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Functional and structural characterization of recombinant dermcidin-1L, a human antimicrobial peptide

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Abstract

Antimicrobial peptides from human skin are an important component of the innate immune response and play a key role as a first line of defense against infections. One such peptide is the recently discovered dermcidin-1L. To better understand its mechanism and to further investigate its antimicrobial spectrum, recombinant dermcidin-1L was expressed in *Escherichia coli* as a fusion protein and purified by affinity chromatography. The fusion protein was cleaved by factor Xa protease to produce recombinant dermcidin-1L. Antimicrobial and hemolytic assays demonstrated that dermcidin-1L displayed microbicidal activity against several opportunistic nosocomial pathogens, but no hemolytic activity against human erythrocytes even at concentrations up to 100 μM. Structural studies performed by circular dichroism spectroscopy indicated that the secondary structure of dermcidin-1L was very flexible, and both α-helix and β-sheet structures might be required for the antimicrobial activity. Our results confirmed previous findings indicating that dermcidin-1L could have promising therapeutic potentials and shed new light on the structure–function relationship of dermcidin-1L.

Keywords: Antimicrobial peptide; Dermcidin-1L; Antimicrobial activities; Hemolytic activity; Circular dichroism

The continuous use of chemical antibiotics has led to the appearance of multi-resistant bacterial strains all over the world [1,2], which resulted in higher mortality of many infectious diseases [3]. Therefore, it is urgent to search for alternatives to traditional antibiotics [4,5]. A large group of antimicrobial peptides produced in the host defense system have been isolated and their functions were characterized [6–8]. Several families of antimicrobial peptides, such as histatins [9,10], defensins [11], and cathelicidins [12], have been isolated from human beings. Dermcidin, a recently discovered, novel antimicrobial peptide family from human skin, was isolated and has no homology to other known antimicrobial peptides [13].

Dermcidin (DCD), composed of 110 amino acids (aa), is proteolytically processed to form a 47 amino acid peptide found in human sweat, corresponding to aa 63–109 of the original translated product. This processed peptide was named DCD-1 and showed to have a broad spectrum of antimicrobial activities [14]. A related peptide, DCD-1L consisting of DCD-1 plus the last leucine (L) residue of the original precursor protein, has stronger antimicrobial activities [13]. DCD, as part of the constitutive innate immune defense of human skin, may help to prevent local and systemic invasion of pathogens by modulating surface colonization [15]. Recently, DCD has been found to kill *Staphylococcus epidermidis*, which indwells medical devices and causes

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nosocomial infections [16,17]. It is also expressed in tubular structures of cutaneous mixed tumours [18] and breast carcinomas [19]. It appears that DCD could be developed into a new class of potential broad-spectrum antimicrobial and anti-tumor drugs. In order to obtain large quantities of highly purified peptide and dissect the mechanism of DCD-1L in killing or inhibiting microorganisms, we have developed a method for the recombinant production of DCD-1L in which the peptide is expressed as a fusion protein, followed by enzymatic cleavage to release active peptide. Furthermore, the antimicrobial activity, hemolytic activity, and the secondary structure of recombinant DCD-1L were investigated.

Materials and methods

Bacterial strains and plasmid. Plasmid pET-32a(+) was purchased from Novagen (EMD Biosciences, Darmstadt, Germany). *Escherichia coli* DH5a and BL21(DE3) were used as host strains in this study. *Staphylococcus aureus CMCC(B)26003*, *Escherichia coli CMCC(B)44102*, and *Candida albicans CMCC(B)98001* were purchased from China Pharmaceutical Analytic System at Shanghai Microorganisms Conservation Center (Shanghai, China). *Pseudomonas sputita* and *Staphylococcus aureus 293*(*oxa*^{r}, *van*^{s}, *tie*^{r}) were kindly provided by Dr. Zhong-Yi Chang in our department and Dr. Hai-Feng Hu at Shanghai Institute of Pharmaceutical Industry, respectively.

Cloning of DCD-1L gene. To assemble a synthetic gene coding for DCD-1L, two oligonucleotide primers, partly complementary to each other, were synthesized with the following sequences [20]: DCD-1La(85 bp), 5′-TCTTCTTTGTTGGAGAAGGGTTTGGACGGTG CTAAGAAGGCTGTTGGTGGTTTGGGTAAGTTGGGTAAGG ACGCTGTTGAGGACT-3′; DCD-1Lb(85 bp), 5′-CAAAACAGA GTCCAAAACGTCCTTAACGTCGTGAACAGCACCCTTACCA ACAGACTCCAAGTCCTCAACAGCGTCCTTACCCAAC-3′.

Elongation of the two primers was carried out using one fragment as the template for the other in the presence of Ex *Taq* Polymerase (TaKaRa, Dalian, China) based on the following conditions: 94 °C for 3 min, 45 °C for 2 min, and 72 °C for 10 min. The dsDNA gene fragment was further amplified using the same enzyme and two new primers, P5 5′-GGC**GGATCC**ATCGAAGGTCGTTCTTCTTTGTT GGAGAAGG-3′ (the *Bam*HI restriction site is bolded and codon for the Factor Xa protease site is underlined) and P3 5′-CCG**GAATTC** TCACAAAACAGAGTCC-3′ (the *Eco*RI restriction site is bolded and the stop codon is underlined). The PCR products were purified by DNA Gel Extraction Kit (Vitagen Shanghai, China) from agarose gel, followed by digestion with restriction endonucleases *Bam*HI and *Eco*RI (MBI Fermentas, Shanghai, China), and cloned into the expression vector pET-32a(+), which was digested by the same two enzymes, to produce the recombinant plasmid pET32a-DCD-1L, containing a N-terminal thioredoxin and hexahistidine tag for purification (see Fig. 1 for the flow diagram of the cloning strategy). The complete sequence of the assembled DCD-1L gene was confirmed by direct sequencing of the plasmid DNA.

Purification of recombinant DCD-1L peptide. The recombinant plasmid pET32a-DCD-1L was transformed into *E. coli* strain BL21(DE3). For expression, a single colony was used to inoculate a 10-mL LB-Amp medium (LB medium supplemented with ampicillin at a final concentration of 100 μg/mL). The culture was grown at 37 °C with vigorous shaking. When the OD_{600} reached 0.6–0.7, the cells were collected by centrifugation and stored at 4 °C overnight. The following morning, the cells were resuspended in 10 mL fresh LB-Amp and used to inoculate 200 mL LB-Amp medium, followed by shaking at 37 °C until the OD_{600} reached 0.6–0.7. Induction was initiated by the addition of 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG), followed by a further 3-h shaking. At the end of shaking, the flask was placed on ice for 5 min and the cells were harvested by centrifugation at 10,000 rpm using the Hitachi R20A2 angle rotor (Hitachi Instruments Service, Ltd., Tokyo, Japan) for 5 min at 4 °C, followed by washing twice with cold NTA-0 buffer (20 mM Tris–HCl, pH 8.0, 0.5 M NaCl, and 10% glycol). The cells were resuspended in 50 mL cold NTA-0 buffer containing 1 mM PMSF and incubated on ice for 30 min. The cells were then sonicated on ice and clarified by centrifugation (12,000 rpm, 10 min). The fusion protein containing the His6-tag was loaded onto 3S SNBC Ni^{2+}–nitrilotriacetic acid resin (NTA) column (Qiagen, Valencia, CA) which was pre-equilibrated in NTA-0 buffer. After extensive washing in the same buffer, the column was eluted with 2 column volumes of gradient NTA buffers with different imidazole concentrations according to the manufacturer's instructions. After SDS–PAGE, the peak fractions containing the fusion protein were pooled and applied onto Sephadex G25 to remove imidazole, which was equilibrated with 50 mM Tris–HCl containing 100 mM NaCl. The fusion protein was then treated with 1.5 U of Factor Xa protease (Novagen)/mg fusion protein to remove the thioredoxin and His6-tag from recombinant DCD-1L peptide (rDCD-1L). This mixture was then incubated with 10 mL Ni^{2+}–NTA resin for 60 min and applied to an empty chromatography column (Qiagen), and flow-through (containing rDCD-1L) and the eluate with NTA-0 buffer were collected. Samples from different stages in the purification were analyzed by SDS–PAGE. The rDCD-1L peptide was further purified and concentrated by ultrafiltration with 10K Amicon Ultra Centrifugal Filter and 3K Centriplus concentrators (Millipore, Billerica, MA). The molecular mass of proteolytic peptide was determined using electrospray ionization mass spectrometry performed on MALDI-TOF/TOF-MS by Proteomics Center at Shanghai Medical College Fudan University. Isoelectric focusing (IEF) of rDCD-1L was performed with 0.8% disc-type polyacrylamide gel (pH gradient ranging from 3.5 to 10). Protein was visualized by Coomassie blue staining. Protein concentrations were determined by Lowry assay.

Antimicrobial assays. The antimicrobial activity of rDCD-1L was analyzed with a colony-forming unit assay as described previously [13]. *S. aureus CMCC(B)26003*, *E. coli CMCC(B)44102*, *C. albicans CMCC(B)98001*, *S. aureus293*, and *P. sputita* were used in this study. All bacteria were grown in LB medium whereas the yeast *C. albicans* was grown in Gaos I medium. The bacterial and yeast concentrations were determined photometrically. Bacterial strains were cultured to an OD_{600} of 0.4–0.5 and the yeast strain to an OD_{450} of 0.4–0.6. Before analysis, we determined the concentration of the organisms in culture by plating different dilutions of cultures. Cells were washed twice with 10 mM phosphate buffer (pH 7.4) and diluted to a concentration of 2×10^6 cells/mL in 10 mM sodium phosphate buffer (pH 6.5) with 100 mM sodium chloride. Cells were incubated in the presence of various concentrations of rDCD-1L at 37 °C for 4 h in 200 μL of 10 mM sodium phosphate buffer (pH 6.5) with 100 mM sodium chloride. After incubation, the cells were diluted 1:100, and 100 μL of the solutions was mixed with 1% molten LB agar and plated in triplicate agar plates. At least two independent experiments for every microorganism were conducted and the mean number of colonies was determined. The bactericidal activities of the tested reagents were plotted using [(cell survival after peptide incubation)/(cell survival without peptide incubation) × 100] versus peptide concentrations, which represented the percentage of survival cells and the toxic potency of rDCD-1L against microorganisms.

Hemolytic assay. The hemolytic activity of rDCD-1L was determined using fresh human erythrocytes. Citrated blood (in mL aliquot) was washed four times with 6 mL PBS buffer (50 mM sodium phosphate buffer, 150 mM NaCl, pH 7.2) and centrifuged at 5000 rpm for 6 min at room temperature. The pellets were resuspended in 3 mL and further diluted to a concentration of 1×10^9 human erythrocytes/mL in

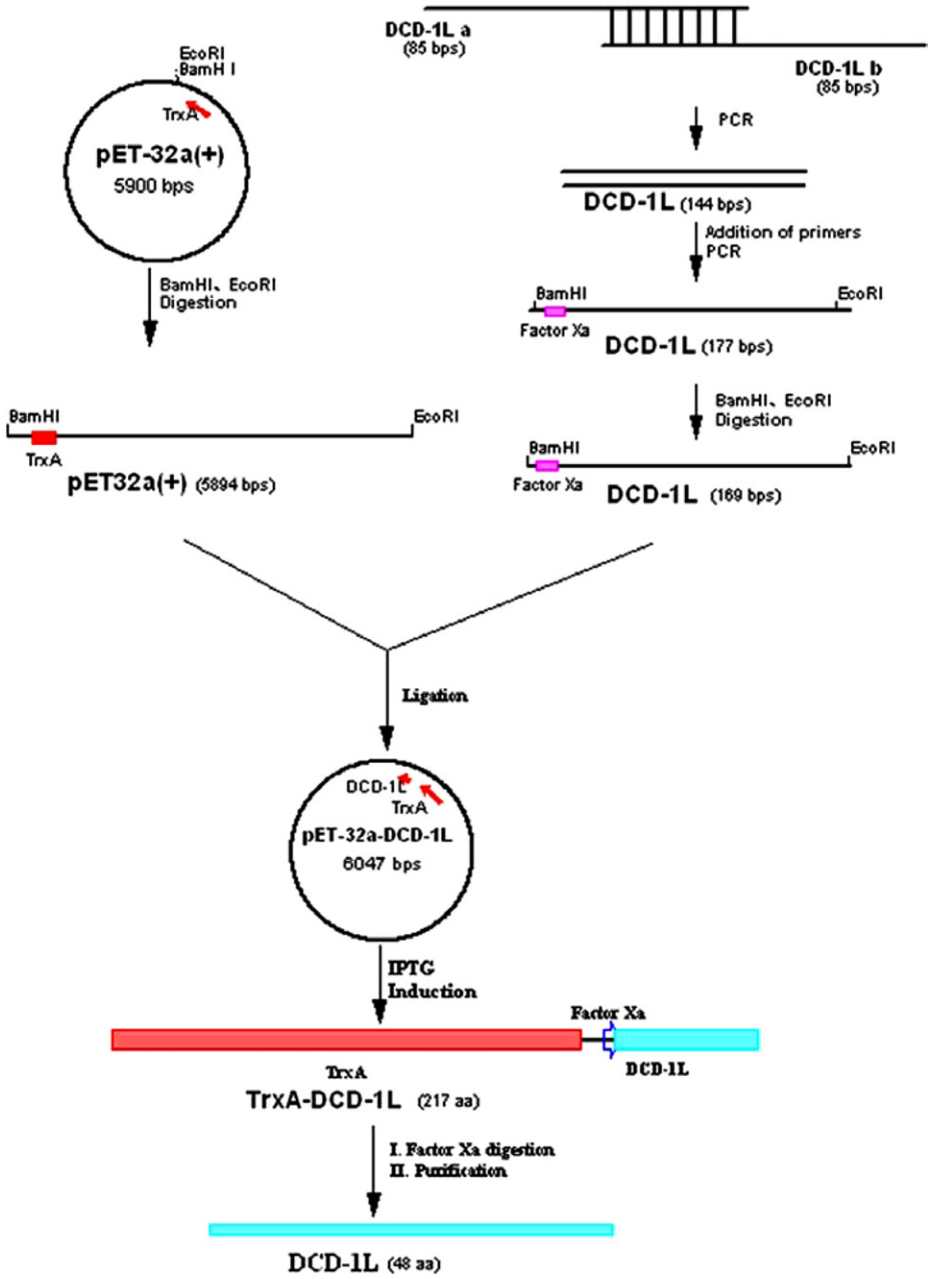

Fig. 1. Strategy for assembly, cloning, and expression of the DCD-1L gene in *E. coli*. This is a schematic representation of the strategy used for the generation of rDCD-1L. Purified rDCD-1L is obtained after affinity chromatography, protease cleavage, and ultrafiltration.

PBS buffer. Lyophilized peptides in various concentrations were dissolved in 200 μL PBS buffer, and 50 μL of human erythrocytes was added, followed by incubation at 37 °C with gentle shaking for 1 h. The samples were then placed on ice and immediately centrifuged at 4 °C. The supernatant was carefully removed and the pellets were resuspended in 1 mL water. Release of hemoglobin was monitored by measuring the absorbance of supernatant at 541 nm in a 1-mL cell. The negative control (0% hemolysis) was 50 μL human erythrocytes in 200 μL PBS buffer, and the positive control (100% hemolysis) was 50 μL human erythrocytes in 200 μL water. Two independent experiments were conducted with triplicated readings for each. The hemolysis percentage was calculated using the following equation: percentage hemolysis of supernatants = [($Abs_{541\ nm}$ in the peptide solution − $Abs_{541\ nm}$ in PBS)/$Abs_{541\ nm}$ in water solution] × 100.

Cirular dichroism spectroscopy. All cirular dichroism (CD) spectrum measurements were made on a JASCO J-715 spectropolarimeter (Jasco, Tokyo, Japan) using a quartz cuvette with 0.1 cm pathlength over a wavelength range of 190–250 nm. The system was purged with N_2 (25 mL/min) and thermostabilized by air condition. Peptide samples were prepared in 10 mM phosphate buffer (pH 6.5), 50% of 2,2,2-trifluoroethanol (v/v) (Sigma, St. Louis, MO), and 30 mM sodium dodecyl sulfate (SDS) (Sigma, St. Louis, MO), respectively. The concentration of the peptide was 55 μM. Data were collected every 0.2 nm and were the average of five scans. The bandwidth was set at 1.0 nm and the sensitivity at 20 mdeg. The response time was 0.5 s. In all cases, baseline scans of aqueous buffer were subtracted from the experimental readings. The results were expressed in units of molar ellipticity per residue (deg cm^2 $dmol^{-1}$) and plotted versus the wavelength. The

contents of different secondary structures were processed by Jasco secondary structure software according to the empirical equation of Chen et al. [21].

Results

Expression and purification of rDCD-1L

Using the genome sequence coding for dermcidin available from GenBank (Accession No. AF144011), a synthetic gene was assembled using oligonucleotide primers and PCR incorporating changes necessary for codon optimization. After cloning into the expression vector pET-32a(+), the recombinant plasmid pET-32a-DCL-1L was introduced into the *E. coli* strain BL21(DE3) for expression. The expressed fusion protein carrying thioredoxin and the His6-tag was purified via Ni^{2+} affinity chromatography, followed by cleavage using Factor Xa protease. The thioredoxin portion and the His6-tag attached to the C-terminus were removed by passing the digestion mixture through the Ni^{2+} resin again. The final purification and concentration of rDCD-1L was achieved by ultrafiltration as shown in Fig. 2. The final yield of rDCD-1L was approximately 2.38% (Table 1). Although the apparent molecular weight of rDCD-1L seen on SDS–PAGE is about 6 kDa, mass spectrometry analysis revealed that the exact mass of the peptide is 4818.1 Da (Fig. 3), which is consistent with the predicted mass calculated from the amino acid sequence of DCD-1L. Isoelectric focusing analysis displayed that the isoelectric point of rDCD-1L was between 5.20 and 5.85 (Fig. 4), which indicates that DCD-1L could be negatively charged at neutral pH. The predicted p*I* varies from 5.07 to 5.45 depending on the software programs used to calculate the p*I*.

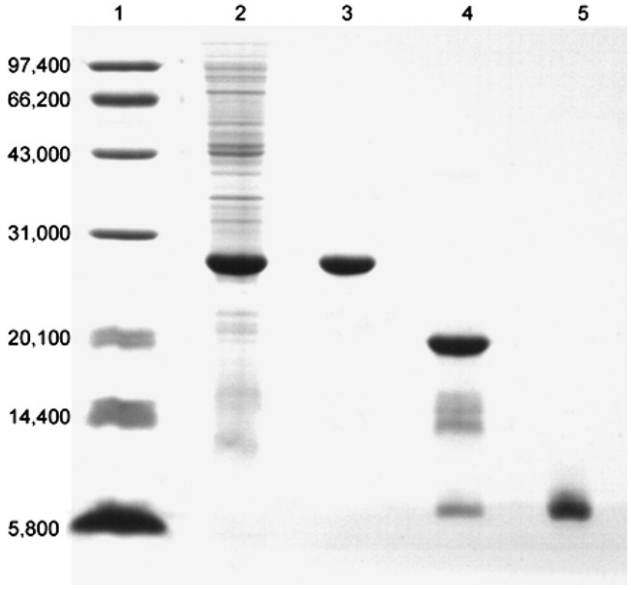

Fig. 2. SDS–PAGE analysis of protein samples at different purification stages. Lane 1, protein marker from DingGuo Biotechnology (Beijing, China); lane 2, total cell lysate of BL21(DE3) containing pET32a-DCD-1L; lane 3, purified fusion protein eluted from the Ni^{2+}–NTA resin; lane 4, purified protein (as in lane 3) treated with Factor Xa protease; and lane 5, the final purified product after removal of thioredoxin and His6-tag using the Ni^{2+}–NTA resin and ultrafiltration.

Analysis of antimicrobial activity and mammalian cell toxicity

The antimicrobial activity of rDCD-1L was assessed in a microbicidal assay in vitro against five different microorganisms. As shown in Fig. 5, rDCD-1L was active against *E. coli CMCC(B)44102*, *P. sputita*, *S. aureus CMCC(B)26003*, and *S. aureus*293 with 50% of bacteria killed at the concentrations of 1.8, 0.5, 8.2, and 17.8 μg/mL, respectively. The effective rDCD-1L concentration for killing 50% of *C. albicans CMCC(B)98001* was 12.6 μg/mL.

To assess the cytotoxicity of the peptides against mammalian cells, the percentage of hemolysis was measured for human erythrocytes at various peptide concentrations (from 0.8 to 100 μM). To our surprise, no hemolytic activity was observed even at the highest peptide concentration. This finding is in contrast to what has been observed with many cationic antimicrobial peptides.

Structural characterization of rDCD-1L

The secondary structure of rDCD-1L was determined by circular dichroism (CD) spectroscopy. Different

Table 1
Purification of rDCD-1L[a]

Fraction	Volume (mL)	Concentration (mg/mL)	Total protein (mg)	Yield (%)
Crude supernatant	90	2.645 ± 0.032	238.089 ± 2.862	100
NTA resin	47	1.407 ± 0.062	66.150 ± 2.935	27.78
Sephadex G-25 coarse	91	0.574 ± 0.057	52.245 ± 5.169	21.94
Ultrafilter	27	1.828 ± 0.012	38.391 ± 0.252	16.12
rDCD-1L	1.15	4.880 ± 0.228	5.612 ± 0.262	2.38

[a] All assays were repeated three times, and the results presented were means ± SEM.

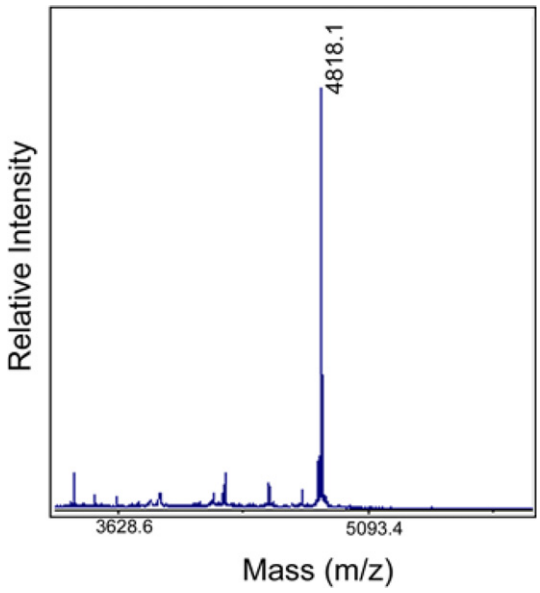

Fig. 3. Molecular mass of rDCD-1L. Mass spectrum obtained from analysis of rDCD-1L on a MALDI-TOF/TOF-MS.

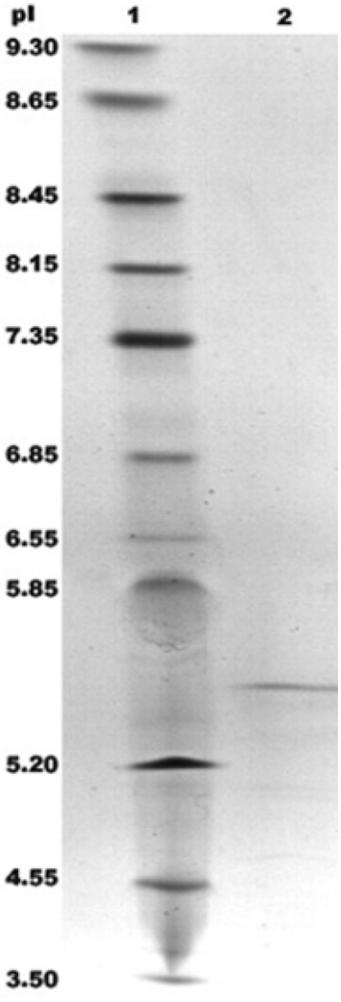

Fig. 4. Determination of pI for rDCD-1L by isoelectric focusing electrophoresis. Lane 1: p*I* Calibration marker (Amersham Biosciences, Piscataway, NJ); lane 2: rDCD-1L.

ultraviolet CD spectra of rDCD-1L in various solvents are shown in Fig. 6. Satisfactory data could be obtained down to 190 nm; below this wavelength the noise levels prevented accurate determination of ellipticity. The CD spectrum exhibits a decrease of random coil and β-sheet character with an increase of α-helix structure on moving from 10 mM PBS to 30 mM SDS. In the 10 mM PBS solution, the peptide shows a spectrum characteristic of a secondary structure in random coil and β-sheet with no α-helix. In 50% TFE, the CD spectrum indicates that some α-helix structure is present. The CD spectrum in 30 mM SDS micelles shows considerably more α-helix character. The comparative summary of these data demonstrates that an overall trend from the predominantly random coil to more significant α-helix can be detected when 10 mM PBS is replaced with 30 mM SDS (Table 2). These results indicated that the secondary structure of rDCD-1L is strongly dependent on the solution environment.

Discussion

As the incidence of bacterial infections has increased in the past two decades because of the development of drug resistant strains, search for alternative antimicrobial agents has resulted in the isolation and identification of a large number of antimicrobial peptides from various natural sources as well as novel synthetic antibacterial peptides [22]. Dermcidin is the newest antimicrobial peptide isolated from human beings. It may have special properties for future application in the treatment of dermatoid diseases, such as acne vulgaris, psoriasis and atopic dermatitis [14], and other cutaneous tumours [18]. In addition, dermcidin has been found to possibly play a role in tumorigenesis by means of enhancing cell growth and survival in a subset of breast carcinomas [19]. In order to further study the role of dermcidin in bacterial pathogenesis and tumorigenesis of breast carcinomas, large quantities of pure and active peptide are needed.

In this study, we have demonstrated an effective way of producing large amount of pure rDCD-1L using a fusion protein expression strategy in *E. coli*. The rDCD-1L had the predicted molecular mass and isoelectric point.

The in vitro antimicrobial properties of rDCD-1L were evaluated in microbicidal assays against a panel of bacterial and fungal organisms. The broad spectrum of rDCD-1L antimicrobial activity was demonstrated in microbicidal assay against Gram-positive bacteria (*S. aureus CMCC(B)26003* and *S. aureus293*), Gram-negative bacteria (*E.coli CMCC(B)44102* and *P. sputita*), and fungus (*C. albicans CMCC(B)98001*). It is especially worth noting that rDCD-1L is effective against drug-resistant *S. aureus* (strain *S. aureus293*). The viability of each

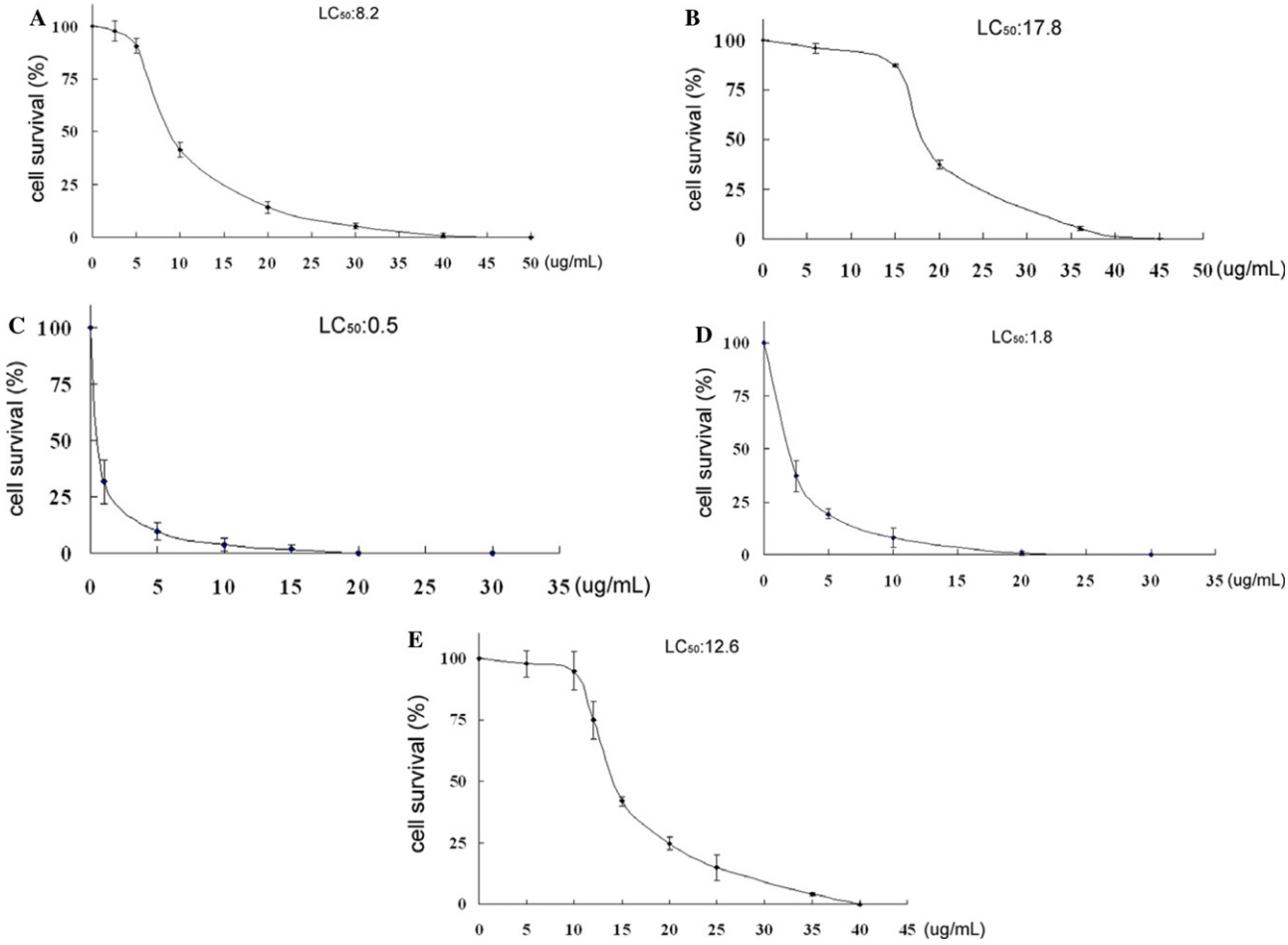

Fig. 5. Determination of antimicrobial activity of rDCD-1L. Bacteria or *C. albicans* were grown to mid-exponential phase, diluted to 1×10^6 colony-forming units, incubated with various concentrations of rDCD-1L for 4 h at 37 °C, and plated for surviving microorganisms. LC_{50} is the lethal concentration (LC) required to kill 50% of the microorganism in the population being tested. (A) *S.aureus CMCC(B)26003*; (B) *S. aureus293*; (C) *P. sputita*; (D) *E. coli CMCC(B)44102*; and (E) *C. albicans CMCC(B)98001*. Data presented here are the average and SEM of at least two independent experiments, each performed in triplicate.

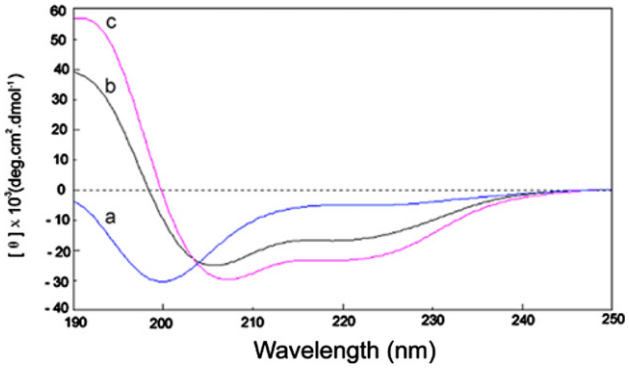

Fig. 6. CD spectra of rDCD-1L in different solutions. CD spectra of rDCD-1L were performed in three solutions: (a) 10 mM PBS; (b) 50% TFE(v/v); and (c) 30 mM SDS.

Table 2
Determination of rDCD-1L secondary structure in different solutions[a]

Solution	% secondary structure		
	Random coil	β-sheet	α-helix
10 mM PBS	55.7	44.3	0
50% TFE	43.2	29.5	27.3
30 mM SDS	36.4	23.9	39.7

[a] All data were processed by Jasco secondary structure estimation software according to the empirical equation of Chen et al. [21].

organism was reduced by more than 99% after 4-h incubations with 15–40 μg/mL rDCD-1L. In collaboration with other group, we also found that rDCD-1L had toxic effect against rifampin- and isoniazid-resistant strain of mycobacterium tuberculosis (data to be published elsewhere). Our results indicate that rDCD-1L has the microbicidal efficacy against several opportunistic nosocomial microorganisms.

Previous studies have demonstrated that the activities of DCD-1 and DCD-1L are dependent on the ionic strength of the incubation buffer [13]. In this context, we have tested the efficacy of rDCD-1L in different PBS buffers and found that PBS buffer (pH 6.5) with

100 mM sodium chloride is optimal for rDCD-1L activity.

To be clinically meaningful, antimicrobial peptides must have an effective concentration that does not exhibit toxicity to human cells [23]. The hemolytic activity of antimicrobial peptides against human erythrocytes is often used as a measure for their cytotoxicity and to estimate their therapeutic index [24,25]. In this study, we have shown that rDCD-1L had no hemolytic activity at all when assayed in PBS. It has been suggested that the abundance of negatively charged sialic acids on the erythrocytes [26] would protect the cell against lysis by electrostatic repulsion of negatively charged molecules, such as DCD-1L, which has a net negative charge. The lack of cytotoxicity of rDCD-1L against erythrocytes suggests that it has potential as a therapeutic substance in the clinical setting.

In this study, we have also determined the secondary structure of rDCD-1L in three different solutions. Our findings suggest that rDCD-1L may adopt two conformations, i.e., β-sheet and α-helix structure on membranes on the basis of the CD spectrum measured in 50% TFE and 30 mM SDS micelles, both of which are used to mimic the biological membrane environments. CD spectroscopy of the peptide indicates a structural transition from a random coil structure in 10 mM PBS, a β-sheet conformation to an α-helix structure on addition of the helicogenic solvent TFE and 30 mM SDS micelles, which is different from the presumption that DCD-1 adopts amphipathic α-helix during its action on cell membrane [13].

In summary, we have described here a recombinant approach for the production of highly pure and active rDCD-1L, which can be used in future studies on the mechanism of this new antimicrobial peptide. We have demonstrated the efficacy of rDCD-1L against several common nosocomial infectious agents and the potential of using rDCD-1L in a clinical setting due to its lack of cytotoxicity against human red blood cells. Our study also highlights the flexible nature of the peptide and shows that the secondary structure is highly dependent on the solutions surrounding the peptide. Further studies are required to unravel the detailed microbicidal mechanism of rDCD-1L.

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References

[1] S.H. Marshall, G. Arenas, Antimicriobial peptides: a natural alternative to chemical antibiotics and a potential for applied biotechnology, Electron. J. Biotechnol. 6 (2003) 271–284.
[2] A.G. Mainous III, C. Pomeroy, Management of Antimicrobials in Infectious Diseases: Impact of Antibiotic Resistance, Humana Press, Totowa, NJ, 2000.
[3] M.L. Cohen, Changing patterns of infectious disease, Nature 406 (2000) 762–767.
[4] R.E. Hancock, R. Lehrer, Cationic peptides: a new source of antibiotics, Trends Biotechnol. 16 (1998) 82–88.
[5] R.E. Hancock, Host defense (cationic) peptides: what is their future clinical potential? Drugs 57 (1999) 469–473.
[6] R.M. Epand, H.J. Vogel, Diversity of antimicrobial peptides and their mechanisms of action, Biochim. Biophys. Acta 1462 (1999) 11–28.
[7] W.L. Maloy, U.P. Kari, Structure–activity studies on magainins and other host defense peptides, Biopolymers 37 (1995) 105–122.
[8] A. Tossi, L. Sandri, A. Giangaspero, Amphipathic alpha-helical antimicrobial peptides, Biopolymers 55 (2000) 4–30.
[9] D. Andreu, L. Rivas, Animal antimicrobial peptides: an overview, Biopolymers 47 (1998) 415–433.
[10] M. Zasloff, Magainins, A class of antimicrobial peptides from *Xenopus* skin: isolation characterization of two active forms, and partial cDNA sequence of a precursor, Proc. Natl. Acad. Sci. USA 84 (1987) 5449–5453.
[11] J. Harder, J. Bartels, E. Christophers, J.M. Schroder, A peptide antibiotic from human skin, Nature 387 (1997) 861.
[12] M. Zanetti, R. Gennaro, D. Romeo, Cathelicidins a novel protein family with a common proregion and a variable C-terminal antimicrobial domain, FEBS Lett. 374 (1995) 1–5.
[13] B. Schittek, R. Hipfel, B. Sauer, J. Bauer, H. Kalbacher, S. Stevanovic, M. Schirle, K. Schroeder, N. Blin, F. Meier, G. Rassner, C. Garbe, Dermcidin: a novel human antibiotic peptide secreted by sweat gland, Nat. Immunol. 2 (2001) 1133–1137.
[14] T. Flad, R. Bogumil, J. Tolson, B. Schittek, C. Garbe, M. Deeg, C.A. Mueller, H. Kalbacher, Detection of dermcidin-derived peptides in sweat by ProteinChip® technology, J. Immunol. Methods 270 (2002) 53–62.
[15] S. Rieg, C. Garbe, B. Sauer, H. Kalbacher, B. Schittek, Dermcidin is constitutively produced by eccrine sweat glands and is not induced in epidermal cells under inflammatory skin conditions, Br. J. Dermatol. 151 (2004) 534–539.
[16] M. Lyte, P.P. Freestone, C.P. Neal, B.A. Olson, R.D. Haigh, R. Bayston, P.H. Williams, Stimulation of *Staphylococcus epidermidis* growth and biofilm formation by catecholamine inotropes, LANCET 361 (2003) 130–135.
[17] C. Vuong, J.M. Voyich, E.R. Fischer, K.R. Braughton, A.R. Whitney, F.R. Deleo, M. Otto, Polysaccharide intercellular adhesion (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system, Cell. Microbiol. 6 (2004) 269–275.
[18] Y. Minami, K. Uede, K. Sagawa, A. Kimura, T. Tsuji, F. Furukawa, Immunohistochemical staining of cutaneous tumours with G-81, a monoclonal antibody to dermcidin, Br. J. Dermatol. 151 (2004) 165–169.
[19] D. Porter, S. Weremowicz, K. Chin, P. Seth, A. Keshaviah, J. Lahti-Domenici, Y.K. Bae, C.L. Monitto, A. Merlos-Suarez, J. Chan, C.M. Hulette, A. Richardson, C.C. Morton, J. Marks, M. Duyao, R. Hruban, E. Gabrielson, R. Gelman, K. Polyak, A

neural survival factor is a candidate oncogene in breast cancer, Proc. Natl. Acad. Sci. USA 100 (2003) 10931–10936.
[20] Y.-P. Lai, Y.-F. Peng, Y.-Z. Yu, J. Huang, Z.-R. Wu, Cloning and expression of DCD-1L in *Pichia pastoris*, China Biotechnol. 2 (2004) 61–65.
[21] Y.H. Chen, J.T. Yang, K.H. Chau, Determination of the helix and beta form of proteins in aqueous solution by circular dichroism, Biochemistry 13 (1974) 3350–3359.
[22] M.-J. Choi, S.H. Kang, S. Kim, J.-S. Chang, S.S. Kim, H. Cho, K.-H. Lee, The interaction of an antimicrobial decapeptide with phospholipids vesicles, Peptides 25 (2004) 675–683.
[23] M. Zasloff, Antimicrobial peptides of multicellular organisms, Nature 415 (2002) 389–395.
[24] B.W. Gibson, D.Z. Tang, R. Mandrell, M. Kelly, E.R. Spindel, Bombinin-like peptides with antimicrobial activity from the skin of *Bombina orientalis*, J. Biol. Chem. 266 (1991) 23101–23111.
[25] S. Ando, H. Nishikawa, H. Takiguchi, S. Lee, G. Sugihara, Antimicrobial specificity and haemolytic activity of cyclized basic amphiphilic β-structural model peptides and their interactions with phospholipids bilayers, Biochim. Biophys. Acta 1147 (1993) 42–49.
[26] G. Saberwal, R. Nagaraj, Cell-lytic and antibacterial peptides that act by perturbing the barrier function of membranes: facets of their conformational features, structure function correlations and membrane perturbing abilities, BBA-Rev. Biomembranes 1197 (1994) 109–131.