Chapter 2
Gramicidin Channels as Cation Nanotubes

Roger E. Koeppe II, Sigrid E. Schmutzer, and Olaf S. Andersen

Abstract The linear gramicidins constitute a family of peptide antibiotics produced by the soil bacterium Bacillus brevis. The first antibiotics to be used in clinical practice, the linear gramicidins exert their antibacterial activity by forming bilayer-spanning channels that increase the monovalent cation permeability of target bacterial plasma membranes. Gramicidin channels are synthesized by non-ribosomal peptide synthesis on large protein complexes and contain both D- and L-amino acid residues; they were the first channels of known chemical composition to be studied. The channels effectively serve as cation-selective organic nanotubes that span lipid bilayer membranes and provide a basis for examining many aspects of ion-channel function and channel-lipid bilayer interactions. The nanotube properties can be tuned by means of mutations or chemical changes to the subunit architecture, as well as by altering the channels' bilayer environment (e.g., the bilayer thickness). Indeed, many analogue sequences within the extended peptide family have been prepared by semi-synthesis or total synthesis. Diverse applications of gramicidin channels have enhanced our understanding of the microphysics of ion permeation, lipid-protein interactions and membrane protein function.

2.1 Introduction

The linear gramicidins constitute a family of peptide antibiotics produced by the soil bacterium *Bacillus brevis* [1, 2]. Analogue sequences within the extended peptide family also can be prepared by semi-synthesis or total synthesis [3–6]. The linear gramicidins were the first antibiotics to be used in clinical practice [7]. They exert their antibacterial activity by forming bilayer-spanning channels [8] that increase the monovalent cation permeability of the target bacterial plasma membranes [9].

Among the ion-selective channels that span lipid bilayer membranes, gramicidin channels can be considered as robust organic nanotubes. The linear gramicidins are peptides containing both D- and L-amino acids that are linked in specific sequences within bacteria such as *Bacillus brevis* by means of a non-ribosomal synthesis in which the sequence is specified by the order of the of the domains that couple

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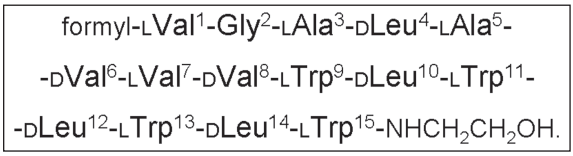

Fig. 2.1 Amino acid sequence of gramicidin A, the primary linear gramicidin produced by the soil bacterium *Bacillus brevis*. The N-terminal is blocked with a formyl group; the C-terminal is blocked with an ethanolamide group. Because the sequence strictly alternates between residues of L- and D-chirality, with Gly^2 considered as an "honorary" D-residue, the sequence can fold to accommodate a β-helical fold, The four tryptophans at positions 9, 11, 13 and 15 serve as "anchors" at the membrane/water interface to orient the respective subunits of a bilayer-spanning dimeric channel

amino acids to the nascent chain [10, 11]. The prototypical member of the gramicidin family is gramicidin A ("gA"), whose sequence includes glycine-2 (which is achiral) and otherwise all D-amino acids at the even-numbered sequence positions, interspersed between all L-amino acids at the odd-numbered sequence positions (Fig. 2.1). The strictly alternation between residues of L and D chirality confers resistance to proteases and allows the native channel structure to fold as a helix with a secondary structure similar to β-sheets, in which the side chains are on the outside of the helix and the peptide backbone lines the ion permeable pore.

The N-formyl blocking group of gramicidin A is added biosynthetically [10] and is required for channel activity [12]. By contrast, the C-aminoethanol blocking group, which derives biosynthetically from glycine, can be modified rather extensively with retention of channel activity [13–17].

In lipid bilayer membranes of appropriate thickness, namely those whose phospholipid acyl chains contain 12–18 carbons, the linear gramicidins fold into single-stranded dimeric membrane-spanning channels. In these channels, the formyl-L-valines meet at the bilayer center and the Trp indole rings anchor the subunits to the respective membrane/water interfaces (Fig. 2.2). The channel lumen is about 0.4 nm in diameter and permits the single-file passage of water molecules and alkali cations [18]. Anions are rejected because the peptide backbone residues do not solvate anions well, as can be deduced from bulk phase free energies of transfer [19] and computational studies on gA channel models [20]. Divalent cations are rejected because the electrostatic barrier for ion entry into the pore becomes forbidding, e.g. [21]. When the Trp interfacial interactions are broken or stressed – namely in organic solvents [22], or in thin or very thick membranes (those whose lipid acyl chains have less than 10 or more than 20 more carbons) [23, 24], or by selected substitution of Trp residues by less polar/amphiphilic residues [25] – the gramicidin dimer refolds away from the single-stranded, functional channel structure into alternative double-stranded dimer conformations (Fig. 2.3) [26], All of the folded dimers are β-helices, with a central backbone, with side chains projecting to the outside [26, 27], as depicted for the single-stranded dimeric "nanotube" in Fig. 2.2 and the double-stranded structure in Fig. 2.3. Usually the double-stranded conformations (also known at π helices [13]) are inert, i.e. not measurably permeable to

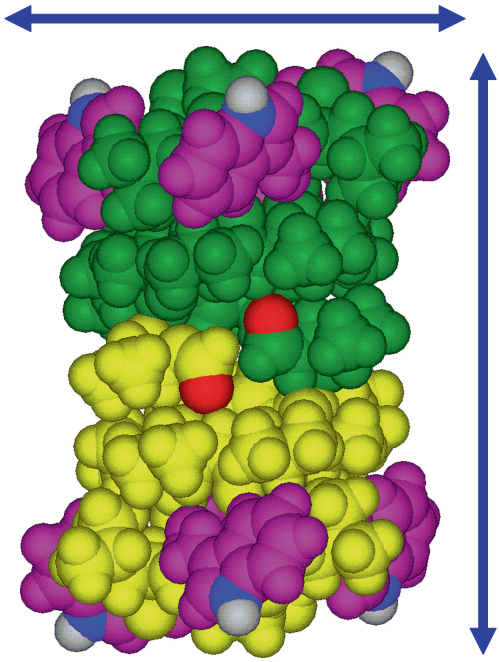

Fig. 2.2 Dimensions of the gramicidin channel, depicted using a CPK model. The calibration *arrows* represent 2.6 nm (*vertical*) and 2.0 nm (*horizontal*). The membrane-spanning channel surrounds a water-filled, cation-selective pore of about 0.4 nm diameter (see also Figs. 2.3, 2.4, and 2.5)

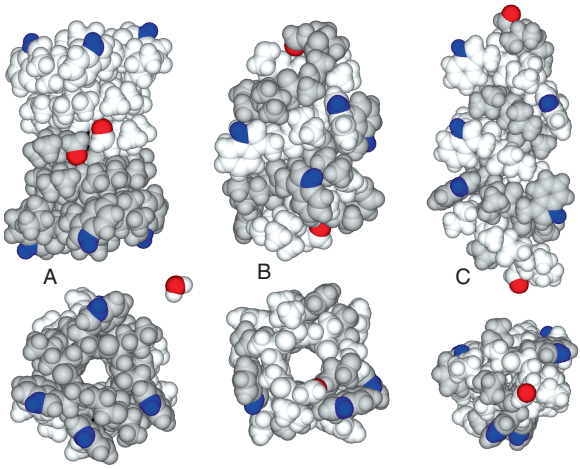

Fig. 2.3 Folding of the gramicidin channel. Side views (*upper*) and end views (*lower*) showing: (**a**) the membrane-spanning channel, $\beta^{6.3}$-helical dimer with 6.3 residues per helical turn; and (**b**, **c**) two members of the set of double-stranded dimer conformations that are observed in organic solvents. Formyl oxygens are *red* and Trp indole NH groups *blue*. A water molecule is shown for reference. Structures **a** and **b** each enclose a pore of about 0.4 nm diameter, whereas structure **c** does not

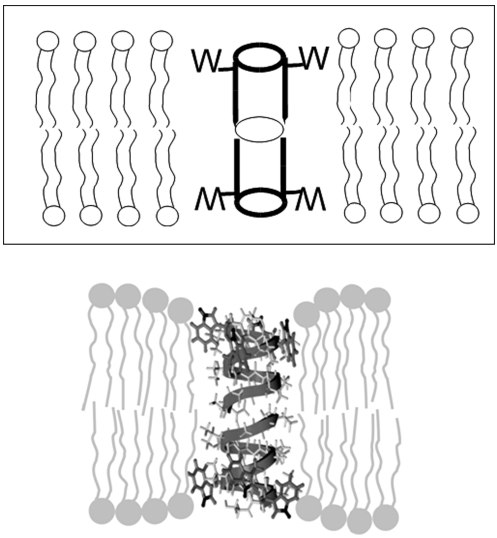

Fig. 2.4 Tryptophan anchoring of the gramicidin channel. The Trp indoles rings ("W" in the upper cartoon) anchor each subunit of the membrane-spanning, cation-conducting channel to its respective membrane/water interface

ions [28], but selected combinations of subunit sequences can sometimes exhibit double-stranded channel activity [29, 30]. In the single-stranded channel conformation, the tryptophans of each gramicidin subunit serve as anchors to the membrane/water interface (Fig. 2.4), holding each respective subunit within the lipid leaflet on the side of the membrane to which it was added [31]. In the interfacial attachment-stabilized conformation, the folded β-helical backbone surrounds a pore of about 4 Å, allowing for the single-file transport of monovalent cations and water molecules (Figs. 2.3 and 2.4) [32].

Gramicidin channels have served as prototypical channels in the development of many physical approaches toward understanding the structure and function of membrane proteins [33]. The main physical approaches have included: (a) single-channel analysis using, e.g., the bilayer punch ("patch clamp") method [34, 35], (b) circular dichroism (CD) spectrosopy to detect the channel's secondary structure [22], (c) size-exclusion chromatography [25, 36] to distinguish weak from strong subunit interactions, (d) fluorescence spectroscopy to assess conformational preference and tryptophan positions and motions [37–39], (e) solution magnetic resonance (NMR) spectroscopy for complete structure determination using detergent-encapsulated (membrane-mimetic) samples [40, 41], and (f) solid-state NMR for complete structure determination using bilayer-incorporated samples [42, 43]. Some of the issues concerning conformational polymorphism and structural heterogeneity have been summarized elsewhere [28, 39, 44]. Although methods for the structural characterization of monomers have remained elusive, an in-plane X-ray scattering study of a gramicidin analogue for which dimer formation is blocked[1] has indicated that membrane-incorporated monomers assume a similar β-helical fold as the individual subunits within the native dimeric channels [45].

[1] When the N-formyl group is replaced by N-*t*-butoxycarbonyl, the dimer/monomer equilibrium is shifted by $\sim 10^{-5}$ [45].

When considered as cation nanotubes, the canonical single-stranded gramicidin channel can be used in diverse ways to investigate the properties of lipid bilayer membranes as well as the physics of ion transport through narrow tubes. This chapter will address fundamental properties and selected practical applications of these nanotubes which assemble from a pair of head-to-head single-stranded gramicidin molecules. The properties of these peptide nanotubes can be tuned by altering the amino acid side chains [46]. Furthermore, the two subunits that compose a given nanotube can be different (in heterodimers) or identical (in homodimers). Each of these assemblies offers advantages for particular applications, and it is possible to measure the energetic consequences of altering the amino acid sequence at the subunit interface.

2.2 Tuning the Channel Properties

Cation-conducting gramicidin channels assemble in lipid bilayer membranes when subunits from opposing bilayer leaflets diffuse laterally, to "line up" and then dimerize; the bilayer-spanning channels are stabilized by six intermolecular hydrogen bonds [31]. Dimer formation (and disappearance) is detected as a step change in the current across a high-resistance "black" lipid[2] membrane (Fig. 2.5) [8, 35]. That is, gramicidin channels do not "open" and "close" – except in a few special

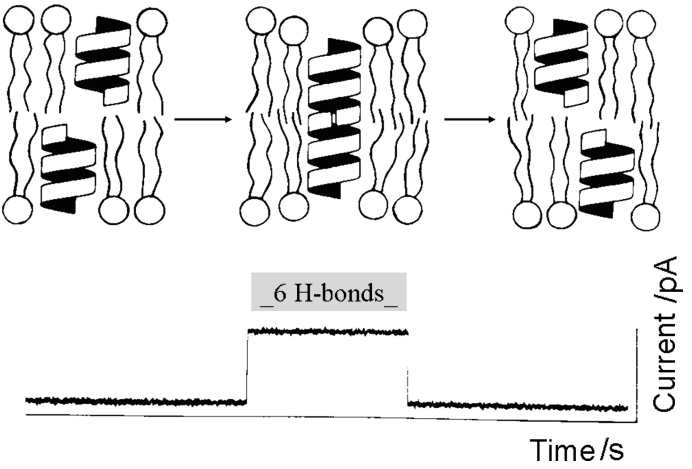

Fig. 2.5 Formation of a gramicidin channel. When subunits in opposing lipid leaflets dimerize, the dimer is stabilized by six hydrogen bonds, and a step change in the transmembrane current can be observed. The step size is typically about 3 pA for Na^+ or about 10 pA for Cs^+ permeation through a channel of gramicidin A (1.0 M salt, diphytanoylphosphatidylcholine/*n*-decane bilayers, 200 mV applied potential, 25°C). The channel lifetime is typically about 1 s. See also Figs. 2.7 and 2.9

[2] A "black" lipid membrane or "bilayer" lipid membrane ("BLM") is only one lipid bilayer thick and is optically black [80].

cases [47, 48] – but rather appear and disappear by a process of transmembrane association and dissociation. In the right-handed β-helical channel, the six hydrogen bonds link the carbonyl oxygens of L-residues 1, 3 and 5 to the backbone NH groups of L-residues 5, 3 and 1 on the respective subunits [49]. This hydrogen-bonded subunit arrangement was first proposed by Urry [27], albeit for a left-handed channel. The right-handed helix sense was established using magnetic resonance (NMR) methods [40, 50, 51].

Gramicidin channels are remarkably conductive. Even though the small pore radius restricts ions and water to move in single file [32, 52], the rate of ion movement through a channel is rapid: in 1.0 M NaCl the single-channel conductance is only about fivefold less than would be predicted for free diffusion through a 2.6 × 0.4 nm pore (Fig. 2.2) that offers no "excess" resistance [53]. Because gramicidin channels are seemingly ideally selective for monovalent cations [54], single-channel measurements provide direct information about the net cation flux through the channel. Gramicidin channels therefore are not just water-filled pores. Favorable short-range ion-channel interactions effectively compensate for the electrostatic barrier for ion movement through the low-dielectric bilayer core. Like other membrane-spanning ion channels, gramicidin channels catalyze ion movement across a lipid bilayer by providing a reaction path that obviates the ion's passage through the lipid bilayer hydrophobic core per se. For this reason, gramicidin channels (and other ion channels) belong to a special class of enzymes in which no covalent bonds are made or broken during the catalytic cycle.

Both the single-channel conductance and the mean channel lifetime of gramicidin channels can be regulated by engineered changes in the amino acid sequence [46].

A. *Conductance*: The cation conductance of gramicidin channels is sensitive to the introduction of many different side chains into the amino acid sequence (Fig. 2.6). For example, the substitution of amino acids with more polar sulfur- or fluorine-containing polar side chains for the hydrophobic valine at position one decreases the single-channel conductance. Notably, substituting trifluorovaline at position one alters the ion selectivity, as this substitution causes a 3-fold reduction in current in the presence of 1.0 M CsCl but a 6-fold reduction for 1.0 M NaCl[3] [55]. Replacing any of the four tryptophan indole dipoles with a nonpolar (and non-dipolar) side chain, such as Phe, decreases the single-channel conductance by about 25% [56] – with the deeper substitutions causing the larger conductance changes. Conversely, enhancing the Trp dipole by 5-fluorination will increase the conductance by about 20% [57]; in this instance through-space ion/5-F-Trp dipole interactions over a distance of several nm serve to attract cations toward the channel entrance and lower the energy barrier for ions to pass the bilayer center [58]. Even the aliphatic "spacer" residues between the tryptophans are important, as replacing

[3]Gramicidin A channels are about 3-fold selective for K^+, Rb^+ or Cs^+ over Na^+ [54]. Amino acid substitutions can serve to "tune" this cation selectivity [55]. Experiments with different cations are chosen for mechanistic studies.

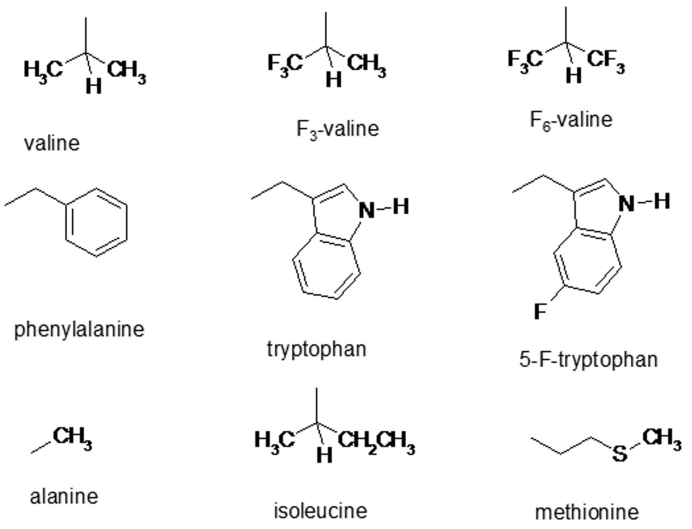

Fig. 2.6 Selected side chains of amino acids that have been introduced into gramicidin channels

D-Leu^{10,12,14} by D-Ile^{10,12,14} or D-Ala^{10,12,14} decreases the single-channel conductance for 1.0 M CsCl by 25–33% [59]. Furthermore, the cation conductance can be tuned by more subtle alterations in the amino acid sequence [49, 55]. These conductance changes occur within the framework provided by the β-helical fold of the membrane-spanning channel, which depends on maintaining the pattern of alternating residues of L- and D-chirality within the gramicidin channel sequence [4, 47, 57, 60].

To illustrate how subtle changes can modulate channel function, Fig. 2.7 shows the influence of glycine and alanine replacements at positions 1 and 2 on the properties of gramicidin channels [49]. [Gly^1-Gly^2]gA channels conduct, with a single-channel current of about 2 pA in the presence of 1.0 M NaCl (and 200 mV applied potential), and exhibit a mean channel lifetime of about 70 ms (Fig. 2.7a, b). Replacing Gly^1 by Ala^1 causes an approximate doubling of the Na^+ current and a 2.5-fold increase in lifetime, whereas a Gly→Ala substitution at position 2 causes no change in the single-channel current but a >10-fold increase in the lifetime (Fig. 2.7c, d). Similar changes in single-channel lifetimes are observed when Cs^+ is the permeant ion, though the current changes are less [49]. Remarkably, the effects of alanines on the channels' ion permeability and lifetimes at the two positions are largely uncoupled.

B. *Channel lifetimes*: As noted above, gramicidin channel lifetimes also are sensitive to the nanotube's amino acid sequence and to the overall channel length, specifically how well the length of the channel's hydrophobic exterior matches the thickness of the host lipid bilayer (Fig. 2.8), a concept known as *hydrophobic matching* [61, 62]. The results shown in Fig. 2.8 illustrate that neither the absolute tube length nor the absolute lipid bilayer thickness, but rather their difference, regulates

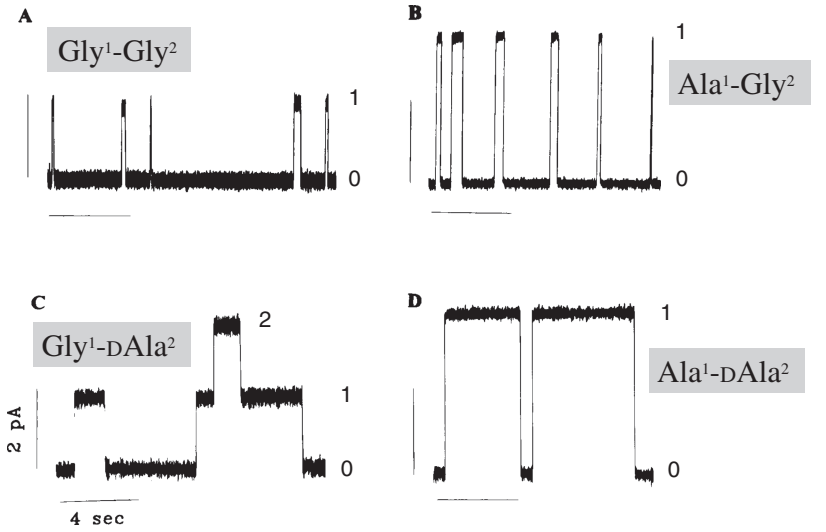

Fig. 2.7 Examples of single-channel current transitions that accompany the dimerization of gramicidin subunits that have glycine or alanine at positions one and two. Current level "1" or "2" denotes one or two conducting channels, whereas level "0" denotes the baseline when there are zero conducting channels. Diphytanoylphosphatidylcholine/*n*-decane bilayers, 1.0 M NaCl, 200 mV applied potential, 25°C. Modified from [49]

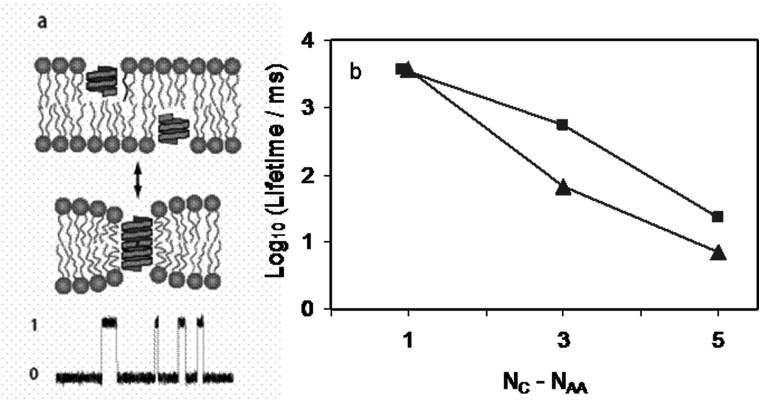

Fig. 2.8 Effect of a bilayer-channel hydrophobic mismatch on gramicidin channel lifetimes. (**a**) Gramicidin channels form by means of transmembrane dimerization of nonconducting subunits (cf. Fig. 2.5), which is observable as discrete current transition between two levels: 0, in which there is no conducting channel, and 1, in which there is one conducting channel. Because channel formation causes a local bilayer thinning, the average channel lifetimes vary as a function of the hydrophobic mismatch. (**b**) Gramicidin channel lifetimes as a function of a hydrophobic mismatch parameter ($N_C - N_{AA}$), where N_C denotes the number of carbon atoms in the acyl chains of the bilayer-forming lipids (monounsaturated phosphatidylcholines), and N_{AA} denotes the number of amino acids in one gramicidin subunit. The *squares* (■) denote experiments in which N_{AA} was varied while the lipid acyl chains had 16 carbons. The *triangles* (▲) denote experiments in which N_C was varied at constant N_{AA} of 15 amino acids per subunit

Table 2.1 Variation of gramicidin channel lifetimes as function of the nanotube amino acid sequence[a]

Residue 1	Residue 2	Dimer lifetime (ms)
Val	Gly	810
Norval	Gly	220
Leu	Gly	210
Norleu	Gly	280
Met	Gly	200
F_3-Val	Gly	180
F_6-Val	Gly	50
Phe	Gly	770
Gly	Gly	70
Ala	Gly	190
Gly	D-Ala	1,100
Ala	D-Ala	2,200

[a]Data from [55, 65] and [49]. Experimental conditions: 1.0 M NaCl, diphytanoylphosphatidyl choline/n-decane, 200 mV, 25°C. Day-to-day variations of ±10% are observed for the mean channel lifetimes [65]. Residues 1 and 2 are located at the subunit/subunit junction in a gramicidin dimer (Fig. 2.2). Channel lifetimes also vary significantly if the tryptophans near the C-terminal are substituted [56, 57]. These tryptophans are located away from the subunit junction but are critical anchors of the channel subunits to the bilayer/electrolyte interface [31] and therefore also influence channel lifetimes, often in highly dramatic ways [56].

the channel lifetime. The concept holds true regardless of whether the tube length is varied within a fixed bilayer or, conversely, a fixed-length tube is examined in bilayers of different thicknesses (Fig. 2.8b) [63].

Substitutions in the amino acid sequence also influence the channel lifetime. For example, polar residues at position one or two – at the subunit interface and near the bilayer center – decrease the channel lifetime (Table 2.1). Remarkably, branched hydrocarbon side chains (Val, Phe) confer significantly longer lifetimes than do straight side chains (Norval, Norleu, Met). Furthermore, D-Ala^2 yields significantly longer channel lifetimes than does Gly^2, independent of the identity of residue one (Table 2.1) [49]. Away from the subunit interface, tryptophans anchor the subunits to the respective membrane water interfaces. Substituting one or more of the Trp indole rings by phenylalanine or glycine, and even varying the intervening "spacer" residues, also have dramatic effects on the channel lifetime [56, 59, 64].

2.3 Applications of Heterodimers

Heterodimer experiments involve the random and independent associations of subunits when two chemically different gramicidin subunits are added to the same lipid bilayer membrane (Fig. 2.9). It thus becomes possible to measure the energetics of different subunit combinations [65, 66]. In varying experimental protocols, the

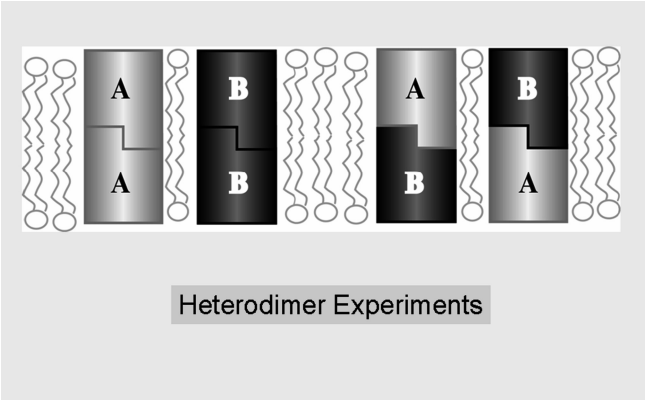

Fig. 2.9 Heterodimer formation. When two chemically different gramicidin subunits A and B are added to both sides of a bilayer, four types of channels may be able to assemble

respective subunits can be added to the same unique side of a membrane, to different sides, or to both sides. The Trp-anchored single-stranded subunits that constitute standard gramicidin channels tend to remain within the leaflet on the side of the membrane to which they are added initially [31]. This "anchoring" by the Trp indole rings at the membrane/water interface is effective for at least 30 min after single-sided addition of gramicidin subunits to only one side of a lipid bilayer membrane [31], meaning that gramicidin channels usually are observed only after adding gramicidins to both sides of a bilayer. If one or more of the Trp side chains are progressively substituted by other aromatic rings such as Phe, naphthyl or 1-Me-indole, the modified gramicidins have an increased probability of forming channels when added to only one side of the bilayer indicating that individual subunits can more easily cross the membrane [25, 67]. In contrast to the canonical single-stranded channels, the double-stranded channels that are formed only in rare cases tend to assemble when both subunits come from the same side of the bilayer membrane [24, 30].

Figure 2.10 shows results from a heterodimer experiment with single-stranded subunits. In such an experiment, subunits A and B (sequence analogues of gramicidin, in this case [Ala^1-D-Ala^2]gA and gA, respectively) were added to both sides of a planar lipid bilayer. The single-channel current trace (top of Fig. 2.10) shows current steps resulting from the formation of homodimeric AA and BB channels, and heterodimeric (or hybrid) channels. The heterodimeric channels will exist in two different orientations (relative to the applied potential), one in which the current is in the A→B direction and one in which the current is in the B→A direction. These two orientations are, in principle, distinguishable because the potential of mean force for ion movement will differ for the two channel orientations [68]. In this case, however, the hybrid channel orientation does not influence the channel properties because the A→B and B→A channels contribute to the same peak – of intermediate magnitude – in the current transition amplitude histogram (middle of

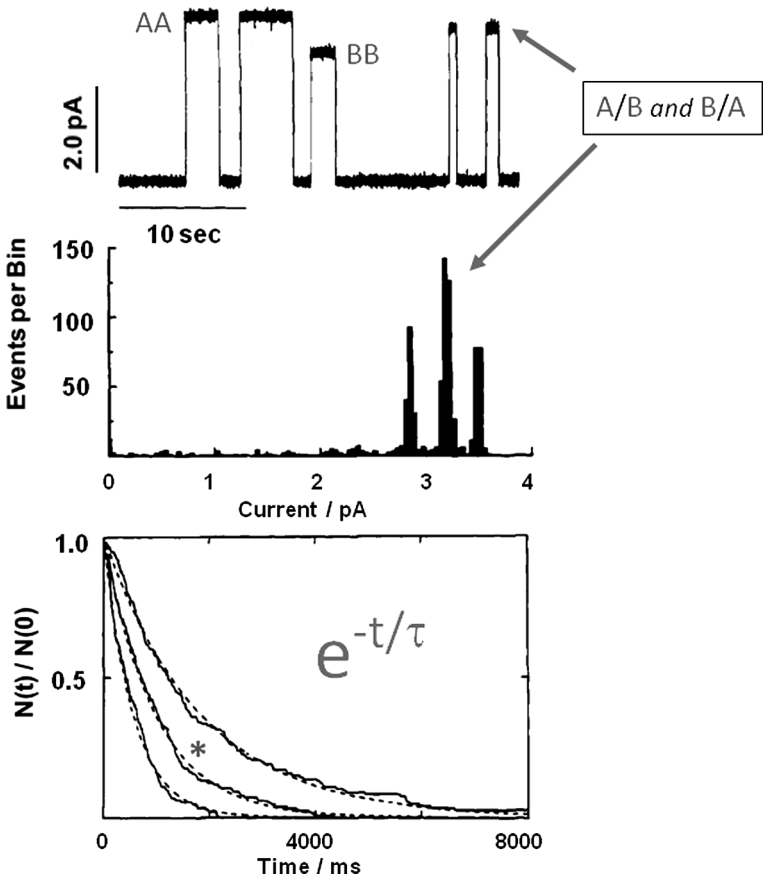

Fig. 2.10 Examples of heterodimeric channels. From top to bottom, the figure depicts a single-channel current trace, a current transition amplitude histogram, and a lifetime survivor plot that depicts the probability of channel disappearance as a function of time after formation. In this example, the heterodimers A→B and B→A exhibit nearly identical single-channel currents (*) and lifetimes (*) which, in both cases, are intermediate between the corresponding properties of the homodimeric AA and BB channels. Subunit A is [Ala^1-D-Ala^2]gA; subunit B is gA. Diphytanoylphosphatidylcholine/*n*-decane bilayers, 1.0 M CsCl, 200 mV applied potential, 25°C. Modified from [49]

Fig. 2.10). (The case where the two orientations can be distinguished will be illustrated below.) The hybrid channels also exhibit a single exponential probability of dissociating and a mean channel lifetime that is intermediate between those of the respective homodimers in a channel lifetime histogram or survivor plot (* in lower section of Fig. 2.10).

Heterodimer experiments reveal the (energetic) consequences of interrupting the pattern of alternating L- and D-residues that constitute the gramicidin channel backbone. For example, the pattern can be interrupted by deletion or insertion of one

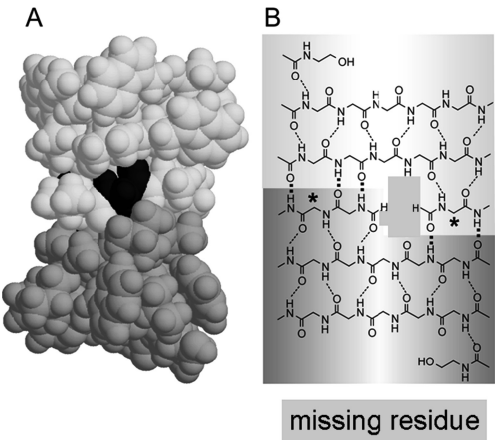

Fig. 2.11 Missing residue at the subunit interface of a gramicidin channel. Adjacent to Gly^2 (*black* in structure **a**; * in net diagram **b**), a residue can be deleted from only one subunit of a gramicidin channel (with "repair" of the N-formyl group). The residue deletion leaves a defect in the wall of the channel and lowers the mean lifetime of the heterodimeric channel, from which one can deduce that the "gap" incurs an energetic cost of about 10 kJ mol^{-1} [66]

residue, or by insertion of extra carbon atoms (CH_2 groups) into the backbone. If a single residue is deleted from only one subunit of a gramicidin channel, but the crucial N-formyl groups are retained on both subunits (Fig. 2.11), then there remains an effective "gap" or defect in the wall of the channel. As a consequence, the hybrid channel conductances and lifetimes are no longer intermediate between those of the respective homodimeric channels. Instead of the situation depicted in Fig. 2.10, the hybrid channels exhibit much *lower* single-channel currents and much *shorter* mean channel lifetimes than either of the corresponding homodimers (c.f. figures 3 and 4 in [66]). The consequence is that, when two gramicidin subunits differ in length by $\pm$ one residue, the channel's backbone structure and helix sense are not affected, but the resulting hybrid channels are destabilized by $\sim$10 kJ mol^{-1} [66]. The destabilization of the heterodimeric nanotube is caused by the de facto "gap" – and loss of one intersubunit hydrogen bond – in the peptide backbone at the junction between the two subunits. This "missing residue" or "gap" analysis gives remarkably consistent results, regardless of whether a residue is added or removed from the N-terminal of gramicidin. (The symmetry of a dimer dictates that the initial residues where the respective subunits meet each other should be of the same chirality, while the β-helical fold dictates that residue chirality should alternate thereafter.) This consistency suggests that the transition state for dimer dissociation is reached when two hydrogen bonds are broken, when the subunits move apart in a rotating/sliding motion [66, 69, 70].

Gramicidin channels also can be used to examine general questions relating to the structural and functional consequences of inserting non-genetic amino acids into the amino acid sequence of a well-folded structure. What are the consequences of

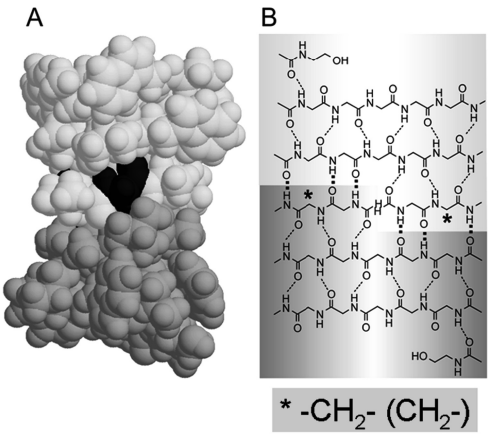

Fig. 2.12 Peptide backbone modification at the subunit interface of a gramicidin channel. Adjacent to Gly^2 (*black* in structure **a**; * in net diagram **b**), one (or two) extra CH_2 group(s) can be inserted into the backbone of a gramicidin channel. See text for details

inserting "extra" atoms into the channel backbone structure? Figure 2.12 illustrates the concept, with Gly^2 shown in black in panel A. Next to the backbone CH_2 of Gly^2 (* in Fig. 2.12b), we have incorporated one or two extra CH_2 groups. Remarkably, the extra methylene groups in the backbone of the nanotube are well tolerated, such that the subunits retain the ability to assemble into conducting channels. Figure 2.13

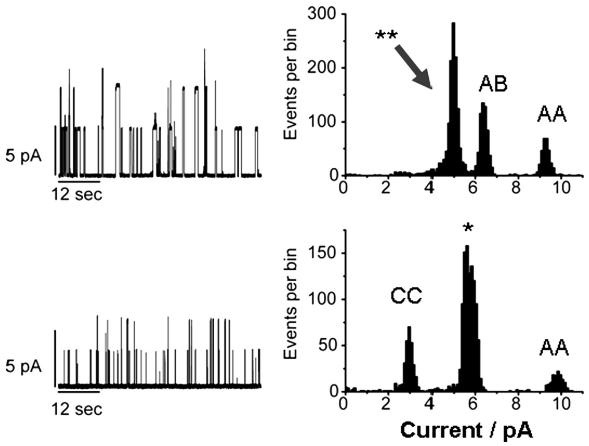

Fig. 2.13 Current reduction due to extra methylene groups at the subunit interface in a gramicidin channel. Subunits A, B and C have 0, 1, or 2 extra $–CH_2–$ groups, respectively, inserted next to the $–C_\alpha H_2–$ of Gly^2 in the native gramicidin sequence. *Left*: single-channel current transitions for mixtures of A/B subunits (*upper*) or A/C subunits (*lower*). *Right*: the respective current transition histograms. In the upper histogram AA homodimers and AB heterodimers exhibit distinct currents, whereas the BB homodimers and BA heterodimers exhibit the same average current (**→ peak). In the lower histogram, AA and CC homodimers exhibit distinctive high and low currents, while the oppositely oriented AC and CA heterodimers exhibit the same intermediate current (* peak). Experimental conditions: 1.0 M CsCl, diphytanoylphosphatidyl choline/n-decane, 200 mV, 25°C

illustrates that one extra CH_2 group (subunit B; upper panels) and even two extra CH_2 groups (subunit C; lower panels) still permit conducting channels to assemble. Interestingly, when only one extra CH_2 is present in only one subunit, the hybrid channel orientation – B→A or A→B – makes a difference, such that the current is different in the two directions. In one of the orientations, the current through the hybrid channel is indistinguishable from the current through the symmetric BB channels (peak ** in upper right of Fig. 2.13). By contrast, the heterodimers in which one subunit incorporates two extra CH_2 groups exhibit no such asymmetry, and the currents through the CA and AC channels are indistinguishable (peak * in lower right of Fig. 2.13).

2.4 Double-Barreled Nanotubes

As noted above, gramicidin channel formation is associated with a local bilayer deformation that varies with the (hydrophobic) mismatch between the bilayer thickness and channel length (see above). Further information about the importance of hydrophobic mismatch can be gained by forming covalent crosslinks between adjacent subunits in a bilayer leaflet, such that double-barreled channels may assemble [71]. Figure 2.14 illustrates the result of adding such tandem subunits – linked at their C-terminals, using a flexible hydrophilic linker that extends from the membrane/water interface out into the aqueous solution. When a channel forms, one first observes some initial "bursting" activity (red ellipse in Fig. 2.14), as the first barrel makes several apparent attempts to open, and then finally succeeds. Almost concerted with the ultimate opening of barrel one – or within a few milliseconds thereafter [71] – one observes that a second barrel also is conducting a cation current. The opening of the second barrel occurs without a prelude of bursting activity and is likely cooperative with the opening/stabilization of the first barrel. Together, the double-barreled assembly remains open for, typically, minutes

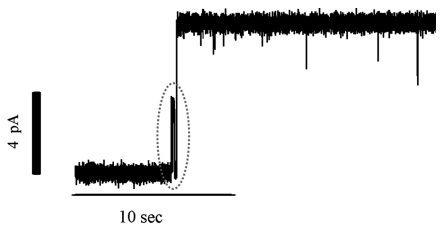

Fig. 2.14 Formation of a double-barreled gramicidin channel. Single-channel current trace obtained with tandem gramicidin subunits that are pairwise linked at the C-terminals. Channel formation is preceded by bursting channel activity (*dotted ellipse*), and is very quickly followed by an apparently concerted or cooperative formation of the second barrel of a double-barreled gramicidin channel. The double-barreled ensemble then remains stable for many tens of seconds (or even minutes). For further details, see Goforth et al. [71] Diphytanoylphosphatidylcholine/*n*-decane bilayers, 1.0 M CsCl, 200 mV applied potential, 25°C

(Fig. 2.14), much longer than the ~0.8 s lifetimes of standard gramicidin channels having unlinked subunits. The tandem dimers are stabilized some 100,000-fold relative to two independent channels [71].

The decisive results with the double-barreled channels suggest strongly that there indeed is a bilayer deformation energy is associated with gramicidin channel formation (because the channel length is somewhat less than the bilayer thickness). One infers that the bulk of the energy cost is associated with the formation of the first barrel (witness the associated bursting behavior), that the cost is much less for forming the second barrel, and that the double-barreled assembly is remarkably stable within the lipid bilayer membrane [71].

2.5 Energetics of Channel-Bilayer Hydrophobic Coupling

The properties of the double-barreled nanotubes provide additional experimental evidence for the notion of an energetic coupling between gramicidin channel formation and lipid bilayer deformation [69, 72–74]. Further experiments have been designed to measure the actual deformation energy, or rather changes in bilayer deformation energy. These types of experiments typically employ gramicidin subunits of different lengths [75]. A design concept is illustrated in Fig. 2.15. When the hydrophobic length (l) of a bilayer-spanning gramicidin nanotube differs from the average thickness (d_0) of the host bilayer (Fig. 2.15), the bilayer thickness will vary locally in the vicinity of the channel dimer in order to "match" the length of the nanotube's hydrophobic exterior to the thickness of the bilayer hydrophobic core. Such bilayer deformations incur an energetic cost, the bilayer deformation energy (ΔG^0_{def}), which will vary as a function of the tube shape, the inclusion/bilayer hydrophobic mismatch (d_0–l), the lipid bilayer elastic properties and the lipid intrinsic curvature [72, 73, 76, 77].

To explore these issues further, we have developed single-molecule methods to measure ΔG^0_{def} and to probe changes in bilayer elastic properties using gramicidins as molecular force transducers [75]. The basis for these experiments is to construct nanotubes of different lengths by using gramicidin subunits of different lengths. Three fundamental approaches to measuring the deformation energy include: first, measurements of changes in channel lifetimes and appearance rates as the lipid bilayer thickness or channel length are varied; second, measurements of the equilibrium distribution among channels of different lengths, formed by homo- and heterodimers between gramicidin subunits of different lengths; and third, measurements of the ratio of the appearance rates of heterodimer channels relative to parent homodimer channels formed by gramicidin subunits of different lengths [75]. Each of these methods contributes an independent measure of the bilayer deformation energy. The best estimates to date (for hydrocarbon-containing bilayers) are 10–20 kJ mol^{-1} nm^{-2} for monoglyceride-based bilayers and 35–45 kJ mol^{-1} nm^{-2} for phosphatidylcholine-based bilayers [69, 75].

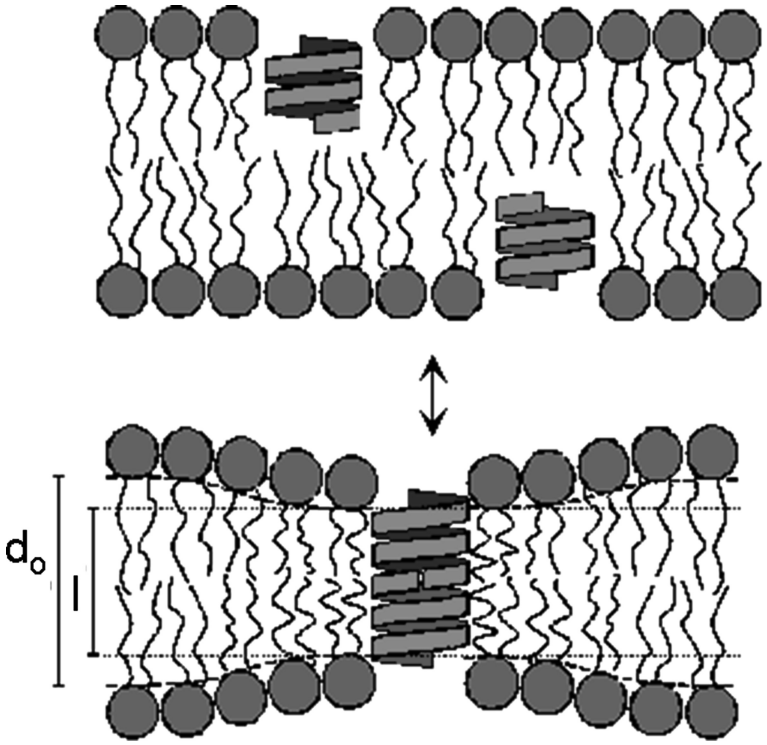

Fig. 2.15 Bilayer deformation energy. gA channel formation by the transbilayer dimerization of subunits may incur an energetic cost. When the channel's hydrophobic length (l) differs from the average thickness of the unperturbed bilayer hydrophobic core (d_o), channel formation will be associated with a bilayer deformation [78, 79], due to the compression and bending of the two bilayer leaflets, which is caused by the hydrophobic couple between the bilayer-spanning channel and the host bilayer. Figure from [53]

2.6 Summarizing Perspective

Gramicidin channels were the first channels of known chemical composition to be studied [8]. These channels serve as cation-selective nanotubes that span lipid bilayer membranes, and continue to provide a robust basis for examining many different aspects of ion channel function and channel-lipid bilayer interactions [39]. In the present context, the nanotube properties can be tuned by means of mutations or chemical changes to the subunit architecture. Diverse applications of gramicidin channels, in proof-of-principle studies and in their own right, have enhanced our understanding of the microphysics of ion permeation, lipid–protein interactions and membrane protein function.

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