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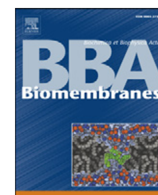

Membrane interactions and dynamics of a 21-mer cytotoxic peptide: A solid-state NMR study

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ABSTRACT

We have investigated the membrane interactions and dynamics of a 21-mer cytotoxic model peptide that acts as an ion channel by solid-state NMR spectroscopy. To shed light on its mechanism of membrane perturbation, ^{31}P and ^{2}H NMR experiments were performed on 21-mer peptide-containing bicelles. ^{31}P NMR results indicate that the 21-mer peptide stabilizes the bicelle structure and orientation in the magnetic field and perturbs the lipid polar head group conformation. On the other hand, ^{2}H NMR spectra reveal that the 21-mer peptide orders the lipid acyl chains upon binding. ^{15}N NMR experiments performed in DMPC bilayers stacked between glass plates also reveal that the 21-mer peptide remains at the bilayer surface. ^{15}N NMR experiments in perpendicular DMPC bicelles indicate that the 21-mer peptide does not show a circular orientational distribution in the bicelle planar region. Finally, ^{13}C NMR experiments were used to study the 21-mer peptide dynamics in DMPC multilamellar vesicles. By analyzing the ^{13}CO spinning sidebands, the results show that the 21-mer peptide is immobilized upon membrane binding. In light of these results, we propose a model of membrane interaction for the 21-mer peptide where it lies at the bilayer surface and perturbs the lipid head group conformation.

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1. Introduction

The study of membrane-active peptides has become the focus of many research groups since they represent potential antimicrobial agents acting as substitutes to ineffective antibiotics for which bacteria have developed resistance mechanisms [1–5]. Part of the defense system of many living organisms, natural membrane-active peptides share common properties such as their small size, and their cationic and amphipathic characters, the latter being essential to their interactions with amphipathic lipid bilayers [6–9]. When interacting with membranes, antimicrobial peptides can adopt different secondary structures, such as α-helix, β-sheet, and extended conformations [10,11], and their membrane interactions can be modulated by varying peptide structural parameters such as helicity, charge, hydrophobicity, and amphipathicity [7,12].

Most of antimicrobial peptides possess a broad spectrum of activity, i.e., acting on viruses, bacteria, and fungi [6,9,13]. However, peptides such as melittin, magainin, lactoferricin B, cecropin B, and citropin 1.1 show cytotoxic properties against mammalian cells by lysing erythrocytes and killing cancer cells [14–21]. Based on extensive studies of natural and synthetic membrane-active peptides, general modes of action are reported in the literature, namely, the carpet-like, the barrel-stave, and the toroidal models [22–25]. Because these mechanisms of membrane perturbation are not fully understood and still under debate, the focus is then turned on the studies of antimicrobial peptides in interaction with model and biological membranes to get more information on the interactions underlying these mechanisms [1,24,26,27]. A better understanding of how membrane-active peptides interact with bilayers opens the way to the design of novel membrane-active agents that possess the desired specific biological action, being cytotoxic and/or antimicrobial [28–30].

Inspired by the great potentials of membrane-active peptides as novel therapeutic agents, many research groups have focused on the design of synthetic ion channels with the aim to better understand both structural features and the types of interactions involved in the channel activity [17,31–33]. In this way, we have designed and synthesized a synthetic helical amphipathic peptide composed of 21 amino acids [34–36]. The 21-mer peptide is a trimer of a repeating unit of five leucine residues and two synthetic 21-crown-7-phenylalanines judiciously positioned so that the hydrophilic crown ethers align on one side of the hydrophobic helix under a helical conformation, giving rise to an amphipathic peptide structure (Fig. 1).

Previous studies revealed that the 21-mer peptide acts as an artificial ion channel when incorporated into lipid bilayers [35,36]. To shed light on its membrane properties, we have studied the interactions between the 21-mer peptide and model membranes by solid-state nuclear magnetic resonance (NMR) spectroscopy. Solid-state NMR is a well-suited technique to study both the lipid bilayers and peptides by taking advantage of the orientational dependence of

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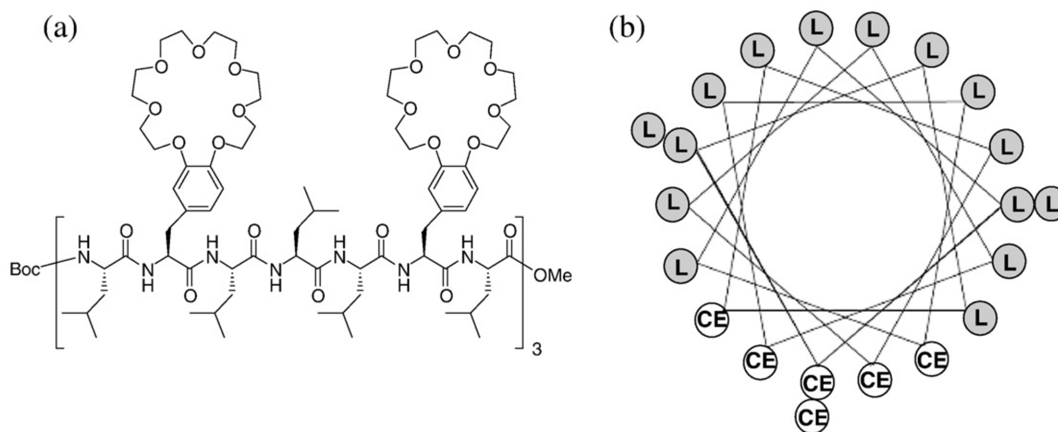

Fig. 1. (a) Diprotected 21-mer peptide structure used in this study. (b) Edmundson wheel projection showing the amphipathic character of the 21-mer peptide under a helical conformation.

anisotropic nuclear spin interactions in the static mode [37–39]. Alternatively, magic-angle spinning (MAS) is commonly used to obtain high-resolution NMR spectra that allow the study of peptide structure and dynamics [40–42]. More specifically, we report the membrane interactions of the 21-mer peptide with oriented model membranes. Static ^{31}P NMR spectroscopy has been used to determine the magnetic orientation of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) bicelles, and the lipid polar head group conformation upon the 21-mer peptide binding, whereas static ^{2}H NMR experiments have been used to investigate DMPC and DPPC lipid acyl chain orientational order. Subsequently, ^{15}N NMR experiments have been performed on both DMPC bicelles and bilayers stacked between glass plates to determine both the peptide rotational diffusion and membrane orientation. Finally, ^{13}C NMR experiments have allowed the study of the 21-mer peptide dynamics when incorporated to multilamellar DMPC vesicles by the analysis of spinning sideband intensity. The results are in agreement with a model of membrane interaction where the helicoidal peptide binds parallel to the bilayer surface, does not show a circular orientational distribution around the bilayer normal, and perturbs the lipid polar head group conformation.

2. Materials and methods

2.1. Materials

DMPC with protonated and deuterated (d_{54}) acyl chains, dihexanoylphosphatidylcholine (DHPC), and DPPC were purchased from Avanti Polar Lipids (Alabaster, AL) and used without purification. Oxime resin was prepared by a standard procedure using polystyrene beads (100–200 mesh 1% DVB, Advanced ChemTech, Louisville, KY) [43]. Resins with substitution levels around 0.5 mmol/g of oxime group were used. Boc-protected amino acids were purchased from Advanced ChemTech (Louisville, KY). The ^{15}N and ^{13}CO leucine residues were purchased from Cambridge Isotope Laboratories (Andover, MA). All solvents were Reagent, Spectro, or HPLC grade quality purchased commercially and used without any further purification except for DMF (degassed with N_2), dichloromethane (distilled), and diethyl ether (distilled from sodium and benzophenone). Water used throughout the studies was distilled and deionized using a Barnstead NANOpurII system (Boston, MA) with four purification columns. All other reagents were purchased from Sigma Aldrich Co. (Milwaukee, WI).

2.2. Peptide synthesis

The unlabeled and ^{15}N, ^{13}C-labeled diprotected 21-mer peptides (Fig. 1a) were synthesized and purified according to published procedures [34]. The N-terminal region of the 21-mer peptide is Boc protected while the C-terminal region is acetylated (Fig. 1a). Previous membrane activity studies have been done on various 21-mer peptides with different N- and C-terminal groups, and the results indicate that the N-Boc and C-acetylated 21-mer peptide show the greatest ion channel activity (Biron et al., unpublished results).

2.3. Sample preparation

2.3.1. Bicelles

Bicelle samples were prepared by mixing 20 mg of long-chain phospholipids (DMPC or DPPC) with DHPC to achieve the desired long-chain:short-chain phospholipid ratios ($q = 3.5$ for DMPC, $q = 3.0$ for DPPC) before sample hydration [44]. For ^{2}H NMR experiments, 5 mg of deuterated DMPC-d_{54} and DPPC-d_{62} was used to replace 5 mg of undeuterated lipids. A total of 80 μL of deuterium-depleted water was used, giving a total proportion of 20% (w/w) lipids in water. This corresponds to 155 water molecules per lipid molecule and such system can be qualified as fully hydrated. The pH was ~6.5 in all samples. The 21-mer peptide was added after the bicelle formation and hydration in a lipid/peptide molar ratio of 60:1. The bicelle samples then underwent at least three freeze (liquid N_2)/thaw (37 °C for DMPC; 50 °C for DPPC)/vortex shaking cycles, and were stored at −20 °C until analysis. Three additional freeze/thaw/vortex cycles were performed before the acquisition of NMR spectra.

2.3.2. Oriented bilayers stacked between glass plates

The DMPC bilayers were prepared by dissolving 30 mg of phospholipids in 120 μL of chloroform, and the solution was deposited onto 18 thin cover glasses. The glass plates were allowed to dry in air for 24 h, and then stacked and hydrated with deionized water in a closed chamber for at least 24 h at 70 °C. Subsequently, the plates were wrapped in Parafilm before use. For the preparation of the peptide-containing bilayers, the dry 21-mer peptide was co-dissolved with dry lipids in chloroform at a lipid/peptide molar ratio of 60:1. Since the lipids found in natural membranes are in the fluid phase, the NMR spectra of mechanically oriented bilayers have been obtained at a temperature above the lipid phase transition, i.e., at 37 °C for DMPC bilayers.

2.3.3. Multilamellar vesicles

Multilamellar vesicles were prepared by co-dissolving 30 mg of DMPC and 10 mg of doubly labeled [^{15}N]Leu$_{15}$-[^{13}CO]Leu$_{18}$-21-mer peptide in chloroform to ensure homogeneization of the lipid/peptide mixture. The solvent was removed under nitrogen gas, followed by storage under vacuum overnight to remove all traces of organic solvent. The dry sample was hydrated with 46 μL of deionized water

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