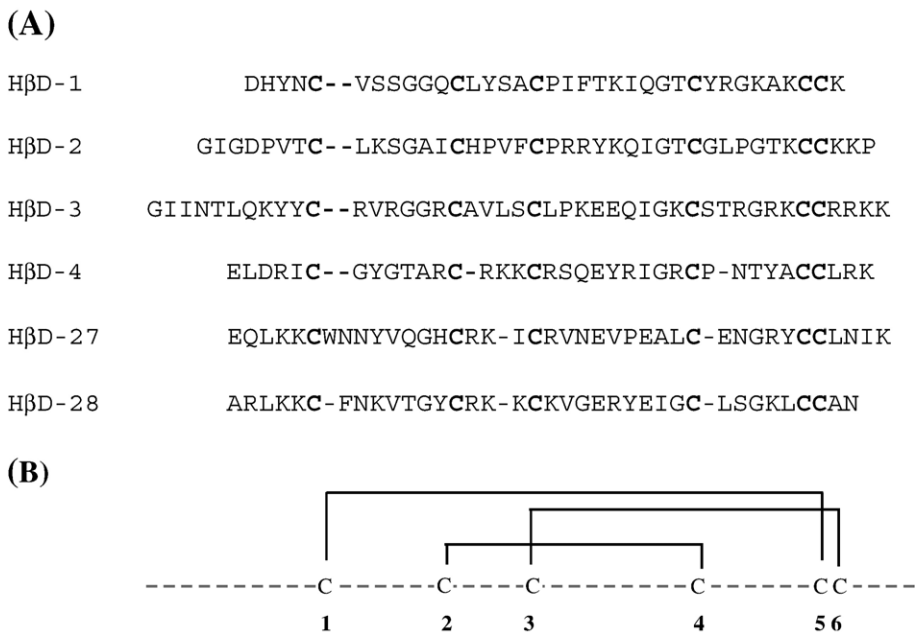

Fig. 1. Amino acid sequence and cysteine connectivities in HβD's. (A) Primary sequences of human beta-defensins. A gap (marked by −) was introduced in all peptides for the sequence alignment as the spacing between second and third cysteines is observed to be three residues as compared to the consensus of four residues in HβD-1-3. (B) The consensus disulfide connectivities among beta-defensins.

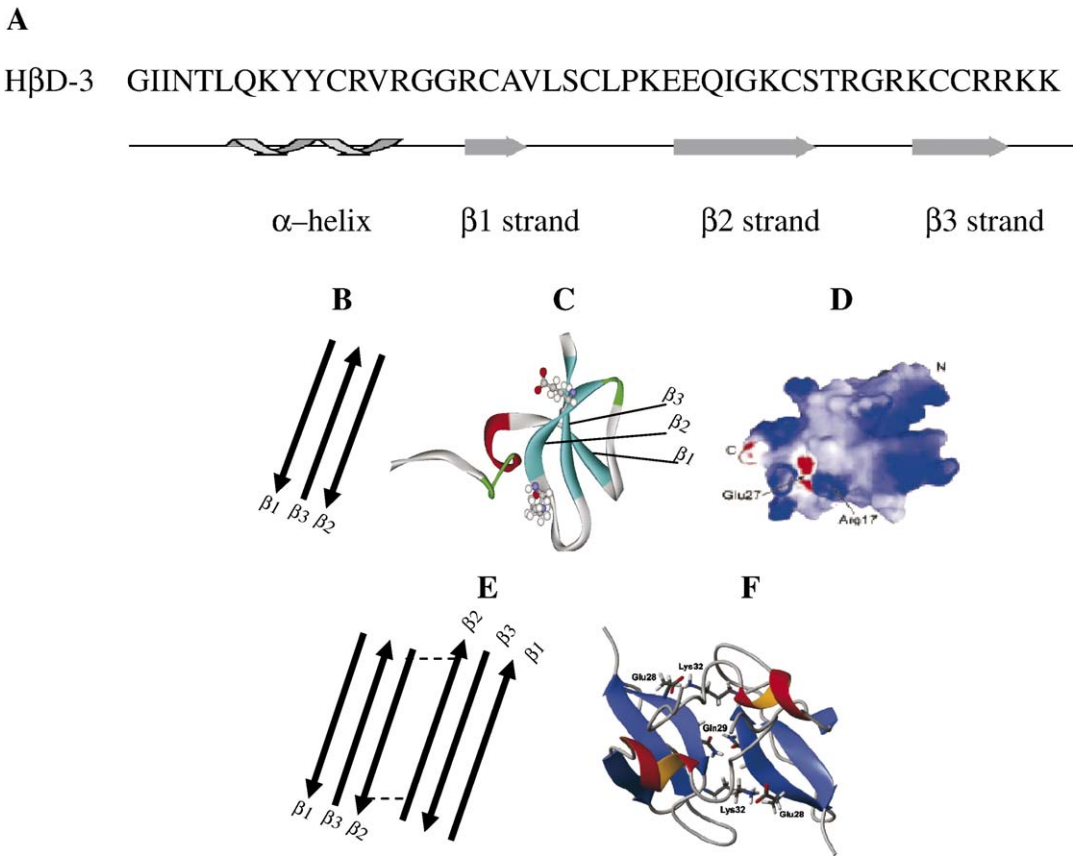

Fig. 2. Structural aspects of HβD-3. (A) Primary sequence alignment and the secondary structural features spanning the peptide as observed by the NOE connectivity [49]. (B) Cartoon model for the interaction among the three beta strands for the stabilization of monomers. The three β-strands (β1, β2, and β3) stack in an antiparallel manner. (C) A tertiary structure of the monomer. The Glu and Lys residues on the β-2 sheet are shown. (D) Electrostatic surface plot. The basic regions are colored in blue and the acidic regions are in red (49). (E) The interaction between the two β2 strands is stabilized by salt bridge formation involving residues Glu-28 and Lys-32 in an antiparallel manner as shown by the dashed lines. (F) A three-dimensional view across two β2 strands in a dimer (62).

β-defensins (Fig. 2B). The tertiary structure determined using the protein data bank (PDB) data file and electrostatic surface plot of monomeric form is shown in Fig. 2C and D. The studies involving native gel migration method, dynamic and static light scattering, and NMR diffusion measurement of the radius of hydration demonstrated that only HβD-3 has the ability to form an amphipathic symmetrical dimer structure through the β2 strand, which exhibits increased positive surface charge upon folding as compared to HβD-1 and HβD-2 (Fig. 2E and F). This property of HβD-3 has been speculated to be responsible for increased anti-*S. aureus* activity and salt insensitivity; therefore this molecule has been possibly implicated in diseases like cystic fibrosis where many of the host defense peptides are inactivated due to increased salt concentration [22,70–72].

In an attempt to understand the role of dimerization in antibacterial activity, an analog ftkHβD-3 was synthesized in which the three residues, Lys_{26}–Glu_{27}–Glu_{28} involved in the dimerization of HβD-3 were replaced by Phe–Thr–Lys, the residues present in HβD-1 [16]. A synthetic hcβD-3 peptide found in *Hylobates concolor* has also been studied [16]; the amino acid residues at 2 (Leu), 3 (Met) and 17 (Trp) sites of this peptide differ from that of HβD-3. These two analogs did not undergo oligomerization as observed on sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis under non-reducing and reducing conditions but their antibacterial activity was comparable against the microorganisms tested. Though the analogs ftkHβD-3 and hcβD-3 differ considerably in the formation of higher ordered structures compared to native HβD-3, their antibacterial activities in the presence of salt, kinetics of killing and membrane permeabilization of Gram-negative and Gram-positive bacteria did not distinguish the native peptide from these two analogs. This observation indicates that the ability to undergo dimerization is not an essential requirement for the antibacterial activity towards *S. aureus* and also for the antibacterial activity in the presence of salt. However, the fact that Hβd-3 undergoes dimerization and/or forms higher order structures imply that this molecule also plays an important role in other biological functions where the interaction of Hβd-3 with cells could be receptor mediated.

The crystal structure of human neutrophil peptide-3 (HNP-3) revealed an amphiphilic dimeric beta-sheet assembly (15). This structure has been suggested to be important in binding and permeabilization of membranes in order to exhibit its antibacterial activity [15]. Other members of the same family, HNP-1 and HNP-2, also have been shown to undergo dimerization/oligomerization in solution or in unilamellar lipid vesicles [73,74]. The solution NMR structure of bovine neutrophil beta-defensin-12 (BNBD-12) showed a 'defensin-like fold' involving three β-strands identical to that of HNP-1–3, even though BNBD-12 and HNP-1–3 have different disulfide connectivities [75,76]. Several studies reported the structure of human beta-defensins (HβD-1–3) using NMR and X-ray crystallography approaches [13,77,78]. In the concentration range of 0.5–2.4 mM HβD-2, the solution NMR and static light scattering studies did not provide any evidence for the oligomerization of the peptide [77]. On the other hand, the formation of HBD-2 dimers was observed using dynamic light scattering studies at concentrations above 6.9 mM [78]. HβD-2 has also been crystallized and in the crystalline state it was found to be in a dimeric form [78]. Interestingly, the mode of dimerization was found to enable the formation of an octameric form of the protein [78]. The higher-order oligomeric structures were found to be stable due to the burial of the hydrophobic surface area and the uniform surface distribution of positively charged residues. Thus, the structural and electrostatic properties of HβD-2 neither favor the insertion of the peptide or nor the formation of pores in membranes. Thereby, the proposed model suggests an electrostatic interaction of HβD-2 with the polar lipid head group to disrupt the bacterial membrane integrity for membrane permeabilization [78].

A crystal structure of HβD-1 revealed a dimeric form of the peptide with a typical human 'β-defensin-like fold' (an N-terminal α-helical segment and three antiparallel β-sheets), however, unlike for HβD-2, no higher-order oligomeric forms were found [13]. Also, the topology of HβD-1 dimers formed between monomers in the asymmetric units is different from that of HβD-2 suggesting a little support for the formation of membrane-embedded pores unlike the dimeric forms of HβD-2 and HNP-3 [13]. Since the human β-defensins share a common 'β-defensin-like fold', the activities of these peptides are believed to be dictated by their quaternary structures. It is speculated that Hβd-3 functions via carpet-type mechanism of membrane-disruption as it is highly cationic and has an ability to form dimerization and/or oligomerization, which is in agreement with a recent study [63].

Although the molecular size of chemokines, such as CCL20/MIP-3á, are larger than β-defensins, their tertiary structure, cationicity and antibacterial activity are similar to that of β-defensins. On the other hand, it is surprising to note that β-defensins interact with chemokine receptors like, CCR6 [79,80]. The peptide derived from interleukin-8 (IL-8) has been shown to exhibit antibacterial activity but lacks the proinflammatory effect. This finding suggests that IL-8 contains structural elements that are capable of interacting with membranes, even though the full-length IL-8 do not possess antibacterial activity [81]. A recent study on the structural congruence among membrane-active host defense peptides/proteins highlights the importance of the conserved structural motif called γ-motif in diverse cysteine-containing molecules, like chemokines, antibacterial peptides, and toxins [82]. Thus, the function of such effector molecules is governed by specific configurational modules associated with a common γ-core motif that have evolved by parallel and divergent process [82].

5. Biological activities of HβD-3

Though HβD-3, unlike other defensin peptides, has been primarily shown to possess a broad spectrum of antibacterial activity against many pathogens and drug resistant microbes [25,61,63,83,84], a recent study suggests that HβD-3 is also involved in several other biological functions such as chemoattraction, thereby connecting innate and adaptive immunity [63]. Antibacterial and biochemical properties of the recombinant and chemically synthesized HβD-3 are indistinguishable

from that of the isolated native peptide [25]. There are several reports in the recent past on the validation of antibacterial activity of this molecule towards array of bacteria including the resistant strains using different experimental protocols. The kinetics of bactericidal activity and bactericidal activity in presence of human serum has been evaluated for peptide, HβD-3 against multidrug-resistant clinical isolates of common nosocomial pathogens, such as *Staphylococcus aureus*, *Enterococcus faecium*, and *Pseudomonas aeruginosa*, and clinical isolates of emergent pathogens, such as *Stenotrophomonas maltophilia* and *Acinetobacter baumannii.* The concentration required for the bactericidal effect monitored after 1.5 h of incubation found to be in the range 4–8 μg/ml but the activity lowered in presence of 20% heat inactivated serum [85]. The Table 2 summarizes a few of the antibacterial activity of this peptide for antibacterial towards different microorganisms. A majority of the experiments are done for the determination of minimum inhibitory concentration (MIC) and lethal concentration (LC) and these assays have merits on their own. However, it is worth mentioning two methods for the evaluation of antibacterial activity of defensin peptides. The determination of virtual lethal doses (VLD) is more informative as the kinetics of growth/killing can be monitored directly [86] The second method, a quantitative flow cytometric assay using a membrane sensitive dye is developed to monitor antibacterial activity of HβD-3 against different bacterial species [87].

Unlike several cationic peptides, HβD-3 did not exhibit cytotoxic activity against eukaryotic cells. It exhibited a very low or <0.5% hemolytic activity towards human erythrocytes when a high amount of peptide (up to 500 μg/ml) was used at physiological salt concentrations. A higher hemolytic activity was observed in the presence of 10 mM sodium phosphate buffer containing 0.34 M sucrose [25]. Similar studies on human erythrocytes showed <10% hemolytic activity at 100 μg/ml and ~30% at 500 μg/ml [88]. When the cytotoxic activity was measured against monocytic human THP-1 cells, peptide HβD-3 had no effect on the viability at a concentration of 10 μg/ml but the viability of these cells was reduced significantly when incubated with a peptide concentration of 50 μg/ml which is about 4–5 fold excess than required for the antibacterial activity [88].

Table 2
Antimicrobial activity of HβD-3 against different microbes

Microorganism	MIC/LC_{90} (μg/ml)	Reference
E. coli ATCC 11303	6.0	[25]
E. coli ATCC 25922	6.0	[61]
E. coli D5α	3.13	[83]
S. aureus ATCC 6538	12.0	[25]
S. aureus ATCC 29213	12.0	[61]
S. aureus ATCC 6538 and 150 mM salt	12.0	[25]
S. aureus multi drug resistant clinical isolate	~25.0	[25]
E. faecium vancomycin resistant	12.0	[25]
P. aeruginosa ATCC 27853	13.0	[25]
S. pyogenes ATCC 12344	12.0	[25]
C. albicans clinical isolate	6.0	[25]
C. albicans 99788, amphotericin B resistant	18.0	[61]
F. nucleatum (ATCC 25586)	>100.0	[83]
P. gingivalis (ATCC 33277)	12.5	[83]

MIC=minimum inhibitory concentration LC_{90}=lethal concentration required for 90% mortality.

A majority of antibacterial peptides are known to act by permeabilization of the bacterial membrane via different types of mechanisms that include membrane depolarization and creation of physical holes in membrane [89–99]. Also, membrane permeabilization studies indicate that membrane composition modulates the activity [98,100–102]. Several models have been proposed for membrane interactions that include carpet, barrel stave, or torroidal pore formation by various biophysical studies [103–112]. These studies have led to the design of peptides exhibiting selective activity [101,113–116]. Apart from membrane permeabilization, it is also known that antibacterial peptides activate the induction of hydrolases, which degrade the cell wall or interfere with the distribution of lipids in the two leaflets of the bilayer. This results in non-maintenance of the membrane integrity and function [117,118]. These molecules are also found to translocate across the membrane and interfere with critical cellular functions that lead to cell death [119].

Studies using transmission electron microscopy demonstrated that HβD-3 has the ability to induce morphological changes, like perforation of the peripheral cell wall and explosion-like liberation of the plasma membrane within about 30 min of interaction with the *S. aureus* membrane suggesting that the plasma membrane is the site of action [25]. Similar studies on *C. jejuni*, bacteria known to cause diarrhea in human, showed thinning of cell wall with the formation of membrane-enclosed blebs that lead to subsequent loss of cytoplasmic contents when incubated with HβD-3. This observation also suggests that the disruption of cell wall integrity is the cause for the bactericidal effect [120]. The membrane-permeabilization kinetics studies measured as β-galactosidase enzyme activity towards *E. coli* ML-35 pYC and *S. aureus* 710A strains indicated that the peptide is capable of initiating permeabilization of the cytoplasmic membrane of *E. coli* within 20–30 min whereas the permeabilization of the cytoplasmic membrane is much slower for the Gram-positive microorganism [16]. These results complemented with the studies by the same group on the kinetics of bacterial killing of both Gram-negative and Gram-positive microorganism. HβD-3 has been shown to kill *E. coli* cells efficiently in less than 30 min while similar effect was observed after 120 min against Gram-positive *S. aureus*. These studies imply the possible roles of each membrane component to act as a barrier towards this peptide as the chemical composition of these bacterial membranes are different. HβD-3 also has been shown to have the ability to form non-selective ion-channels in the oocyte membranes of *Xenopus laevis* [23].

Several of the cationic antibacterial peptides have been found to possess anti-endotoxin properties by binding to lipopolysaccharide (LPS) and lipoteichoic acid (LTA) and thereby prevent the sepsis or septic shock that is associated with the presence of pathogenic Gram-negative and Gram-positive bacteria [121–128]. Similarly, human β-defensin HβD-2, belonging to a class of cationic antimicrobial peptides, exhibited the ability to block the LPS interaction and is correlated with

their ability to block LPS-induced TNF-α production that is responsible for inflammation [127]. Due to polycationic nature of HβD-3 (+11 in monomeric form), it can effectively interact with the anionic charges on the LPS molecule and neutralize the endotoxic activity. Recent studies using atomic force microscope (AFM), electrical measurements on interaction of HβD-3 with LPS monolayers suggest this peptide has the potential to intercalate and also form lesions [129]. Their results correlated with the antibacterial activity and thereby it is hypothesized that lipid–peptide specific interactions are involved in biological activity of HβD-3. Thus, HβD-3 has the ability to bind, neutralize LPS and thereby exhibit anti-endotoxin property like HβD-2. However, the exact mechanism of action for the membrane interaction is yet to be carried out in detail. Wu et al., have suggested a carpet model for the mode of action and destruction of the membrane that is aided by a high number of positive charges in the amphiphilic structure [63].

Apart from the antibacterial activity, HβD-3 has been shown to possess immunomodulatory properties such as chemoattraction of T-lymphocytes and immature dendritic cells, thus playing an important role in adaptive immunity. The optimal concentration for the migration of monocytes and CCR6-transfected human embryonic kidney (HEK) 293 cells was found to be 100 ng/ml and 10 ng/ml respectively as assayed by microchemotaxis chamber technique method [130]. HβD-3 is known to interact with chemokine receptor CCR6 by direct binding [63]. This molecule has been also reported to induce secretion of IL-18, a proinflammatory cytokine in human keratinocytes [131]. The ability of HβD-3 to kill bacteria and activate immune cells indicates that these molecules are involved in innate and adaptive immunity like other defensin molecules [132–134]. Additional biological activities include: HβD-3 has been shown to exhibit the inhibition of HIV-1 replication *in vitro* similar to peptide HβD-2, induction of tissue remodeling proteins such as matrix metalloproteinases (MMPs) and reduction of MMP's inhibitors (TIMP-1/-2) in human cartilage [45,135,136]. These biological activities demonstrate multifunctional roles of HBD-3.

6. Correlation of structure and function of HβD-3 analogs

In order to exploit the structure–function relationships of HβD-3 and to understand the rationale for the design of potent active molecules, several peptide analogs that differ in disulfide connectivities, length, cationic charges, hydrophobicity have been generated [61,63,88]. The sequences and the biological activities of HβD-3 (peptide-1) and its analogs (peptides 2–28) are summarized in Table 3. It should be noted that three independent measurements on antibacterial assay included in Table 3 cannot be compared directly. Moreover, the antibacterial activity also depends on the type of experiment, nature of medium, ionic strength of the medium, and the type of species used in assay conditions.

Important results from the structure–function correlation studies are summarized below. A synthetic peptide wherein the disulfide linkages are misfolded (or differing in the S–S connectivity) showed comparable antibacterial activity to the native peptide towards *E. coli* [63]. For example, peptides 2–6 that differ in disulfide connectivities among themselves as well as with the native HβD-3 peptide (peptide-1) (see Table 3) but exhibit similar antibacterial activity. Analogs devoid of 5 (peptides 8, 9, 10), 7 (peptide-14) and 9 (peptide-15) N-terminal amino acids but containing all the three native or non-native disulfide connectivities also exhibited the same antibacterial activity as that of HβD-3 indicating the misfolding of three disulfides and deletion of 9 N-terminus residues did not affect the antibacterial activity. However, the linear analogs in which all cysteines were substituted by α-aminobutyric acid (peptide-7 in Table 3) and peptide-11 with a deletion of five N-terminal residues in which Cys were carboxymethylated (peptide-11) also exhibited antibacterial activity. These results suggest that the correct location of Cys–Cys connectivities and the formation of a tertiary structure are not essential for the exhibition of antibacterial activity. This observation is again supported by 5 N-terminal deleted analogs (peptides-2 and peptide-13 that span all the three disulfide region in HβD-3) where all Cys residues replaced by Ala and Trp did not affect the antibacterial activity towards Gram-negative and Gram-positive bacteria.

The studies on shorter peptides containing one or two disulfide bridges did not clearly establish the role of Cys–Cys connectivity in the native molecule. Peptide-16, that is devoid of 18 N-terminal residues with two non-native disulfide connectivities, and peptide-25, containing 17 residues with single non-native disulfide connectivity, showed a drastic drop in antibacterial activity. As these analogs (having single and two disulfides) vary in length, cationic charges, hydrophobicity and span different regions of the HβD-3, it is difficult to conclude which of the property is important in modulating the antibacterial activity.

Interestingly, peptides as short as 9 amino acids (peptide-22) corresponding to the C-terminal region exhibited antibacterial activity towards *E. coli*. Addition of cationic charged residues enhanced the activity as observed in peptides 20 and 21. However, further increase in length (peptide-19) lowered the antibacterial activity as compared to its shorter analog, peptide-20 indicating the importance of cationic charges as observed in several cationic peptides. Two short analogs peptides (23 and 24), corresponding to N-terminal region were also active towards *E. coli*. However, all these shorter analogs exhibited lower activity towards Gram-positive bacteria.

The effect of hydrophobicity towards antibacterial activity was studied by the generation of peptides wherein Cys have been replaced by Trp. Peptide-13, that lacks 5 N-terminal residues and all Cys replaced by Trp, showed lower activity compared to their three Cys–Cys bridged analogs, peptides 8, 9 and 10 towards Gram-positive and Gram-negative bacteria. Peptide-12, that lacks 5 N-terminal residues and all Cys replaced with Ala, had similar activity as the Trp replaced analog (peptide-13). However, similar replacement in shorter analogs in peptides 18 and 27 significantly increased the antibacterial activity as compared to their disulfide analogs, peptides 16 and 25 containing two and one Cys–Cys connectivities and corresponding to different regions of the native peptide 1, HβD-3. Even the Cys replacements by residue

Table 3
Overview of the primary sequence alignment and biological activities of HβD-3 and its analogs studied

Peptide	Primary sequence	C-C bridge/changes	Antibacterial activity		Chemotaxis Activity		References
			A	B	C	D	
1	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–5, 2–4, 3–6	0.08,* 6.0#9.4$	10.0#, 3.13$	100	10	[61,63,16]
2	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–5, 2–6, 3–4	0.06*	--	10	100	[63]
3	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–5, 2–3, 4–6	0.03*	--	1000	1000	[63]
4	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–6, 2–4, 3–5	0.08*	--	≥10000	1000	[63]
5	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–6, 2–3, 4–5	0.05*	--	1000	100	[53]
6	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–6, 2–5, 3–4	0.02*, 5.0#	14.0#	1	100	[61,63]
7	GIINTLQKYY**a**RVRGG**a**AVLS**a**LPKEEQIGK**a**STRGRK**aa**RRKK	C replaced by a	0.04* 6.0#	5.0#	@	@	[61,63]
8	LQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–5, 2–4, 3–6	12.5$	3.13$	--	--	[88]
9	LQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–5, 2–3, 4–6	9.4$	4.7$	--	--	[88]
10	LQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–2, 3–6, 4–5	9.4$	4.7$	--	--	[88]
11	LQKYY**C**RVRGGR**C**AVLS**C**LPKEEQIGK**C**STRGRK**CC**RRKK	all C are Cam	12.5$	12.5$	--	--	[88]
12	LQKYY**A**RVRGGR**A**AVLS**A**LPKEEQIGK**A**STRGRK**AA**RRKK	C replaced by A	18.75$	12.5$	--	--	[88]
13	LQKYY**W**RVRGGR**W**AVLS**W**LPKEEQIGK**W**STRGRK**WW**RRKK	C replaced by W	18.75$	12.5$	--	--	[88]
8/14	KYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–5, 2–4, 3–6	6.0#	16.0#	--	--	[61]
9/15	YCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	1–5, 2–4, 3–6	6.0#	17.0#	--	--	[61]
16	AVLSCLPKEEQIGKCSTRGRKCCRRKK	1–4, 2–3	150$	100$	--	--	[88]
17	AVLS**A**LPKEEQIGK**A**STRGRK**AA**RRKK	C replaced by A	75$	75$	--	--	[88]
18	AVLS**W**LPKEEQIGK**W**STRGRK**WW**RRKK	C replaced by W	9.4$	9.4$	--	--	[88]
10/19	KEEQIGK**S**STRGRK**S**SRRKK	C replaced by S	20.0#	>20.0#	--	--	[61]
11/20	K**S**STRGRK**S**SRRKK	,,	1.0#	>20.0#	--	--	[61]
12/21	RGRK**S**SRRKK	,,	4.0#	>20.0#	--	--	[61]
13/22	RGRK**S**SRRK	,,	10.0#	>20.0#	--	--	[61]
14/23	KYY**S**RVRGGR**S**AVLS**S**LPK	,,	9.0#	>20.0#	--	--	[61]
15/24	GIINTLQKYY**S**RVRGGR	,,	19.0#	>17.0#	--	--	[61]
25	LQKYYCRVRGGRCAVLS	1–2	100$	200$	--	--	[88]
26	LQKYY**A**RVRGGR**A**AVLS	C replaced by A	150$	150$	--	--	[88]
27	LQKYY**W**RVRGGR**W**AVLS	C replaced by W	12.5$	12.5$	--	--	[88]
28	GIINTLQKYYCRVRGGRCAVLSCLP**FTK**QIGKCSTRGRKCCRRKK	KEE by FTK	8.0&	0.4&	--	--	[16]

X–Y numbers indicate the Cys connectivity; for example, 1–5 indicates first Cys from the amino terminal connected to the fifth Cys. a=α-aminobutyric acid, Cam=carboxamidomethyl, bold letters indicate the change of amino acid from the native peptide. Antibacterial activity against (A) *E. coli* and (B) *S. aureus* either at *=LC_{50} or #=LC_{90} μg/ml. LC_{50} or LC_{90} is the concentration of protein at which 50 or 90% of the viable cells are killed. $=minimum inhibitory concentration (MIC) in μg/ml measured in ¼ MHB culture medium. &=MIC in μM in 5% tryptic soya broth while the MICs of native peptide towards each of these organisms were 1 μM. Chemotactic activity against (C) monocytes and (D) HEK in ng/ml. @=not active at >10,000 ng/ml.

Ala (peptides 17 and 26) increased the antibacterial activity compared to their disulfide bridged ones (peptides 16 and 25). Hence, the increase in hydrophobicity enhanced the antibacterial potency in shorter analogs containing 17 and 27 residues than to its longer analog (peptide-13).

Primary structure–function analysis on these analogs suggests that the C-terminal segment with higher cationic charges is important for antibacterial activity towards Gram-negative bacteria while the native peptide exhibited optimal antibacterial activity towards both Gram-negative and Gram-positive bacteria. This finding possibly, indicates the importance of N-terminus and hydrophobic residues in modulating antibacterial activity of HβD-3 against Gram-positive bacteria, *S. aureus* and hence, useful in the design of peptides that exhibit selective inhibition of growth of either Gram-negative or Gram-positive bacteria.

Though the role of reduced form of disulfide linkages in native peptide was not established, Campopiano et al., observed that the native folding is necessary for higher antibacterial activity in defensin-related peptide Defr1 [137]. Reduced peptides (i.e., without S–S bonds) were less active compared to oxidized peptides. Reduced and oxidized forms showed different mobility patterns on non-denaturing 16% tricine gel, indicating the ability to form higher ordered structures and their possible role in folding and biological activity. We observed that the folded form of HβD-3 is protected to a greater extent from *in vitro* degradation by serine–threonine proteases like, trypsin as compared to the linear one which is reduced and alkylated. It has been supported by the observation that the peptides 8, 9, and 10 that have three disulfide folding exhibited higher anti-*S. aureus* activity as compared to its linear counterparts in peptides 11, 12 and 13 (Table 3). This implies the importance of disulfides in the formation of tertiary structures to overcome the degradation by proteases secreted by several bacteria.

The analogs were also tested for the ability to interact with immune cell lines. The analogs having the non-native three disulfide connectivities also exhibited chemotaxis at different concentrations. On the other hand, the replacement of all Cys by α-aminobutyric acid completely abolishes the chemotactic activity of HβD-3 [63]. This observation clearly suggests that three disulfide bond (either native or non-native) induced tertiary structure are important for the binding and activation of receptors for chemotaxis.

There is only one report on cytotoxic activity of HβD-3 analogs towards human erythrocytes and monocytic THP-1 cells [88]. Peptides with 5 N-terminal residues with three disulfide connectivities (peptides 8, 9 and 10) and Cys substitution by Ala (peptide-12) or Trp (peptide-13) were assessed for their cytotoxic activity. The N-terminal deleted peptides with native or non-native Cys–Cys connectivities showed higher viability as compared to its full length HβD-3 towards the THP-1 cell line. The alanine substitution did not change the viability while the Trp substitution leads to show a higher potency in reducing the viability of the same cells at concentrations 100 μg/ml. A similar activity profile was also observed in human erythrocytes. Peptides with N-terminal deleted analogs with three disulfide connectivities were less active compare to the native peptide-1. Substitution of Ala did not change the lytic activity whereas Trp substitution enhanced the hemolysis when compared to the peptides of same length.

The structural determination by CD in water of HβD-3 derivatives (peptides 8, 9, 10, 12, 13, 16 and 25), exhibited similar spectrum as native peptide 1 indicating disulfide bridges, substitution of Cys by Ala or Trp residues has no effect towards formation of secondary structures and biological activities like antibacterial and hemolytic activities [88]. It is important to mention that the aqueous structure do not clearly reflect the biological activity of peptides as majority of the membrane active peptides have random conformation and transform to ordered secondary structures in the presence of lipid media/ membrane mimicking environment.

The structure–function correlation studies indicate that the antibacterial activity of HβD-3 depends on cationic charges while an increase in the hydrophobicity of designed analogs enhanced the activity towards Gram-positive and Gram-negative bacteria and induced cytotoxic activity. Cationic Hβd-3 derivatives, as short as 9–14 residues in length, from the C-terminal region can exhibit potent anti-*E. coli* activity. The studies on HβD-3 and its full length analogs suggest that the structures induced by the disulfide bonds are dispensable for both antibacterial and chemotaxis activities, whereas the absence of disulfide bonds leads to antibacterial activity only. This finding gives a flexibility in designing potent antimicrobials (either linear or cyclized analogs) as non-chemotactic therapeutic agents. The development of such molecules is a promise for non-immunogenic antibiotics to humans in combating pathogens. These findings are also in agreement with a recent study that investigated the role of Cys–Cys connectivities in tachyplesin [138].

Thus, the structure–function analysis of defensin peptides indicates that their consensus cysteine sequence, connectivity and tertiary structures are optimized towards modulation of biological functions other than antibacterial activity. HβD-3, being also an inducible peptide expressed upon challenge, is important in fighting the bacterial invasion and it appears that its constitutive expression is important in controlling other biological functions. The concentrations required for the antibacterial activity is about 100–1000 fold excess compared to the immunomodulatory activity shown by this peptide. To consolidate, this peptide is involved in several biological functions like, antimicrobial activity against an array of microorganisms, interaction with immune cells, inhibition of HIV replication, anti-endotoxin activity, interactions with membrane components, induction of tissue modeling proteins and so on. Thus, involvement of HβD-3 in diverse activities is recognized as possessing multiple functions that belongs to a class of β-defensin peptides.

7. Future scope

Recently published papers report on the details of mammalian and human defensins, evolution of β-defensins, and the antibiotic activity of HβD-3 [17,92]. Another recent review article, published during the preparation of our article, presents

the synthesis and structure–activity relationship of β-defensins and multi-functional peptides of the immune system [4]. Therefore, this article focused on HβD-3 emphasizing on the importance of its structure and function.

Though constitutive or inducible production of antibacterial peptides is a host defense strategy used by various species, pathogens also have evolved with resistance mechanisms to host defense. Several resistance mechanisms operate in the pathogen to resist the killing effect by the host antibacterial peptide. Presently it is not well understood whether a lower expression or a deficiency in expression of these host defense peptides would lead to a successful survival of the pathogen and a progress of disease. However, *in vitro* studies have indicated that microbes develop resistance towards these host defense molecules when pretreated with sublethal concentrations [83]. Our preliminary work in collaboration with Dr. Shelburne's research group using 2D-HPLC indicated a change in the total protein expression profile of an anaerobe, *P. gingivalis*, as compared to the untreated one (unpublished data). This intriguing observation would help us to understand the possible roles of new proteins towards the development of resistance or the survival mechanisms that the microbes undergo against the host defense molecules. This finding opens a new area of research for the development of drugs towards combating the microbes that are resistant towards host defense molecules.

Research in our laboratory is also aimed at understanding the mechanism of action of diverse biological functions of HβD-3 and the design of shorter peptide analogs of HβD-3 for biophysical studies. Though, the involvement of HβD-3 in innate and adaptive immunity is well established, it is not clear which of the parameters (e.g. primary structure, charge, hydrophobicity, tertiary or quaternary structure) in the native molecule are important for its biological function. Our preliminary investigations into the structure–function relationships via biophysical studies involving cyclic and linear analogs of 20–25 residues, GRCAVLSWLPKEEQIGKCSTR and LSCLPKEEQIGKWSTRGRKSCRRKK spanning two different regions of HβD-3 indicates that these analogs have the ability to interact selectively with anionic liposomes and exhibit moderate inhibition of growth in Gram-negative and Gram-positive bacteria with MICs values in the range 25–150 μg/ml. These molecules did not lyse sheep erythrocytes and were non-toxic to cultured human monocytic THP-1 cells to a concentration of 150–200 μg/ml indicating their non-toxic property towards mammalian cells. It is unlikely that such analogs are immunogenic, as they are part of the native peptide and are not expected to interfere in immunomodulatory function in humans, as they do not form tertiary structures like the native peptide involving three disulfides. The Biophysical studies involving the outer-membrane components are also under investigation to understand the neutralizing ability of these peptides towards endotoxic components like, LPS and LTA of both Gram-negative and Gram-positive bacteria and thereby control the LPS induced inflammation. Our studies in this direction would lead towards the design of potent antibacterial, non-immunogenic short peptides that might have therapeutic importance. We believe that HβD-3 is presently underexplored and there is a tremendous amount of research on this exciting molecule needs to be carried out to further understand its multiple functions that play a vital role in the maintenance of human health.

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References

[1] R. Bals, Epithelial antimicrobial peptides in host defense against infection, Respir. Res. 1 (2000) 141–150.
[2] D. Yang, A. Biragyn, D.M. Hoover, J. Lubkowski, J.J. Oppenheim, Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense, Annu. Rev. Immunol. 22 (2004) 181–215.
[3] M. Selsted, A.J. Ouellette, Mammalian defensins in the antimicrobial immune response, Nat. Immunol. 6 (2005) 551–557.
[4] E. Kluver, K. Adermann, A. Schulz, Synthesis and structure–activity relationship of β-defensins, multi-functional peptides of the immune system, J. Pept. Sci. 12 (2006) 243–257.
[5] K. DeSmet, R. Contreras, Human antimicrobial peptides: defensins, cathelicidins, and histatins, Biotechnol. Lett. 27 (2005) 1337–1347.
[6] T. Ganz, Defensins: antimicrobial peptides of innate immunity, Nat. Immunol. 3 (2003) 710–720.
[7] R.D. Yedery, K.V.R. Reddy, Antimicrobial peptides as microbicidal contraceptives: prophecies for prophylactics—A mini review, Eur. J. Contracpt. Reprod. Health Care 10 (2005) 32–42.
[8] J.J. Oppenheim, A. Biragyn, L.W. Kwak, D. Yang, Roles of antimicrobial peptides such as defensins in innate and adaptive immunity, Ann. Rheum. Dis. 62 (2003) 17–21.
[9] P. Kougias, H. Chai, P.H. Lin, Q. Yao, A.B. Lumsden, C. Chen, Defensins and cathelicidins: neutrophil peptides with roles in inflammation, hyperlipidemia and atherosclerosis, J. Cell. Mol. Med. 9 (2005) 3–10.
[10] J. Wehkamp, K. Fellermann, K.R. Herrlinger, C.L. Bevins, E.F. Stange, Mechanisms of disease: defensins in gastrointestinal diseases, Nat. Clin. Pract. Gastroenterol. Hepatol. 2 (2005) 406–415.
[11] E.F. Stange, M. Schmid, K. Fellermann, J. Wehkamp, Chronic inflammatory bowel diseases (IBD): novel pathophysiological concepts and their clinical relevance, Schweiz. Rundsch. Med. PRAXIS 94 (2005) 1429–1432.
[12] J.-M. Schroder, J. Harder, Molecules in focus human beta-defensin-2, Int. J. Biochem. Cell Biol. 31 (1999) 645–651.
[13] D.M. Hoover, O. Chertov, J. Lubkowski, The structure of human β-defensin-1: new insights into structural properties of β-defensins, J. Biol. Chem. 276 (2001) 39021–39026.
[14] D.M. Hoover, K.R. Rajashankar, R. Blumenthal, A. Puri, J.J. Oppenheim, O. Chertov, J. Lubkowski, The structure of human β-defensin-2 shows evidence of higher order oligomerization, J. Biol. Chem. 275 (2000) 32911–32918.
[15] C.P. Hill, J. Yee, M.E. Selsted, D. Eisenberg, Crystal structure of defensin HNP-3, an amphiphilic dimer: mechanisms of membrane permeabilization, Science 251 (1991) 1481–1485.
[16] M. Boniotto, N. Antcheva, I. Zelezetsky, A. Tossi, V. Palumbo, M.V. Verga Falzacappa, S. Sgubin, L. Braida, A. Amoroso, S. Crovella, A study of host defence peptide β-defensin 3 in primates, Biochem. J. 374 (2003) 707–714.
[17] S. Crovella, N. Antcheva, I. Zelezetsky, M. Boniotto, S. Pacor, M.V.V. Falzacappa, A. Tossi, Primate β-defensins — structure, function and evolution, Curr. Pro. Pepti. Sci. 6 (2005) 7–21.
[18] K.M. Huttner, C.L. Bevins, Antimicrobial peptides as mediators of epithelial host defense, Pediatr. Res. 45 (1999) 785–794.
[19] R.E.W. Hancock, G. Diamond, The role of cationic antimicrobial peptides in innate host defences, Trends Microbiol. 8 (2000) 402–410.

[20] B.C. Schutte, J.P. Mitros, J.A. Bartlett, J.D. Walters, H.P. Jia, M.J. Welsh, T.L. Casavant, P.B. McCray Jr., Discovery of five conserved β-defensin gene clusters using a computational search strategy, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2129–2133.
[21] K.W. Bensch, M. Raida, H.-J. Magert, P. Schulz-Knappe, W.-G. Forssmann, hBD-I: a novel β-defensin from human plasma, FEBS Lett. 368 (1995) 331–335.
[22] R. Bals, X. Wang, Z. Wu, T. Freeman, V. Bafna, M. Zasloff, J.M. Wilson, Human β-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung, J. Clin. Invest. 102 (1998) 874–880.
[23] J.-R.C. García, F. Jaumann, S. Schulz, A. Krause, J. Rodríguez-Jiménez, U. Forssmann, K. Adermann, E. Klüver, C. Vogelmeier, D. Becker, R. Hedrich, W.-G. Forssmann, R. Bals, Identification of a novel, multifunctional β-defensin (human β-defensin 3) with specific antimicrobial activity. Its interaction with plasma membranes of *Xenopus* oocytes and the induction of macrophage chemoattraction, Cell Tissue Res. 306 (2001) 257–264.
[24] J.-R.C. Garcia, A. Krause, S. Schulz, F.-J. Rodriguez-Jimenez, E. Kluver, K. Adermann, U. Forssmann, A. Frimpong-Boateng, R. Bala, W.-G. Forssmann, Human β-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity, FASEB J. 15 (2001) 1819–1822.
[25] J. Harder, J. Bartels, E. Christophers, J.-M. Schroder, Isolation and characterization of human β-defensin-3, a novel human inducible peptide antibiotic, J. Biol. Chem. 276 (2001) 5707–5713.
[26] Y. Yamaguchi, T. Nagase, R. Makita, S. Fukuhara, T. Tomita, T. Tominaga, H. Kurihara, Y. Ouchi, Identification of multiple novel epididymis-specific β-Defensin isoforms in humans and mice, J. Immunol. 169 (2002) 2516–2523.
[27] S. Krisanaprakornkit, A. Weinberg, C.N. Perez, B.A. Dale, Expression of the peptide antibiotic human β-defensin 1 in cultured gingival epithelial cells and gingival tissue, Infect. Immun. 66 (1998) 4222–4228.
[28] D.A. O'Neil, E.M. Porter, D. Elewaut, G.M. Anderson, L. Eckmann, T. Ganz, M.F. Kagnoff, Expression and regulation of the human β-Defensins hBD-1 and hBD-2 in intestinal epithelium, J. Immunol. 163 (1999) 6718–6724.
[29] J. Harder, U. Meyer-Hoffert, L.M. Teran, L. Schwichtenberg, J. Bartels, S. Maune, J.-M. Schröder, Mucoid *Pseudomonas aeruginosa*, TNF-α, and IL-1β, but Not IL-6, induce human β-defensin-2 in respiratory epithelia, Am. J. Respir. Cell Mol. Biol. 22 (2000) 714–721.
[30] D. Yang, O. Chertov, S.N. Bykovskaia, Q. Chen, M.J. Buffo, J. Shogan, M. Anderson, J.M. Schroder, J.M. Wang, O.M.Z. Howard, J.J. Oppenheim, β-Defensins: linking innate and adaptive immunity through dendritic and T cell CCR6, Science 286 (1999) 525–528.
[31] H. Ishimoto, H. Mukae, Y. Date, T. Shimbara, M.S. Mondal, J. Ashitani, T. Hiratsuka, S. Kubo, S. Kohno, M. Nakazato, Identification of hBD-3 in respiratory tract and serum: the increase in pneumonia, Eur. Respir. J. 27 (2006) 253–260.
[32] Q. Lu, L.P. Samaranayake, R.P. Darveau, L. Jin, Expression of human β-defensin-3 in gingival epithelia, J. Periodontal Res. 40 (2005) 474–481.
[33] D. Sawamura, M. Goto, A. Shibaki, M. Akiyama, J.R. McMillan, Y. Abiko, H. Shimizu, Beta defensin-3 engineered epidermis shows highly protective effect for bacterial infection, Gene Ther. 12 (2005) 857–861.
[34] A. Dunsche, Y. Acil, H. Dommisch, R. Siebert, J.M. Schroder, S. Jepsen, The novel human beta-defensin-3 is widely expressed in oral tissues, Eur. J. Oral Sci. 110 (2002) 121–124.
[35] R.S. McIntosh, J.E. Cade, M. Al-Abed, V. Shanmuganathan, R. Gupta, A. Bhan, P.J. Tighe, H.S. Dua, The spectrum of antimicrobial peptide expression at the ocular surface, Invest. Ophthalmol. Visual Sci. 46 (2005) 1379–1385.
[36] Q.X. Chen, M. Book, X.M. Fang, A. Hoeft, F. Stuber, Screening of copy number polymorphisms in human β-defensin genes using modified real-time quantitative PCR, J. Immunol. Methods 308 (2006) 231–240.
[37] B.A. Dale, S. Krisanaprakornkit, Defensin antimicrobial peptides in the oral cavity, J. Oral Pathol. & Med. 30 (2001) 321–327.
[38] K. Fellermann, E.F. Stange, Defensins-innate immunity at the epithelial frontier, Eur. J. Gastroenterol. Hepatol. 13 (2001) 771–776.
[39] B.C. Schutte, P.B. McCray Jr., β-Defensins in lung host defense, Annu. Rev. Physiol. 64 (2002) 709–748.
[40] A.E. King, H.O.D. Critchley, R.W. Kelly, Innate immune defences in the human endometrium, Reprod. Biol. Endocrinol. 1 (2003) 116–123.
[41] A.E. King, D.C. Fleming, H.O. Critchley, R.W. Kelly, Differential expression of the natural antimicrobials, beta-defensins 3 and 4, in human endometrium, J. Reprod. Immunol. 59 (2003) 1–16.
[42] H.P. Jia, B.C. Schutte, A. Schudye, R. Linzmeier, J.M. Guthmiller, G.K. Johnson, B.F. Tack, J.P. Mitros, A. Rosenthal, T. Ganz, P.B. McCray Jr., Discovery of new human β-defensins using a genomics-based approach, Gene 263 (2001) 211–218.
[43] S. Jolya, C.C. Organa, G.K. Johnsonb, P.B. McCray Jr., J.M. Guthmiller, Correlation between b-defensin expression and induction profiles in gingival keratinocytes, Mol. Immunol. 42 (2005) 1073–1084.
[44] Z. Feng, B. Jiang, J. Chandra, M. Ghannoum, S. Nelson, A. Weinberg, Human beta-defensins: differential activity against candidal species and regulation by *Candida albicans*, J. Dent. Res. 84 (2005) 445–450.
[45] M.E. Quinones-Mateu, M.M. Lederman, Z. Feng, B. Chakraborty, J. Weber, H.R. Rangel, M.L. Marotta, M. Mirza, B. Jiang, P. Kiser, K. Medvik, S.F. Sieg, A. Weinberg, Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication, AIDS 17 (2003) F39–F48.
[46] A. Weinberg, M.E. Quinones-Mateu, M.M. Lederman, Role of human β-defensins in HIV infection, Adv. Dent. Res. 19 (2006) 42–48.
[47] O.E. Sørensen, D.R. Thapa, A. Rosenthal, L. Liu, A.A. Roberts, T. Ganz, Differential regulation of β-defensin expression in human skin by microbial stimuli, J. Immunol. 174 (2005) 4870–4879.
[48] J. Harder, R. Siebert, Y. Zhang, P. Matthiesen, E. Christophers, B. Schlegelberger, J.-M. Schroder, Mapping of the gene encoding human β-defensin-2 (DEFB2) to chromosome region 8p22–p23.1, Genomics 46 (1997) 472–475.
[49] L. Liu, L. Wang, H.-P. Jia, C. Zhaoa, H.H.Q. Heng, B.C. Schutte, P.B. McCray Jr., T. Ganz, Structure and mapping of the human β-defensin HBD-2 gene and its expression at sites of inflammation, Gene 222 (1998) 237–244.
[50] R. Linzmeier, C.H. Ho, B.V. Hoang, T. Ganz, A 450-kb contig of defensin genes on human chromosome 8p23, Gene 233 (1999) 205–211.
[51] A.J. Ouellette, D. Pravtcheva, F.H. Ruddle, M. James, Localization of the cryptdin locus on mouse chromosome 8, Genomics 5 (1989) 233–239.
[52] C.L. Bevins, D.E. Jones, A. Dutra, J. Schaffzin, M. Muenke, Human enteric defensin genes: chromosomal map position and a model for possible evolutionary relationships, Genomics 31 (1996) 95–106.
[53] L. Iannuzzi, D.S. Gallagher, G.P. Di Meo, G. Diamond, C.L. Bevins, J. E. Womack, High-resolution FISH mapping of beta-defensin genes to river buffalo and sheep chromosomes suggests a chromosome discrepancy in ffttle standard karyotypes, Cytogenet. Cell Genet. 75 (1996) 10–13.
[54] L. Liu, C. Zhao, H.H.Q. Heng, T. Ganz, The human β-defensin-1 and α-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry, Genomics 43 (1997) 316–320.
[55] L.A. Duits, M. Rademaker, B. Ravensbergen, M.A.J.A. van Sterkenburg, E. van Strijen, P.S. Hiemstra, P.H. Nibbering, Inhibition of hBD-3, but Not hBD-1 and hBD-2, mRNA expression by corticosteroids, Biochem. Biophys. Res. Commun. 280 (2001) 522–525.
[56] P.J. Barnes, Anti-inflammatory actions of glucocorticoids : molecular mechanisms, Clin. Sci. 94 (1998) 557–572.
[57] G. Maisetta, G. Batoni, S. Esin, G. Raco, D. Bottai, F. Favilli, W. Florio, M. Campa, Susceptibility of *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans* to bactericidal activity of human β-defensin 3 in biological fluids, Antimicrob. Agents Chemother. 49 (2005) 1245–1248.
[58] C.C. Taggart, C.M. Greene, S.G. Smith, R.L. Levine, P.B. McCray Jr., S. O'Neill, N.G. McElvaney, Inactivation of human β-defensins 2 and 3 by elastolytic cathepsins, J. Immunol. 171 (2003) 931–937.
[59] M.J. Binks, B.A. Fernie-King, D.J. Seilly, P.J. Lachmann, K.S. Sriprakash, Attribution of the various inhibitory actions of the streptococcal inhibitor of complement (SIC) to regions within the molecule, J. Biol. Chem. 280 (2005) 20120–20125.

[60] B.A. Fernie-King, D.J. Seilly, P.J. Lachmann, Inhibition of antimicrobial peptides by group A streptococci: SIC and DRS, Biochem. Soc. Trans. 34 (2006) 273–275.
[61] D.M. Hoover, Z. Wu, K. Tucker, W. Lu, J. Lubkowski, Antimicrobial characterization of human β-defensin 3 derivatives, Antimicrob. Agents Chemother. 47 (2003) 2804–2809.
[62] D.J. Schibli, H.N. Hunter, V. Aseyev, T.D. Starner, J.M. Wiencek, P.B. McCray Jr., B.F. Tack, H.J. Vogel, The solution structures of the human β-defensins lead to a better understanding of the potent bactericidal activity of HBD3 against *Staphylococcus aureus*, J.Biol. Chem. 277 (2002) 8279–8289.
[63] Z. Wu, D.M. Hoover, D. Yang, C. Boulegue, F. Santamaria, J.J. Oppenheim, J. Lubkowski, W. Lu, Engineering disulfide bridges to dissect antimicrobial and chemotactic activities of human β-defensin 3, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 8880–8885.
[64] L. Huang, J. Wang, Z. Zhong, L. Peng, H. Chen, Z. Xu, P. Cen, Production of bioactive human beta-defensin-3 in *Escherichia coli* by soluble fusion expression, Biotechnol. Lett. 28 (2006) 627–632.
[65] S. Chen, F.T. He, Y.L. Dong, R.F. Li, H.G. Gao, M. Chen, J.H. Peng, The cloning, high level expression in *Escherichia coli* of human beta-defensin 3 and its antimicrobial activity analysis, Shengwu Gongcheng Xuebao 20 (2004) 490–495.
[66] A. Schulz, E. Kluver, S. Schulz-Maronde, K. Adermann, Engineering disulfide bonds of the novel human beta-defensins hBD-27 and hBD-28: differences in disulfide formation and biological activity among human beta-defensins, Biopolymers 80 (2005) 34–49.
[67] J. Harder, J.-M. Schroder, Psoriatic scales: a promising source for the isolation of human skin-derived antimicrobial proteins, J. Leukocyte Biol. 77 (2005) 476–486.
[68] Y.-Q. Tang, M.E. Selsted, Characterization of the disulfide motif in BNBD-12, an antimicrobial β-defensin peptide from bovine neutrophils, J. Biol. Chem. 268 (1993) 6649–6653.
[69] F. Bauer, K. Schweimer, E. Kluever, J.-R. Conejo-Garcia, W.-G. Forssmann, P. Roesch, K. Adermann, H. Sticht, Structure determination of human and murine β-defensins reveals structural conservation in the absence of significant sequence similarity, Protein Sci. 10 (2001) 2470–2479.
[70] M.J. Goldman, G.M. Anderson, E.D. Stolzenberg, U.P. Kari, M. Zasloff, J.M. Wilson, Human β-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis, Cell 88 (1997) 553–560.
[71] P.K. Singh, H.P. Jia, K. Wiles, J. Hesselberth, L. Liu, B.A. Conway, E.P. Greenberg, E.V. Valore, M.J. Welsh, T. Ganz, B.F. Tack, P.B. McCray Jr., Production of β-defensins by human airway epithelia, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 14961–14966.
[72] W.B. Guggino, Cystic fibrosis and the salt controversy, Cell 96 (1999) 607–610.
[73] A. Pardi, X.L. Zhang, M.E. Selsted, J.J. Skalicky, P.F. Yip, NMR studies of defensin antimicrobial peptides. 2. Three-dimensional structures of rabbit NP-2 and human HNP-1, Biochemistry 31 (1992) 11357–11364.
[74] W.C. Wimley, M.E. Selsted, S.H. White, Interactions between human defensins and lipid bilayers: evidence for formation of multimeric pores, Protein Sci. 3 (1994) 1362–1373.
[75] Y.Q. Tang, M.E. Selsted, Characterization of the disulfide motif in BNBD-12, an antimicrobial β-defensin peptide from bovine neutrophils, J. Biol. Chem. 268 (1993) 6649–6653.
[76] G.R. Zimmermann, P. Legault, M.E. Selsted, A. Pardi, Solution structure of bovine neutrophil β-defensin-12: the peptide fold of the β-defensins is identical to that of the classical defensins, Biochemistry 34 (1995) 13663–13671.
[77] M.V. Sawai, H.P. Jia, L. Liu, V. Aseyev, J.M. Wiencek, P.B. McCray Jr., T. Ganz, W.R. Kearney, B.F. Tack, The NMR structure of human β-defensin-2 reveals a novel R-helical segment, Biochemistry 40 (2001) 3810–3816.
[78] D.M. Hoover, R.R. Kanaghalagatta, R. Blumenthal, A. Puri, J.J. Oppenheim, O. Chertov, J. Lubkowski, The structure of human β-defensin-2 shows evidence of higher order oligomerization, J. Biol. Chem. 275 (2000) 32911–32918.
[79] J.M. Perez-Canadillas, A. Zaballos, J. Gutierrez, R. Varona, F. Roncal, J. P. Albar, G. Marquez, M. Bruix, NMR solution structure of murine CCL20/MIP-3 α, a chemokine that specifically chemoattracts immature dendritic cells and lymphocytes through its highly specific interaction with the β-chemokine receptor CCR6, J. Biol. Chem. 276 (2001) 28372–28379.
[80] D.M. Hoover, C. Boul`egue, D. Yang, J.J. Oppenheim, K. Tucker, W. Lu, J. Lubkowski, The structure of human macrophage inflammatory protein-3 α/CCL20, J. Biol. Chem. 277 (2002) 37647–37654.
[81] A. Bjorstad, H. Fu, A. Karlsson, C. Dahlgren, J. Bylund, Interleukin-8 derived peptide has antibacterial activity, Antimicrob. Agents Chemother. 49 (2005) 3889–3895.
[82] N.Y. Yount, M.R. Yeaman, Structural congruence among membrane-active host defense 3 polypeptides of diverse phylogeny, Biochim. Biophys. Acta 1758 (2006) 1373–1386 (this issue). doi:10.1016/j.bbamem.2006.03.027.
[83] C.E. Shelburne, W.A. Coulter, D.A. Olguin, M.S. Lantz, D.E. Lopatin, Induction of β-defensin resistance in the oral anaerobe *Porphyromonas gingivalis*, Antimicrob. Agents Chemother. 49 (2005) 183–187.
[84] T.D. Starner, B. Agerberth, G.H. Gudmundsson, P.B. McCray Jr., Expression and activity of β-defensins and LL-37 in the developing human lung, J. Immunol. 174 (2005) 1608–1615.
[85] G. Maisetta, G. Batoni, S. Esin, W. Florio, D. Bottai, F. Favilli, M. Campa, In vitro bactericidal activity of human β-defensin 3 against multidrug-resistant nosocomial strains, Antimicrob. Agents Chemother. 50 (2006) 806–809.
[86] B. Ericksen, Z. Wu, W. Lu, R.I. Lehrer, Antibacterial activity and specificity of the six human α-defensins, Antimicrob. Agents Chemother. 49 (2005) 269–275.
[87] S. Nuding, K. Fellermann, J. Wehkamp, H.A.G. Mueller, E.F. Stange, A flow cytometric assay to monitor antimicrobial activity of defensins and cationic tissue extracts, J. Microbiol. Methods 65 (2006) 335–345.
[88] E. Klüver, S. Schulz-Maronde, S. Scheid, B. Meyer, W.-G. Forssmann, K. Adermann, Structure–activity relation of human β-defensin 3: influence of disulfide bonds and cysteine substitution on antimicrobial activity and cytotoxicity, Biochemistry 44 (2005) 9804–9816.
[89] H.V. Westerhoff, D. Juretic, R.W. Hendler, M. Zasloff, Magainins and the disruption of membrane-linked free-energy transduction, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 6597–6601.
[90] I. Marcotte, K.-L. Wegener, Y.H. Lam, B.C.S. Chia, M.R.R. de Planque, H. Bowie, M. Auger, F. Separovic, Interaction of antimicrobial peptides from Australian amphibians with lipid membranes, Chem. Phys. Lipids 122 (2003) 107–120.
[91] K.A. Henzler-Wildman, D.K. Lee, A. Ramamoorthy, Mechanism of lipid bilayer disruption by the human antimicrobial peptide, LL-37, Biochemistry 42 (2003) 6545–6558.
[92] H.-G. Sahl, U. Pag, S. Bonness, S. Wagner, N. Antcheva, A. Tossi, Mammalian defensins: structures and mechanism of antibiotic activity, J. Leukocyte Biol. 77 (2005) 466–475.
[93] A. Tossi, L. Sandri, A. Giangaspero, Amphipathic, α-helical antimicrobial peptides, Biopolymers 55 (2000) 4–30.
[94] A. Mecke, D.K. Lee, A. Ramamoorthy, B.G. Orr, M.M. Banaszak Holl, Membrane thinning due to antimicrobial peptide binding: an atomic force microscopy study of MSI-78 in lipid bilayers, Biophys. J. 89 (2005) 4043–4050.
[95] D. Andreu, L. Rivas, Animal antimicrobial peptides: an overview, Biopolymers 47 (1998) 415–433.
[96] Z. Oren, Y. Shai, Mode of action of linear amphipathic α-helical antimicrobial peptides, Biopolymers 47 (1998) 451–463.
[97] M. Dathe, T. Wieprecht, Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells, Biochim. Biophys. Acta 1462 (1999) 71–87.
[98] Y. Chen, C.T. Mant, S.W. Farmer, R.E. Hancock, M.L. Vasil, R.S. Hodges, Rational design of alpha-helical antimicrobial peptides with enhanced activities and specificity/therapeutic index, J. Biol. Chem. 280 (2005) 12316–12329.
[99] H.W. Huang, Action of antimicrobial peptides: two-state model, Biochemistry 39 (2000) 8347–8352.

[100] A.G. Rao, Conformation and antimicrobial activity of linear derivatives of tachyplesin lacking disulfide bonds, Arch. Biochem. Biophys. 361 (1999) 127–134.
[101] Y. Shai, From innate immunity to de-novo designed antimicrobial peptides, Curr. Pharm. Des. 8 (2002) 715–725.
[102] R.F. Epand, A. Ramamoorthy, R.M. Epand, Membrane lipid composition and the interaction of pardaxin: the role of cholesterol, Prot. Peptide Letters 13 (2006) 1–5.
[103] K.J. Hallock, K.A. Henzler-Wildman, D.K. Lee, A. Ramamoorthy, An innovative procedure using a sublimable solid to align lipid bilayers for solid-state NMR studies, Biophys. J. 82 (2002) 2499–2503.
[104] Y. Shai, Mode of action of membrane active antimicrobial peptides, Biopolymers 66 (2002) 236–248.
[105] R. Mani, J.J. Buffy, A.J. Waring, R.I. Lehrer, M. Hong, Solid-state NMR investigation of the selective disruption of lipid membranes by Protegrin-1, Biochemistry 43 (2004) 13839–13848.
[106] E. Strandberg, A.S. Ulrich, NMR methods for studying membrane-active antimicrobial peptides, Concepts Magn. Reson. 23 (2004) 89–120.
[107] K. Matsuzaki, O. Murase, N. Fujii, K. Miyajima, An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation, Biochemistry 35 (1996) 11361–11368.
[108] S. Abu-Baker, X.Y. Qi, J. Newstadt, G.A. Lorigan, Structural changes in a binary mixed phospholipid bilayer of DOPG and DOPS upon saposin C interaction at acidic pH utilizing 31P and 2H solid-state NMR spectroscopy, Biochim. Biophys. Acta 1717 (2005) 58–66.
[109] B. Bechinger, Detergent-like properties of magainin antibiotic peptides: A 31P solid-state NMR spectroscopy study, Biochim. Biophys. Acta 1712 (2005) 101–108.
[110] P.C. Dave, E. Billington, Y.L. Pan, S.K. Straus, Interaction of alamethicin with ether-linked phospholipid bilayers: oriented circular dichroism, ^{31}P solid-state NMR, and differential scanning calorimetry studies, Biophys. J. 89 (2005) 2434–2442.
[111] R. Mani, A.J. Waring, R.I. Lehrer, M. Hong, Membrane-disruptive abilities of β-hairpin antimicrobial peptides correlate with conformation and activity: a ^{31}P and 2H NMR study, Biochim. Biophys. Acta 1716 (2005) 11–18.
[112] J.P.S. Powers, A. Tan, A. Ramamoorthy, R.E.W. Hancock, Solution structure and interaction of the antimicrobial polyphemusins with lipid membranes, Biochemistry 44 (2005) 15504–15513.
[113] K. Matsuzaki, K. Sugishita, N. Fujii, K. Miyajima, Molecular basis for membrane selectivity of an antimicrobial peptide, magainin 2, Biochemistry 34 (1995) 3423–3429.
[114] W.L. Maloy, U.P. Kari, Structure–Activity studies on magainins and other host defense peptides, Biopolymers 37 (1995) 105–122.
[115] Z. Oren, J. Hong, Y. Shai, A repertoire of novel antibacterial diastereomeric peptides with selective cytolytic activity, J. Biol. Chem. 272 (1997) 14643–14649.
[116] R.E.W. Hancock, D.S. Chapple, Peptide antibiotics, Antimicrob. Agents Chemother 43 (1999) 1317–1323.
[117] G. Bierbaum, H.-G. Sahl, Induction of autolysis of Staphylococci by the basic peptide antibiotics pep5 and nisin and their influence on the activity of autolytic enzymes, Arch. Microbiol. 141 (1985) 249–254.
[118] K. Matsuzaki, Why and how are peptide–lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes, Biochim. Biophys. Acta 1462 (1999) 1–10.
[119] G. Kragol, S. Lovas, G. Varadi, B.A. Condie, R. Hoffmann, L. Otvos Jr., The antibacterial peptide pyrrhocoricin inhibits the ATPase actions of DnaK and prevents chaperone-assisted protein folding, Biochemistry 40 (2001) 3016–3026.
[120] M. Zilbauer, N. Dorrell, P.K. Boughan, A. Harris, B.W. Wren, N.J. Klein, M. Bajaj-Elliott, Intestinal innate immunity to *Campylobacter jejuni* results in induction of bactericidal human beta-defensins 2 and 3, Infect. Immun. 73 (2005) 7281–7289.
[121] H.G. Boman, D. Wade, I.A. Boman, B. Wihlint, R.B. Merrifield, Antibacterial and antimalarial properties of peptides that are cecropin-melittin hybrids, FEBS Lett. 259 (1989) 103–106.
[122] K.L. Piers, M.H. Brown, R.E. Hancock, Improvement of outer membrane-permeabilizing and lipopolysaccharide-binding activities of an antimicrobial cationic peptide by C-terminal modification, Antimicrob. Agents Chemother. 38 (1994) 2311–2316.
[123] M. Gough, R.E.W. Hancock, N.M. Kelly, Antiendotoxin activity of cationic peptide antimicrobial agents, Infect. Immun. 64 (1996) 4922–4927.
[124] R.E.W. Hancock, Peptide antibiotics, Lancet 349 (1997) 418–422.
[125] M.G. Scott, H. Yan, R.E.W. Hancock, Biological properties of structurally related alpha-helical cationic antimicrobial peptides, Infect. Immun. 67 (1999) 2005–2009.
[126] M.G. Scott, M.R. Gold, R.E.W. Hancock, Interaction of cationic peptides with lipoteichoic acid and gram-positive bacteria, Infect. Immun. 67 (1999) 6445–6453.
[127] M.G. Scott, A.C.E. Vreugdenhil, W.A. Buurman, R.E.W. Hancock, M.R. Gold, Cutting edge: cationic antimicrobial peptides block the binding of lipopolysaccharide (LPS) to LPS binding protein, J. Immunol. 164 (2000) 549–553.
[128] M. Zasloff, Antimicrobial peptides of multicellular organisms, Nature 415 (2002) 389–395.
[129] A. Böhling, S.O. Hagge, S. Roes, R. Podschun, H. Sahly, J. Harder, J.-M. Schröder, J. Grötzinger, U. Seydel, T. Gutsmann, Lipid-specific membrane activity of human α-Defensin-3, Biochemistry 45 (2006) 5663–5670.
[130] W. Falk, R.H. Goodwin Jr., E.J. Leonard, A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration, J. Immunol. Methods 33 (1980) 239–247.
[131] F. Niyonsaba, H. Ushio, I. Nagaoka, K. Okumura, H. Ogawa, The human β-defensins (-1, -2, -3, -4) and cathelicidin LL-37 induce IL-18 secretion through p38 and ERK, MAPK activation in primary human keratinocytes, J. Immunol. 175 (2005) 1776–1784.
[132] O. Chertov, D.F. Michiel, L. Xu, J.M. Wang, K. Tani, W.J. Murphy, D.L. Longo, D.D. Taub, J.J. Oppenheim, Identification of Defensin-1, Defensin-2, and CAP37/Azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils, J. Biol. Chem. 271 (1996) 2935–2940.
[133] D. Yang, O. Chertov, S.N. Bykovskaia, Q. Chen, M.J. Buffo, J. Shogan, M. Anderson, J.M. Schroder, J.M. Wang, O.M.Z. Howard, J.J. Oppenheim, β-Defensins: linking innate and adaptive immunity through dendritic and T cell CCR6, Science 286 (1999) 525–528.
[134] D. Yang, Q. Chen, O. Chertov, J.J. Oppenheim, Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells, J. Leukocyte Biol. 68 (2000) 9–14.
[135] D. Varoga, T. Pufe, J. Harder, J.M. Schröder, R. Mentlein, U. Meyer-Hoffert, M.B. Goldring, B. Tillmann, J. Hassenpflug, F. Paulsen, Human β-defensin-3 mediates tissue remodeling processes in articular cartilage by increasing metalloproteinases and reducing their endogenous inhibitors, Arthritis Rheum. 52 (2005) 1736–1745.
[136] L. Sun, C.M. Finnegan, T. Kish-Catalone, R. Blumenthal, P. Garzino-Demo, G.M.L.T. Maggiore, S. Berrone, C. Kleinman, Z. Wu, S. Abdelwahab, W. Lu, A. Garzino-Demo, Human beta-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection, J. Virol. 79 (2005) 14318–14329.
[137] D.J. Campopiano, D.J. Clarke, N.C. Polfer, P.E. Barran, R.J. Langley, J.R.W. Govan, A. Maxwell, J.R. Dorin, Structure–Activity relationships in defensin dimmers: a novel link between beta-defensin tertiary structure and antimicrobial activity, J. Biol. Chem. 279 (2004) 48671–48679.
[138] A. Ramamoorthy, S. Thennarasu, A. Tan, K. Gottipati, S. Sreekumar, D.L. Heyl, F.Y.P. An, C.E. Shelburne, Deletion of all cycteines in Tachyplesin I abolishes hemolytic activity and retains antimicrobial activity and LPS selective binding, Biochemistry 45 (2006) 6529–6540.