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NMR Structure and Localization of the Host Defense Antimicrobial Peptide Thanatin in Zwitterionic Dodecylphosphocholine Micelle: Implications in Antimicrobial Activity

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Running Title: Structure of Thanatin in DPC Micelle

Abstract

Antimicrobial peptides (AMPs) are potentially vital as the next generation of antibiotics against multidrug resistant bacterial pathogens. Thanatin, an insect derived pathogen inducible 21-residue long antimicrobial peptide, demonstrates antimicrobial activity toward broad range of pathogens. Thanatin is an excellent candidate for antibiotics development due to potent in vivo activity in animal model and low toxicity to human cells. Recent studies indicated mode of action of thanatin could be intriguing and may comprise bacterial membrane permeabilization and interactions with periplasmic proteins. In order to better understand selectivity and membrane disruption, here, we determined 3-D structure of the thanatin in zwitterionic DPC-d_{38} micelle by NMR spectroscopy. The depth of insertion of thanatin into micelle structure was investigated by spin labelled doxyl lipids, 5-DSA and 16-DSA. DPC-bound structure of thanatin is defined by a β-hairpin structure and an extended and turn conformations, for residues G1-I8, at the N-terminus. The β-hairpin structure is delineated by two antiparallel β-strands, residues I9-C11 and residues K17-R20, which is connected by loop consisted of residues N12-G16. There are cross β-strands sidechain-sidechain packing interactions among hydrophobic and aromatic residues. Spin labelled lipid studies revealed a set of spatially proximal residues V6, I8, Q19, R20 and M21 may be deeply inserted into the hydrophobic core of the DPC micelle. Whilst, residues including those at the turn/loop are merely surface localized. The atomic resolution structure and orientation of thanatin in zwitterionic DPC micelle may be utilized for understating mode of action in lipid membrane and further development of non-toxic analogs.

Keywords: Antimicrobial peptides, Host defense antimicrobial peptide, thanatin, NMR, structure, DPC

Abbreviations: AMPs, antimicrobial peptides, NMR, nuclear magnetic resonance, DPC, dodecyl phosphocholine, PRE, paramagnetic relaxation enhancement

1. Introduction

Host defense antimicrobial peptides (AMPs) serve a dominant role in protecting against invading pathogens in all forms of life [1-3]. The global upsurge of drug resistant, multidrug resistant (MDR) and extremely drug resistant (XDR) bacteria is a serious threat to human health [4-6]. Centers for disease control and prevention (CDC) of USA described an annual 2.8 million antibiotic-resistant infections causing death of 35,000 people. The ability of AMPs to kill drug resistant bacteria has been thought to be utilized for the development of novel antibiotics [7-11]. Recent studies demonstrated that AMPs can lower bacterial load in *in vivo* murine models of infection for ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*) group of pathogens [12-19]. Currently, several AMPs and derived analogs are in clinical trials, whereas some AMPs like MSI-78 or pexiganan, omiganon had been tested in past clinical trials [20-23]. As a mode of bacterial cell killing, most cationic AMPs disrupt bacterial membrane and potentially limit the development of bacterial resistance [24, 25]. Several mechanisms e.g. barrel stave, toroidal pore, carpet, are put forward explaining membrane disruption process of AMPs [26-28]. Atomic resolution structure of AMPs in membrane or in membrane mimic environment could in principle help in engineering novel analogs with lower host toxicity, improved bacterial potency and enhanced in vivo stability [29-33]. AMPs can be classified into three major structural classes namely α-helical, β-sheet or β-hairpin and extended/flexible [34, 35]. Protegrins, α and β defensins, tachyplesins are well investigated examples of β-sheet or β-hairpin AMPs [36-42]. β-sheet structures of these AMPs are stabilized by multiple disulfide bonds.

Thanatin a 21-residue long potent AMP isolated from the hemolymph of bacterially challenged insect *Podisus maculiventris* [43]. The secondary structure of thanatin contains a β-hairpin at the C-terminus, stabilized by a single disulfide bond between residues Cys 11

and Cys 18, encompassing a small ring of 8 amino acids. Thanatin is largely non hemolytic and has demonstrated bactericidal and fungicidal activity against a wide range of strains at physiological concentrations [43]. Thanatin was found to be active in murine model of septic shock and drug resistant pathogens [44-48]. Further, thanatin derived peptides are reported to be in clinical or preclinical trials against systemic fungal infections in immunocompromised patients and multi-drug resistant bacterial infections [49]. Bacterial membrane permeabilization and cell agglutinations have been proposed to be mode of action of thanatin [50-52]. A recent study showed that thanatin bind to proteins LptA and LptD of *E. coli* which are involved in transport of LPS to the outer membrane [53]. 3-D structures of thanatin are reported in free solution and in negatively charged outer membrane LPS [51]. Significant differences were observed in the structures of thanatin obtained in aqueous solution and in LPS micelle [51]. In free solution, thanatin assumed β-hairpin conformation at the C-terminal half whereas the N-terminal region was largely in random conformations [51]. By contrast, in LPS micelle, a dimeric structure of thanatin was sustained by four stranded β-sheet with a less flexible N-terminal region [51]. In order to gain further insights into mechanism and activity of thanatin, here, we have solved atomic resolution structure of thanatin in zwitterionic detergent micelle DPC-d_{38} and have investigated micelle localization using NMR spectroscopy. The current results shed new insights toward membrane specificity and activity of thanatin.

2. Materials and Methods

2.1 Materials

Thanatin was commercially synthesized by GLBiochem^{TM} (Shanghai, China) and purified by reverse phase HPLC using a linear gradient of acetonitrile/water, 1% trifluoroacetic acid (TFA) solvent system with purity >95%. Mass of the peptide was confirmed by mass

spectrometry. Deuterated DPC-d_{38}, D_2O and deuterated methanol were purchased from Cambridge Isotope Inc (Massachusetts USA). Paramagnetic lipids 5-DSA and 16-DSA, TFA were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 NMR spectroscopy

All NMR experiments were carried out in a Bruker AVANCE II 600 MHz spectrometer equipped with a cryoprobe and pulse field gradient. Lyophilized powder of thanatin (0.25 mM) was dissolved either in aqueous solution containing 125 mM perdeuterated DPC-d_{38} that is equivalent to 2.5 mM concentration of micelle. NMR experiments were done at peptide/detergent ratio of 1:10. Two-dimensional (2-D) ^1H-^1H TOCSY and NOESY spectra of the peptide samples were acquired at a temperature of 308 K, pH 5.5. All 2-D experiments were performed with 2K (t2)×400 (t1) data points with a relaxation delay of 1s for 88 (NOESY) and 32 (TOCSY) scans. Mixing time of 2-D NOESY and TOCSY experiments were set to 150 ms and 80 ms, respectively. NMR chemical shifts were referenced to 40 μM DSS as an internal standard. WATERGATE procedure was used for water signal suppression. Sweep Width (SW) was fixed to 13.02 ppm in both dimensions. For probing localization and insertion of thanatin in DPC micelle, PRE (paramagnetic relaxation enhancement) studies were achieved by acquiring 2-D TOCSY spectra of thanatin (0.25 mM) either in the absence or in the presence of 2 mM of 5-DSA and 16-DSA. Stock solutions of 5-DSA and 16-DSA were prepared in deuterated methanol. The intensities of the αH/NH cross-peaks (for Pro residues δH/αH) of individual amino acid residues in TOCSY spectra were estimated before and after addition of the paramagnetic probes and remaining amplitude in intensity was determined. TOCSY spectra were acquired using a spin-lock MLEV 17 sequence with a mixing time of 80 ms. NMR data were processed using TopSpin 3.0 (Bruker). After zero

filling along the t1 dimension, 2 K (t2) × 2 K (t1) data matrices were obtained. Spectral analysis and peak picking were done using SPARKY software.

2.3 Structure calculation

The assigned NOEs were classified as strong, medium or weak and translated to an upper bound distance constraints: 2.8, 3.5 and 5.0 Å, respectively. Backbone dihedral angles ϕ and ψ, were estimated by Wishart Preditor using $^{\alpha}$H chemical shifts [54]. The estimated dihedral constraints ϕ and ψ values were further varied by ± 25˚. An ensemble of DPC-bound structure of thanatin was calculated using CYANA (version 2.1) [55]. The structure calculation was done in a step wise fashion to obtain the final ensemble with low RMSD and target function.

3. Results

3.1 Mapping Influence of DPC in Structural Changes of Thanatin

We examined resonance perturbation of thanatin upon interactions with DPC detergent micelle. Fig. 1 shows superposition of NH/C$^{\alpha}$H correlations of 2-D TOCSY spectra of thanatin in free solution and in presence of DPC-d_{38} micelle. Resonances of all the residues, except for S2, of thanatin in micelle bound state were assigned using standard sequential walk method [56]. Most of the residues of thanatin demonstrated significant changes in chemical shift of NH and $^{\alpha}$H resonances. Notably, the amide proton chemical shift of thanatin in DPC micelle showed a wider dispersion, ranging from 8.98 ppm to 7.59 ppm, compared to that of free solution. Further, overlapping resonances of residues e.g. T15 and K17, in free solution became non-degenerate in DPC micelle solution. The well resolved NMR spectra of thanatin in DPC solution implied absence of any occurrence of potential fusion or agglutination among the micelles. Such processes were likely to yield broad NMR spectra resulting from chemical exchange and high molecular weight. Fig. 2 shows secondary

chemical shift, deviation from random coil value, of $^{\alpha}$H resonances of residues of thanatin in DPC micelle. The secondary chemical shift of $^{\alpha}$H of I9, Y10, C11, K17, C18, Q19 and R20 delineated significant positive deviation that would indicate β-sheet conformations of these residues [57]. The intervening residues, except for T15, showed a negative deviation in secondary chemical shift, potentially indicating turn or loop conformations. Also, the secondary chemical shift of the N-terminal residues encompassing K3-I8 did not show any discernable pattern of regular secondary structure. Taken together, the aforementioned data indicate that thanatin binds to zwitterionic DPC micelle and residues, I9-C11 and K17-R20, showed stable β-sheet conformation.

3.2 Analyses of 2-D NOESY Spectra of Thanatin in DPC Micelle

2-D NOESY spectra of thanatin in DPC micelle is of high quality exhibiting large number of NOEs among sidechain/sidechain, backbone/sidechain and backbone/backbone resonances (Fig. 3). Apart from sequential and medium range NOEs, residues surrounding the disulfide bridge delineated several long-range NOE contacts. For example, NOE contacts were unambiguously detected involving the aromatic sidechain and backbone amide proton of residue Y10 with the backbone amide proton of residue M21 and backbone amide proton of residue Q19. Long-range NOEs could be seen between residues N12/Q19, C18/C11, C18/N12 (Fig. 3, Table 1). N-terminus residues, away from disulfide bond, exhibited sequential and medium range NOEs. Fig. 4 summarizes number and type of NOEs for individual residues of thanatin in DPC micelle. Note, aromatic residue Y10 showed as many as 38 NOE contacts including 13 long-range NOEs.

3.3 Atomic resolution Structure of Thanatin in DPC Micelle

An ensemble of structures of thanatin bound to DPC micelle was determined using NOE driven distance and backbone dihedral angle constrains using CYANA. Fig. 5A shows superpositions of twenty lowest energy structures of thanatin in DPC micelle. Close superposition can be seen for the residues I9-M21, whereas N-terminal residues G1-I8 exhibited more flexibility. Backbone and all heavy atom RMSDs of the superposed structures were estimated to be 0.77Å and 1.29Å, respectively (Table 2). In DPC micelle, 3-D structure of thanatin is defined by a β-hairpin for the residues I9-R20 whereby β1 strand encompasses residues I9-Y10-C11 and β2 strand includes residues K17-C18-Q19-R20 (Fig. 5B). The two adjacent antiparallel β-strands are connected by a well-structured loop consisted of residues N12-R13-R14-T15-G16 (Fig. 5B). There are mutual sidechain/sidechain packing and potential hydrogen bonding interaction between the two antiparallel β-strands. Cross β-strands sidechain/sidechain packing can be realized by the close proximity of the aromatic sidechain of residue Y10 with the sidechains of residues M21 and Q19 (Fig. 5B). The sole disulfide bond between residues C11 and C18 of thanatin occupies the opposite face of the β-hairpin structure and in proximity with the aliphatic sidechain of residue I9. There are potential hydrogen bonding interactions between the β-strands whereby C=O and NH groups of residue Y10 of β1 strand are within hydrogen bond distance, (O to N distant <3 Å), with the NH and C=O groups of residue Q19 of β2 strand, respectively (Fig. 5C). The five-residue loop of the β-hairpin is well defined in the structural ensemble (Fig. 5A). Residues R13 and R14 occupy the center of the loop of the β-hairpin structure (Fig. 5B). Note, backbone dihedral angles of residue R13, ~ ϕ = -50.8^o ψ = -32^o and residue R14 ~ ϕ = -56^o ψ = -42.8^o are found to be at the α_R conformational space. Further, backbone dihedral angle, ϕ = 47.2^o ψ = 51.4^o, of residue G16 delineated α_L conformation that perhaps is important for the reversal of the loop structure. Furthermore, conformational characteristics of loop may involve a long

range hydrogen bond between C=O of residue N12 and NH of residue G16. There are also polar interactions between the sidechains of residue N12 and T15 in the loop. While mixed conformations can be seen for the residues, S2-I8, at the N-terminal region. A turn structure is realized by residues P5-V6-P7 and residues S2-K3-K4 are found to be in extended conformations. Further, the sidechains of residues K4, I8 and M21 are spatially close defining an orientation of the N-terminus with the β-hairpin structure.

The electrostatic potential of the 3-D structure of thanatin in DPC micelle displayed a large cationic surface maintained by residues K3, K4, R13, R14, K17 and R20 (Fig. 5D). There are two distinct patches of the cationic surface. Although spatially distal, residues K3, K4, at the extended N-terminus are oriented along the same face of residues R13, R14 of the loop of β-hairpin. The second basic patch is formed by residues K17 and R20 located at β2 strand of the structure.

3.4 Localization of Thanatin in DPC Micelle

Quantitation of PRE-mediated broadening of resonances was employed to examine micelle insertion and localization of thanatin. In separate experiments, intensity changes of $^{\alpha}$H/NH cross-peaks of residues in 2-D TOCSY spectra were estimated either in the absence or in presence of 2 mM spin labelled paragenetic lipids 5-DSA and 16-DSA. 5-DSA would influence resonances that are close to the head group of the detergent micelles whereas resonances of residues inserted into micelle will be perturbed by 16-DSA [58]. The PRE effect of thanatin has been summarized in Fig. 6. As seen, most of the residues of thanatin experienced moderate to high PRE in the presence of spin labelled lipids, indicating association with the micelle. In particular, in the presence of 5-DSA, residues K4, P5, V6, P7, I8, R14, T15, Q19, R20 and M21 delineated moderate PRE effect whereas PRE effect were estimated to be higher for residues I9, Y10, C11, R13, G16, K17 and C18 (Fig. 6, top panel).

High PRE effect in the presence of 16-DSA can be detected for residues K4, V6, I8, Y10, C11, R13, K17, C18, Q19, R20 and M21 (Fig. 6, middle panel). Notably, residues V6, I8, Q19, R20 and M21 are more perturbed by 16-DSA compared to 5-DSA. Fig. 6 (lower panel) shows ratio of remaining amplitude of residues in 5-DSA and 16-DSA. Note, residues whose resonances were not visible in PRE TOSCY spectra were excluded. Residues K4, Y10, K17, Q19 and R20 appeared to be more influenced by 16-DSA. On the other hand, residues K3, P5, I9, R14, T15 and G16 were more affected by 5-DSA. The PRE effect has been mapped onto the structure of thanatin (Fig. 7). As seen, the distal part of the C-terminal β-hairpin and few residues of the extended N-terminus could be deeply inserted into the core of DPC micelle. Whereas, most part of the β-hairpin including residues at the β1 strand and in the loop are located close to the micelle head group (Fig. 7).

4. Discussion

Disulfide bonds are important for antimicrobial activity and structural stability of β-sheet and β-hairpin AMPs [34, 35, 59]. Multiple disulfide bonds are found in some of the well investigated potent AMPs including tachyplesins, protegrins and different classes of defensins (α, β, θ). A small number of AMPs are known to contain a single disulfide bond but can exert potent antimicrobial activity [59]. Typical examples of single disulfide bonded AMPs are frog skin derived tigerinins, bovine bactenecin, marine worm derived arenicin 1 and 2 and lactoferricin B, fragment of the protein lactoferrin [60-63]. AMPs with single disulfide bond may differ depending on the position of the disulfide bond in the sequence. Tigerinin-2 and bactenecin, both 12-residue long, contain S-S bridge within nine residue ring. Whereas, a longer 18-residue ring, Cys3-Cys20, is found for 21-residue arenicin-1 and 25-residue lactoferricin B. Atomic-resolution structures of the single disulfide bonded AMPs in membranous environment are poorly investigated. However, 3-D structures would

significantly pave the way to understand membrane perturbation mechanisms and develop potent analogs. NMR studies established that arenicins assume β-hairpin structure including residues at the N and C-termini within the S-S ring [64, 65]. The β-hairpin structure of arenicins appeared to show dimers in DPC micelle and dimers/oligomers in POPC lipid bilayer [64, 65]. The 21-residue thanatin is unique among single disulfide bonded AMPs as characterized by a short eight residue disulfide ring at the C-terminus [43]. Interestingly, frog AMPs 24-residue brevinins contain seven residue disulfide ring at its C-terminus shares 50% sequence homology with thanatin [43]. Notably, brevinins assumed helical or helix-hinge-helix conformations in detergent solutions [66]. Thanatin demonstrated broad spectrum antimicrobial activity against Gram negative, Gram positive bacteria and several strains of fungi [43]. However, mode of action of thanatin is not clearly understood and appears to be complex with more than one target. As a mode of Gram negative bacterial cell killing, thanatin disrupts integrity of the LPS-outer membrane of the Gram negative bacteria and permeabilizes inner plasma membrane [43, 50-52]. Interactions of thanatin with LPS outer membrane may cause bacterial cell agglutination [43, 51]. In addition to LPS interactions, recent studies demonstrated binding of Gram negative bacterial proteins with thanatin. Periplasmic proteins LptA and LptD, involved in LPS transport system, can form high affinity complex with thanatin that may lead to defective biogenesis of outer membrane in *E. coli* [53, 67]. Thanatin demonstrated inactivation of the periplasmic metallo-β-lactamase in drug resistant Gram negative bacteria by displacing Zn^{2+} ions from at the active site of the enzyme [52]. However, the board spectrum activity of thanatin may require permeabilization of bacterial plasma membrane and interactions with membrane lipids [51, 52]. Therefore, in order to fully comprehend mechanistic insights, atomic-resolution structures of thanatin in membrane mimic environment could be useful as a starting point for establishing structure-activity correlation. Atomic-resolution structure and interactions of thanatin are reported as a

complex of negatively charged LPS micelle [51]. The LPS bound structure of thanatin demarcated a topology of four stranded β-sheet resulting from dimerization of two β-hairpin subunits. In order to determine influence of membrane environment and to gain potential correlation with activities, here, we determined 3-D structure of thanatin stably bound to zwitterionic DPC-d_{38} micelle. Although, detergent micelles are known to be poor model membrane, however, determination of atomic resolution structure of AMPs either embedded in lipid bilayers or nanodiscs, bicelles has met only limited success due to difficulties associated with quality of NMR spectra or stability of the complexes [68, 69]. DPC has been serving a prototypical detergent for elucidation of 3-D structures of number of potent AMPs [64, 70-72]. Notably, AMPs demonstrated higher order oligomeric structures in DPC micelle. Such oligomeric structures of AMPs considered to be vital for membrane disruption processes [64, 70-72]. Further, bacterial cytoplasmic membrane contains mixture of both zwitterionic and anionic lipids [73]. Binding of cationic AMPs can cause clustering of anionic lipids and domain formation as a mode of action of membrane disruption [74, 75]. The zwitterionic lipids in bacterial membrane play important roles in charge separation mechanism of AMPs [74, 75]. 3-D structure of thanatin in DPC micelle revealed two stranded antiparallel β-sheet, β1 strand: residues I9-C11, β2 strand: residues K17-R20, centering the eight residue disulfide ring C11-C18. Two antiparallel β-stands are juxtaposed by a polar/cationic loop forming a β-hairpin. On the other hand, the N-terminal residues G1-I8 of thanatin assumed an extended and turn conformations in DPC micelle that is oriented along β1 strand of the hairpin structure. Two Pro residues, P5 and P7 at the N-terminus yielded a bend or turn conformation at the N-terminal region of thanatin. PRE studies demonstrated that residues both from the extended N-terminal and β-hairpin structure of thanatin could be localized within DPC micelle. Notably, nonpolar residues V6, I8 from the N-terminal half and residues Q19, R20 and M21 from the C-terminal region appeared to be

experienced a deeper insertion towards the centre of DPC micelle. Whereas, other residues of thanatin including polar and cationic residues may be located away from the centre of the micelle. Thanatin was found to be assuming dimeric β-sheet topology in negatively charged LPS micelle. The dimeric structure of thanatin was deduced from NOE contacts among residues of the N-terminal β1 strand of the β-hairpin [51]. Thanatin appeared to be largely monomeric in DPC micelle as we have not detected any diagnostic NOEs pertaining to dimerization. 3-D structure of thanatin has also been reported in free solution [76]. Structures of thanatin obtained in DPC micelle, in aqueous solution and in LPS micelle are compared (Fig. 8). As seen, DPC bound structure of thanatin shows significant differences with that of in aqueous solution in terms of backbone and sidechain dispositions. The RMSD of backbone atoms between two structures was estimated to be 3.76 Å. Notably, the N-terminal extended portion, the β1 strand and loop structure showed substantial differences between the structures (Fig. 8A). It may be noteworthy that more long range NOE connectivities (26 NOEs) for thanatin structure were identified in DPC micelles as compared to the free solution (14 NOEs). Superposition of DPC bound structure with a subunit of LPS bound structure of thanatin yielded backbone RMSD of 5.0 Å (Figure 8B). Although the β-hairpin structure of thanatin is conserved in LPS and DPC micelle, however, the high RMSD value indicates relative orientation of the 3-D topologies have significant differences. These observations suggest that membrane environments play important roles in stabilizing disparate active conformations of thanatin. In order words, the dimeric structure of thanatin in LPS micelle appeared to be relevant for cell agglutinations and outer membrane disruption of Gram negative bacteria. Whereas, we surmise that the monomeric β-hairpin structure of thanatin deduced in DPC micelle perhaps can be implicated for binding to bacterial inner membrane (*vide supra*). Notably, residues R13 and R14 of thanatin were found to be important for antibacterial activity against Gram negative and positive bacteria [51]. Replacement of

residues R13 and R14 of thanatin with Ala had resulted marked loss of antibacterial activity [51]. Both residues are found to be in close contact with LPS [51] and DPC micelles (Fig. 6). Taken together, the atomic resolution structure and localization of thanatin in DPC micelle can provide mechanistic insights of the cell membrane binding and perturbation. The micelle-bound structure of thanatin revealed two cationic surfaces composed by residues K3, K4, R13, R14, K17 and R20. Based on PRE, the cationic face consisted of residues K3, K4, R13, R14 is surface localized are amenable for interactions with the target membrane. Thanatin may be embedded onto the surface of the plasma membrane whereby these cationic residues, by salt bridges and/or hydrogen bonds, interact with the negatively charged phosphate head groups of the plasma membrane. The peptide/lipid interactions may be further reinforced by insertion of non-polar residues into the hydrophobic milieu of the membrane. Such binding interaction of thanatin with the target membrane is likely to cause destabilization of the head group by charge clustering and impart defect in packing of the lipid chains leading to a perturbation of the bilayer structure.

5. Conclusions

AMPs containing single disulfide bond could be attractive templates for the development of less complex or low-cost antibiotics compared to multiple disulfide bonded AMPs. A high-throughput screening study of bactenecin based library has reported generation of short AMPs with improved antibacterial activity. The current work and associated investigations of the single disulfide bonded non-hemolytic AMP thanatin could pave the way for the development of potent and novel analogs that are effective against drug resistant bacteria. In particular, the β-hairpin structure of thanatin appeared to be a conserved feature either in terms of binding with periplasmic proteins or with negatively charged LPS and zwitterionic

DPC micelles. The future work may entail designing analogs of thanatin with stable β-sheet structures for improved antimicrobial activity.

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Figure Legend

Figure 1: Overly of the finger-print region of the 2-D TOCSY spectra of thanatin in DPC-d_{38} micelle (in red) and in aqueous solution (in yellow) showing $^{\alpha}$H/NH correlations of amino acid residues. Backbone $^{\alpha}$H/NH correlations of residues K3-M21, excluding residues P5 and P7, of thanatin are marked in DPC-d_{38} micelle bound state. Backbone $^{\alpha}$H/NH correlation of residue S2 could not be assigned presumably due to overlapping resonances.

Figure 2: Bar diagram showing secondary chemical shift of $^{\alpha}$H atom of amino acid residue of thanatin in DPC-d_{38} micelle. The positive deviation of secondary chemical shift of residues I9-C11 and K17-R20 indicated β-strand conformation.

Figure 3: Selected sections of 2-D NOESY spectra of thanatin acquired in solution containing DPC-d_{38} micelle showing NOE connectivity among backbone NH/NH, downfield sidechain/NH resonances (lower panel) and NH/$^{\alpha}$H and aliphatic sidechain resonances (upper panel).

Figure 4: Bar diagram summarizing number and type of NOE contacts, identified from the analysis of 2-D NOESY spectra, of individual residue of thanatin bound to DPC-d_{38} micelle. Residue Y10 showed as many as 38 NOEs including 13 long range NOE contacts.

Figure 5: (panel A) Superposition of twenty low energy structure of thanatin in DPC-d_{38} micelle solution determined using CYANA. (panel B) Ribbon representation of a representative structure of micelle bound thanatin showing the β-hairpin structure at the C-terminus and an extended/turn structure at the N-terminal. The aromatic sidechain of residue is in close packing interactions with the sidechain of residues M21 and Q19. (panel C) The backbone topology of thanatin highlighting potential hydrogen bonds between the β-strands of β-hairpin. (panel D) Electrostatic potential diagram of DPC-d_{38} micelle bound thanatin in two orientations showing extended cationic patches in the 3-D structure.

Figure 6: Bar diagrams showing estimated remaining amplitude of $^{\alpha}H$/NH ($^{\alpha}H/^{\delta}H$ for Pro) cross peak intensity obtained from 2-D TOCSY spectra of individual residue of thanatin in the presence of 2 mM 5-DSA (upper panel), 2 mM 16-DSA (middle panel) and a ratio of the remaining amplitude of two spin labelled probes (lower panel) in DPC-d_{38} micelle solution.

Figure 7: Ribbon representation of the DPC-micelle bound 3-D structure of thanatin highlighting residues highlighting PRE effect. Residues V6, I8, Q19, R20 and M21 found to be significantly perturbed by 16-DSA are marked by sphere whereas residues K3, K4, Y10, R13, R14 and K17 affected by 5-DSA are represented as stick.

Figure 8: Superposition of 3-D structures of thanatin determine in DPC micelle and in free solution (panel A) and with the dimeric structure in LPS micelle (panel B). The atomic resolution structure in DPC-d_{38} micelle differs significantly, in terms of backbone and sidechain dispositions, from structures in free solution and LPS micelle.