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Importance of indole N–H hydrogen bonding in the organization and dynamics of gramicidin channels

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ABSTRACT

The linear ion channel peptide gramicidin represents an excellent model for exploring the principles underlying membrane protein structure and function, especially with respect to tryptophan residues. The tryptophan residues in gramicidin channels are crucial for the structure and function of the channel. In order to test the importance of indole hydrogen bonding for the biophysical properties of gramicidin channels, we monitored the effect of N-methylation of gramicidin tryptophans, using a combination of steady state and time-resolved fluorescence approaches along with circular dichroism spectroscopy. We show here that in the absence of the hydrogen bonding ability of tryptophans, tetramethyltryptophan gramicidin (TM-gramicidin) is unable to maintain the single stranded, head-to-head dimeric channel conformation in membranes. Our results show that TM-gramicidin displays a red-shifted fluorescence emission maximum, lower red edge excitation shift (REES), and higher fluorescence intensity and lifetime, consistent with its nonchannel conformation. This is in agreement with the measured location (average depth) of the 1-methyltryptophans in TM-gramicidin using the parallax method. These results bring out the usefulness of 1-methyltryptophan as a fluorescent tool to examine the hydrogen bonding ability of tryptophans in proteins and peptides. We conclude that changes in the hydrogen bonding ability of tryptophans, along with coupled changes in peptide backbone structure induce the loss of single stranded $\beta^{6.3}$ helical dimer conformation. These results agree with earlier results from size-exclusion chromatography and single-channel measurements for TM-gramicidin, and confirm the importance of indole hydrogen bonding for the conformation and function of ion channels and membrane proteins.

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1. Introduction

Biological membranes represent complex two-dimensional, non-covalent assemblies of a diverse variety of lipids and proteins. They provide an identity to the cell and facilitate cellular communication and information processing. Membrane proteins are workhorses of the cellular machinery. About 30% of all proteins are predicted to be membrane proteins and ~50% of all proteins are membrane proteins for eukaryotic cells [1,2]. The crystallization efforts of membrane proteins in their native conditions are often complicated, and pose considerable challenge due to the intrinsic dependence of membrane protein structure on surrounding membrane lipids [3]. Approaches based on NMR and fluorescence spectroscopy have proved useful in elucidating the organization, topology and orientation of membrane proteins and peptides [4,5]. An additional advantage of spectroscopic approaches is that the information obtained is dynamic in nature, necessary for understanding membrane protein function.

Transmembrane proteins and peptides have characteristic stretches of amino acids capable of interacting with the membrane bilayer and are reported to have a significantly higher tryptophan content than soluble proteins [6]. Tryptophan residues are believed to be crucial in the structure and function of membrane proteins and peptides [7–12]. A major observation is that tryptophans in membrane proteins and peptides are not uniformly distributed, but tend to be localized toward the membrane interface. Interestingly, the interfacial region in membranes is characterized by unique motional and dielectric properties, distinct from both the bulk aqueous phase and the hydrocarbon-like interior of the membrane [12,13]. A unique feature of tryptophan is its ability to participate in both hydrophobic and polar interactions. Among the naturally occurring amino acids, tryptophan shows the highest tendency to localize at the interface, based on partitioning of model peptides to membrane interfaces. Besides aromaticity and ring shape, hydrogen

Abbreviations: TM-gramicidin, tetramethyltryptophan gramicidin; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; 5-PC, 1-palmitoyl-2-(5-doxyl)stearoyl-*sn*-glycero-3-phosphocholine; 12-PC, 1-palmitoyl-2-(12-doxyl)stearoyl-*sn*-glycero-3-phosphocholine; 2-AS, 2-(9-anthroyloxy)stearic acid; 12-AS, 12-(9-anthroyloxy)stearic acid; REES, red edge excitation shift; SUV, small unilamellar vesicles; CD, circular dichroism; LED, light emitting diode

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bonding could play a role in the partitioning of the indole ring [8,14,15]. The overall role of tryptophan residues in the structure and function of membrane proteins and peptides is apparent from the observation that substitution or deletion of tryptophans often results in reduction or loss of their function [16–20].

The linear peptide gramicidin forms prototypical ion channels specific for monovalent cations and has been extensively used to explore the organization, dynamics, and function of membrane-spanning channels [21,22]. Gramicidin is a multi-tryptophan peptide (Trp-9, 11, 13, and 15; see Fig. 1a) which serves as an excellent model for transmembrane channels due to a number of reasons such as small size, ready availability and the relative ease with which chemical modifications can be performed. These special features make gramicidin unique among small membrane-active peptides and provide the basis for its use to explore the principles that govern the folding and function of membrane-spanning channels [21–23]. Interestingly, gramicidin channels share vital structural features involving ion selectivity with complex ion channels such as KcsA potassium channels [24]. Gramicidin assumes a wide range of environment-dependent conformations due to its unique sequence of alternating L- and D-chirality. Two major conformations adopted by gramicidin in various environments are: (i) the single stranded $\beta^{6.3}$ helical dimer (the 'channel' form), and (ii) the double stranded intertwined helix (collectively known as the 'nonchannel' form) [22]. The amino terminal-to-amino terminal single-stranded $\beta^{6.3}$ helical dimer form is the thermodynamically preferred conformation in membranes and membrane-mimetic media. In this conformation, the tryptophan residues remain clustered at the membrane–water interface [25–28]. Interestingly, the membrane interfacial localization of tryptophan residues is absent in 'nonchannel' conformations and the tryptophan residues are distributed along the membrane axis [10,22,25]. Nonchannel conformations have been shown to exist in membranes with polyunsaturated lipids [29], and in membranes with increased acyl chain lengths under hydrophobic mismatch conditions [30,31].

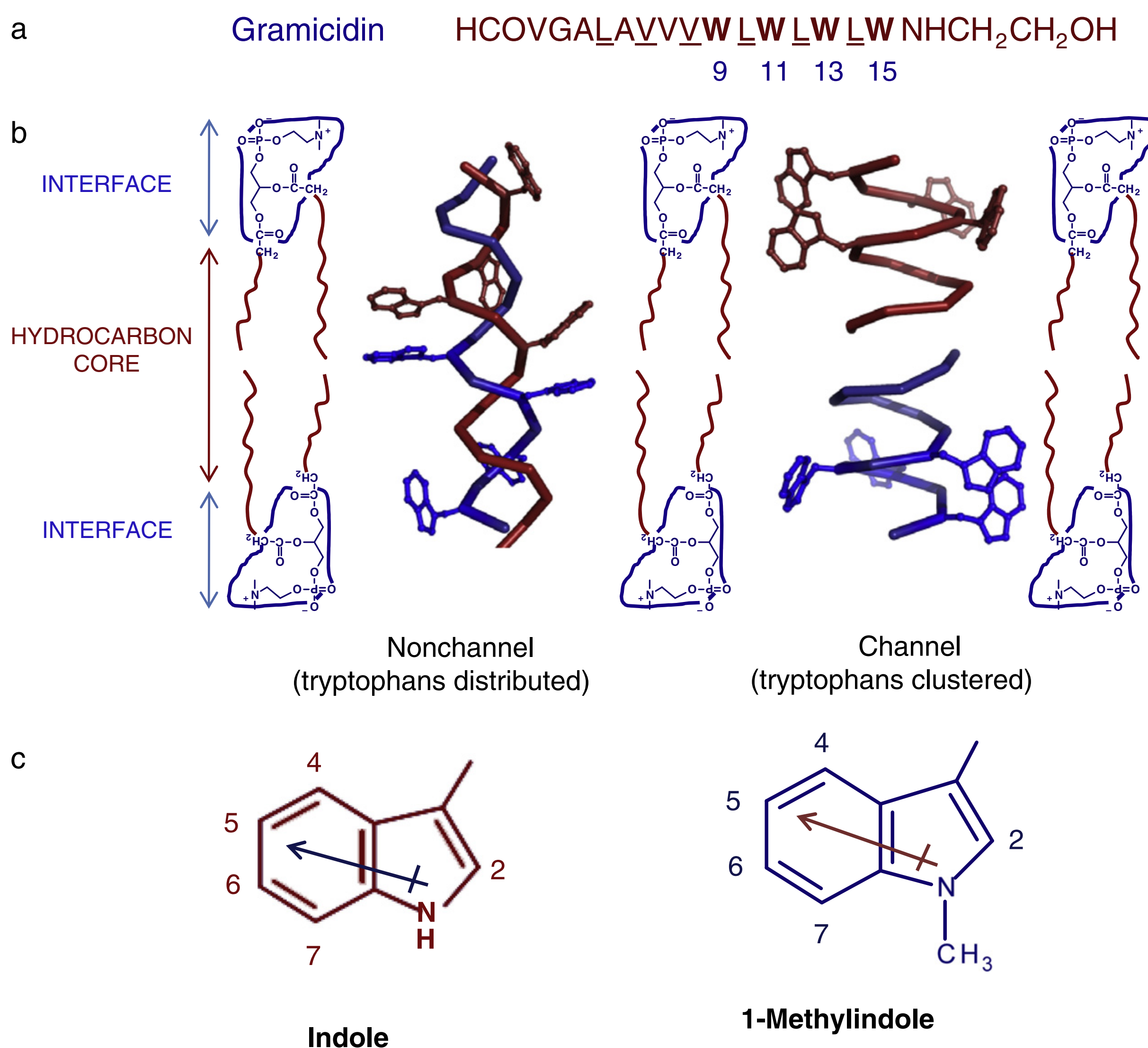

Fig. 1. (a) Amino acid sequence of gramicidin A highlighting the positions of the four tryptophans. Alternating D-amino acid residues are underlined. In the analog (TM-gramicidin) used in this study, four tryptophan residues in gramicidin are replaced by 1-methyltryptophan residues. (b) A schematic representation of the nonchannel and channel conformations of gramicidin showing the localization of tryptophan residues in the membrane bilayer. Tryptophans are clustered toward the membrane interface in the channel conformation. In contrast, tryptophans are distributed along the bilayer normal in the nonchannel conformation. See text for other details. Adapted and modified from Ref. [12]. (c) Chemical structures of indole and 1-methylindole. The major difference between indole and 1-methylindole is in their ability to form hydrogen bonds. While indole can form hydrogen bond with its –NH group, this ability is lost in 1-methylindole. Interestingly, the dipole moments of indole and 1-methylindole (shown as a vector) are similar in direction and magnitude. See text for other details. Adapted and modified from Ref. [43].

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