Structure and interactions of magainin antibiotic peptides in lipid bilayers: A solid-state nuclear magnetic resonance investigation

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INTRODUCTION

Many organisms use antibiotic peptides as a defense mechanism, thereby supplementing or replacing the immune system (1). Magainins are a family of 21 to 26 residue peptides found in the skin and other organs of frogs (2, 3) with a broad spectrum of antibacterial, antifungal, and tumorcidal activity (2–4). They disrupt the electrochemical ionic gradient across cell membranes, probably by forming oligomeric peptide ion channels (4–6). Whereas these peptides show strong interactions with bacterial and acidic model membranes (2, 7, 8), they do not lyse formed circulating vertebrate blood cells (3, 4). Multidimensional solution nuclear magnetic resonance (NMR) experiments show that magainin is unstructured in aqueous solution and exhibits a high α-helix content in trifluoroethanol/water (9) and in micelles (Shon, K., M. Zasloff, and S. J. Opella, unpublished data). Helical wheel analysis indicates that such a helix is amphipathic. Solid-state NMR spectroscopy was used to study the structure, dynamics and interactions of magainins associated with lipid bilayers.

MATERIALS AND METHODS

Specifically ^{15}N or ^{2}H labeled magainin 2s with the following sequence GIGKFLHSAKKFGKAFVGEIMNSamide were synthesized by means of solid-phase peptide synthesis (t-BOC chemistry). Peptides, buffered to neutral pH, and phospholipids (Avanti Polar Lipids, Alabaster, AL) were cosolubilized in organic solvent, dried onto cover glasses (11 × 22 mm), and equilibrated at 93 to 98% relative humidity (rh). The resulting bilayers were oriented by stacking the glass plates on top of each other. A flat-coil double-resonance probe enabled NMR spectroscopy of oriented membrane samples at high sensitivity (10). Proton-decoupled ^{15}N-NMR spectra were obtained with the help of a cross-polarisation MOIST sequence (11). Deuterium NMR spectra were acquired by using a 90_{o}-τ-90_{π}-τ pulse echo sequence with phase cycling and quadrature detection.

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Abbreviations used in this paper: NMR, nuclear magnetic resonance; POPC, 1-polmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol.

RESULTS AND DISCUSSION

Magainins, ^{15}N labeled at single sites between residues 2 and 20, were oriented in model membranes of variable lipid composition and the ^{15}N-NMR spectra recorded (Fig. 1). All samples investigated exhibit amide resonances at the highfield end of the chemical shift range indicating that the magainin α-helix is oriented parallel to the bilayer surface (±35°) and extends over major parts of the bilayer-associated polypeptide. This is in contrast to M2δ, the amphipathic pore-forming fragment of the nicotinic acetylcholine receptor channel, that exhibits a "classical" transmembrane alignment with its amphipathic helix axis parallel to the bilayer normal (12). Membrane lipid composition has a small effect on magainin structure. The ^{15}N resonance exhibits a small downfield shift in the presence of 50 mol% cholesterol compared with pure phospholipid bilayers.

Addition of magainin to phospholipid membranes containing $^{2}H_{54}$-DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) causes the width of the lipid deuterium NMR spectra to decrease in a concentration dependent manner (Fig. 2, *A* and *B*). This decrease in order parameter of the lipid fatty acyl chains indicates intercalation of the peptide into the bilayer. Distinct changes in the magainin-lipid interactions can be observed at cholesterol concentrations ≥ 40 mol % (Fig. 2, *C* and *D*), suggesting a more superficial association of magainin with these membranes.

Fig. 3 depicts magainin and M2δ peptides in their orientation parallel or perpendicular to the bilayer surface, respectively. Whereas only one subunit of each peptide is shown, they probably occur as oligomers in their active conformation. The magainin molecule has been located in the bilayer interface in agreement with its amphipathic properties and its disordering effect for the fatty acyl chains. The ensemble of biochemical, biophysical, and structural properties of magainins suggests that these peptides belong to a class of so far uncharacterized membrane channels.

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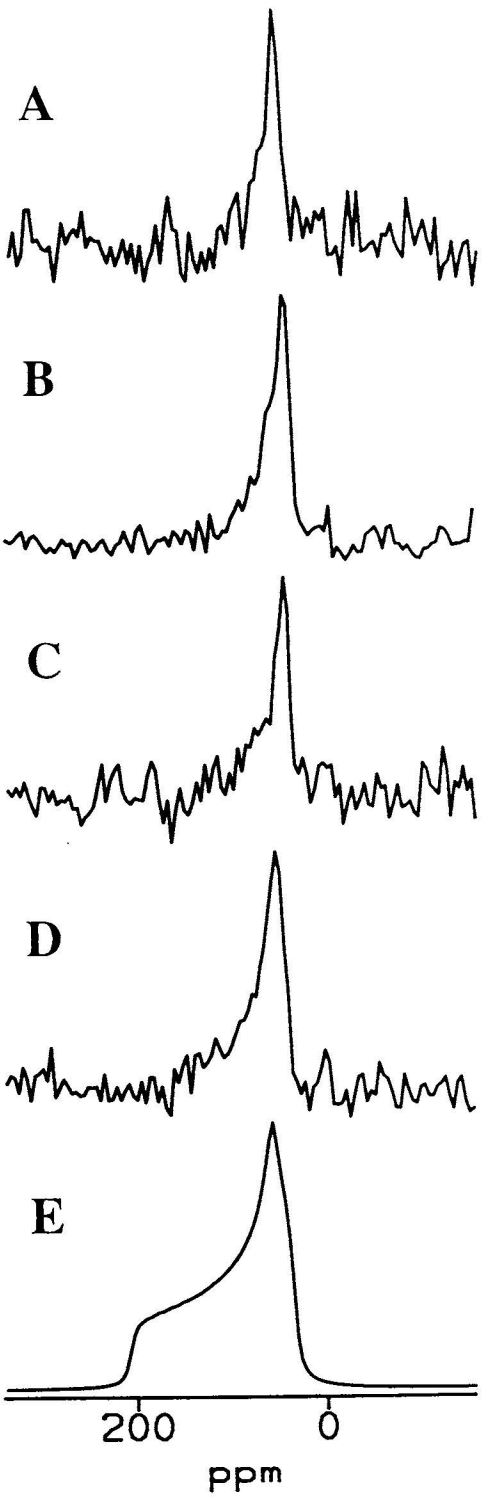

FIGURE 1 Experimental ^{15}N-NMR spectra of ^{15}N-Ile^{2} magainin, oriented in *A*, POPE/POPG 3:1 (molar ratio); *B*, POPC/POPG 3:1; *C*, POPC; *D*, POPC/POPG/Cholesterol 3:1:4; and *E*, simulated rigid lattice ^{15}N amide powder pattern.

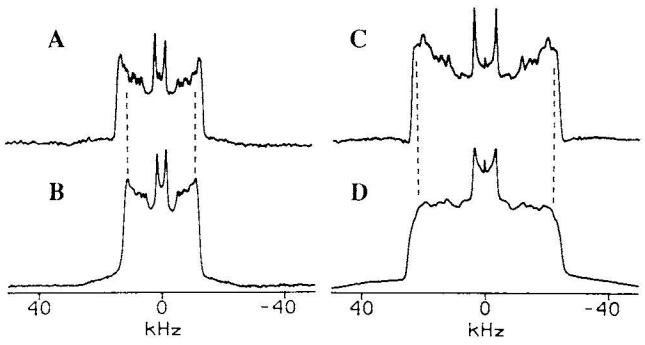

FIGURE 2 ^{2}H-NMR spectra of *A*, $^{2}H_{54}$-DMPC/POPC/POPG 3:3:2; *B*, same as *A* in the presence of 7.5 mol % magainin; *C*, $^{2}H_{54}$-DMPC/POPC/POPG 3/3/2 in the presence of 40 mol % cholesterol; and *D*, same as *C* in the presence of 7.5 mol % magainin. (150 mM NaCl, 20 mM Tris, pH 7.5, 30°C).

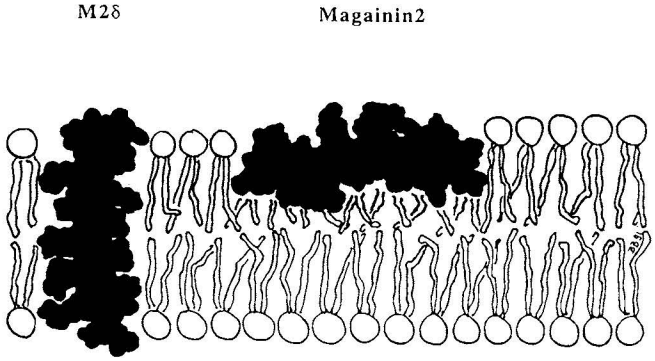

FIGURE 3 Schematic drawing of magainin and M2δ in lipid bilayers.

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