Received: 10 January 2018 | Revised: 6 April 2018 | Accepted: 10 April 2018

DOI: 10.1002/psc.3087

RESEARCH ARTICLE

WILEY Journal of PeptideScience

Influences of disulfide connectivity on structure and antimicrobial activity of tachyplesin I

Juan Shi[1,3] | Lok-Yan So[2] | Fangling Chen[1,3] | Jiazhen Liang[1,3] | Ho-Yin Chow[2] | Kwok-Yin Wong[2] | Shengbiao Wan[1,3] | Tao Jiang[1,3] | Rilei Yu[1,3]

[1] Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China

[2] Department of Applied Biology and Chemical Technology and the State Key Laboratory of Chirosciences, The Hong Kong Polytechnic University, Hong Kong

[3] Laboratory for Marine Drugs and Bioproducts of Qingdao National Laboratory for Marine Science and Technology, Qingdao 266003, China

Correspondence
Rilei Yu, Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China.
Email: ryu@ouc.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 81502977; Central Universities, Grant/Award Numbers: 201762011 and 201512007; Qingdao National Laboratory for Marine Science and Technology, Grant/Award Number: 2015ASKJ02

Tachyplesin I is a potent antimicrobial peptide with broad spectrum of antimicrobial activity. It has 2 disulfide bonds and can form 3 disulfide bond isomers. In this study, the structure and antimicrobial activity of 3 tachyplesin I isomers (tachyplesin I, 3C12C, 3C7C) were investigated using molecular dynamic simulations, circular dichroism structural study, as well as antimicrobial activity and hemolysis assay. Our results suggest that in comparison to the native peptide, the 2 isomers (3C12C, 3C7C) have substantial structural and activity variations. The native peptide is in the ribbon conformation, while 3C12C and 3C7C possess remarkably different secondary structures, which are referred as "globular" and "beads" isomers, respectively. The substantially decreased hemolysis effects for these 2 isomers is accompanied by significantly decreased anti-gram-positive bacterial activity.

KEYWORDS

antimicrobial activity, antimicrobial peptide, disulfide connectivity, secondary structure, tachyplesin I

1 | INTRODUCTION

Tachyplesin antimicrobial peptides belong to a family of polypeptides present in the limulus lymphocyte granulosa cells of the horseshoe crabs.[1] Tachyplesin peptides consist of tachyplesin I, tachyplesin II, tachyplesin III, as well as polyphemusin I and polyphemusin II. The size of the tachyplesin peptide is relatively small, containing 17 to 18 amino acids and a C-terminal amide group. All tachyplesin antibacterial peptides are amphiphilic, consisting of 3 tetrameric tetrapeptide repeats and 2 disulfide bonds that constrain the peptide into a β-hairpin structure (Figure 1). Tachyplesin I was first isolated from the oriental horseshoe blood cell acid extracts in 1988.[2] It has a broad spectrum of antimicrobial activity against gram-positive bacteria, gram-negative bacteria, and fungi, with MIC of 0.2 to 0.9, 0.3 to 1, and 0.5 to 0.9 μM, respectively.[3]

Of interest of this study is tachyplesin I. Native tachyplesin I has a disulfide connectivity of Cys^{I}—Cys^{IV} and Cys^{II}—Cys^{III} (Figure 1B). Previously performed structure elucidation by 1H NMR spectroscopy shows the adoption of an antiparallel β-hairpin structure in water or in dodecophosphatecholine micelles.[4,5] The determined NMR structures of tachypelsin I show that its secondary structure in water (PDB ID: 1WO0) and in dodecylphosphocholine micelles is similar to each other (PDB ID: 1WO1).[6]

Disulfide bonds are clearly key contributors to stabilizing the secondary structures. Disulfide bond engineering of the tachyplesin I (TPI I) is an interest of previous studies. Laederach et al[7] determined NMR structures of tachypelsin I and TPA4 (all Cys residues substituted by Ala) in aqueous solution. The results showed that TPA4 is in a conformation of random coil, and its antimicrobial activity was considerably weakened in comparison to the wild-type peptide. The MIC of

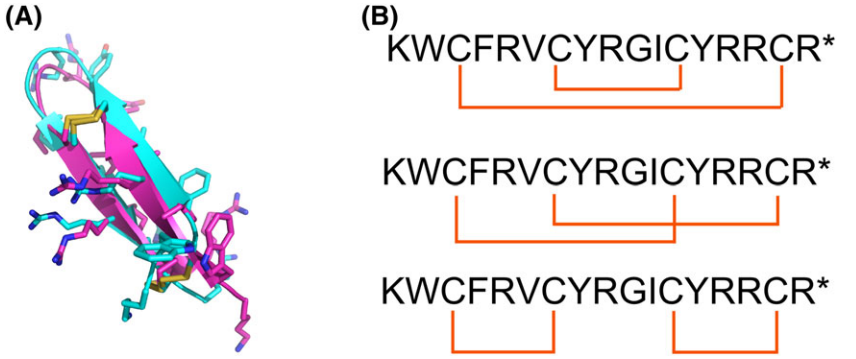

FIGURE 1 Schematic representation of tachyplesin I, 3C7C, and 3C12C. A, The secondary structure of tachyplesin I in water (cyan) and in dodecylphosphocholine micelles (magenta). B, The sequence of tachyplesin I (ribbon), 3C12C (globular), and 3C7C (beads), respectively. The "*" represents the amide group of the C-termini

TPA4 against *Escherichia coli* was increased to 3 μmol/L and that of antipathogenic fungi was more than 35 μmol/L. This result suggests that there might be a link between the antimicrobial activity and the disulfide bond connecting format.

However, the disulfide bonds that play an essential role in sustaining the β-hairpin secondary structure are not prerequisite for the activity of TPI I. A TPI I variant with Cys residues at 3,16 replaced by Ala possessed substantially improved therapeutic indexes, although its β-hairpin structure was significantly perturbed.[8] Moreover, the CDT analog which was produced by tachyplesin I that its 4 Cys residues were deleted showed a random coil conformation in solution but remained a β-hairpin-like structure with the presence of lipids and possessed comparable antimicrobial activity to the wild-type peptide, The CDT analog showed no hemolysis activity up to 200 μg/mL, indicating that removal of the 4 Cys residues could significantly decrease the hemolytic effects but maintain the antimicrobial activity.[9,10] Therefore, the disulfide bond is not the prerequisite determinant for the activity of TPI I. Replacement of the disulfide bonds using other types of covalent bonds was also performed for the purposes of improving the stability of the peptide. Holland-Nell et al[11] used triazoles for replacement of the disulfide bonds in TPI I, and in another study, the biscarba diaminodiacids were used as the disulfide bond surrogate of TPI I.[12] The produced analogs maintained comparable or even had better antimicrobial activity than the native peptide.

TPI I has 2 disulfide bonds and potentially can form 3 disulfide isomers. Then, a question arises on the influences of the varied disulfide connectivity to the structure and function of this peptide. Different disulfide bond isomers of the bioactive peptides usually have varied structures and activities. The α-conotoxin AuIB isomers have substantial difference in their secondary structures and bioactivity, especially in their way of inhibiting nicotinic acetylcholine receptors (nAChRs).[13] Structure and activity relationship for the disulfide isomers of αO-conotoxin GeXIVA,[14] μ-conotoxins KIIIA, μ-conotoxin KIIIB,[15] μ-conotoxin PIIIA,[16] and α-conotoxin GI[17,18] has been investigated, and the results suggested that in some cases, certain disulfide isomers could retain the bioactivity of the native peptide despite of their significantly varied secondary structures.

In this study, 2 disulfide bond isomers, 3C7C (Figure 1D) and 3C12C (Figure 1C) of TPI I, were synthesized, including TPI I. And their structure and antimicrobial activity were investigated for exploring their structure-activity relationship.

2 | METHOD AND MATERIAL

2.1 | Molecular dynamic simulations

The initial models of 3C7C and 3C12C were created using the NMR structure of TPI I (PDB ID: 1WO0) as the template in Modeller (version 9v12).[19,20] MD simulations were implemented on TPI I, 3C7C, and 3C12C, respectively, using the Amber 16 package as previously described.[21] The FF14SB force field was applied for the simulation.[22] The peptide was solvated into an octagon box of TIP3P water molecules and neutralized using Cl^- ahead of the MD simulations. Subsequently, the unfavorable van der Waals interactions were eliminated via 2 steps of minimization, including 3000 steps of steepest descent minimization and 3000 steps of conjugate gradient minimization. During the energy minimization process, the cutoff of the nonbonded interactions was limited to 12 Å. After minimization, MD was performed. In MD, the solute was restrained and the temperature of the whole system was gradually increased from 10 to 300 K in 100 picoseconds in the NVT ensemble. Afterwards, the system was equilibrated in the NPT ensemble where the temperature and pressure were kept at 300 K and 1 atm, respectively. Eventually, in the production process, the whole system was relaxed and a 100-nanosecond molecular dynamic process was carried out. For all MD steps, the time step was set to 0.002 picosecond, the particle mesh Ewald (PME)[23] method was applied to deal with long-range electrostatic interactions, and the lengths of the bonds involving hydrogen atoms were fixed with the SHAKE algorithm. MD trajectories were analyzed using VMD,[24] and molecular structures were shown using PyMol (Schrödinger, LLC).

2.2 | Peptide synthesis

All peptides (TPI I, 3C7C, and 3C12C) were synthesized by FMOC-SPPS as described previously.[25] The first disulfide bond was oxidized in 0.1 M NH_4HCO_3 (pH 8~8.5) at a peptide concentration of 0.3 mg/mL with stirring for 24 hours at room temperature. After the oxidation, the products were purified by reversed-phase HPLC (RP-HPLC) on a Phenomenex C_{18} column (250 × 10 mm, 10 μm) using a gradient of 0% to 75% solvent B (see composition below) and a flow rate of 6 mL/minute in 75 minutes, with the UV wavelength monitored at 214 nm. The oxidized peptide mass was monitored by electrospray mass spectroscopy (Figure S1). Afterwards, the peptide was isolated for lyophilization.

Formation of the second disulfide bond was performed by dissolving 10 mg peptide in 10 mL (c = 0.415 mM) solvent A/B (solvent A: H_2O/0.05% trifluoroacetic acid; solvent B: 90% CH_3CN/10% H_2O/0.045% trifluoroacetic acid), dropwise adding 0.2 mL I_2 solution (5 mg I_2 dissolved in 1 mL CH_3CN) with stirring for 30 minutes, then quenching with ascorbic acid (5 mg ascorbic acid dissolved in 1 mL H_2O). After the oxidation, the products were purified by RP-HPLC on a Phenomenex C_{18} column using the same gradient setup as described in the first disulfide oxidation. After confirming the product peak using mass spectrometry, the solid was obtained by lyophilization. Analytical RP-HPLC with a gradient of 0% to 80% buffer B and a flow rate of 1 mL/minute in 40 minutes and electrospray mass spectroscopy were used to confirm the purity and molecular mass of the synthesized peptides (Figure S2 and Table S1).

2.3 | Circular dichroism study

Circular dichroism (CD) spectra were performed on a Jasco J-810 spectropolarimeter over the wavelength range of 250 to 190 nm using a 1.0-mm path length cell, a bandwidth of 1.0 nm, a response time of 2 seconds, and averaging over 3 scans. Spectra were recorded at room temperature under nitrogen atmosphere. TPI I, 3C7C, and 3C12C were dissolved in 2 mL methanol to get a concentration of 0.24 mM. The spectra are expressed as molar ellipticity [θ]: [θ] = 1000 × mdeg / (l × c). The mdeg is the raw CD data, c is the peptide molar concentration (mM), and l is cell path length (mm).

TABLE 1 The disulfide bonds of TPI, 3C7C, and 3C12C

Name	First Disulfide Bond	Second Disulfide Bond
TPI I	Cys^{II}—Cys^{III}	Cys^{I}—Cys^{IV}
3C12C	Cys^{I}—Cys^{III}	Cys^{II}—Cys^{IV}
3C7C	Cys^{I}—Cys^{II}	Cys^{III}—Cys^{IV}

2.4 | Antimicrobial activity

Minimum inhibitory concentration (MIC) is the lowest concentration of a chemical that inhibits visible growth of a bacterium. Minimum inhibitory concentration of the peptides (TPI I, 3C7C, and 3C12C) against gram-negative *E. coli* K-12 and gram-positive *Staphylococcus aureus* ATCC 29213 was determined using the described method.[26] Briefly, the experiment was performed in the 96-well plates with serial twofold dilution of peptides across the columns. Each dilution was performed in duplicate. Each well contains 80 μL of medium, 10 μL of peptide, and 10 μL of bacterial culture (to give a final CFU/mL of ca. 5 × 10^6). Controls with bacterium and medium only were included to ensure the viability of bacterium and the sterility of medium. The plate was then incubated at 37°C for 18 hours before recording the results.

2.5 | Hemolysis test

Human erythrocytes and hemolysis kit were purchased from HaemoScan (The Netherlands). Aliquots of human blood sample were

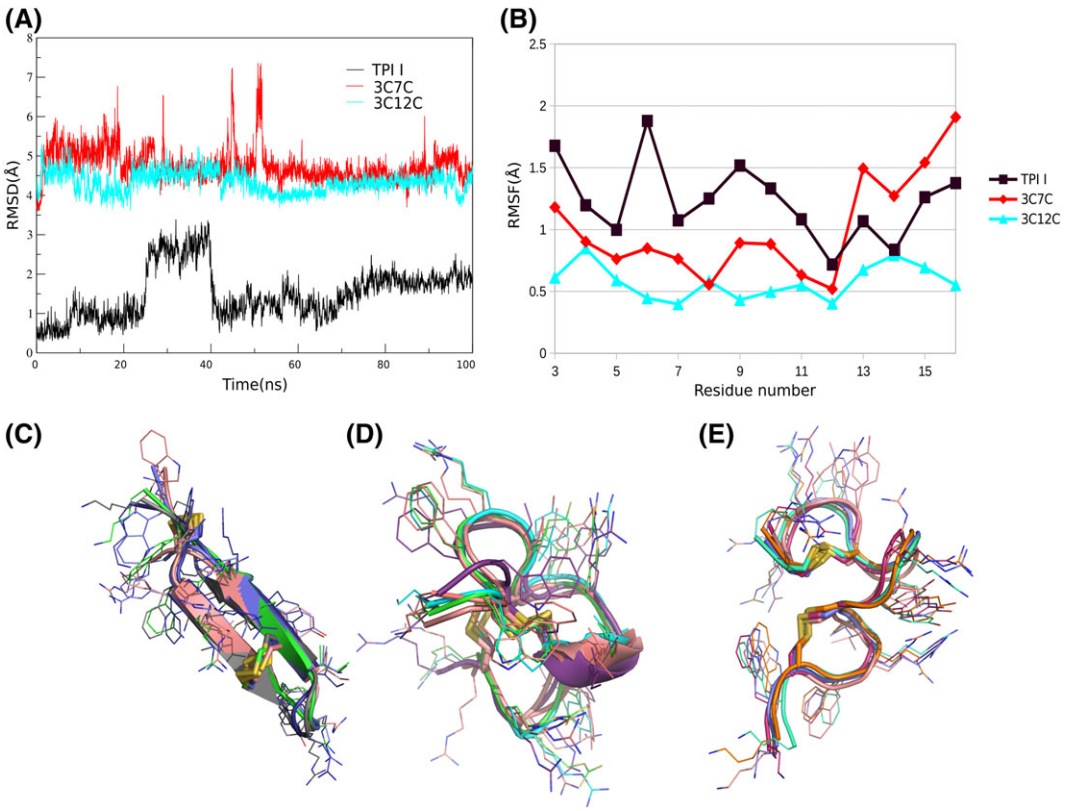

FIGURE 2 Molecular dynamic simulations of TPI I and its 2 isomers. A, Evolution of the root mean square deviation (RMSD) for the backbone of the TPI I (black), 3C7C (red), and 3C12C (cyan). B, Root mean square fluctuation (RMSF) of TPI I (black), 3C7C (red), and 3C12C (cyan). C, D, E, 5 Conformational frames of TPI I, 3C12C, and 3C7C extracted from the largest cluster of frames obtained from the last 50-nanosecond molecular dynamic simulations. Over 100-nanosecond molecular dynamic simulations were performed on the 3C7C, 3C12C, and TPI I to explore their conformation changes in MD. The RMSD was calculated using the Cα backbone between C3 and C16 of the peptide sequence

washed twice with 5 mL washing buffer and centrifuged at 1200×*g* for 10 minutes at 4°C. This procedure was repeated twice with 5 mL dilution buffer, and the last sediment was resuspended in 5 mL dilution buffer to a final concentration of 5% erythrocyte. One hundred microliter tested peptides were added to 100 μL diluted erythrocyte suspension. Hemolysis of peptides was tested at 7 concentrations (8, 16, 32, 64, 128, 256, and 512 μg/mL). One hundred microliter MilliQ water was added to 100 μL erythrocyte suspension as a negative control (0% hemolysis), while 100 μL 2% Triton X-100 was added to 100 μL erythrocyte suspension as a positive control (100% hemolysis). The assay mixtures were then incubated at 37°C for 1 hour under slow rotation (100 rpm). After incubation, the samples were centrifuged at 5000 rpm for 1 minute, and hemolysis was quantified by measuring the absorbance of supernatant by spectrometry at 415 nm, and 450 nm as a reference wavelength.

3 | RESULTS AND DISCUSSION

3.1 | Peptide synthesis

For TPI I, the protecting group of Cys^{II} and Cys^{III} is rityl (trt); the other protecting group of Cys^{I} and Cys^{IV} is acetamidomethyl (Acm). The trt protection group of Cys^{II} and Cys^{III} can be deprotected in NH_4HCO_3, whereas the Acm protection group can only be removed in I_2 solvent. Thus, in the first round oxidation in NH_4HCO_3, only the disulfide bond Cys^{II}—Cys^{III} was formed, whereas the second disulfide bond Cys^{I}—Cys^{IV} is formed in oxidation using I_2 solvent. In a similar way, the 2 disulfide bonds of 3C7C and 3C12C were selectively oxidized and formed in 2 steps of oxidation. The disulfide connectivity of TPI 1, 3C12C, and 3C7C is shown in Table 1.

3.2 | Molecular dynamic simulation

Molecular dynamic simulations were performed to investigate the conformational evolution and structural stability of the TPI I and its 2 disulfide isomers. As shown in Figure 2, the RMSD values for TPI I are around 2 Å in most of the simulation time. By contrast, the RMSD values for both 3C7C and 3C12C are more than 4 Å suggesting that their conformation significantly deviates the initial conformation of the TPI I. Hence, the conformation of the peptide is determined by the disulfide connectivity format. For better quantification of the peptide flexibility of TPI I, 3C7C, and 3C12C, the root mean fluctuation of their backbone was calculated. As shown in Figure 2B, the root mean square fluctuation values for 3C12C are much smaller than that of the TPI I and 3C7C indicating that the backbone of 3C12C is more rigid than that of TPI I and 3C7C. The globular disulfide connectivity imposes more constrains to the backbone of the peptide than the ribbon and beads disulfide connectivity, which is responsible for the higher rigidity of the 3C12C than 3C7C and TPI I. Interestingly, the structure of the 3C7C is similar to 3C12C, and both peptides contain the random coil and helix fragments despite of their different disulfide connectivity. By contrast, the secondary structure of TPI I is in a β-sheet conformation. In 3C7C, the sequence between the 2 disulfide constrained fragments is flexible, which might easily allow it to refold into conformation similar to 3C12C.

3.3 | Secondary structure

Alternative disulfide connectivity could lead to substantially different CD spectra for the 3 TPI I disulfide isomers. The CD spectra of TPI I and its 2 disulfide isomers, 3C7C and 3C12C, are shown in Figure 3. In previous studies, the TPI I exhibited a strong negative band near 210 nm, and a strong positive band near 198 nm; these bands are because of a β-sheet structure.[27] In line with previous studies, both the negative and positive bands were also observed in our CD spectra for the TPI I. By contrast, the strong positive band near 198 nm is absent at the CD spectra of both 3C7C and 3C12C indicating a substantial conformation deviation from the native peptide. Such a result is consistent with the above MD simulations. Indeed, the NMR structure of the TPI I consists of an antiparallel β-strand connected by a β-turn and stabilized by 2 disulfide bonds. However, models of both 3C7C and 3C12C could not sustain the antiparallel β-strand structure in 100-nanosecond MD simulations (Figure 2). For 3C12C, its absorption peak is similar to 3C7C in which a negative band between 193 and 210 nm is presented (Figure 3) suggesting that both of them are formed by random coil.

3.4 | Retention time in HPLC

The CD spectra study clearly show that varied disulfide connectivity leads to substantially different structures and hence to the presentation of different molecular surfaces as well as different retention times in HPLC. The retention time for 3C7C (Figure 4A), 3C12C (Figure 4B), and TPI I (Figure 4C) is 11.227, 11.013, and 33.747 minutes,

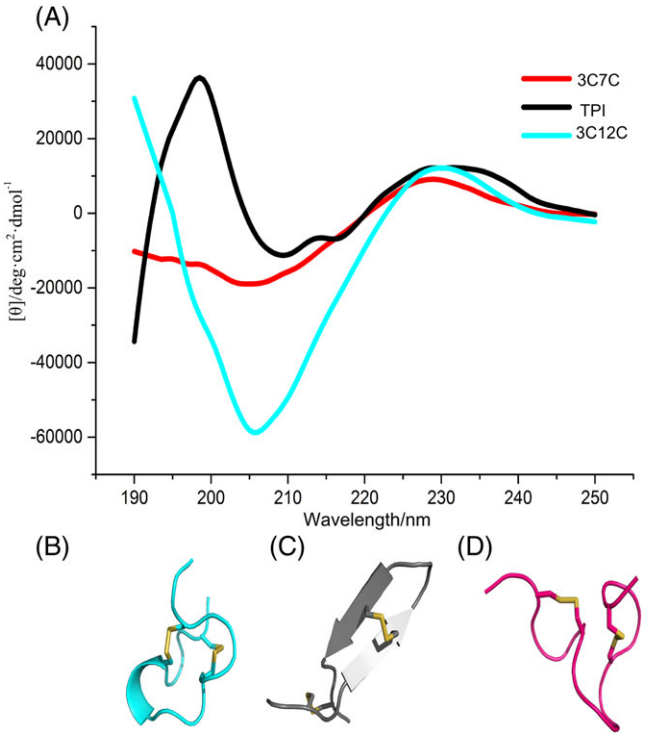

FIGURE 3 Circular dichroism spectra and structural skeletons of the TPI I and its 2 disulfide isomers 3C7C and 3C12C. A, Circular dichroism spectra of TPI I (black), 3C7C (red), 3C12C (cyan). B, Structural skeleton of 3C12C. C, Structural skeleton of TPI I. D, Structural skeleton of 3C7C

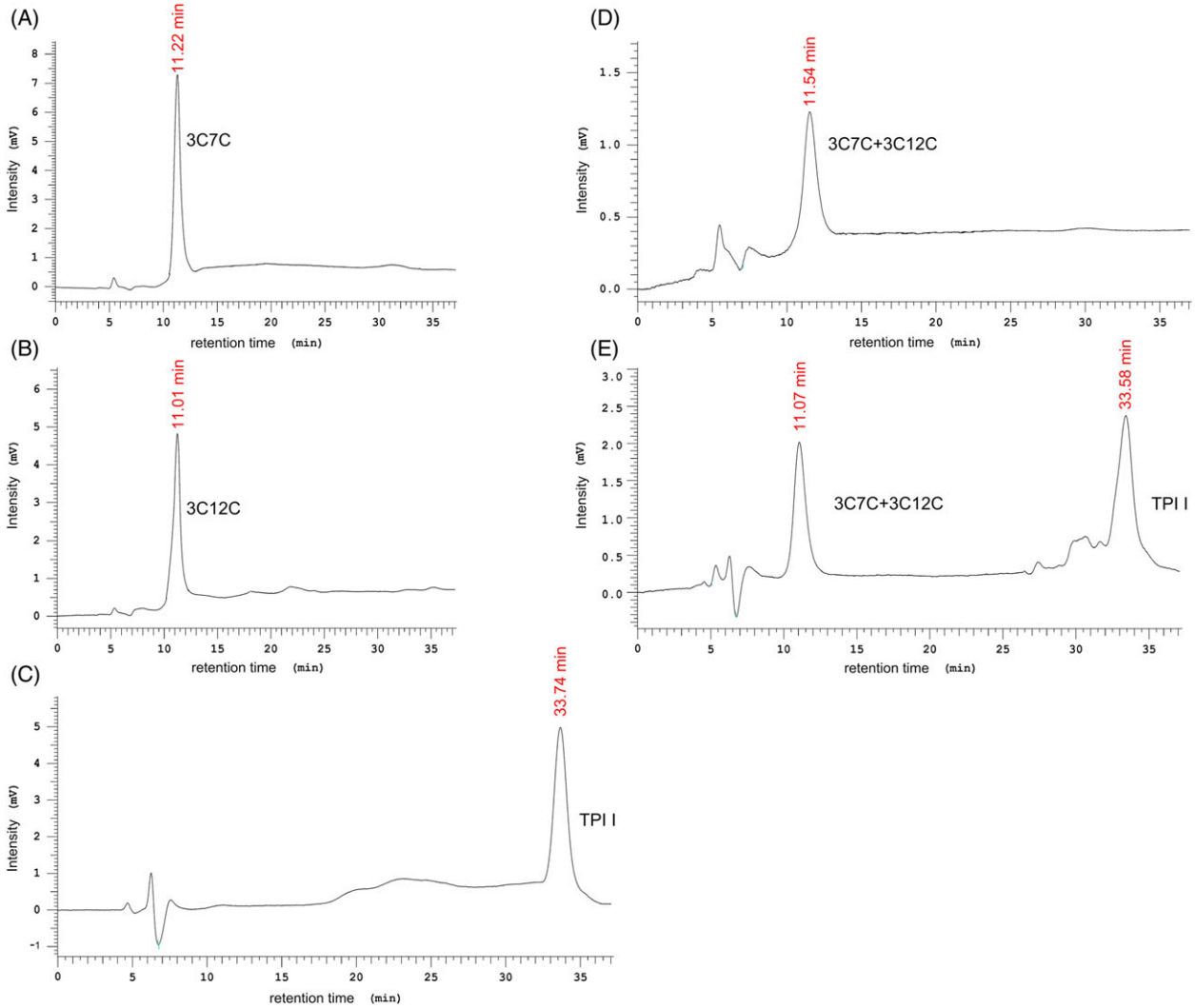

FIGURE 4 The retention time of TPI and its 2 disulfide isomers. (A), (B), (C), (D), and (E) show the analytical RP-HPLC profile of 3C7C, 3C12C, TPI I, the mixture of 3C7C and 3C12C, and the mixture of 3C7C, 3C12C, and TPI I, respectively

respectively. The comparable retention time between 3C7C and 3C12C suggests that both peptides apparently have very similar Connolly surfaces (Figure 4D). Indeed, the main conformation of the 3C7C is very similar to that of 3C12C, and hence, they are expected to have similar Connolly surfaces. The α-conotoxin TxIA also has 3 isomers, and likewise, the RP-HPLC results show that the retention time of the 2 isomers (beads and globular) is essentially the same.[28] Therefore, it is not surprising to observe comparable retention time in the analytical RP-HPLC between 3C7C (beads format) and 3C12C (globular format). By contrast, the native TPI I is in an antiparallel β-strand conformation and has much longer retention time (Figure 4E). These results indicate that 3C7C and 3C12C isomers might have similar hydrophobicity, and both are less hydrophobic than native TPI I.

3.5 | Antimicrobial activity and hemolytic activity

Disulfide connectivity significantly affects the antimicrobial activity as well as the hemolysis activity of the 3 peptide isomers. The MICs of TPI I, 3C7C, and 3C12C against the gram-negative and gram-positive bacteria are given in Table 2. All peptides exhibited activities against both gram positive and negative bacteria. TPI I possesses the same antimicrobial activity against *E. coli* K-12 and *S. aureus* ATCC 29213 with a MIC of 8 μg/mL consistent with previously reported values.[3] The 3C7C and 3C12C share the same antimicrobial activity against the gram-negative bacteria *E. coli* K-12, with a MIC of 16 μg/mL approximately 2 times of the native peptide. On the other hand, the antimicrobial activity of 3C7C and 3C12C against the gram-positive bacteria is significantly decreased with the former decreased by 8 times and the latter decreased by 4 times comparing to the native peptide. The 3C7C and 3C12C become more specific to the gram-negative bacteria than to the gram-positive bacteria, which might result from the different lipid composition and distribution of gram positive and negative bacteria. The significant decreasing of the antimicrobial activity further highlights an essential role played by the antiparallel hairpin structure in the activity of the antimicrobial

TABLE 2 The MIC of TPI I, 3C12C, and 3C7C

Name	*E. coli* K-12	*S. aureus* ATCC 29213
TPI I	8 μg/mL	8 μg/mL
3C12C	16 μg/mL	64 μg/mL
3C7C	16 μg/mL	32 μg/mL

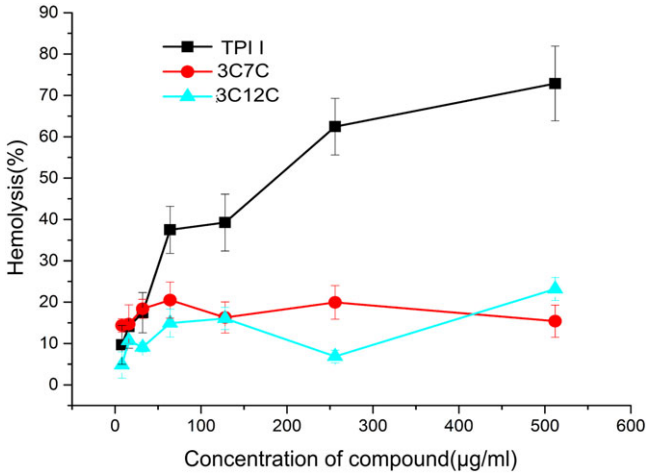

FIGURE 5 Hemolytic activity of TPI I, 3C7C, and 3C12C. Variation of the hemolysis activity of TPI I, 3C7C, and 3C12C with their increased concentrations

peptides in line with the results given by Sivanesam et al.[29] The reduced antimicrobial activity for 3C7C and 3C12C isomers can be attributed to their substantial conformation deviation from the native peptide. Besides, the disruption of the hydrophobic-hydrophilic balance of the native isomer surface can be another reason for the decreased antimicrobial activity of the 3C7C and 3C12C isomers. Similar to TPA,[30] its mean hydrophobicity value is lower than TPI I and its antimicrobial activity was declined. In analytical RP-HPLC analysis, the retention time for the TPI I is about 3 times of 3C7C and 3C12C isomers suggesting that the TPI I is more hydrophobic than its 2 disulfide isomers.

Comparing to the native peptide TPI I, the hemolysis activities of 3C7C and 3C12C are significantly decreased (Figure 5). At peptide concentration from 8 to 512 μg/mL, the hemolysis of the native isomer is increased substantially, from 9.6% to 72.8%, whereas the hemolysis of 3C12C and 3C7C is only slightly increased, from 14.3% (8 μg/mL) to 20.5% (64 μg/mL) for 3C7C, and from 4.8% (8 μg/mL) to 14.9% (64 μg/mL) for 3C12C. In previous study, the peptide CDT was found to possess substantially decreased hemolysis activity, because of the lack of hydrophobicity and amphipathicity in comparison to the wild-type peptide.[10] The substantial conformation change together with hydrophobicity and hydrophilic variations of the peptide surfaces can together contribute to the significantly reduced the hemolysis of the 3C12C and 3C7C isomers.

4 | CONCLUSION

Disulfide connectivity significantly impacts the secondary structure, antimicrobial bioactivity, and hemolysis of the TPI I disulfide isomers. The manner of disulfide bond connectivity determines the secondary structure of the disulfide isomers, which could in turn affect their Connolly surfaces and the hydrophobicity. It is likely that changing the Connolly surfaces of the peptide can impact their interactions with the bacteria membranes, their antimicrobial activities, and the hemolysis effects. However, more effects remain to be performed for further studying the interaction mechanism between varied TPI I disulfide isomers and the bacteria membranes at molecular level to obtain more explicit explanations in future studies.

Intriguingly, both 3C7C and 3C12C possess comparable antimicrobial activity against the gram-negative bacteria, but has significantly decreased hemolysis activity than the native isomer. These 2 isomers could potentially be used as novel peptide scaffold for design of novel antimicrobial peptides against the gram-negative bacteria with low hemolysis effects in future.

ACKNOWLEDGEMENTS

This work was supported by the Fundamental Research Funds for the Central Universities (Nos. 201762011 and 201512007 for R.Y.), the grant from National Natural Science Foundation of China (NSFC) (No. 81502977 for R.Y.), the Scientific and Technological Innovation Project Financially Supported by Qingdao National Laboratory for Marine Science and Technology (No. 2015ASKJ02).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest with the content of this article.

ORCID

Rilei Yu http://orcid.org/0000-0002-9611-3514

REFERENCES

1. Nakamura T, Furunaka H, Miyata T, et al. Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (Tachypleus tridentatus). Isolation and chemical structure. *J Biol Chem*. 1988;263(32):16709-16713.
2. Miyata T, Tokunaga F, Yoneya T, et al. Antimicrobial peptides, isolated from horseshoe crab hemocytes, tachyplesin II, and polyphemusins I and II: Chemical structures and biological activity. *J Biochem*. 1989;106(4):663-668.
3. Tam JP, Lu YA, Yang JL. Marked increase in membranolytic selectivity of novel cyclic tachyplesins constrained with an antiparallel two-beta strand cystine knot framework. *Biochem Biophys Res Commun*. 2000;267(3):783-790.
4. Kawano K, Yoneya T, Miyata T, et al. Antimicrobial peptide, tachyplesin I, isolated from hemocytes of the horseshoe crab (Tachypleus tridentatus). NMR determination of the beta-sheet structure. *J Biol Chem*. 1990;265(26):15365-15367.
5. Han E, Lee H. Structural effects of tachyplesin I and its linear derivative on their aggregation and mobility in lipid bilayers. *J Mol Graph Model*. 2015;59:123-128.
6. Boughton AP, Nguyen K, Andricioaei I, Chen Z. Interfacial orientation and secondary structure change in tachyplesin I: Molecular dynamics and sum frequency generation spectroscopy studies. *Langmuir the Acs Journal of Surfaces & Colloids*. 2011;27(23):14343-14351.
7. Laederach A, Andreotti AH, Solution FDB. Micelle-bound structures of tachyplesin I and its active aromatic linear derivatives. *Biochemistry*. 2002;41(41):12359-12368.
8. Edwards IA, Elliott AG, Kavanagh AM, Blaskovich MAT, Cooper MA. Structure-activity and -toxicity relationships of the antimicrobial peptide tachyplesin-1. *ACS Infect Dis*. 2017;3(12):917-926.
9. Saravanan R, Mohanram H, Joshi M, et al. Structure, activity and interactions of the cysteine deleted analog of tachyplesin-1 with lipopolysaccharide micelle: Mechanistic insights into outer-membrane permeabilization and endotoxin neutralization. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2012;1818(7):1613-1624.

10. Ramamoorthy A, Thennarasu S, Tan AM, et al. Deletion of all cysteines in tachyplesin I abolishes hemolytic activity and retains antimicrobial activity and lipopolysaccharide selective binding. *Biochemistry*. 2006;45(20):6529-6540.
11. Holland-Nell K, Meldal M. Maintaining biological activity by using triazoles as disufide bond mimetics. *Angew Chem Int Edit*. 2011;50(22):5204-5206.
12. Cui HK, Guo Y, He Y, et al. Diaminodiacid-based solid-phase synthesis of peptide disulfide bond mimics. *Angew Chem Int Edit*. 2013;52(36):9558-9562.
13. Grishin AA, Wang CIA, Muttenthaler M, Alewood PF, Lewis RJ, Adams DJ. Alpha-conotoxin AuIB isomers exhibit distinct inhibitory mechanisms and differential sensitivity to stoichiometry of alpha 3 beta 4 nicotinic acetylcholine receptors. *J Biol Chem*. 2010;285(29):22254-22263.
14. Zhangsun D, Zhu X, Kaas Q, et al. αO-Conotoxin GeXIVA disulfide bond isomers exhibit differential sensitivity for various nicotinic acetylcholine receptors but retain potency and selectivity for the human α9α10 subtype. *Neuropharmacology*. 2017;127:243-252.
15. Khoo KK, Gupta K, Green BR, et al. Distinct disulfide isomers of muconotoxins KIIIA and KIIIB block voltage-gated sodium channels. *Biochemistry*. 2012;51(49):9826-9835.
16. Tietze AA, Tietze D, Ohlenschlager O, et al. Structurally diverse muconotoxin PIIIA isomers block sodium channel NaV 1.4. *Angew Chem Int Ed Engl*. 2012;51(17):4058-4061.
17. Gehrmann J, Alewood PF, Craik DJ. Structure determination of the three disulfide bond isomers of alpha-conotoxin GI: A model for the role of disulfide bonds in structural stability. *J Mol Biol*. 1998;278(2):401-415.
18. Jiang N, Ma J. Influence of disulfide connectivity, electrostatics, and hydrophobicity on the conformational variations of alpha-conotoxin GI single-disulfide analogues: Simulations with polarizable force field. *J Phys Chem B*. 2010;114(34):11241-11250.
19. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol*. 1993;234(3):779-815.
20. Eswar N, Webb B, Martirenom MA, et al. Comparative protein structure modeling using MODELLER. *Curr Protoc Protein Sci*. 2014;47(1137). 5.6.1.
21. Case, DA, Cerutti DS, Cheatham T, Darden T, Duke R,Giese TJ, et al. 2017, University of California, SanFrancisco2017.
22. Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of multiple amber force fields and development of improved protein backbone parameters. *Proteins*. 2006;65(3):712-725.
23. Darden T, York D, Pedersen L. Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems. *Journal of Chemical Physics*. 1993;98(12):10089-10092.
24. Humphrey W, Dalke A, Schulten KVMD. Visual molecular dynamics. *J Mol Graph Model*. 1996;14(1):33-38.
25. Yu R, Kompella SN, Adams DJ, Craik DJ, Kaas Q. Determination of the α-conotoxin Vc1.1 binding site on the α9α10 nicotinic acetylcholine receptor. *J Med Chem*. 2013;56(9):3557-3567.
26. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc*. 2008;3(2):163-175.
27. Tamamura H, Ikoma R, Niwa M, Funakoshi S, Murakami T, Fujii N. Antimicrobial activity and conformation of tachyplesin-I and its analogs. *Chem Pharm Bull*. 1993;41(5):978-980.
28. Wu Y, Wu XS, Yu JP, Zhu XP, Zhangsun DT, Luo SL. Influence of disulfide connectivity on structure and bioactivity of alpha-conotoxin TxIA. *Molecules*. 2014;19(1):966-979.
29. Sivanesam K, Kier BL, Whedon SD, Chatterjee C, Andersen NH. Hairpin structure stability plays a role in the activity of two antimicrobial peptides. *FEBS Lett*. 2016;590(24):4480-4488.
30. Rao AG. Conformation and antimicrobial activity of linear derivatives of tachyplesin lacking disulfide bonds. *Arch Biochem Biophys*. 1999;361(1):127-134.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Shi J, So L-Y, Chen F, et al. Influences of disulfide connectivity on structure and antimicrobial activity of tachyplesin I. *J Pep Sci*. 2018;24:e3087. https://doi.org/10.1002/psc.3087