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Solvent-dependent structure of two tryptophan-rich antimicrobial peptides and their analogs studied by FTIR and CD spectroscopy

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Abstract

Structural changes for a series of antimicrobial peptides in various solvents were investigated by a combined approach of FTIR and CD spectroscopy. The well-characterized and potent antimicrobial peptides indolicidin and tritrpticin were studied along with several analogs of tritrpticin, including Tritrp1 (amidated analog of tritrpticin), Tritrp2 (analog of Tritrp1 with Arg→Lys substitutions), Tritrp3 (analog of Tritrp1 with Pro→Ala substitutions) and Tritrp4 (analog of Tritrp1 with Trp→Tyr substitutions). All peptides were studied in aqueous buffer, ethanol and in the presence of dodecylphosphocholine (DPC) micelles. It was shown that tritrpticin and its analogs preferentially adopt turn structures in all solvents studied. The turn structures formed by the tritrpticin analogs bound to DPC micelles are more compact and more conformationally restricted compared to indolicidin. While several peptides showed a slight propensity for an α-helical conformation in ethanol, this trend was only strong for Tritrp3, which also adopted a largely α-helical structure with DPC micelles. Tritrp3 also demonstrated along with Tritrp1 the highest ability to interact with DPC micelles, while Tritrp2 and Tritrp4 showed the weakest interaction.
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Keywords: Antimicrobial peptide; CD spectroscopy; FTIR spectroscopy; Indolicidin; Tritrpticin

1. Introduction

Extensive worldwide use of conventional antibiotics over the last few decades has resulted in the emergence of new strains of bacteria that are resistant to most and sometimes even all available antibiotics [1]. As a result, the development of new classes of antibiotics, which can overcome such resistance, has become an increasingly important issue. In this context, antimicrobial peptides have garnered significant interest in recent years. These peptides play a major role in the innate immune system of all living organisms, including vertebrates, insects, bacteria and plants (e.g. [1–5]). It has been shown that antimicrobial peptides exhibit significant activity against a broad spectrum of microbial organisms, including Gram-positive and Gram-negative bacteria, protozoa, fungi and, in some cases, enveloped viruses as well as virally infected cells and tumor cells (e.g. [3]). However, they exhibit a broad range of activity that in some cases unfortunately includes healthy eukaryotic cells like the erythrocytes (e.g. [2,4,6,7]). The hemolytic and general cytotoxic activity of antimicrobial peptides is a drawback for future clinical applications and many studies are aimed at improving their therapeutic indices [8].

Despite the wide variety of structures, antimicrobial peptides have several common features: they are generally composed of up to 60 amino acid residues, they are highly cationic, they are hydrophobic and amphiphatic, and in most cases they are membrane active [4]. The mechanism of action of antimicrobial peptides is still a subject of considerable debate. It was originally believed that most antimicrobial peptides act by disrupting bacterial membranes either by forming pores or by dissolving the membrane in a detergent-like manner [2,4,6]. This conclusion is supported by the fact that most of antimicrobial peptides are amphiphatic containing cationic and hydrophobic elements, which enables them to interact with or insert into negatively charged bacterial membranes. However, it has recently been reported that several peptides show no direct correlation between antimicrobial activity and

Abbreviations: Indo, indolicidin; Trp, tritrpticin; FTIR, Fourier transform infrared spectroscopy; ATR, attenuated total reflectance; CD, circular dichroism; TFE, trifluoroethanol; DMSO, dimethyl sulphoxide; DPC, dodecylphosphocholine; TFA, trifluoroacetate

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their ability to disrupt a membrane. Therefore, it has been suggested that some antimicrobial peptides can spontaneously enter cells and interact with DNA, proteins or other intracellular targets and thus interfere with macromolecular synthesis (e.g. [2,9–14]).

Based on the secondary structure, antimicrobial peptides can be categorized into four major groups: β-sheet structures stabilized by two or three disulfide bridges, α-helices, extended structures with a predominance of one or more amino acids, and loop structures containing only one disulfide bridge [3,10]. In the present study we focused on two peptides falling into the category of peptides with a predominance of one or more amino acids, namely indolicidin and tritrpticin.

Both indolicidin and tritrpticin are relatively short peptides consisting of only 13 residues (Table 1) and both are members of the cathelicidin family of peptides, which are synthesized as larger precursor molecules in bone marrow [15]. Another common feature of both peptides is the very high content of tryptophan (39% in indolicidin and 23% in tritrpticin) and arginine residues (15% and 31%, respectively). Both peptides also possess 3 and 2 proline residues, respectively (Table 1).

Indolicidin was first isolated from the cytoplasmic granules of bovine neutrophils [16]. Tritrpticin is thought to be released from a cathelicidin found in porcine leukocytes [17]. Both peptides have a broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi [16–18] and in the case of indolicidin activity against protozoa has also been observed [19]. Unfortunately, indolicidin and tritrpticin exhibit relatively high hemolytic activity (e.g. [20–22]). Therefore, over the last decade much effort was directed towards investigating their secondary structure, antimicrobial and hemolytic activity and elucidating the mechanism of action for indolicidin (e.g. [9,13,20,23–25]) and tritrpticin [14,21,26–28]. The NMR solution structures have been solved for both indolicidin [24] and tritrpticin [26] and significant progress has been made in understanding the role of each amino acid residue on the activity of indolicidin by means of selected substitutions of one residues [9,22,29–31]. However, much less is known about the role of particular amino acid residues in tritrpticin and only a few studies have been published in this matter [21,27,32]. Recently, the solution NMR structures for an extensive set of tritrpticin analogs with various amino acid substitutions have been solved and antimicrobial and hemolytic activity have been determined in our laboratory [14] (Table 1). We are currently expanding this investigation by employing FTIR and CD spectroscopy to investigate the secondary structure of tritrpticin and its analogs Tritrp1, Tritrp2, Tritrp3 and Tritrp4 (Table 1) in water, ethanol, TFE, DMSO and in an aqueous solution containing DPC micelles in order to determine the range of the possible conformations in different membrane mimetic conditions. Finally, we also included in our studies indolicidin as a control peptide considering that extensive information on its structure in various environments is available from the literature.

Table 1
Sequences and net charges for the six peptides used in the study

Peptide	Sequence	Net charge
Indolicidin	ILPWKWPWWPWRR-NH_2	+4
Tritrpticin	VRRFPWWWPFLRR-COO^-	+4
Tritrp1	VRRFPWWWPFLRR-**NH_2**	+5
Tritrp2	V**KK**FPWWWPFL**KK-NH_2**	+5
Tritrp3	VRRF**A**WWW**A**FLRR-**NH_2**	+5
Tritrp4	VRRFP**YYY**PFLRR-**NH_2**	+5

The substitutions in tritrpticin analogs are indicated in bold.

Both FTIR (e.g. [33–43]) and CD (e.g. [44–46]) spectroscopy have been widely used to obtain information on protein and peptide secondary structure. While these techniques cannot provide 3-dimensional structures of the molecules at atomic resolution, they provide an overview of the secondary structures of peptides and proteins. In addition, a significant advantage of FTIR spectroscopy is the ability to use a wide variety of solvents, including micelles and lipid vesicles, whereas the use of CD spectroscopy is limited due to light scattering. Finally, FTIR often allows simultaneous detection of the changes both in the peptide secondary structure and in the side chain interactions.

Organic solvents have been extensively used for structural characterization of peptides and proteins [36,47–49]. Ethanol and trifluoroethanol (TFE) are known to induce helical structures in proteins and peptides if they possess a propensity to form helices [36,44,49–51]. Dimethyl sulphoxide (DMSO) is known to disrupt the secondary structure of peptides and proteins due to the formation of hydrogen bonds between the peptide N–H groups and S=O groups of DMSO, which are stronger hydrogen bond acceptors than C=O peptide groups [36,47,48,52]. Therefore, FTIR studies of peptides in DMSO allow a determination of the solvent accessibility as well as the presence of any strong intramolecular hydrogen bonds in the peptides, which cannot be disrupted by interaction with DMSO (e.g., in cyclic structures) [47,48].

Frequently, aqueous suspensions of micelles are used to mimic a membrane. The most widely used systems are dodecylphosphocholine (DPC) and sodium dodecylsulfate (SDS) [21,24,26,53]. While SDS micelles are negatively charged they can be used to mimic the negatively charged bacterial membranes, whereas DPC micelles are zwitterionic, thus modelling mammalian cells. DPC and SDS micelles are also well suited membrane models for NMR experiments due to their relatively small size [14,24,26].

The main objective of the present study is to establish the range of possible peptide structural variations depending on the environment. Unlike some of the cyclic peptides used in previous studies of peptide–membrane interactions (e.g., gramicidin S [7]), which do not undergo any conformational changes upon the interaction with lipid bilayer and all spectral changes arise from the actual peptide–lipid interaction, the peptides used in the present investigation can change their structure under the influence of factors such as solvent polarity, ability to form H-bonding and other environmental effects. Structural change can also occur upon peptide partitioning into lipid bilayer. Thus, we need to know the range of possible variations in the peptide structure before attempting any peptide–lipid interaction studies. Knowing the range of possible

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