Marked Increase in Membranolytic Selectivity of Novel Cyclic Tachyplesins Constrained with an Antiparallel Two-β Strand Cystine Knot Framework

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We have developed a highly constrained 18-residue cyclic peptide template based on the antimicrobial peptide tachyplesin-1 that features an end-to-end peptide backbone and a cystine knot-like motif with three evenly spaced disulfide bonds to cross-brace the anti-parallel β-strands and to approximate an amphipathic “β-tile”-like structure. Six β-tile analogs were prepared to correlate different topological patterns with membranolytic specificity. Their conformations and antimicrobial and hemolytic activities were compared with tachyplesin-1 and the recently discovered Rhesus monkey theta defensin (RTD) which contains similar β-tile structural elements. The β-tile peptides and RTD retained broad spectrum antimicrobial activities. In general, they were less active than tachyplesin-1 in 10 tested organisms but their activity increased under high-salt (100 mM NaCl) rather than in low-salt conditions. The β-tile peptides are highly nontoxic to human erythrocytes with EC_{50} ranging from 600 to 4000 μM. Collectively, our results show that the design of a highly rigid peptide template is useful for further analog study to dissociate antimicrobial activity from cytotoxicity which would be helpful in discovering clinical applications for peptide antibiotics.© 2000 Academic Press

Antimicrobial peptides are important components of the innate immunity against microbial infections (1-6). These peptides are usually cationic and their mechanisms of action generally involve membranolytic disruption, permeability, or pore formation against bacteria, fungi or viruses. Structurally, their forms differ. Open-chained antimicrobial peptides are usually found in eukaryotes and higher organisms from insects to humans and, thus far, >100 such peptides have been identified. In contrast, prokaryotes often produce closed-chain antimicrobial peptides, including the earliest known cyclic peptide antibiotic the gram-acids and tyrocidines (7, 8). In addition, these cyclic peptides often contain unnatural amino acids and extensive structural modifications that may confer resistance to proteolytic degradation and additional specificity to target macromolecules other than lipid membranes. We have therefore explored the design of highly constrained cyclic peptides derived from eukaryotic sources in the hope of developing metabolically-stable synthetic analogs which can dissociate antimicrobial specificity from cytotoxicity to yield potential therapeutic leads. In particular, we are interested in developing a highly rigid cyclic peptide template with two β-strands cross-braced by multiple disulfide bonds to facilitate structure-function study and to minimize the uncertainty of conformational changes in analog studied.

The 17-amino acid tachyplesin TP-1 (Fig. 1), a broad-spectrum antimicrobial cationic peptide of the tachyplesin family, is a suitable starting point for our design strategy. TP-1 was first isolated from acid extracts of the Japanese horseshoe crab (Tachypleus tridentatus) hemocytes (3) and, since then, five other tachyplesins have been identified (9). They share significant size and structural similarities with another antimicrobial family, the protegrins. NMR studies (10, 11) reveal that TP-1 adopts an anti-parallel β-sheet structure constrained by two disulfide bridges and connected by a four-residue type I β-turn. In this planar conformation, bulky hydrophobic side groups are localized in one side of the plane and four of the six cationic side groups are clustered at both ends of the molecule to form an amphipathic β-sheet. More importantly, its disulfide connectivity places the N- and C-terminals in close proximity, making it amenable to an N-to-C cyclization

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to form a backbone peptide bond on a two-β strand framework.

To implement our design for a highly rigid cyclic template based on TP-1, we first transformed the odd-numbered residue TP-1 to an even-numbered tachyplesin by adding a Gly at its COOH-terminus. The resulting 18-residue peptide TP18 favors end-to-end circularization (12). Cyclizing this 18-residue tachyplesin to form an end-to-end peptide backbone cTP 1 involves an additional disulfide since the Arg^14^ pair positioned in the middle of the antiparallel strands is replaced by a Cys^5^ pair to afford a cyclic tripeptide cTP 2 (cc, cyclic cystine) as a prototypic design template. This triple disulfide-bonded template contains a two-fold symmetry with amino acid spacings of the disulfide pairs following the 4n rule, i.e., the parallel disulfide pairs and the two reverse turns are evenly spaced by four amino acid residues. The disulfide pattern with the center Cys II-V pair threading through the other two cystine pairs is conceptually similar to the cystine-knot motifs that are found in peptides or proteins with three or more β strands (13). The design of three parallel disulfide bonds rigidifying a two-β strand structure also approximates a “β-tile”-like structure as four fused cyclic hexapeptides. In addition, such a design maintains the amphipathic character with an invariant face containing three hydrophobic cystine pairs and a variable face containing clusters of hydrophobic and charged amino acids.

During the course of our study, a cyclic peptide termed RTD (rhesus theta defensin) that bears similar β-tile structural elements was discovered by Tang et al. (14). RTD 7 (Fig. 1), an 18-amino acid cyclic peptide with three evenly spaced disulfide bonds, is structurally similar to the design of cCTP 2. This naturally occurring cyclic peptide is generated by posttranslational head-to-tail ligation of two truncated α-defensin-like gene products. However, the distributions of hydrophobic and charged clusters of cCTP 2 and RTD differ topologically. RTD has an alternating hydrophobic and charged motif whereas cCTP 2 has a central hydrophobic cluster and two charged clusters at the two four-residue reverse turns. In this report, we describe the design and syntheses of five β-tile analogs 2-6 with different topological patterns of charged and hydrophobic clusters on the variable face to determine their antimicrobial specificity and cytotoxicity. Their conformations and antimicrobial activity in 10 microorganisms were compared with tachyplesin-1 and the structurally similar RTD. A significant finding is that the conformational rigidity of these β-tile peptides confers a marked increase in membrane-lytic selectivity and strong dissociation of antimicrobial activity from cytotoxicity to human cells.

**FIG. 1.** Amino acid sequences of cyclic tachyplesin cTP 1, tricystine cCTP 2 and RTD 7. TP-1, X = NH_2; TP18, X = Gly-NH_2. The conserved residues are shown in bold face.

**MATERIALS AND METHODS**

Boc-(t-butoxycarbonyl) amino acids derivatives and N-hydroxybenzotriazole (HOBt) were obtained from Chem-impex International Inc. All solvents, including acetonitrile (CH_3CN), dichloromethane (DCM), dimethylformamide (DMF), dimethylsulfoxide (DMSO) and 1-methyl-2-pyrrolidinone (NMP) used without additional distillation were obtained from EM Science. N.N’-diisocyclohexylcarbodiimide (DCC), N.N’-diisopropylpropylamine (DIEA) and p-cresol were purchased from Aldrich Chemical Co. α-Chymotrypsin, trypsin and α-cyano-4-hydroxycinnamic acid were purchased from Sigma Chemical Co. Trifluoroacetic acid (TFA) was obtained from Halocarbon. Tris(carboxyethyl)-phosphine (TCEP) was from Calbiochem. Ultra pure urea was obtained from ICN Biomedics. Dialysis membranes were from Spectrum Medical.

Ten organisms obtained from the American Type Culture Collection (ATCC, Rockville, MD) were used for antimicrobial assays. Four Gram-negative bacteria included Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Klebsiella oxytoca ATCC 49131, and Proteus vulgaris ATCC 49132. The three Gram-positive bacteria were Staphylococcus aureus 29213, Micrococcus luteus ATCC 49732 and Enterococcus faecalis ATCC 29212. The three fungi were Candida albicans ATCC 37092, Candida kafyr ATCC 37095, and Candida tropicalis ATCC 37097. The strains were incubated in trypticase soy broth (TSB) which was prepared in double distilled water and autoclaved for sterilization. TSB was purchased from Becton-Dickinson (Cockeysville, MD).

Peptide syntheses and purification. Automated solid-phase peptide synthesis on an ABI 430A peptide synthesizer was performed using Boc-chemistry and a single coupling protocol with DCC/HOBt in DMF/NMP (1:1, v/v). Analytical reverse-phase high performance liquid chromatography (RP-HPLC) was conducted on a Shimadzu LC-6A system with an C18 Vydac column (4.6 x 250 mm). A linear gradient of 10–90% buffer B run for 30 min at 1 ml/min with detection at 225 nm. Eluent A: 0.04% TFA/H_2O; B: 0.04% TFA/60% CH_3CN. Preparative RP-HPLC was performed on a Waters 600 system with an C18 Vydac column (20 x 250 mm). Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) was measured on a PerSeptive Systems Voyager instrument. Samples were dissolved in 1 μl of a 1:2 mixture of H_2O-CH_3CN. Measurements were made in a linear model, with 2-cyano-4-hydroxycinnamic acid as the matrix.

Preparation of N-terminal cysteine thioester precursors. All precursors of cyclic peptide assembly by Boc-chemistry solid-phase synthesis (15, 16) on Boc-Val-SCH_2CH_2CO-MBHA resin (1 g, 0.26 mmol/g) (17, 18). The six cysteiny1 protecting groups of these linear precursors were designed with two different protecting group schemes: method A chemoselective protection-Cys 5 and 14 were protected with acetamidomethyl (Acm) and the remaining four with...
4-methylbenzyl (MeBzl) and method B uniform protection—all six cysteines were protected with MeBzl. The assembled peptides were cleaved from the resin (250 mg) by with hydrofluoric acid (HF) treatment (HF/p-cresol, 9:1, v/v, 12 mL) for 75 min at 0°C. After removal of HF by vacuum and washing with ether to remove the organic scavenger, the crude and deprotected peptides collected on a glass filter-funnel were extracted into an 8 M urea solution (pH 7.8, 100 ml) containing TCEP (100 mg). Alliquots of crude unprotected peptide thioesters were purified to confirm their identity before cyclization (e.g., the intermediate linear thioester form 2a (Fig. 2) showed MW cal. 2448.8, found M + H+ 2449.5; Rf = 20.9 min and 2b MW cal. 2306.7, found M + H+ 2307.5; Rf = 21.3 min).

End-to-end cyclization of N-terminal cysteiny thioester peptide. The 8 M urea solution (150 ml) was dialyzed (MW cut off 1000) by sequentially lowering the urea concentration (2000 ml) to 4 M, and then 2 M to permit concurrent cyclization which was monitored by analytical C18 reversed-phase HPLC and MALDI-MS (e.g., the tetra cysteine, cyclic intermediate 2c MW cal. 2343.8, found M + H+ 2344.6; Rf = 22.6 min; 2d MW cal. 2201.7, found M + H+ 2202.5; Rf = 22.1 min). In general, all cyclization reactions were complete during the 12–18 h dialysis sequence. The dialyzed solution was then diluted by water to 1 M urea (300 ml) for disulfide formation based during the 12–18 h dialysis sequence. The dialyzed solution was then added dropwise until a brown color persisted. The reaction, maintained in a nitrogen atmosphere and in a darkened vessel, was completed in 45 min, as monitored by HPLC. The solution was cooled in an ice-bath, and excess iodine was quenched by ascorbic acid. The peptide was purified on preparative HPLC in the same manner as described above and again characterized by MS (e.g., ccTP 2 MW cal. 2195.7, found M + H+ 2196.8; Rf = 23.2 min). From method B, all three disulfide bonds were formed in 10% DMSO/1 M urea solution for 24–40 h. MALDI–MS measurements were used to determine the identity of these purified cyclized peptides and gave the expected values (e.g. ccTP 2, MW cal. 2195.7, found M + H+ 2197.3; Rf = 23.2 min).

Disulfide connectivities of cyclic peptide intermediates prepared from method A with two disulfide bonds and two Cys(Acm) 2e were determined by α-chymotrypsin digestion. α-Chymotrypsin cleaves the C-terminal side of aromatic amino acid residues and based on the favorable placements of the four aromatic residues, two peptide segments C(Acm)RC(CF)RGKW, (MW cal. 1245.4, found M + H+ 1247.1) and C(Acm)VC(YCIGR)Y (MW cal. 1165.8, found M + H+ 1167.1) with the desired parallel cross-strand disulfide motif (Cys I–VI and Cys III–IV) were obtained.

Circular dichroism (CD) measurements. CD spectra were recorded on a Jasco J-720 spectropolarimeter over the wavelength range of 250–190 nm using a 1.0-mm path length cell, a bandwidth of 1.0 nm, a response time of 2 s, and averaging over three scans. The spectra are expressed as molar ellipticity [θ].

Antimicrobial and hemolytic assays. A sensitive and reproducible two-stage radial diffusion antimicrobial assay of Lehrer et al. (19) was employed. Antimicrobial activities were expressed in units (0.1 mm = 1 U), and the MICs were determined from the x intercepts of the dose-response curves. The hemolytic activity was determined using fresh human erythrocytes. Peptide concentrations causing 25% hemolysis (EC25) were derived from the dose-response curves (20). The membranolytic selectivity index is expressed as EC25/MIC.

RESULTS AND DISCUSSION

β-tile design and synthesis. Removing the Arg6,14 pairs of cationic charges by the Cys5,14 disulfide constraint in ccTP 2 resulted in a continuous cluster of eleven hydrophobic amino acids. Six of these are located on the variable face, with Trp3, Phe6, Val8 and Tyr13 on one β-strand and Ile11 and Tyr13 on the opposite strand together with five half-cystines on the invariable face. The four cationic charges are clustered near both ends, with a linear array of three cationic charges at one corner and the lone remaining Arg at the opposite corner (Table 1). This topology of a dense hydrophobic cluster may favor aggregation in aqueous solutions. Thus, subsequent analogs were designed to disperse cationic charges and to segregate hydrophobic clusters. [Arg13]ccTP 3 and [Arg18]ccTP 4 contained aromatic amino acid replacements, respectively, by one or two Arg residues. Analog 3 with Tyr13 → Arg replacement preserves the single continuous hydrophobic strand of seven amino acids, Trp3-Tyr8, and the single linear array of three cationic charges found in ccTP 2. Analog 4, with a dual replacement of Tyr8,13 → Arg breaks up this continuous stretch and shifts the cross-strand Arg18 pair to near the middle of the variable face. Analogs 5 and 6 were designed so that, topologically, the patterns of cationic charges and hydrophobic clusters are in an alternating slanted parallel pattern, with analog 6 forming a nearly perfectly symmetrical alternating cluster pattern. This alternating parallel pattern is also found in the naturally occurring cyclic peptide RTD 7. Thus, for comparative purposes, we grouped the β-tiles into two topological categories: (i) ccTP 2-like analogs 2–4, and (ii) RTD-like analogs 5–7.

Syntheses of β-tile peptides and RTD have been facilitated by a novel but simple method recently developed in our laboratory to form cyclic peptides in aqueous solutions (21, 22) and a detailed description is described under Materials and Methods. This method,
known as thia-zip cyclization is assisted by the multiple thiols of unprotected linear peptide thioesters form an end-to-end peptide bond without the use of any coupling reagents (23). The cyclization of the 18-residue macrocyclic peptide was found to be facile and completed in 24 h as monitored by RP-HPLC. Oxidative formation of the three cystine pairs was achieved essentially in a one-pot condition after the cyclization by the addition of 10% DMSO to the peptide solution and was complete in 24–48 h as monitored by HPLC and MS. This random disulfide oxidation to form the desired product was highly successful, presumably due to the preformed structures of the \( \beta \)-tiles. Selected analogs with random disulfide formations were also compared with a two-step disulfide bond scheme in which the formation of the disulfide pairs was based on chemoselectivity and was unambiguous (24–26). The results showed that both approaches gave the desired \( \beta \)-tile peptides with the correct parallel disulfide pairings. With either approach, the disulfide bond connectivity was further confirmed by trypsin/chymotrypsin digestions coupled with MALDI mass spectral analyses.

### Table 1

<table>
<thead>
<tr>
<th>ccTP analogs( ^a )</th>
<th>Compound number</th>
<th>Sequence( ^b )</th>
<th>Cationic charge</th>
<th>Topology</th>
</tr>
</thead>
<tbody>
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<td>2</td>
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</tr>
<tr>
<td>[Arg( ^{13} )]</td>
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<td><img src="image" alt="Sequence Diagram" /></td>
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<td>[Arg( ^{13} )]</td>
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<td><img src="image" alt="Sequence Diagram" /></td>
<td>Slanted parallel</td>
<td></td>
</tr>
<tr>
<td>[Arg( ^{13,11} )]</td>
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<tr>
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<td>7</td>
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<td>Slanted parallel</td>
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</tbody>
</table>

\( ^a \) Sequence mutations by either Arg or Lys for hydrophobic amino acids are based on the parent compound ccTP 2.

\( ^b \) ○, Lys; ●, Arg; ..., disulfide bond.

Fig. 3A. All three compounds exhibited non-classical CD spectra of \( \beta \)-strand structures. Overall, they showed two positive bands near 200 and 230 nm and a weak negative band near 208 nm. However, there are substantial differences in their magnitudes as measured by ellipticity and band shifts in their minima and maxima. The two bands near 200 and 207 nm have been associated with \( \beta \)-sheet peptides connected by a reverse turn (27) and are consistent with the reported CD spectra of TP-1 (11, 28). The CD spectra of membrane-associated \( \beta \)-proteins such as porins also have a strongly positive band (\( \pi-\pi^* \) transition) around 195 nm and a weaker negative band (\( n-\pi^* \) transition) around 220 nm (27). However, the strong positive band near 230 nm is usually not observed in these \( \beta \)-proteins but has been observed in cystine-knot \( \beta \)-strand peptides (24). Other cyclic tachyplesins 3 to 6 (Fig. 3B and 3C) retained the negative band near 208 nm and the positive band near 230 nm, but showed variations in ellipticity below 200 nm. Although there is insufficient information to deconvolute these CD spectra of these two-\( \beta \)-strand cyclic cystine-knot structures due to the various contributions by \( \beta \)-turns, aromatic residues, disulfides and \( \beta \)-sheet structures, it is reasonable to
conclude that all cyclic tachyplesins 2-6 as well as RTD display ordered structures in methanol. Furthermore, because the CD profiles of the cyclic tachyplesins in this study and RTD of Tang et al. (14) are very similar even though their amino acid sequences differ the rigidity of the cyclic two-strand cystine-knot templates will likely retain a similar ordered structure.

We also determined the cyclic tachyplesin structures in 50% TFE-phosphate buffer at pH 7.2 and found no substantial differences in their CD spectra in these two media (data not shown). However, in the absence of TFE, most cyclic tachyplesins, particularly ccTP 2, showed flattened differential absorption spectra (Fig. 3D). For example, near 208 nm the molar ellipticity of

![CD spectra of tachyplesins and its tricystine analogs in methanol. (A) Tachyplesin (--), ccTP (···), RTD (--). (B) 5 (--), RTD (···), 6 (--). (C) 4 (--), ccTP (···), 3 (--). (D) In water: tachyplesin (--), ccTP (···), 5 (--). The concentration of each peptide was 100 µM.](image-url)
Comparison of Antimicrobial Activity of TP-1, RTD and Cyclic Tachyplesins 2 to 6

<table>
<thead>
<tr>
<th>Organism</th>
<th>TP-1</th>
<th>ccTP 2</th>
<th>ccTP 3</th>
<th>ccTP 4</th>
<th>ccTP 5</th>
<th>ccTP 6</th>
<th>RTD 7</th>
</tr>
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<tbody>
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<td>Gram-negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.4</td>
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<td>6.9</td>
<td>10.4</td>
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<td>9.8</td>
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<tr>
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<td>1.0</td>
<td>6.0</td>
<td>14.4</td>
<td>16.4</td>
<td>28.9</td>
<td>26.7</td>
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<td>0.5</td>
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<td>7.8</td>
<td>4.0</td>
<td>7.6</td>
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<tr>
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</tr>
<tr>
<td>S. aureus</td>
<td>0.4</td>
<td>0.5</td>
<td>1.0</td>
<td>0.8</td>
<td>1.3</td>
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<tr>
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<td>1.9</td>
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<tr>
<td>Fungi</td>
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<tr>
<td>C. albicans</td>
<td>0.7</td>
<td>0.9</td>
<td>5.1</td>
<td>17.2</td>
<td>24.2</td>
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<tr>
<td>C. kefyr</td>
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<td>1.3</td>
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<td>1.1</td>
<td>4.3</td>
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<td>1.0</td>
<td>5.2</td>
<td>11.0</td>
<td>12.4</td>
<td>3.9</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*M* Experiments were performed in radial diffusion assay with underlay gel containing 1% agarose, 10 mM phosphate buffer with (high-salt) or without (low-salt) 100 mM NaCl. Activities against multiple strains are expressed as the minimum inhibitory concentration (MIC, *µ*M).

ccTP 2 and analog 5 decreased from −17,000 and 60,000 in methanol to −4,000 and 14,000 deg cm² dmol⁻¹, respectively. A similar decrease in ellipticity was also observed at about 230 nm. The extent of the flattening has been attributed to light scattering particle formation in the solution and is a function of particles size and a combination of different chromophores (29, 30). However there were no significant changes in the CD spectra of TP-1 in methanol and water and this tachyplesin is known to behave as a soluble monomeric peptide without any tendency to aggregate in aqueous solutions. The difference of β-tile CD spectra observed in water and in methanol suggests the possibility of strong aggregation of ccTP 2 and, to a lesser extent, analogs 3–6 in low ionic aqueous environments.

Structure-function study. The minimum inhibitory concentrations (MICs) of six β-tile peptides and RTD against four Gram-negative bacteria, three Gram-positive bacteria and three fungi were determined by the two-stage radial diffusion assay (19) in both low- and high-salt (with 100 mM NaCl) conditions (Table 2). In general, most of the activity of β-form antimicrobial peptides such as defensins or defensin-like peptides is abrogated when tested in high-salt conditions with the exceptions of RTD, protegrins, and tachyplesins. However, to be clinically relevant, antimicrobial activity must be retained under physiological conditions of 120–150 mM NaCl which is simulated by the high-salt assay.

TP-1 and its corresponding linear 18-residue TP18 analog showed MICs of 0.2 to 1.3 *µ*M and were generally more active against the test organisms in low-salt than in high-salt assays. Comparing with TP-1, all six β-tile tachyplesins and RTD retained comparable activity, particularly under high-salt conditions, against the three Gram-positive bacteria, S. aureus, M. luteus and E. faecalis, but were less active against two of the four Gram-positive bacteria, K. oxytoca and, in particular, P. vulgaris, as well as one of the test fungi, C. albicans. The RTD-like group consisting of RTD 7 and β-tile analogs 5 and 6 displayed relatively similar activity spectrum activity against E. coli, S. aureinosa, C. kefyr and C. tropicalis. However, their activities in low- and high-salt conditions were markedly different. RTD was generally more active in low- than in high-salt conditions, whereas the activity of β-tile analogs 5 and 6 was reversed. The most dramatic difference of this activity reversal was observed against E. coli in which the MIC of RTD was 2.0 *µ*M in the low-salt assay and 28.4 *µ*M, a 14-fold decrease, in the high-salt assay. In contrast, the MICs of β-tile analogs 5 and 6 were 5.5 and 6.1 *µ*M under low-salt conditions but improved about five fold to 1.2 and 1.2 *µ*M, respectively, under high-salt conditions. This unusual behavior in the high-salt condition against E. coli was also observed in the ccTP 2-like group consisting of β-tile analogs 2–4. In general, the antimicrobial profiles of ccTP 2 and RTD 7 were similar despite their differences in topological arrangement of cationic charges.

Hemolytic activity. All β-tile-like peptides including RTD were highly nonhemolytic to human erythrocytes and the conventional EC₅₀ could not be determined because it would require unrealistic high concentra-
tions of peptides. However, their effective concentrations (EC_{25}) causing 25% red blood cell hemolysis were found to range from 590 μM in analog 3 to 3,900 μM in analog 6 compared to 29 μM for TP-1 (Table 3). Based on their EC_{25}, we have shown that it is possible to dissociate antimicrobial activity from hemolytic activity using the β-tile design of TP-1 to create analogs with high selectivity index (EC_{25}/MIC) as high as 13,000. For example analogs 2 to 6 showed selective indexes from 311 to 13,000 ranging as compared to 29 to 97 in TP-1, against the three test Gram-positive organisms (Table 2).

**CONCLUSION**

Based on the two β-strand tachyplesins, we have successfully developed a highly constrained symmetric, closed-chain structure with three evenly spaced disulfide bonds to approximate a β-tile template. CD measurements in methanol provide support for the rigidity of this design. A unique aspect of such a design is its amphiphilic character consisting of a sulfur-rich face and an opposite face with charged and hydrophobic clusters which can be used for analog design. Five β-tile analogs showed interesting broad activity profiles that are distinctly different from tachyplesin. More importantly, they are not only salt-insensitive, but also exhibit increased activity in high-salt conditions. This unusual property coupled with their lack of toxicity to human erythrocytes may provide a useful and novel scaffolding for developing peptide antibiotics with a high therapeutic index and to study their mechanism of actions for membranolytic selectivity toward microbial and human lipid membranes.

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