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Short Communication

Structures of cyclic, antimicrobial peptides in a membrane-mimicking environment define requirements for activity‡

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Abstract: New antimicrobial compounds are of major importance because of the growing problem of bacterial resistance. In this context, antimicrobial peptides have received a lot of attention. Their mechanism of action, however, is often obscure. Here, the structures of two cyclic, antimicrobial peptides from the family of arginine- and tryptophan-rich peptides determined in a membrane-mimicking environment are described. The sequence of the peptides has been obtained from a cyclic parent peptide by scrambling the amino acids. While the activity of the peptides is similar to that of the parent peptide, the structures are not. The peptides do, however, all adopt an amphiphilic structure. A comparison between the structures helps to define the requirements for the activity of these peptides. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: antimicrobial peptides; NMR spectroscopy; peptide structure; RW peptides

The identification of new antimicrobial agents has gained increasing importance in recent years, since the growing bacterial resistance to existing drugs poses a significant threat to human health [1–3]. Since the development of new antibiotics has declined, antibacterial and antifungal peptides are becoming more interesting as a potential new generation of therapeutic agents [4,5]. These peptides are characterized by an activity against a wide range of microbes. In addition, even though they are evolutionary ancient weapons of higher animals [4], resistance has rarely been reported. A drawback is their fairly low activity and that their mechanism of action is still not clear, thus preventing an improvement in activity and a further development as pharmaceutical compounds.

We have recently determined the structure of the antimicrobial peptide *cyclo*-(Arg-Arg-Trp-Trp-Arg-Phe) (c-RW) and several analogues using solution NMR spectroscopy and have described their potential interactions with a biological membrane using extensive molecular dynamic simulations [6,7]. The membrane-mimicking environment, in our case DPC micelles, leads to a change in the structure of the peptides as compared to their conformation in aqueous solution. An amphipathic structure is induced by the micelles where the hydrophobic part is formed by the aromatic side chains while the hydrophilic part is made up of the peptide backbone. The backbone faces the outside of the membrane, the guanidine groups form contacts to charged lipid head groups and the aromatic side chains protrude into the lipid chains of the membrane. To explore the effects of amino acid replacement on structure and activity, several changes have been introduced. A replacement of tryptophan by tyrosine or arginine by lysine preserves the structure of the peptide but changes the activity considerably [6,8]. Since these findings point to the importance of tryptophan and arginine, the next step was the change in sequence while preserving the type of amino acid side chain. Scrambling of the original sequence in a way that the three aromatic side chains are next to each other (*cyclo*-(Arg-Arg-Trp-Trp-Phe-Arg) (c-RW2) and *cyclo*-(Arg-Arg-Trp-Phe-Trp-Arg) (c-RW3)) did not affect the activity of the peptides [9]. Since the structure of the parent peptide in a membrane-mimicking environment was induced by the interaction of the amino acid side chains with the micelles, it was interesting to determine the structure of those two peptides with scrambled sequence as well to see whether the structures are comparable.

The structure of c-RW2 and c-RW3 were therefore determined in detergent micelles using a methodology described before using solution NMR methods [6]. The resulting structures are shown in Figure 1 in comparison with the parent peptide. While the sequence of the two peptides is scrambled as compared to the parent peptides, the only difference between the two peptides is a change in the position of Phe and Trp. The structures, however, differ from each other in that c-RW3 adopts a structure with two regular β-turns, while c-RW2 exhibits one regular turn and one irregular

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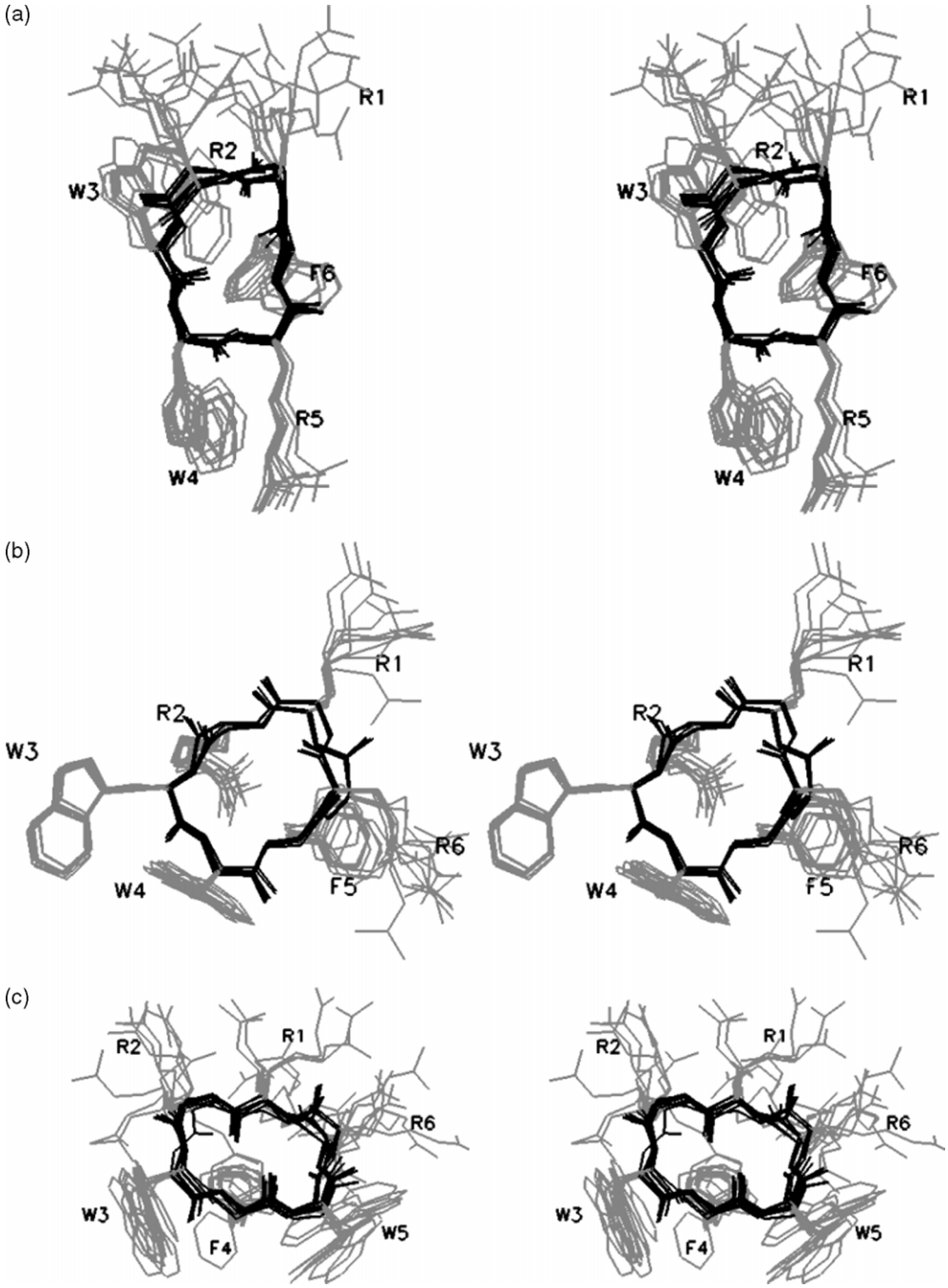

Figure 1 Structures of three tryptophane and arginine rich peptides. (a) The structure of *cyclo*-(Arg-Arg-Trp-Trp-Arg-Phe) has been determined previously; it consists of two regular turns. (b) The structure of *cyclo*-(Arg-Arg-Trp-Trp-Phe-Arg) exhibits one regular turn and one irregular structural arrangement. (c) The backbone structure of *cyclo*-(Arg-Arg-Trp-Phe-Trp-Arg) is comparable to the structure of the parent peptide (a) despite the fact that the amino acid side chains are distributed in an entirely different way.

structural arrangement. A comparison between c-RW3 and the parent peptide, c-RW, shows that both have a comparable structure but that the distribution of amino acids is quite different. Obviously the structures are dictated by the different needs of the amino acid side chains regarding space and a suitable environment.

A comparison of the hydrophobicity of the three peptides is shown in Figure 2. This shows that despite the structural differences all three peptides are amphipatic, and that the common features can be detected in the way this amphipaticity is created. Owing to the interaction of the side chains with the micelles, all the aromatic residues point in the same direction thus establishing the hydrophobic part; the arginine side chains are then distributed in a way to interact with the head groups of the membrane-mimicking compounds.

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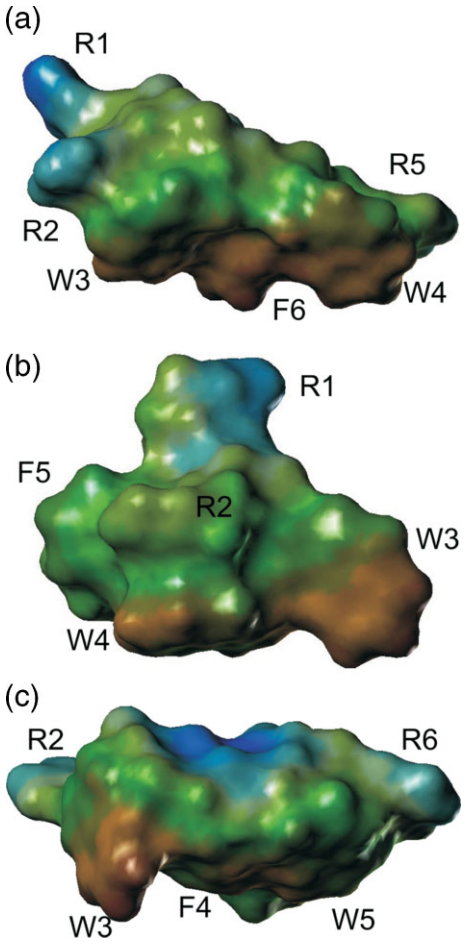

Figure 2 Comparison of the hydrophobicity of the three peptides. All three yield an amphipatic structure, which forces a change in the arrangement of the amino acid side chains and thus in the backbone structure. (a) c-RW (*cyclo*-(Arg-Arg-Trp-Trp-Arg-Phe)). (b) c-RW2 (*cyclo*-(Arg-Arg-Trp-Trp-Phe-Arg)). (c) c-RW3 (*cyclo*-(Arg-Arg-Trp-Phe-Trp-Arg)).

From those findings, we conclude that the peptide backbone merely presents the scaffold for the orientation of the side chains of the amino acids. The scaffold is flexible and will adopt to the need of the amino acid side chains to create an amphipatic molecule and allow for a proper orientation of the side chains. In addition, it can be concluded from prior experiments that the antimicrobial activity requires a sufficient number of bulky aromatic residues (such as indole rings) and guanidinium groups. On the basis of these conclusions, we were able to design an antimicrobial compound with similar activity as the original peptide by using a simpler scaffold that is capable of positioning the amino acids in a proper manner [10].

In conclusion, we have determined the structure of two antimicrobial peptides determined using solution NMR spectroscopy. The peptides have been derived from a parent peptide by scrambling the sequence of the peptide. While all three peptides exhibit similar acitivity, they show differences in structure. Similarities, however, can be found when comparing the amphipaticity and the orientation of the side chains. This reinforces our prior conclusion that the peptidic nature of the compounds is irrelevant but that the amphipatic nature and a proper orientation of the side chains or arginine and tryptophan are sufficient for the activity.

MATERIALS AND METHODS

The synthesis of the peptides and the test of their activity has been described previously [8,9]. For the preparation of the samples used for NMR spectroscopy, the peptides were dissolved in H_2O/D_2O (9 : 1, 600 µl) to yield a final sample concentration of 2.5 mM. Perdeuterated DPC (purchased from Cambridge Isotope Laboratories, Inc., Andover, MA) was added from a stock solution to yield a final concentration of 50 mM and pH 6.3.

All the NMR spectra were recorded on a Bruker DRX600 spectrometer. DQF-COSY [11], TOCSY (14, 28, 56 and 128 ms mixing time) [12], and NOESY (mixing time 80 ms) [13], were recorded at 300 K. Water suppression was achieved using a WATERGATE sequence [14]. The number of data points in F_2 and F_1 dimension were 4096 and 512, respectively. Spectra were multiplied by a squared cosine function and zero-filled to 4K × 2K using XWIN-NMR (Bruker, Karlsruhe, Germany).

The 2D NMR spectra were evaluated using SPARKY [15]. NOESY cross-peaks were fitted with a gaussian function and interproton distances were derived from the peak volumes. Upper and lower restraint boundaries were obtained by defining a tolerance for the calculated distances, ±0.7 Å. Restraint corrections were applied for pseudoatom assignments. Prochiral assignments for β-protons were derived as described by Wagner [16]. Structures were calculated by molecular dynamics using Amber 6.0 [17]. The simulated annealing protocol included an unrestrained high-temperature step for randomization of the initial structure. Restraints were applied in an additional high-temperature stage. The simulated annealing was concluded by restrained cooling and energy minimization. Out of 100 runs the ten lowest-energy structures were kept as final structures. For structural analysis, the program MOLMOL was used [18]. The average of the ten lowest-energy structures was calculated and the one with the lowest RMS deviation from the average was chosen as the representative structure. The lipophilic potential surfaces were created using Sybyl 6.9 (Tripos Inc., St Louis, Missouri, USA).

The structures have been deposited in the PDB (c-RW2: 2OX2, c-RW3: 2OTQ).

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