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Conformational study of melectin and antapin antimicrobial peptides in model membrane environments

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ABSTRACT

Antimicrobial peptides have long been considered as promising compounds against drug-resistant pathogens. In this work, we studied the secondary structure of antimicrobial peptides melectin and antapin using electronic (ECD) and vibrational circular dichroism (VCD) spectroscopies that are sensitive to peptide secondary structures. The results from quantitative ECD spectral evaluation by Dichroweb and CDNN program and from the qualitative evaluation of the VCD spectra were compared. The antimicrobial activity of the selected peptides depends on their ability to adopt an amphipathic α-helical conformation on the surface of the bacterial membrane. Hence, solutions of different zwitterionic and negatively charged liposomes and micelles were used to mimic the eukaryotic and bacterial biological membranes. The results show a significant content of α-helical conformation in the solutions of negatively charged liposomes mimicking the bacterial membrane, thus correlating with the antimicrobial activity of the studied peptides. On the other hand in the solutions of zwitterionic liposomes used as models of the eukaryotic membranes, the fraction of α-helical conformation was lower, which corresponds with their moderate hemolytic activity.

1. Introduction

The increasing resistance of bacteria to the currently used antibiotics is a global problem, which complicates the treatment of infectious diseases. This situation requires a search for alternative antimicrobial agents which kill bacteria with fundamentally different modes of action than traditional antibiotics [1,2]. Among these agents, antimicrobial peptides represent a family of anti-infective compounds that have been studied as potential candidates to fight bacterial infection for the recent decades [3,4].

Antimicrobial peptides are in fact short chains of amino acids (mostly 12–50 residues) and are widely found in the animal and plant kingdom [5]. They are differentiated on the basis of various aspects, their secondary structure being one of the most common. The most studied compounds include linear α-helical antimicrobial peptides, for instance melittin [6], cecropins [7], dermaseptins [8], magainins [9], cathelicidins [10] and also short peptides isolated in our laboratory from the venom of various bees, such as melectin [11–13], lasioglossins [14,15], panurgines [16], antapin [17], and others. The antimicrobial activity of these cationic peptides depends on their ability to adopt an amphipathic α-helical conformation in a bacterial membrane environment which is a result of their attraction to the negatively charged surface of bacterial membrane through electrostatic interactions. The infiltration of peptides into the lipid bilayer of the membrane and the disruption of the membrane structure which follows [18] leads to the leakage of cytoplasmic components and to the cell death [1,2,19,20].

In the present work, we focused on secondary structure of a previously described antimicrobial peptide melectin [11] (MEP) and of a newly identified peptide antapin [17] (ANTP) in different membrane-mimicking environments. The MEP peptide consisting of 18 amino acid residues (H-Gly-Phe-Leu-Ser-Ile-Leu-Lys-Lys-Val-Leu-Pro-Lys-Val-Met-Ala-His-Met-Lys-NH_2) was isolated from the venom of the cleptoparasitic bee *Melecta albifrons* [11] and the ANTP peptide consisting of 19 amino acids (H-Gly-Leu-Leu-Ser-Ala-Leu-Arg-Lys-Met-Ile-Pro-His-Ile-Leu-Ser-His-Ile-Lys-Lys-NH_2) was isolated from

Abbreviations: ANTP, antapin; D_2O, deuterium oxide; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol); DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol); ECD, electronic circular dichroism; FT-IR, Fourier transform infrared; IR, infrared; LUV, large unilamellar vesicles; MEP, melectin; SDS, sodium dodecyl sulfate; SPM, sphingomyelin; TFE-d_3, deuterated trifluoroethanol; VCD, vibrational circular dichroism.

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another wild bee *Anthophora plumipes* [17]. The presence of a Pro residue in position 11 makes these two cationic peptides structurally unique due to the Pro-kink [11] introduced to their α-helical structure, which influences their biological activities as was already described for MEP [11]. Besides of this structural peculiarity, both peptides contain the same amino acids at specific conservative positions similarly to different well described α-helical antimicrobial peptides [21]: Gly-1, Ser-4, Leu-6, Lys-8, and His and Lys residues localized towards the C-terminus. The amino acid sequence of both MEP and ANTP is beneficial to the formation of amphipathic α-helical structures with hydrophobic and hydrophilic faces, which is an important property that also represents the basis for their lytic activity.

Generally, the activity of antimicrobial peptides strongly depends on the composition of biological membranes. While bacterial membranes are composed predominantly of negatively charged phospholipids (phosphatidylglycerol, phosphatidylserine, cardiolipin), eukaryotic membranes consist of zwitterionic lipids (phosphatidylethanolamine, phosphatidylcholine, sphingomyelin) and cholesterol [1,22]. The antimicrobial activity of peptides is determined against a set of different bacteria, while their toxicity on eukaryotic cells is commonly tested using erythrocytes and is expressed as hemolytic activity [11,14–16,23,24]. The high hemolytic activity of antimicrobial peptides is obviously undesirable. Hence, the general aim is to find such a peptide, which would exhibit high antimicrobial and simultaneously low hemolytic activity [5,21]. The MEP and ANTP peptides have previously shown antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [11,17]. MEP also showed low and ANTP moderate hemolytic activity [11,17]. As the level of biological activities of antimicrobial peptides depends on the content of α-helical conformation [25–28] when interacting with the membrane, we focused on MEP and ANTP secondary structure in several different solutions of liposomes and micelles mimicking biological membranes. Moreover, we considered several other parameters, such as peptide's cationicity, hydrophobicity and amphipathicity, which also influence the peptide interactions with biological membranes. Our membrane-mimicking models were designed to reflect the composition of eukaryotic and bacterial cell membranes. The eukaryotic membranes were simulated by the liposomes made from zwitterionic 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and DOPC/cholesterol. The liposomes simulating bacterial membrane were prepared from the mixture of negatively charged dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DMPG) and zwitterionic DOPC, and from 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) with 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DOPG). Zwitterionic sphingomyelin (SPM) liposomes were used as a simple model of the nerve cell membrane. In addition, we analyzed the secondary structure of MEP and ANTP in negatively charged sodium dodecyl sulfate (SDS) micelles and in an aqueous solution of trifluoroethanol (TFE), which is known to promote the α-helical conformation in peptides [29].

The conformational study of these two peptides was carried out using electronic (ECD) and vibrational circular dichroism (VCD). These spectroscopic techniques are inherently sensitive to molecular chirality [21] and sensitively reflect peptide secondary structure as different secondary structures are characterized by different spectral patterns in both the VCD and ECD spectra [12,13,15,30,31]. ECD has long been established as one of the main spectroscopic techniques to study the solution structure of antimicrobial peptides and is in fact generally used in peptide and protein structural studies. As such, methods to quantitatively estimate the content of different conformations have been developed based on a database of ECD spectral sets and mathematical algorithms. On the other hand, the use of VCD spectroscopy for the studies of antimicrobial peptides is quite new, especially in the studies with membrane-mimicking environments. Therefore, we have employed both techniques to compare the results from the well-established ECD spectroscopy with the qualitative observations from VCD to see whether this technique with generally better resolution of different secondary structures can be applied in such studies.

2. Materials and methods

2.1. Materials

The lipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) sodium salt (DOPG) and sphingomyelin (SPM) were purchased from Avanti Polar Lipids (USA). The 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) sodium salt (DMPG) was purchased from CordenPharma (Switzerland). The sodium dodecyl sulfate (SDS) surfactant and cholesterol were purchased from Sigma Aldrich (Germany). The antimicrobial peptides MEP and ANTP were synthesized, purified and characterized in our laboratory as described previously [11,17]. Their purity and identity were verified by HPLC and mass spectroscopy, respectively.

2.2. Preparation of liposomes and micelles

A solution with the SDS concentration of 160 $g \cdot L^{-1}$, which is higher than its critical micellar concentration, was used as a solution of the SDS micelles. The appropriate amount of SDS was dissolved in a phosphate buffer (10 $mmol \cdot L^{-1}$, pH 7.5), vortexed and used as such.

The liposomes were prepared by standard procedures [32,33]. The appropriate amount of a dried lipid was weighed and dissolved in chloroform (analytical grade, Lach-Ner, Czech Republic) or a chloroform/methanol (analytical grade, Penta, Czech Republic) mixture (2:1, v/v). The solvent was evaporated on a rotatory evaporator and remaining solvent molecules were removed under high vacuum (5 mbar), where the sample was left at room temperature for additional 4 h. Throughout this process, a thin film was obtained. The film was hydrated by the addition of a phosphate buffer (10 $mmol \cdot L^{-1}$, pH 7.5) and vortexed extensively to obtain a solution of multilamellar liposomes. The liposomes were unified to large unilamellar vesicles (LUV) in the extrusion process by passing twenty-three times through a polycarbonate membrane with the mean pore diameter of 100 nm (Whatman, USA) using a Mini-Extruder (Avanti Polar Lipids, USA). The concentration of lipids in the resulting solution was 100 $g \cdot L^{-1}$.

The dynamic light scattering method (ZetaSizer Nano-ZS, Malvern Instruments, USA) confirmed a quite narrow size distribution of the prepared LUVs with the maximum at 123 ± 2 nm. After the extrusion, the LUVs were allowed to equilibrate for at least 2 h before use.

To test the scattering effects, larger DOPC liposomes of 150 ± 9, 210 ± 10 and 412 ± 12 nm were prepared by extrusion and smaller DOPC liposomes of 80 ± 8, 52 ± 8 and 38 ± 6 nm were prepared by sonication.

2.3. Preparation of peptide samples

The purified antimicrobial peptides were obtained as salts with a trifluoroacetate anion, which was unsuitable for the VCD measurements due to a strong absorption of trifluoroacetate ions in the region overlapping the peptide signal (amide I′). Hence, the anion needed to be exchanged to hydrochloride before the spectral measurements. Peptides were therefore dissolved in a 0.1 $mol \cdot L^{-1}$ HCl solution (analytical grade, Lach-Ner, Czech Republic), frozen and lyophilized. The procedure was repeated two times.

The peptide samples (50 $g \cdot L^{-1}$) were prepared by dissolving the peptide in an appropriate amount of liposomal solution, deuterium oxide (D_2O, 99.9% D, Merck KGaA, Germany) or a 40% solution of deuterated trifluoroethanol (TFE-d_3, 99.5% D, Sigma, Germany) in D_2O. Typically 2.5 mg of the peptide was dissolved in 50 μL of the solvent. The samples with the SDS micelles were prepared first by dissolving the peptide in a phosphate buffer (10 $mmol \cdot L^{-1}$, pH 7.5, 25 μL) and then the stock solution of the SDS micelles was added (25 μL) to obtain the solution of peptide concentration of 50 $g \cdot L^{-1}$. Such samples were used to measure the ECD, VCD and IR spectra.