J. Membrane Biol. 174, 207–212 (2000)

The Journal of
Membrane
Biology

Gating and Permeation in Ion Channels Formed by Gramicidin A and Its Dioxolane-linked Dimer in Na^+ and Cs^+ Solutions

E.P. Quigley[1], **D.S. Crumrine**[2], **S. Cukierman**[1]

[1]Department of Physiology, Loyola School of Medicine, Maywood, IL 60153, USA
[2]Department of Chemistry, Loyola University Chicago, Chicago, IL 60626, USA

Received: 17 September 1999/Revised: 21 December 1999

Abstract. The association of two gramicidin A (gA) peptides via H-bonds in lipid bilayers causes the formation of an ion channel that is selective for monovalent cations only. In this study, two gAs were covalently linked with a dioxolane group (SS dimer). Some functional properties of natural gA channels were compared to that synthetic dimer in Na^+- or Cs^+-containing solutions. The SS dimer remained in the open configuration most of the time, while natural gA channels had a relatively brief mean open time. Single channel conductances to Na^+ (g_{Na}) or Cs^+ (g_{Cs}) in the SS dimer were smaller than in natural gA. However, g_{Na} was considerably more attenuated than g_{Cs}. This probably results from a tight solvation of Na^+ by the dioxolane linker in the SS channel. In Cs^+ solutions, the SS had frequent closures. By contrast, in Na^+ solutions the synthetic dimer remained essentially in the open state. The mean open times of SS channels in different solutions ($T_{open,Na} > T_{open,Cs} > T_{open,H}$) were inversely proportional to the single channel conductances ($g_H > g_{Cs} > g_{Na}$). This suggests that ion occupancy inside the pore stabilizes the open configuration of the gA dimer. The mean closed time of the SS dimer was longer in Cs^+ than in H^+ solutions. Possible mechanisms for these effects are discussed.

Key words: Single ion channels — Gramicidin — Gating — Permeation — Dioxolane — Chirality

Introduction

Gramicidin A (gA) is a pentadecapeptide formed by an alternating sequence of D- and L-amino acids (Sarges & Witkop, 1965). This primary structure defines a right-handed, single-stranded, $\beta^{6.3}$ helix in micelles and solid state (Arseniev et al., 1985; Ketchem et al., 1993, 1997). gA forms ion channels in biological membranes that are selective for monovalent cations only (Hladky & Haydon, 1972). There is an incomparable wealth of biological, physical, and chemical information on gA channels in relation to other ion channels (Busath,1993; Koeppe & Andersen, 1996). Each gA peptide partitions in one monolayer of a lipid membrane. Six intermonomer H-bonds between the amino termini of two gAs causes the formation of an ion channel (Urry, 1971). Previously, gA monomers were covalently linked with a malonyl (Urry et al., 1971) or with a carbonsuboxide group (Bamberg & Janko, 1977). As anticipated, covalently linked gAs have an average open time considerably longer than natural gA channels. Recently, two gA monomers were linked with a dioxolane group (Stankovic et al., 1989). Because the starting compound for the production of the dioxolane is tartaric acid, two different stereoisomers of the dioxolane-linked gA can be synthesized (the SS or the RR dimers corresponding to the D- or L-diethyl ester tartrate, respectively). In the SS dimer, there is a continuous and constrained transition between the two β helices of gAs. Therefore, the basic secondary structure of the SS dimer is essentially the same as in natural gA (Stankovic et al., 1989; Crouzy et al., 1997; Quigley et al., 1999).

In our laboratory, proton conduction in the SS and RR dimers has been studied and compared to its properties in natural gA channels (Cukierman et al., 1997; Quigley et al., 1998, 1999; Cukierman, 1999). The transport of protons in water and in gA channels including the dioxolane gAs, occurs via a Grotthuss mechanism (Myers & Haydon, 1972; Levitt et al., 1978; Finkelstein, 1987). It was previously found that single channel pro-

Correspondence to: S. Cukierman

ton conductances in the SS-dioxolane linked gA dimer, and in natural gA channels have similar properties (Cukierman et al., 1997). Proton transport via a Grotthuss type mechanism in gA channels is a specific type of ion transport that does not involve direct interaction between protons and channel wall (Levitt et al., 1978; Pomès & Roux, 1996; Cukierman, 1999). Because proton transport does not occur hydrodynamically as with other ions, it is of interest to investigate the functional consequences of inserting a dioxolane link between two gA molecules on the transport properties of monovalent cations that interact with natural gA channels. In this study, the basic conduction and gating properties of the SS dioxolane-linked gA peptides were compared to natural gA channels in Na^+ and Cs^+ solutions. These permeating cations were chosen because of their markedly different charge densities. It is shown here that in the SS dimer the single channel conductances to Na^+ or Cs^+ are considerably less than in natural gA. However, the single channel conductance in the SS dimer is significantly more attenuated in Na^+ than in Cs^+ solutions. This is probably due to an increased channel binding affinity for Na^+ in relation to Cs^+. Interestingly, the SS dimer shows closures in Cs^+ but not in Na^+ solutions.

Materials and Methods

gA Channels

The SS dimer of dioxolane-linked gA peptides was synthesized from D-diethyl ester tartrate, purified using HPLC, and characterized by NMR as described before (Cukierman et al., 1997; Quigley et al., 1999). gA peptides used in the synthesis were either purchased from Fluka (Milwaukee, WI), or purified from a mixture of different gramicidin peptides (gramicidin D) using flash chromatography.

Lipid Bilayers

Experimental procedures were the same as described before (Cukierman et al., 1997). Briefly, membranes were formed onto a 0.1 mm diameter hole in a polystyrene cup (*cis*-side) nested inside a plastic chamber that formed the *trans*-side. Membranes were formed from a mixture of PE (1-palmitoyl-2-oleoyl-phosphatidylethanolamine) and PC (1-palmitoyl-2-oleoyl-phosphatidylcholine) in a 4:1 ratio (~60 mM in decane). Phospholipids were purchased from Avanti Lipids (Alabaster, AL). Experiments were performed at room temperature (21–23°C).

Experimental Setup

Both sides of the membrane containing identical salt solutions of unbuffered NaCl or CsCl were connected to an Axopatch 1D amplifier (Axon Instruments, Foster City, CA) via Ag/AgCl wires immersed in solutions. DC-voltages were applied across the bilayer. Experiments were recorded on a VCR tape and analysed offline. Analysis of single channels were performed using pClamp software (Axon Instruments, Foster City, CA).

Results

Figure 1 shows typical single channel recordings of the SS and natural gA channels in different experimental conditions. The left and right panels show recordings of the SS and gA channels, respectively. In the top and bottom panels, recordings were obtained in symmetrical solutions of 1 M CsCl or 1 M NaCl, respectively. The dashed lines in the left panels represent the closed state of the channel (0 pA), while in the right panels, they represent the open state of gA channels (baseline is 0 pA). There were several differences between the SS and gA dimers: (i) The SS dimer gates in a significantly different manner than natural gA channels. Notice that while the SS channel is predominantly in the open state (*see* dwell time distributions in Fig. 3 below), gA channels have brief openings only. In fact, it was this relatively long open time that allowed the identification of the SS dimer in lipid bilayers. Similar long open times like those illustrated in the left panels of Fig. 1 were *not* observed with natural gAs under identical experimental conditions (right panels); (ii) In NaCl solutions, intermittent closures of the SS dimer were not observed. Once the channel incorporated in the bilayer it remained in the open state throughout the duration of the experiment. The bottom recording in the left panel shows a sequence of incorporations of three different single SS dimers in a PEPC bilayer. Because SS dimers did not close in Na^+-containing solutions, the determination of single channel conductances had to follow the strategy illustrated in the left panel in the bottom row. Several incorporations of the SS dimer in the bilayer were followed, and single channel conductances at different voltages determined. This procedure allowed unambiguous determinations of single channel conductances in the SS dimer. Because the single channel conductance to Na^+ in the SS dimer is very small, channel recordings in Na^+ solutions had to be filtered at a low frequency. While it is possible that fast channel closures may exist and the limited frequency response of the system could not have detected them, a careful examination of single channel recordings in NaCl at cutoff frequencies comparable to recordings in CsCl did not clearly reveal channel closures; (iii) The single channel conductances of the SS dimer illustrated in Fig. 1 were approximately 30 pS in 1 M Cs^+, and 1.5 pS in 1 M Na^+ (20-fold difference). By contrast, the single channel conductance of gA in Cs^+ (35 pS) was approximately 3.5-fold larger than in Na^+-solutions (10 pS). Dimerization of two gA molecules with a dioxolane linker creates a channel that is not only less conductive than natural gA, but has conduction and gating properties that depend on the nature of the premeating monovalent cation.

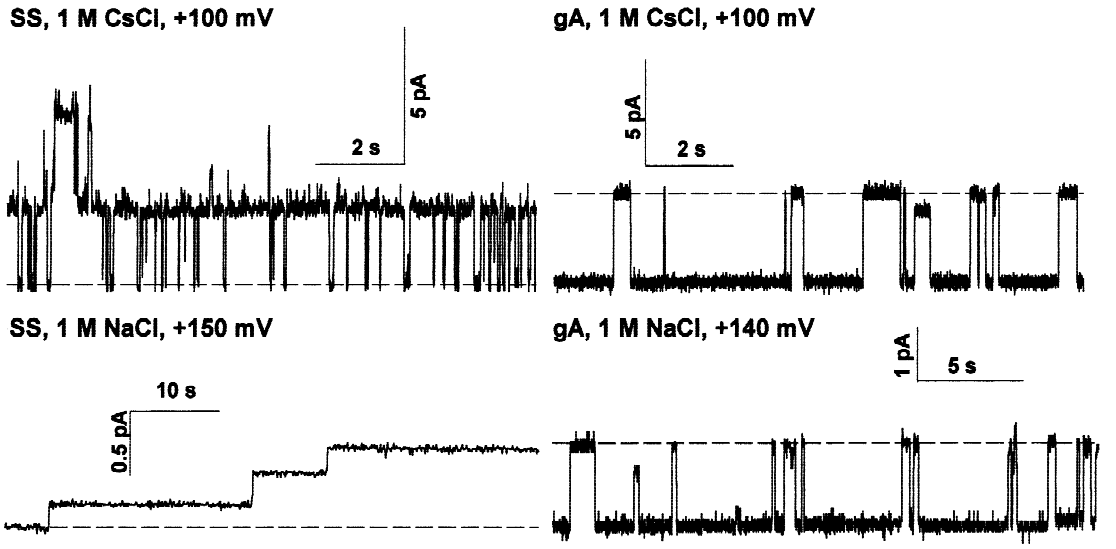

Fig. 1. Single channel recordings of the SS (left panels) and gA (right panels) in 1 M CsCl (upper row) or 1 M NaCl (bottom row). Recordings were low-pass Bessel filtered at 150 Hz and digitized at 400 Hz except for the bottom recordings on the left which was low-pass filtered at 50 Hz and digitized at 100 Hz. For the sake of clarity, the original single channel current in the bottom left panel left was decimated. This consisted in averaging stretches of single channel recordings every 50 msec, and displaying the average. Notice in the upper recording of the SS channel in CsCl a few brief openings of gA channels. It was not possible to completely purify the SS dimers from unreacted gA monomers. However the contamination of single channel recordings of the SS dimer by natural gA channels was minimal and did not interfere with single channel analysis.

In Fig. 2, the single channel conductances to Na^+ (left panel) or Cs^+ (right panel) were plotted against salt concentrations. In this figure, the open and filled circles represent single channel conductances in gA and in the SS dimer, respectively. In the left panel (NaCl) experimental points were fitted to an adsorption isotherm.

$$g_{Na} = g_{max} \cdot (1 + K_D/[\mathrm{Na}])^{-1} \quad (1)$$

in which g_{Na} is the single channel conductance to Na^+, g_{max} is the maximum g_{Na}, and K_D is the dissociation constant of Na^+ from the pore of the channel. g_{max} and K_D were 1.4 pS and 70 mM for the SS, and 16 pS is and 409 mM for the gA channel, respectively. In the right panel, a similar plot is illustrated for both channels in different symmetrical [Cs]. Fitting to Eq. 1 was not attempted in Cs^+ solutions because both gA and SS channels have a behavior that is more complex than in Na^+ solutions and suggestive of multiple ion occupancy (Eisenman et al., 1978; Urban et al., 1980; Finkelstein & Andersen, 1981). Notice that at high [Cs^+], there is a clear decline in single channel conductance for both the gA and SS channels. As mentioned in relation to Fig. 1, the difference in single channel conductances between open and filled circles is considerably less in Cs^+ than in Na^+ solutions for all concentrations.

Dwell time distributions for the SS channel in 1 M CsCl are shown in Fig. 3. Histograms of the number of open and closed events are shown in the left and right panels, respectively. Because a single exponential was fit to each set of experimental points (mean open time (T_{open}) = 235 msec; mean closed time (T_{closed}) = 15 msec the gating of SS dimers is consistent with a simple closed $\leftrightarrow$ open kinetic scheme.

Discussion

The new experimental findings presented in this study are: (i) Dioxolane-linked gA channels (SS) have a smaller g_{Na} and g_{Cs} than natural gA channels. (ii) g_{Na} is considerably more attenuated than g_{Cs}. (iii) When Na^+ is the permating cation, the SS channel remains in the open state, while with 1 M Cs^+ closing events with a mean duration of ~15 msec were recorded. In both solutions however, the mean open time of the gA dimer is considerably longer than in natural gA channels.

Results from this and previous studies demonstrated that the conductivity sequence for the monovalent cations $H^+ > Cs^+ > Rb^+ > Na^+$ is the same as in natural gA channels (Myers & Haydon, 1972; Andersen & Procópio, 1980; Urban et al., 1980; Cukierman et al., 1997). Another gA characteristic that persists in the SS channel is that when Na^+ is the permeating cation (Fig. 2), both channels seem to behave in a single occupancy mode (Andersen & Procópio, 1980; Finkelstein & Andersen, 1981; *see* however, Eisenman et al., 1978; Urban & Hladky, 1979, and Ring & Sandblom, 1983, for different

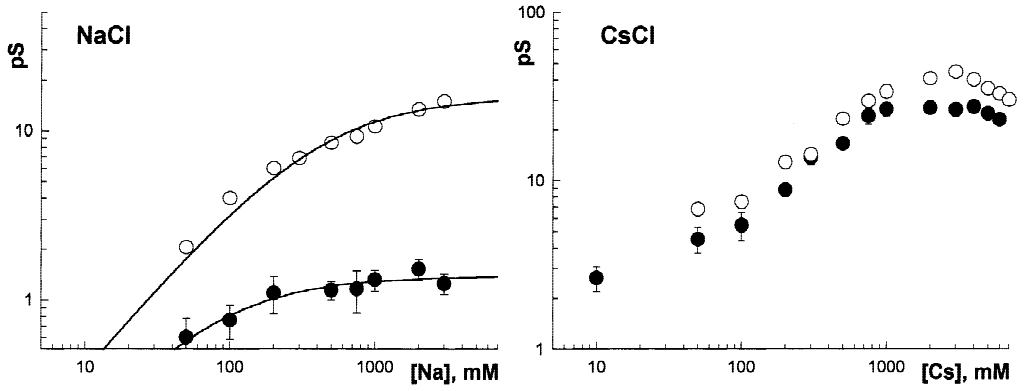

Fig. 2. Relationships between single channel conductances for the SS (filled circles) or gA (open circles) and different [Na] (left panel) or [Cs]. Single channel conductances were measured from linear regressions of current-voltage plots in the range of ±100 mV. Points are the means ± SD of 3 to 7 different measurements (different channels in different bilayers). In the left panel, best fits to Eq. (2) in text were achieved with the following parameters (mean ± SEM): K_D = 409 ± 66 mM (open circles), 70 ± 18 mM (filled circles), and g_{max} = 16 ± 0.9 pS (open circles), and 1.4 ± 0.1 pS (filled circles).

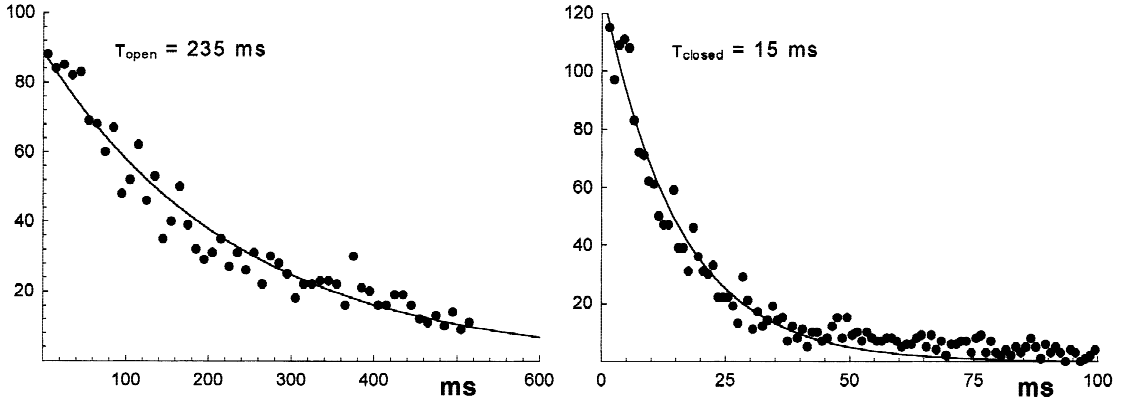

Fig. 3. Dwell time distributions of the open and closed states are shown in the left and right panels, respectively. Experimental points were fitted to single exponential with mean open and closed times of 235 and 15 msec, respectively. The transmembrane potential was 100 mV. Single channel recordings were low-pass at 200 Hz with a Bessel filtered and digitized at 400 Hz. Single channel open and closed events were defined by a threshold located half way between the fully closed and open levels of the channel.

interpretations). By contrast, the relationships between g_{Cs} and [Cs^+] in both channels show signs of multiple ionic occupancy. At high Cs^+ concentrations, multiple channel occupancy causes a decrease in single channel conductance (Eisenman et al., 1978). This is one possible interpretation for the attenuation of the Cs^+ single channel conductance at very high concentrations (Hladky & Haydon, 1972; Eisenman et al., 1978; Finkelstein & Andersen, 1981).

A noticeable difference between natural gA and its dioxolane-linked dimer concerns an overall reduction in single channel conductance for all Ia group monovalent cations (Stankovic et al., 1989, *present results*). Interestingly, the single channel conductance to Na^+ is considerably more attentuated than with other monovalent cations, g_H, on the other hand, is similar in natural gA and SS channels (Cukierman et al., 1997). With the notable exception of protons that cross the SS dimer via a Grotthuss mechanism, the transport of monovalent cations in gA channels occurs via a single file or no-pass diffusion mechanism (Levitt, 1984; Finkelstein, 1987). It has been suggested that there are no major electrostatic barriers for the transport of Na^+ across natural gA channels (Finkelstein & Andersen, 1981; Dani & Levitt, 1981). It was reasoned that the mobility of Na^+ ions inside the pore of gA channels is essentially determined by the mobility of water molecules (Finkelstein & Andersen, 1981; Dani & Levitt, 1981). Water molecules located ahead of Na^+ in the single file must leave the channel in order for Na^+ to exit the pore. Thus, one possible explanation for the markedly reduced Na^+ conductance in the SS dimer would be that water permeabil-

ity in this channel is reduced. This is not likely however. It was previously shown that the single channel proton conductance (g_H) in PEPC bilayers is similar in both the gA and SS channels (Cukierman et al., 1997). The rate-limiting step for proton transfer across the channel is the reorientation of water molecules inside the pore as in a typical Grotthuss's mechanism (Pomès & Roux, 1996; Cukierman, 1999; DeCoursey & Cherny, 1999). The overall mobility and dynamics of water molecules inside the pore are ultimately determined by the energetics of H-bond interactions between waters and the wall of the channel pore (Pomès & Roux, 1996; Quigley et al., 1999). A larger number of (or stronger) H-bond interactions between water molecules and channel wall could in principle decrease both proton transfer and water mobility in the pore. Therefore, g_H can in principle be considered as a qualitative indicator of water mobility inside the channel pore. Because g_H is similar in both the SS and gA channels, water mobility should not be dramatically different in these channels. Moreover, if water mobility were significantly decreased in the SS dimer, a stronger attenuation of Cs^+ could have been noticed. Consequently, it seems more likely that Na^+ ions bind tighter to the pore of the SS dimer than to natural gA channels. Because of its small size Na^+ would tend to lie off-axis in the channel pore and interact with oxygens of the channel wall in order to achieve a more coordinated solvation (Tredgold and Jones, 1979; Mackay et al., 1984). It is likely that in the SS dimer, the oxygen from the dioxolane linker could solvate Na^+, binding it tighter, and reducing its transit time inside the pore. In summary, the addition of a dioxolane linker between gA monomers (SS channel) causes an overall reduction in single channel conductances to different monovalent cations, with the notable exception of protons which do not interact directly with the channel wall (Pomès & Roux, 1996). In relation to Na^+, a significant electrostatic interaction between this cation and the wall of the pore develops in the SS dimer.

Dimerization of gA channels with different linkers causes a major prolongation of the mean open time of natural gA channels (Bamberg & Janko, 1977; Stankovic et al., 1989; Cukierman et al., 1997). However, covalently linked gA channels do not lose the ability to close. The mean dwell times in the SS dimer depend on the nature of the permeating cation. The mean open time in HCl solutions was approximately 30 ms (Cukierman et al., 1997), and in this study it was demonstrated that the mean open time increased to 230 msec in 1 M CsCl. With Na^+ as the permeating species, the SS dimer remains essentially in the open state. The mean open times are inversely proportional to the single channel conductances. This indicates that a long residency time (high binding constant of ion to channel causes low ionic conductance) of an ion inside the channel's pore stabilizes the channel in the open configuration. Similar phenomena have been described before in other ion channels like the delayed rectifier in neurons (Matteson & Swenson, 1986), acetylcholine receptors (Marchais & Marty, 1979), Ca channels (Nelson et al., 1984), and K channels from the sarcoplasmic reticulum of mammalian skeletal muscles (S. Cukierman, *unpublished observations*). It was also demonstrated that in natural gA channels, the mean open time increases with the bulk concentration of the permeant cation (Kolb & Bamberg, 1977). This observation is also consistent with our experimental results. An increase in cation concentration leads to an increased averagic ionic occupancy of the pore thus lengthening the channel's open time.

Ring and Sandblom (1983, 1988, 1992) proposed that the association of gA monomers (open state of channel) in bilayers is stabilized by the channel's ion occupancy. Dissociation of monomers was markedly reduced by the channel's ionic occupancy. Our experimental observations suggest that the open state of the channel is also modulated by ion occupancy of the pore even when two gA monomers are covalently linked, and as such, unable to dissociate in the plane of the bilayer. While the hypothesis advanced by Ring and collaborators is certainly consistent with their experimental and modeling results, it seems that ion occupancy of the pore can favor the open state of the channel by mechanisms other than the stabilization of monomer-monomer association via H-bonds.

By contrast, the mean closed times do not have a simple relationship as a function of single channel conductances with different permeant cations. The mean closed time increased 1,000-fold (from 15 μsec in HCl to 15 msec in CsCl), and with the caveat that recordings in Na^+ had to be dramatically filtered, no clear closing events were detected in the latter condition. Stankovic and collaborators (1989, 1990) have not detected closing flickers in the SS dimer in KCl or HCl solutions. While our experimental results in HCl solutions are in disagreement with theirs (Cukierman et al., 1997; Quigley et al., 1999), it is of interest to note that closing flickers in the SS dimer depend on the nature of the permeating cation. Crouzy et al. (1994) have performed molecular dynamics simulation in both the SS and RR dioxolane-linked dimers. Their results suggested that it is possible for the dioxolane to protrude inside the pore and close the channel. In particular, it was found that one K^+ inside the RR stereoisomer of the gA dimer stabilized the dioxolane linker inside the pore. Gating modulation by permeant cations of the SS dimer is a complex phenomenon in which the interaction of permeant cations with different parts of the pore could favor different states of the channel. Because of its relative simplicity, the SS dimer offers a unique opportunity to investigate at the atomic level the basic rules that govern ion channel gating.

EPQ was supported by the MD/PhD program (Loyola University School of Medicine), and by a Schmidtt fellowship (Graduate School, Loyola University Chicago).

References

Andersen, O.A., Procopio, J. 1980. Ion movement through gramicidin A channels. On the importance of the aqueous diffusion resistance and ion-water interactions. *Acta Physiol. Scand. Suppl.* **481:**27–35

Arseniev, A.S., Barsukov, I.L., Bystrov, V.F., Lonize, A.L., Ovchinikov, Y.A. 1985. Proton NMR study of gramicidin A transmembrane ion channel. Head-to-head right handed, single stranded helices. *FEBS Lett.* **186:**168–174

Bamberg, E., Janko, K. 1977. The action of a carbonsuboxide dimerized gramicidin A on lipid bilayer membranes. *Biochim. Biophys. Acta* **465:**486–499

Busath, D.D. 1993. The use of physical methods in determining gramicidin channel structure and function. *Ann. Rev. Physiol.* **55:**473–501

Crouzy, S., Woolf, T.B., Roux, B. 1994. A molecular dynamics study of gating in dioxolane-linked gramicidin A channels. *Biophys. J.* **67:**1370–1386

Cukierman, S. 1999. Flying protons in gramicidin A channels. *Isr. J. Chem.* **39:**419–426

Cukierman, S., Quigley, E.P., Crumrine, D.S. 1997. Proton conduction in gramicidin A and in its dioxolane-linked dimer in different lipid bilayers. *Biophys. J.* **73:**2489–2502

Dani, J.A., Levitt, D.G. 1981. Water transport and ion-water interaction in the gramicidin channel. *Biophys. J.* **35:**501–508

DeCoursey, T.E., Cherny, V.V. 1999. An electrophysiological comparison of voltage-gated proton channels, other ion channels, and other proton channels. *Isr. J. Chem.* **39:**———

Eisenman, G., Sandblom, J., Neher, E. 1978. Interactions in cation permeation through the gramicidin channel. Cs, Rb, K, Na, Li, Tl, H, and effects of anion binding. *Biophys. J.* **22:**307–340

Finkelstein, A. 1987. Water Movement Through Lipid Bilayers, Pores, and Plasma Membrane. Theory and Reality. John Wiley, New York

Finkelstein, A., Andersen, O.S. 1981. The gramicidin A channel: a review of its permeability characteristics with special reference to the single-file aspect of transport. *J. Membrane Biol.* **39:**155–171

Hladky, S.B., Haydon, D.A. 1972. Ion transfer across lipid membranes in the presence of gramicidin A. I. Studies of the unit conductance channel. *Biochem. Biophys. Acta* **274:**294–312

Ketchem, R.R., Hu, W., Cross, T.A. 1993. High resolution of gramicidin A in a lipid bilayer by solid-state NMR. *Science* **261:**1457–1460

Ketchem, R.R., Roux, B., Cross, T.A. 1997. High-resolution polypeptide structure in a lamellar phase lipid environment from solid state NMR derived orientational constraints. *Struct.* **5:**1655–1669

Koeppe, II R.E., Andersen, O.S. 1996. Engineering the gramicidin channel. *Annu. Rev. Biophys. Biomol. Struct.* **25:**231–258

Kolb, H.A., Bamberg, E. 1977. Influence of membrane thickness and ion concentration on the properties of gramicidin A channel. Autocorrelation, spectral power density, relaxation and single channel studies. *Biochim. Biophys. Acta* **464:**127–141

Levitt, D.G. 1984. Kinetics of movement in narrow channels. *Curr. Topics in Memb. and Transp.* **21:**181–197

Levitt, D.G., Elias, S.R., Hautman, J.M. 1978. Number of water molecules coupled to the transport of sodium, potassium, and hydrogen ions via gramicidin, nonactin or valinomycin. *Biochim. Biophys. Acta* **512:**436–451

Marchais, D., Marty, A. 1979. Interaction of permeant ions with channels activated by acetylcholine in *Aplysia* neurones. *J. Physiol.* **297:**9–45

Mackay, D.H.J., Berens, P.H., Wilson, K.R. 1984. Structure and dynamics of ion transport through gramicidin A. *Biophys. J.* **46:**229–248

Matteson, D.R., Swenson, Jr., R.P. 1986. External monovalent cations that impede the closing of K channels. *J. Gen. Physiol.* **87:**795–816

Myers, V.B., Haydon, D.A. 1972. Ion transfer across lipid membranes in the presence of gramicidin A. *Biochim. Biophys. Acta* **274:**313–322

Nelson, M.T., French, R.J., Krueger, B.K. 1984. Voltage-dependent calcium channels from brain incorporated into planar lipid bilayers. *Nature* **308:**77–80

Pomès, R., Roux, B. 1996. Structure and dynamics of a proton wire: a theoretical study of H^+ translocation along the single-file water chain in the gramicidin A channel. *Biophys. J.* **71:**9–39

Quigley, E.P., Quigley, P., Crumrine, D.S., Cukierman, S. 1999. Proton permeation in different stereoisomers of gramicidin A channels. *Biophys. J.* **77:**2479–2491

Quigley, E.P., Emerick, A., Crumrine, D.S., Cukierman, S. 1998. Proton current attentuation by methanol in a dioxolane-linked gramicidin A dimer in different lipid bilayers. *Biophys. J.* **75:**2811–2820

Ring, A. 1992. Influence of ion occupancy and membrane deformation on gramicidin A channel stability in lipid membranes. *Biophys. J.* **61:**1306–1315

Ring, A., Sandblom, J. 1988. Modulation of gramicidin A open channel lifetime by ion occupancy. *Biophys. J.* **53:**549–559

Ring, A., Sandblom, J. 1983. Measurement of channel life time in artificial lipid membranes: dimerization kinetics of gramicidin A. *J. Membrane Biol.* **16:**319–337

Sarges, R., Witkop, B. 1965. The structure of valine- and isoleucine-gramicidin A. *J. Am. Chem. Soc.* **87:**2011–2019

Stankovic, C.J., Heinemann, S.H., Delfino, J.M., Sigworth, F.J., Schreiber, S.L. 1989. Transmembrane channels based on tartaric acid-gramicidin A hybrids. *Science* **244:**813–817

Stankovic, C.J., Heinemann, S.H., Schreiber, S.L. 1990. Immobilizing the gate of a tartaric-acid gramicidin A hybrid channel molecule by rational design. *J. Am. Chem. Soc.* **112:**3702–3704

Tredgold, R.H., Jones, R. 1979. A study of gramicidin using deuterium oxide. *Biochim. Biophys. Acta* **550:**543–545

Urban, B.W., Hladky, S.B., Haydon, D.A. 1980. Ion movements in gramicidin pores: an example of single-file transport. *Biochim. Biophys. Acta* **602:**331–354

Urban, B.W., Hladky, S.B. 1979. Ion transport in the simplest single file pore. *Biochim. Biophys. Acta* **554:**410–429

Urry, D.W. 1971. Gramicidin A transmembrane channel: a proposed $\Pi_{(L,D)}$ helix. *Proc. Natl. Acad. Sci. USA* **68:**672–676

Urry, D.W., Goodall, M.C., Glickson, J.D., Meyers, D.F. 1971. The gramicidin A transmembrane channel: characteristics of head-to-head dimerized $\Pi_{(L,D)}$ helices. *Proc. Natl. Acad. Sci. USA* **68:**1907–1911