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Gramicidin channel controversy — the structure in a lipid environment

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Membrane-spanning gramicidin channels remain unique because of their small size, well-characterized function and well-defined structure. In organic solvents, the gramicidins are conformationally polymorphic; but a large body of work (summarized in refs 1,2) shows conclusively that the predominant channel form is a head-to-head dimer of two single-stranded (SS) -helices — confirming an early prediction[3]. We therefore take issue with the suggestion in a recent editorial in *Nature Structural Biology*[4] that the major conformer responsible for ion movement across membranes is a double-stranded (DS) dimer. The DS gramicidin crystal structures determined by Duax and collaborators[5], which were presented at the conference described in the editorial, constitute an important advance, but they do not relate to the active channel structure.

The identity of the channel structure was established by 1980 based on experiments that probed the general organization of gramicidin monomers in membrane-spanning channels. The experiment's aim was to distinguish between two different folding motifs (Fig. 1*b*): DS dimers, which were known to exist in organic solvents; and SS dimers, which further could be distinguished by their orientation. Single-channel experiments with gramicidin analogs modified at their N-termini showed that the N-termini are close together in the membrane-spanning channel[6]. The introduction of a charged residue at the N-terminus abolishes channel activity; but such a replacement is tolerated at the C-terminus[7]. By 1978, the evidence was strongly in support of the head-to-head SS dimer[8]. Single-channel experiments using more subtle N-terminal modifications[9] provided additional support for this structure.

Furthermore, combined conductance and spectroscopic measurements showed that the channels are dimers, and that all dimers are conducting channels[10] This allowed for nuclear magnetic resonance experiments, using ^{13}C and ^{19}F NMR to probe the exposure of the N- and C-termini to lipid or water-soluble shift reagents[11], which showed conclusively that membrane-spanning gramicidin channels are head-to-head SS dimers. Later experiments show that circular dichroism spectra of bilayer-incorporated gramicidin differ qualitatively from those of gramicidin in organic solvents[12] and that DS dimers are characteristic for gramicidin in organic solvents[13].

Why are the membrane-spanning channels not DS dimers? Most likely because more Trp residues in the sequence (Fig. 1*a*) would be buried in the bilayer core if the channels were double-stranded (Fig. 1*c*). The energetic cost of burying the amphipathic Trp residues will destabilize the DS channels relative to their SS counterparts[14]; one cannot neglect the importance of the (membrane) environment for the gramicidin's conformational preference.

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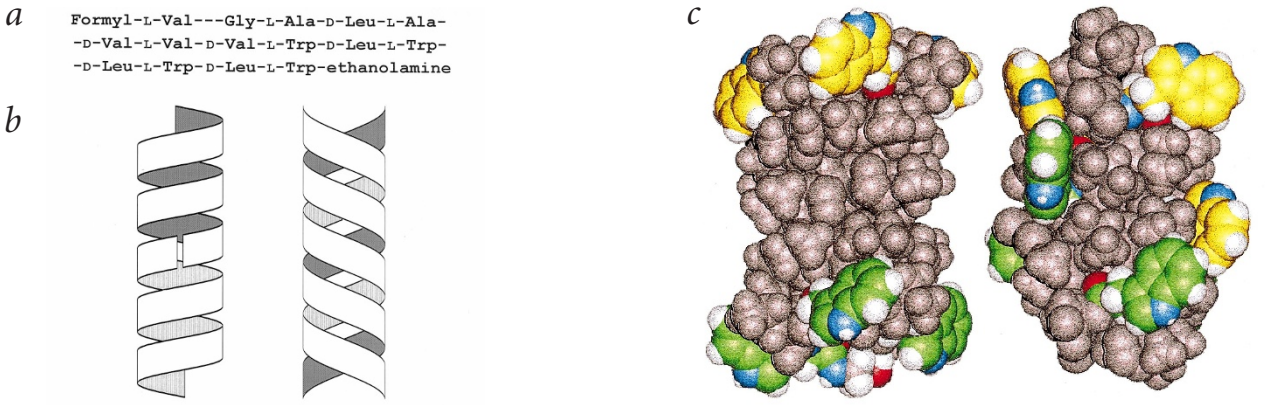

Fig. 1 ***a***, Primary sequence of gramicidin A. ***b***, Schematic structures of SS and DS gramicidin dimers. ***c***, Space-filling models of a SS (left)[15] and a DS (right)[4] gramicidin dimer (PDB codes 1GRM and 1AV2). In each model, the Trp residues in the two monomers are in yellow and green, respectively.

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