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Biochemical and Biophysical Research Communications 360 (2007) 156–162
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Recombinant expression, synthesis, purification, and solution structure of arenicin ☆

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Received 1 June 2007
Available online 14 June 2007

Abstract

Arenicins are 21-residue cationic antimicrobial peptides, isolated from marine polychaeta *Arenicola marina*. In order to determine a high-resolution three-dimensional structure of arenicin-2, the recombinant peptide was overexpressed as a fused form in *Escherichia coli*. Both arenicin isoforms were synthesized using the Fmoc-based solid-phase strategy. Recombinant and synthetic arenicins were purified, and their antimicrobial and spectroscopic properties were analyzed. NMR investigation shows that in water solution arenicin-2 displays a prolonged β-hairpin, formed by two antiparallel β-strands and stabilized by one disulfide and nine hydrogen bonds. A significant right-handed twist in the β-sheet is deprived the peptide surface of amphipathicity. CD spectroscopic analysis indicates that arenicin-2 binds to the SDS and DPC micelles, and conformation of the peptide is significantly changed upon binding. Arenicin strongly binds to anionic lipid (POPE/POPG) vesicles in contrast with zwitterionic (POPC) ones. These results suggest that arenicins are membrane active peptides and point to possible mechanism of their selectivity toward bacterial cells.

Keywords: Antimicrobial peptide; Arenicin; Lugworm; *Arenicola marina*; Marine invertebrate; Expression; Recombinant; Fusion protein; Purification; Folding; Antimicrobial activity; NMR; CD; β-Hairpin

Natural antimicrobial peptides (AMPs) are one of the most important components of the innate immune system. In invertebrates, AMPs are considered to play a key role in host defense against pathogenic bacteria, fungi, and enveloped viruses [1,2]. Marine invertebrate animals live in a microbe laden environment without adaptive immunity system. A variety of AMPs from representatives of marine invertebrates, including marine sponge, cnidarians, annelida, mollusks, chelicerates, crustaceans, and tunicates, were described. Among them are the cysteine-rich peptides from jellyfish (aurelin) [3], mussels (myticin) [4], and horseshoe crabs (tachyplesins and polyphemusins) [5,6], the proline- and cysteine-rich peptides from shrimp (penaeidins) [7], the α-helical peptides from ascidians (clavanins, styelins) [8–10]. Earlier we discovered two novel 21-residue antimicrobial peptides, arenicin-1 and arenicin-2, exhibiting activity against Gram-positive and Gram-negative bacteria and fungi [11]. Arenicins were isolated from coelomocytes of marine polychaeta lugworm *Arenicola marina*. Molecular masses (2758.3 and 2772.3 Da) and complete amino acid sequences (RWCVYAYVRVRGVLVRYRRCW and RWCVYAYVRIRGVLVRYRRCW) were determined for both isoforms. Each arenicin has one disulfide bond (Cys3–Cys20) forming a 18-residue ring. A 202-residue preproarenicin contains a putative signal peptide (25 amino

☆ *PDB and BMRB Accession codes:* The ^{1}H chemical shifts, NMR constraints and derived atomic coordinates (20 models) of Ar-2 have been deposited into the Protein Data Bank (www.pdb.org. Accession code 2JNI).

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doi:10.1016/j.bbrc.2007.06.029

acids) and a long prodomain (156 amino acids). Arenicins have no structure similarity to any previously identified antimicrobial peptides. The high abundance of hydrophobic and cationic residues in both arenicins sequences points to bacterial anionic membranes as a target of the peptide antibiotic action. As a putative membrane-active cationic AMP, arenicin should adopt amphipathicity. To determine a high-resolution three-dimensional structure of these newly discovered AMPs, the recombinant and synthetic arenicins were obtained. Recombinant expression, solid-phase synthesis, purification, folding, antimicrobial and spectroscopic properties, and solution structure of arenicin in water are described in this report.

Materials and methods

Arenicin-2 heterologous expression in Escherichia coli. The recombinant plasmid pET-His8-TrxL-Ar2 was constructed by ligating the 3490 bp BglII/XhoI fragment of pET-20b(+) vector (Novagen) with a PCR-constructed insert containing the T7 promoter, the ribosome-binding site and the sequence encoding the recombinant protein. The last one included octahistidine tag, TrxL carrier protein [*E. coli* thioredoxin A amplified from pET-32a(+) vector (Novagen) in which the M37L mutation was introduced], a methionin residue and the mature arenicin-2 (Fig. 1). Plasmid purification and transformation were performed according to [12]. The BL-21 (DE3) cells transformed with pET-His8-TrxL-Ar2 were grown in LB media with 150 mkg/mL of ampicillin up to OD_{600} 0.5 and then were induced by 0.05–0.2 mM IPTG. The induction was performed at 25–30 °C for 4–6 h with a shaking speed of 200 rpm. The expression level and solubility of His8-TrxL-Ar2 were monitored using Tris–tricine SDS–PAGE [13].

Recombinant fusion protein purification and cleavage. The cells were harvested by centrifugation and sonicated in the buffer containing 100 mM Na_2HPO_4/NaH_2PO_4 (pH 7.8), 0.5 M NaCl, 1% Triton X-100 and 1 mM PMSF. Insoluble fraction was separated by centrifugation and then repeatedly washed with 25 mM phosphate buffer (pH 7.8). The pellet was solubilized (20–40 mg/mL) in 100 mM phosphate buffer (pH 7.8) containing 6 M guanidine hydrochloride and 10 mM imidazole and applied to Ni–NTA column. The recombinant protein was eluted with 0.5 M imidazole. After dialysis the protein of interest was dissolved in 80% TFA (20 mg/mL) and then cleaved by an equal mass of CNBr under standard conditions. The lyophilized products of cleavage reaction were dissolved at a concentration of 10 mg/mL in 10% acetonitrile containing 0.1% TFA.

Recombinant arenicin-2 purification. The clarified solution was loaded to the RP-HPLC semi-preparative column C4 (Waters) equilibrated with solution A2 (5% acetonitrile, 0.1% TFA). The chromatography was performed at a flow rate of 1.5 mL/min in a linear gradient of solution B2 (80% acetonitrile, 0.1% TFA) in solution A2: 10–40% for 60 min, 40–100% for 15 min, and 100–0% for 15 min. The peaks monitored at 214 nm were collected, vacuum dried and applied to Tris–tricine SDS–PAGE. Purified recombinant arenicin-2 was analyzed by MALDI-TOF MS using Reflex III mass-spectrometer (Bruker Daltonics) and by automated Edman degradation using the Procise cLC 491 Protein Sequencing System (PE Applied Biosystems).

Arenicins synthesis. The strategy of the arenicin-1 and arenicin-2 synthesis was based on the standard Fmoc-protocol with the TBTU/DIEA activation using the Wang-resin as the solid-phase. The Trt protecting groups was used for Cys. Deprotection and cleavage were performed with a TFA:anisole:DMS:EDT mixture (94:2:2:2). The peptides were purified by HPLC on a Reprosil-Pur C18 column (5 μm, 10 × 250 mm) in a linear gradient of acetonitrile (10–50% for 40 min) and then were oxidized with an isopropanol-water mixture (1:1) at pH 8.5. The synthetic arenicins were

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1                His8                          thioredoxin
MetSerHisHisHisHisHisHisHisGlySerSerAspLysIleIleHisLeuThr
atgagccatcaccaccaccatcaccatcacggatctagcgataaaattattcacctgact

21
AspAspSerPheAspThrAspValLeuLysAlaAspGlyAlaIleLeuValAspPheTrp
gacgacagttttgacacggatgtactcaaagcggacggggcgatcctcgtcgatttctgg

41                       M37L
AlaGluTrpCysGlyProCysLysLeuIleAlaProIleLeuAspGluIleAlaAspGlu
gcagagtggtgcggtccgtgcaaactgatcgccccgattctggatgaaatcgctgacgaa

61
TyrGlnGlyLysLeuThrValAlaLysLeuAsnIleAspGlnAsnProGlyThrAlaPro
tatcagggcaaactgaccgttgcaaaactgaacatcgatcaaaaccctggcactgcgccg

81
LysTyrGlyIleArgGlyIleProThrLeuLeuLeuPheLysAsnGlyGluValAlaAla
aaatatggcatccgtggtatcccgactctgctgctgttcaaaaacggtgaagtggcggca

101                                          thioredoxin
ThrLysValGlyAlaLeuSerLysGlyGlnLeuLysGluPheLeuAspAlaAsnLeuAla
accaaagtgggtgcactgtctaaaggtcagttgaaagagttcctcgacgctaacctggcc

121                        arenicin-2
GlySerMetArgTrpCysValTyrAlaTyrValArgIleArgGlyValLeuValArgTyr
ggatctatgcgttggtgcgtttacgcttatgtacgcatccgtggtgttctggtgcgttac

141
ArgArgCysTrp.
cgccgttgctggtaa
```

Fig. 1. Nucleotide sequence of pET-His8-TrxL-Ar2 coding region and its translation. Octahistidine tag, substitution introduced into thioredoxin structure and arenicin-2 sequence are highlighted. Thioredoxin part of the fusion protein is represented with an arrow. Abbreviation "M37L" relates to the amino acids numeration of mature thioredoxin that differs from the numeration given here.

purified by HPLC and identified by MALDI-TOF MS using Reflex III mass-spectrometer (Bruker Daltonics) and by automated Edman degradation using the Procise cLC 491 Protein Sequencing System (PE Applied Biosystems).

NMR spectroscopy, spectral assignment and spatial structure calculation. The sample used for structure determination contained 1.5 mg of the recombinant arenicin-2 in 0.6 mL of H_2O (10% D_2O), pH 3.3. All NMR experiments were performed on Varian Unity-600 or Bruker DRX-500 spectrometers. The WATERGATE technique was used to suppress strong solvent resonance. The 2D DQF-COSY, CLEAN-MLEV ($\tau_m = 80$ ms) and NOESY ($\tau_m = 50$, 100, and 150 ms) spectra were recorded in the States-TPPI manner [14]. Unless otherwise stated, all spectra were measured at 15 °C. Proton resonance assignments for arenicin-2 were obtained by the standard procedure [15] using the XEASY program [16]. The $^3J_{HNH^\alpha}$ coupling constants were determined from line shape analysis of NOESY cross peaks in the Mathematica program (Wolfram research). The $^3J_{H^\alpha H^\beta}$ coupling constants were estimated from 2D multiplet patterns in DQF-COSY spectrum. The temperature gradients of amide protons were measured in temperature range 10–40 °C using 2D CLEAN-MLEV spectra. The spatial structure calculation was performed using the simulated annealing/molecular dynamics protocol as implemented in the CYANA program version 2.1 [17]. Interproton distance constraints were derived from the cross-peak intensities measured in the NOESY spectra with $\tau_m = 100$ ms (where spin-diffusion effects might be ignored) via a "$1/r^6$" calibration. The torsion angle constraints were obtained from J coupling constants and NOE intensities. Additional upper/lower restraints were applied to close Cys3–Cys20 disulfide and backbone–backbone hydrogen bonds.

CD spectroscopy. All CD spectra were measured on a Jasco J-810 instrument (Jasco, Japan) at ambient temperature. POPC, POPG, and POPE were products of Avanti Polar Lipids (Alabaster, AL). DPC and SDS were purchased from Anatrace Inc. (Maumee, OH). The POPC and POPE/POPG (7:3) vesicles were prepared using an Avanti Mini-Extruder and 30 nm polycarbonate membranes. Water solution of arenicin-2 was added to vesicle or micelle suspensions for CD measurements.

Antimicrobial assay. Antimicrobial activities of recombinant and synthetic arenicins peptides against *Escherichia coli*, strain ML-35p, *Listeria monocytogenes*, strain EGD, and *Candida albicans 820* were measured in, radial diffusion assays as described in [11]. Antimicrobial activities towards *Bacillus megaterium* strain VKM41, *Micrococcus luteus*, strain B1314, *Staphylococcus aureus*, strain 209p, *Agrobacterium tumefaciens*, strain 281, and *Fusarium solani*, strain VKM F-142, were measured by microspestrophotometry using 96-well microplates with concentration of 2×10^8 cell/mL (2×10^7 cell/mL for *B. megaterium*) which corresponds to OD_{620} 1/1.5. Filter-sterilized protein solutions (10 μL) of different concentrations in 20% AcN, 0.1% TFA were added to aliquots (110 μL) of the exponential culture, tenfold diluted by ½LB (2LB, 1% sucrose for *M. luteus*), and incubated in shaker for 5 h. Bacterial growth was evaluated by measuring the culture OD_{620} using microplate reader Multiscan EX (Thermo). The solvent (20% AcN, 0.1% TFA) was used as a negative control. The MICs were defined as the minimal concentration of peptide required to produce at least 90% inhibition of bacterial growth. *F. solani* was grown on potato-glucose agar plates for 10 days until spores were abundantly produced. The spores of *F. solani* were collected, and the concentration was adjusted to 10^4 spores/mL using double diluted potato-glucose medium. Spore suspension (110 μL) was incubated in microtitre wells with the indicated amount of peptide (10 μL) for 48 h at 25 °C. Germination of the *F. solani* spores was observed with microscope and evaluated by OD_{620} measurement.

Results and discussion

Arenicin-2 heterologous expression in E. coli

In order to achieve higher yield of arenicin-containing fusion protein, we assayed several strain-plasmid combinations (data not shown). *E. coli* strains BL-21 (DE3), BL-21 (DE3) pLysS, BL-21 (DE3) pLysE, and Origami B (DE3) were transformed with recombinant plasmids, containing arenicin-2 sequence fused with different carrier proteins: bacterial ketosteroid isomerase (KSI), cellulose-binding domain (CBD), thioredoxin A. Even using thioredoxin as an expression partner, we obtained the recombinant proteins in the form of inclusion bodies. In all the recombinant plasmids the arenicin sequence was placed downstream of the carrier protein in order to obtain a complete analogue of the natural peptide after CNBr treatment. In some constructions a short anionic fragment was incorporated between the carrier protein and arenicin-2 sequences, but it did not give any significant increase of the peptide yield. BL-21(DE3)/pET-His8-TrxL-Ar2 was proved to be a suitable strain for the laboratory-scale arenicin-2 production. The yield constituted up to 5 mg/L (converting to pure peptide) without much optimization process. Decreasing the induction temperature from the initial 37 °C to 25–30 °C produced the most considerable effect. It resulted in several-fold multiplication of the expression level of His8-TrxL-Ar2 and other arenicin-containing fusion proteins.

Arenicin-2 purification

Following the cells harvesting, sonication and preparative centrifugation of the cell lysate, the insoluble fraction was subjected to consecutive washing steps, solubilization and Ni–NTA affinity chromatography. In our experiments 1 ml of the Ni–NTA resin bound 5–6 mg of the proteins present in the water-insoluble fraction of the cell lysate, mainly His8-TrxL-Ar2. Arenicin was obtained by CNBr cleavage of the fusion protein. MS analysis of the cleavage products demonstrated along with the cyclic arenicin-2 (2772 Da) the presence of a minor satellite peak shifted by +16 Da (2788 Da) that might be hydroxyarenicin. Fine purification of the peptide was performed by RP-HPLC. Under the same conditions the retention time of the recombinant arenicin-2 (33–34 min) matched those of its natural and synthetic analogues.

Characteristics of recombinant and synthetic arenicins

The recombinant and synthetic peptides homogeneity and their identity to natural arenicins were monitored by SDS–PAGE, MALDI-TOF mass-spectrometry, automated microsequencing, and antimicrobial assay. It was shown that the recombinant and synthetic arenicins were identical to the natural peptides in respect of their molecular masses, amino acid sequences, and antimicrobial activities. It is worth mentioning that the electrophoretic patterns of both purified recombinant and synthetic arenicin-2, apart from the expected ~3 kDa band, sometimes showed an additional band around 12 kDa region. Further examination allowed us to hypothesize that the "~12 kDa band" was formed by the peptide oligomer (data not

shown). It seemed that arenicin molecule has a strong oligomerization tendency and spontaneously forms native conformation. This property of arenicin allowed us to produce its fully active recombinant and synthetic analogues without introducing the refolding step into downstream process.

Biological activity of recombinant and synthetic arenicins

Recombinant and synthetic arenicins were shown to be active towards all the test microorganisms: Gram-positive bacteria *L. monocytogenes*, strain EGD, MIC_{90} 0.6 μg/mL; *B. megaterium*, stain VKM41, MIC_{90} 2.6 μg/mL; *M. luteus*, stain B1314, MIC_{90} 2.6 μg/mL; *S. aureus*, stain 209p, MIC_{90} not determined; Gram-negative bacteria *E. coli*, strain ML-35p, MIC_{90} 4.0 μg/mL; *A. tumefaciens*, strain 281, MIC_{90} 5.0 μg/mL; ascomycete *C. albicans* 820, MIC_{90} 4.5 μg/mL. Recombinant and synthetic arenicins in concentration of 50 μg/ml also completely inhibited spore germination of *F. solani*, stain VKM F-142, and caused the fungi spore lysis.

Structure of arenicin-2 in aqueous solution

To investigate the spatial structure of arenicin-2 in aqueous solution we employed the standard methods of NMR spectroscopy [14,15]. A summary of the NMR data obtained for the recombinant Ar-2 is shown in Fig. 2A and B. The values of $^3J_{HNH^\alpha}$ coupling constants, the chemical shift indexes (CSI) for H^α protons and pattern of NOE contacts indicate that the peptide represents a prolonged β-hairpin with Arg11 and Gly12 on its tip. The peptide structure is stabilized by the disulfide bond Cys3–Cys20 and by nine backbone–backbone hydrogen bonds evinced by low temperature gradients ($\Delta\delta HN/\Delta T < 5$ ppb) of corresponding amide protons (Fig. 2A and B). The set of 20 structures of arenicin-2 (Fig. 2C and Table 1) was calculated in the CYANA program. The ribbon representation of the calculated spatial structure of arenicin-2 is shown in Fig. 3A. The peptide represents β-hairpin, formed by two β-strands (Cys3–Ile10 and Val13–Cys20). The tip of the β-hairpin (Arg11–Gly12) is folded in type I' β-turn. Inspection of the obtained structure indicates that the electrostatic π-cation interactions between Tyr5, Tyr7, Tyr17, Trp21 and Arg18, Arg16, Arg19, Arg1, respectively, are possibly contribute to stability of β-hairpin conformation. The disulfide Cys3–Cys20 joins residues opposite one another on the antiparallel β-strands and has the 'short right-handed hook' or *g+npng+* conformation. The two-stranded β-sheet in arenicin-2 structure has significant right-handed twist. This distortion effectively shields the hydrophobic side of the β-sheet from the contact with polar solvent. The maps of electrostatic (Fig. 3B) and molecular hydrophobicity (Fig. 3C) potentials on a surface of the peptide illustrate absence of pronounced amphipathicity of the arenicin-2 in the aqueous solution. The charged and hydrophobic residues are evenly distributed on the peptide surface (Fig. 3).

The influence of membrane environment on the structure of arenicin-2

The amphipathicity (i.e. segregation of polar and hydrophobic side-chains on a peptide surface) is an absolute prerequisite for membrane activity of cationic AMPs [1,2,18]. Thus, if biological membrane is one of the targets for arenicin-2 action, the spatial structure of the peptide should change upon contact with lipid bilayer and become more amphipathic. To investigate possible changes in arenicin-2 conformation upon contact with lipid bilayer, the CD spectra of the peptide were measured in the presence of lipid vesicles and detergent micelles. The CD spectrum of arenicin-2 in aqueous solution (Fig. 4) demonstrates at least four bands: two positive (at ~203 and 230 nm) and two negative (at ~195 and 213 nm). The spectrum looks quite atypical, as for β-structural peptide only two bands (positive at ~195 nm and negative near 220 nm) were expected. This atypical CD spectrum can be explained by distortions (twist) in the β-sheet structure and by CD signals from aromatic residues (Tyr/Trp) and from the disulfide (Cys3–Cys20) that could produce strong bands in the far-UV region (190–250 nm) of CD spectrum [19]. Addition of arenicin-2 to anionic lipid vesicles, that mimic outer bacterial membrane (POPE/POPG 7:3, 20 mM), led to fast and irreversible precipitation of the vesicles at all the peptide concentration studied (L:P from 100 to 1000). Measurement of CD spectra was impossible for these samples due to significant light scattering. Addition of arenicin-2 to zwitterionic lipid vesicles, that mimic eukaryotic membrane (POPC), led to optically clear solutions and allowed us to measure the CD spectrum. The spectrum of arenicin-2 in the presence of POPC vesicles (Fig. 4) is identical to spectrum of the peptide in aqueous solution. This can be explained either by inability of arenicin-2 to bind to the neutral membranes or by preservation of the peptide spatial structure upon membrane binding. To test these hypotheses, the CD spectra of arenicin-2 were measured in the presence of anionic (SDS) and zwitterionic (DPC) detergent micelles that partially mimic lipid bilayers. The obtained spectra (Fig. 4) are also characterized by four bands: one positive (at ~190 nm) and three negative (in the range of 205–230 nm). Although the peptide spectra in the presence of SDS and DPC micelles are dissimilar to each other, they are significantly dissimilar to the spectrum of arenicin-2 in aqueous solution and more close in appearance to canonical spectrum of β-structure. This indicates that the peptide is capable to bind to the membrane-like anisotropic surface of detergent micelles, and conformation of the peptide (particularly, distortions in the β-sheet) changes upon binding. The obtained results confirm inability of arenicin-2 to bind to the zwitterionic POPC membranes. The observed binding to the zwitterionic DPC micelles can be explained by loose packing of detergent micelles as compared to lipid bilayers.

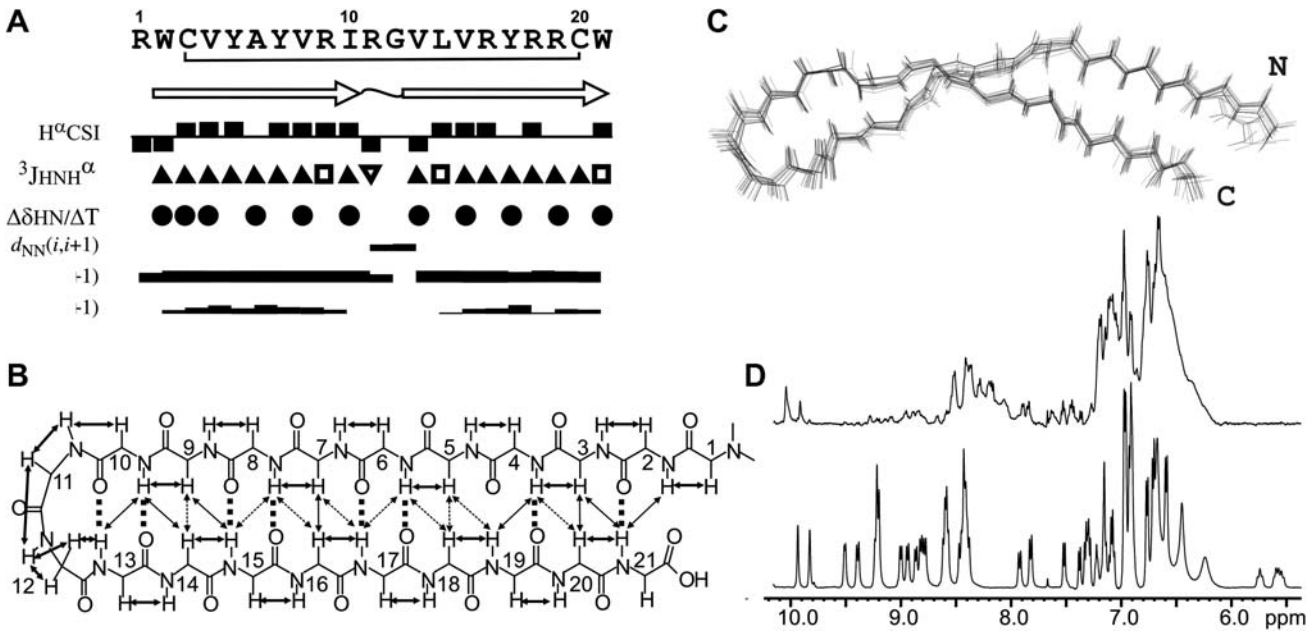

Fig. 2. Results of the NMR investigation of arenicin and its linear analogue. (A) Overview of NMR data defining the arenicin-2 secondary structure in the aqueous solution (pH 3.3, 15 °C). NOE connectivities, $^{3}J_{HNH^{\alpha}}$ coupling constants, H^{α} chemical shift indices (CSIs) and temperature gradients of amide protons (ΔδHN/ΔT) are shown versus arenicin-2 sequence. The widths of the NOE connectivities bars correspond to the relative intensity of cross-peak in the 100 ms NOESY spectrum. The large (>8.5 Hz), small (<5 Hz) and medium (others) $^{3}J_{HNH^{\alpha}}$ couplings are designated by the filled triangles, squares and open triangles, respectively. The filled circles denote amide protons with temperature gradients less than 5 ppb/K. The positive and negative CSIs values denote β-strand and α-helical propensity, respectively. Elements of secondary structure are shown on separate line, the β-strands designated by arrows and β-turn by wavy line. (B) Identification of the pairing scheme of the β-strands in arenicin-2. The observed NOE contacts are shown by arrows. (C) The set of the best 20 arenicin-2 structures, superimposed over the backbone atoms. Only backbone atoms are shown. (D) The fragments of 1D ^{1}H NMR spectra (pH 3.3, 30 °C) for arenicin-1 (lower spectrum) and its linear analogue (upper spectrum).

Structural characterization of the linear analogue of arenicin-1

Structure of the synthetic linear analogue, the reduced form of arenicin-1, was studied in aqueous solution by NMR spectroscopy. To suppress spontaneous oxidation of the disulfide, the spectra were measured at acidic pH (~3). Comparison of 1D ^{1}H NMR spectra for cyclic and linear arenicin-1 variants (Fig. 2D) indicates that reduction of disulfide disrupts the β-hairpin structure. The shift of HN and H^{α} proton signals to random coil regions (near 8.5 and 4.5 ppm, respectively) indicate that linear form of the peptide in aqueous solution are in virtually unfolded state. Moreover, several sets of additional weak signals (Fig. 2D) observed in the NMR spectrum of the reduced arenicin-1 point to the presence of conformation exchange between unfolded and partially folded states of the peptide.

Table 1
Statistics for the best CYANA structures of arenicin-2

Parameter (Unit)	Quantity	CYANA (mean ± SD)
No. of structures	Calculated	100
	Selected	20
Target function (Å^2)		0.19 ± 0.03
No.of distance constraints (upper/lower)	NOE	172
	H-bond	18/18
	SS-bond	3/3
No. of torsion angle constraints	φ	19
	χ^1	14
Upper constraint violations (Å)	Sum	0.9 ± 0.3
	Max	0.24
Lower constraint violations (Å)	Sum	0.1 ± 0.0
	Max	0.11
Van der Waals constraint violations (Å)	Sum	0.5 ± 0.1
	Max	0.18
Angle constraint violations (degree)	Sum	7.2 ± 0.8
	Max	6.1
rmsd of atomic coordinates (Å)	Backbone	0.37 ± 0.11
	Heavy atoms	1.18 ± 0.40

In summary, our results afford further insight into the possible mechanism of action of arenicins. In spite of the hydrophobicity and cationic charge +6, the significantly twisted β-hairpin structure of arenicin-2 is deprived of pronounced amphipathicity in aqueous solution. Nevertheless, the peptide demonstrates membrane activity and strongly binds to detergent micelles and anionic lipid vesicles (POPE/POPG 7:3) that mimic bacterial membrane. The results of CD investigation provide the clues to understand this contradiction. The observed changes of the arenicin-2 conformation upon micelle binding possibly induce additional amphipathicity at the peptide surface, allowing energetically favorable contacts of cationic and hydrophobic side-chains with polar head-groups and hydrophobic micelle interior, respectively. Inspection of the peptide structure reveals that simple untwisting of the β-hairpin

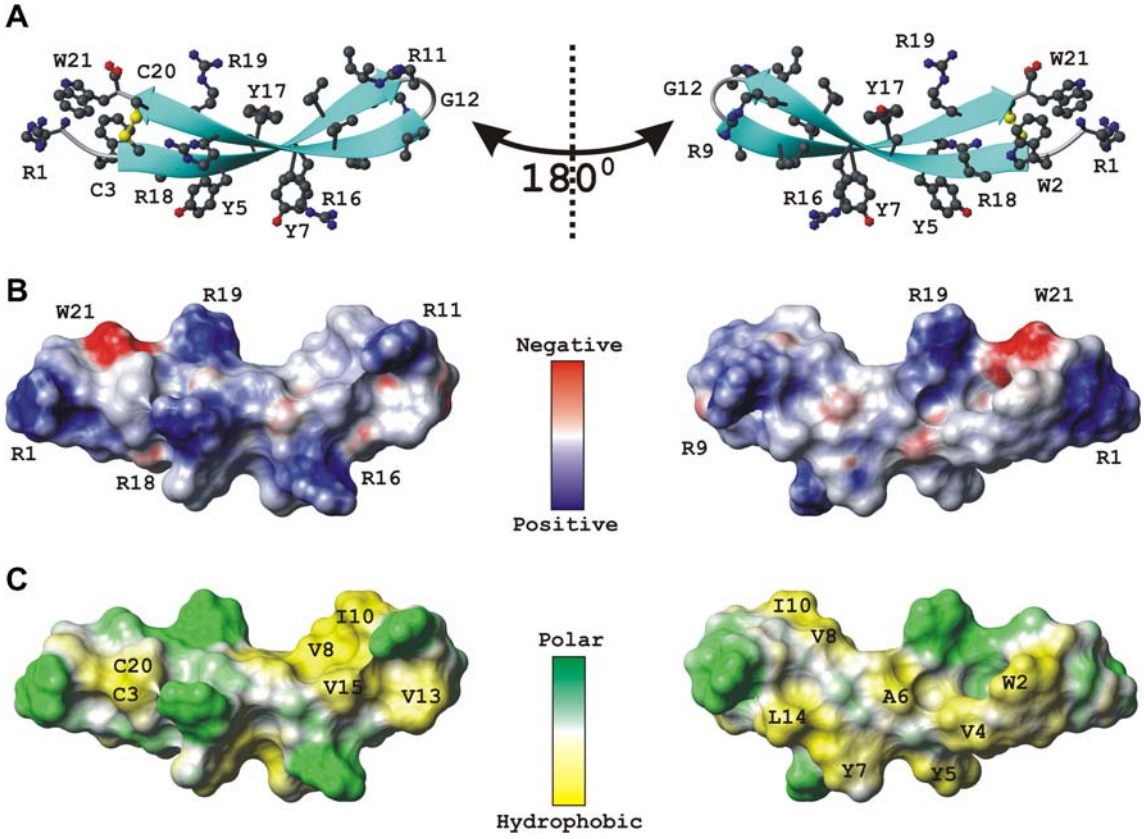

Fig. 3. Two-sided view of the arenicin-2 structure in aqueous solution. (A) Ribbon representation of the peptide. The side chains are shown in ball-and-stick representation. (B) Electrostatic potential on the arenicin-2 surface. The red and blue areas denote negative and positive regions, respectively. (C) Molecular hydrophobicity potential [20] on the arenicin-2 surface. The green and yellow areas denote polar and hydrophobic regions, respectively. The figure was prepared in MolMol [21].

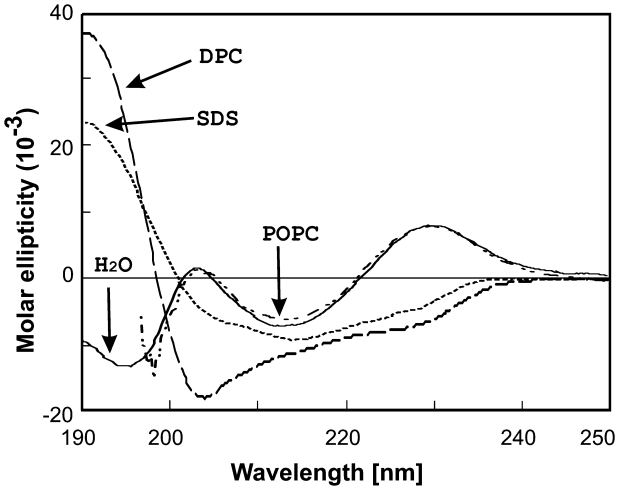

Fig. 4. CD spectra of arenicin-2 in water (0.35 mM), in DPC and SDS micelles (0.35 mM, P:D = 1:60), and in POPC vesicles (0.2 mM, P:L = 1:100). Due to light scattering, the spectrum in vesicles was measured only to 197 nm.

will induce significant amphipathicity at its surface. Arenicin is likely to undergo similar conformational rearrangements upon binding to lipid membrane. The observed inability of arenicin-2 to bind to the neutral bilayers (POPC) might underlie the peptide selectivity toward bacterial cells.

Acknowledgments

This work was supported by the Russian Foundation for Basic Research (RFBR projects 06-04-49409 and 06-04-49416), the Russian Federal Agency for Science and Innovations (state contracts Nos. 02.512.11.2037 of 19.02.2007 and 02.522.11.2007 of 11.05.2007), and the International Association for the Promotion of Cooperation with Scientists from NIS (INTAS project 03-51-4984). The authors thank Svetlana V. Kuznitsova for synthesis of the primers.

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