Design of Regulated Ion Channels Using Measurements of Cis−Trans Isomerization in Single Molecules

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Abstract: A key component in the design of artificial ion channels is the design of regulatory elements. We have synthesized a derivative of the peptide ion channel, gramicidin, in which an amino group is connected to the mouth of the channel by a linker containing a carbamate group. Thermal cis−trans isomerization of the carbamate, which can be detected at the single-molecule level, is found to regulate the flux of Cs^+ ions through the channel. The predictability of the system suggests that design of more sophisticated types of channel regulation will be possible.

Introduction

The goal of producing nanoscale devices[1] subsumes an ability to design and build objects of molecular dimensions with controlled activities. Artificial ion channels are examples of such nanoscale devices which have attracted considerable attention.[2] Catalysis of the movement of ions across membranes has been achieved with several synthetic systems.[3] Ideally, one would like to control this activity—modulate the flow of ions through the channels—in a predictable way.[4]

The technique of single-channel recording[5] provides a powerful aid for molecular design in that it gives information directly about the behavior of single (ion channel) molecules. We have used this technique, and taken the well-established structure of the gramicidin channel as a starting point, for exploring the design of regulated artificial ion channels.

Gramicidin (g, gram) is a 15 amino acid peptide which forms channels when two $\beta^{6.3}$ helical monomers self-associate (N-terminus to N-terminus) in a lipid membrane.[6] The channel passes monovalent cations; anions are impermeant. We reasoned that the placement of flexible, positively-charged groups at the C-terminal end of the peptide (*i.e*, near the mouth(s) of the channel) might modulate the cation conductance (as has been proposed for a variety of protein ion channels[7]) and that the size of the effect would depend on the proximity of the charge to the channel entrance. In addition, if the geometry was suitable, such groups might physically block the channel—in analogy with what has been proposed for the mechanism of inactivation of certain protein ion channels (the "ball and chain" model of channel inactivation[8]).

Experimental Section

Synthesis and Purification of Gramicidin Derivatives. For simplicity, the peptides are named according to the amine portion of the C-terminal extensions. Structures of the extensions are shown in Figure 1; the oxygen of the carbamate bond derives from the C-terminal hydroxyl group of the parent peptide (Scheme 1). Gramicidin−ethylenediamine was synthesized as follows: Commerical gramicidin D (20 μmol) (Sigma, St. Louis, MO) was esterified (1 h, 4 °C) with *p*-nitrophenyl chloroformate (200 μmol) in dry tetrahydrofuran containing triethylamine (TEA). The resulting carbonate ester was either separated by gel filtration or treated directly with an excess of ethylenediamine in dimethylformamide. The product was separated by gel filtration using LH-20 in methanol and then by double passage through a reverse-phase HPLC column (Zorbax Rx-C8; 80% MeOH, 0.1% trifluoroacetic acid, TEA to pH 3). Other derivatives were made by replacing ethylenediamine with the appropriate amine, diamine, or amino acid. All derivatives were characterized by UV, TLC, NMR, and FAB-mass spectrometry. TLC (C/M/W 65/25/4): g−ethylenediamine, $R_f = 0.47$; g−propylamine, $R_f = 0.77$; g−piperazine, $R_f = 0.63$; gramicidin−β-alanine, $R_f = 0.4$; g−*N*,*N*-dimethylethylenediamine, $R_f = 0.52$; g−*N*,*N*,*N*′-trimethylethylenediamine, $R_f = 0.57$; gramicidin, $R_f = 0.73$. All derivatives with free amino groups gave a positive reaction with ninhydrin on TLC plates. FAB-MS: g−ethylenediamine (MH^+ 1969), g−propylamine (M^+ 1968, MNa^+ 1991), g−piperazine (MH^+ 1995), g−β-alanine (M^+ 1998, MNa^+ 2020), g−*N*,*N*-dimethylethylenediamine (MH^+ 1996), g−*N*,*N*,*N*′-trimethylethylenediamine (MH^+ 2010). NMR signals (^{1}H-NMR, 500 MHz, DMSO-d_6) were observed at 3.9−4.0 ppm (DMSO reference, 2.49 ppm) corresponding to protons on the carbon adjacent to oxygen in the carbamate linkage for each derivative. No signals were observed at this position in the parent compound. The near identity of the indole regions of the spectra confirm the absence of any degradation of tryptophan residues in the peptide.

Circular Dichroism. CD spectra of the peptides in dioleoylphosphatidylcholine sonicated vesicles (1:20 mole ratio) were recorded with a Jasco J-700A spectropolarimeter using quartz cells of either 0.01 or

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(1) (a) Spach, G.; Duclohier, H.; Molle, G.; Valleton, J. M. *Biochimie* **1989**, *71*, 11−21. (b) Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1990**, 29, 1304−1319. (c) Drexler, K. E. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 377−405.

(2) (a) Stankovic, C. J.; Heinemann, S. H.; Delfino, J. M.; Sigworth, F. J.; Schreiber, S. L. *Science* **1989**, *244*, 813−817. (b) Lear, J. D.; Wasserman, Z. R.; DeGrado, W. F. *Science* **1988**, *240*, 1177−1181. (c) Pregel, M. J.; Jullien, L.; Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1637−1640. (d) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. *Nature* **1994**, *369*, 301−304.

(3) (a) Fyles, T. M. In *Bioorganic Chemistry Frontiers*; Dugas, H., Ed.; Springer-Verlag: Heidelberg, 1990; Vol. 1, pp 71−114. (b) Akerfeldt, K. S.; Lear, J. D.; Wasserman, Z. R.; Chung, L. A.; DeGrado, W. F. *Acc. Chem. Res.* **1993**, *26*, 191−197. (c) Voyer, N. *J. Am. Chem. Soc.* **1991**, *113*, 1818−1821.

(4) (a) Stankovic, C. J.; Heinemann, S. H.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 3702−3704. (b) Woolley, G. A.; Epand, R. M.; Kerr, I. D.; Sansom, M. S. P.; Wallace, B. A. *Biochemistry* **1994**, *33*, 6850−6858. (c) Stankovic, C. J.; Heinemann, S. H.; Schreiber, S. L. *Biochim. Biophys. Acta* **1991**, *1061*, 163−170.

(5) *Single-Channel Recording*; Sakmann, B., Neher, E., Eds.; Plenum Press: New York, 1983.

(6) (a) Woolley, G. A.; Wallace, B. A. *J. Membr. Biol.* **1992**, *129*, 109−136. (b) Finkelstein, A.; Andersen, O. S. *J. Membr. Biol.* **1981**, *59*, 155−171. (c) Bamberg, E.; Apell, H. J.; Alpes, H.; Gross, E.; Morell, J. L.; Harbaugh, J. F.; Janko, K.; Lauger, P. *Fed. Proc.* **1978**, *37*, 263−2638.

(7) (a) Jordan, P. C. *Biophys. J.* **1987**, *51*, 297−311. (b) Schofield, P. R.; Darlison, M. G.; Fujita, N.; Burt, D. R.; Stephenson, F. A.; Rodriguez, H.; Rhee, L. M.; Ramachandran, J.; Reale, V.; Glencorse, T. A.; Seeburg, P.; Barnard, E. A. *Nature* **1987**, *328*, 221−227.

(8) Hille, B. *Ionic Channels of Excitable Membranes*, 2nd ed.; Sinauer: Sunderland, MA, 1992.

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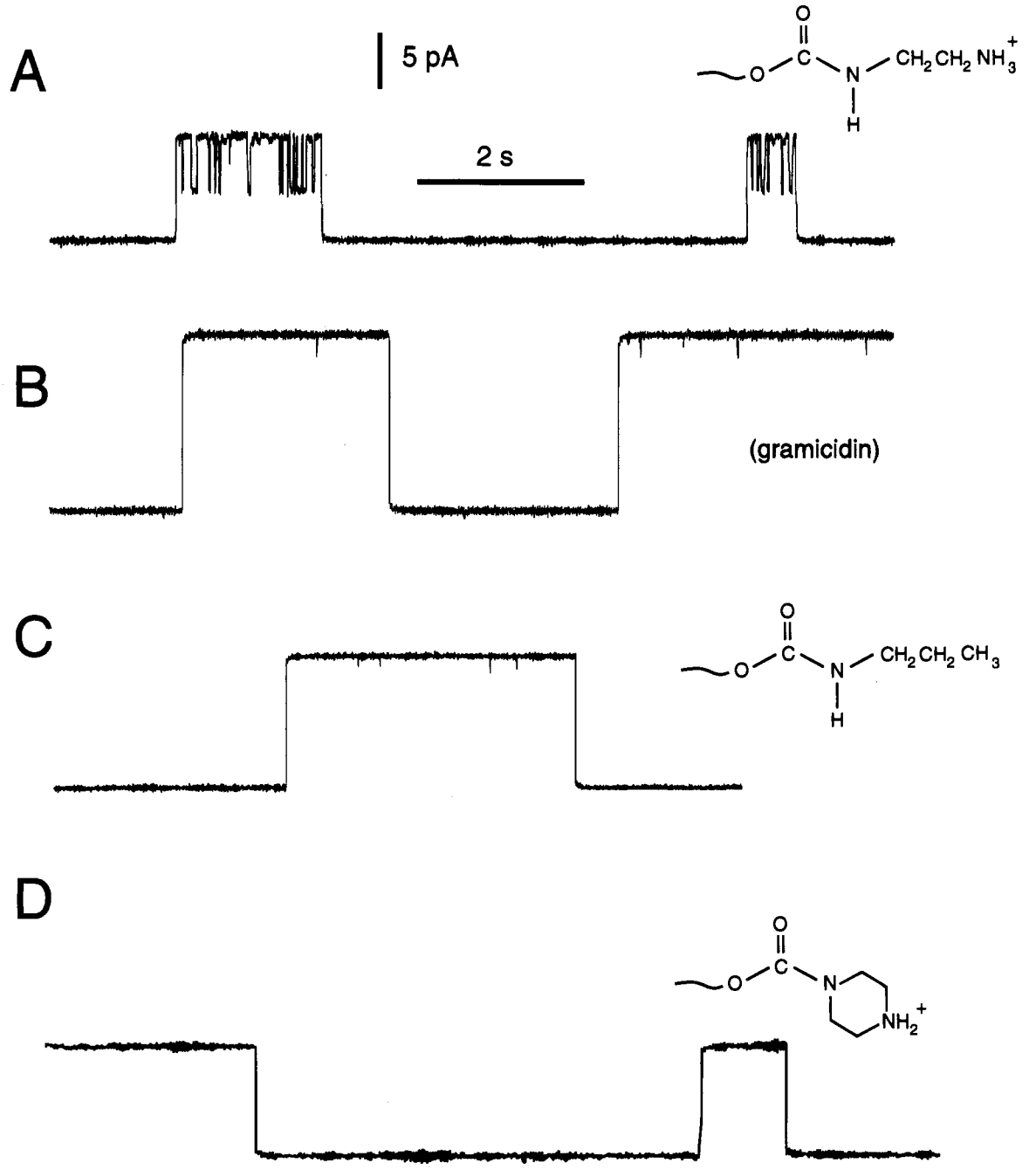
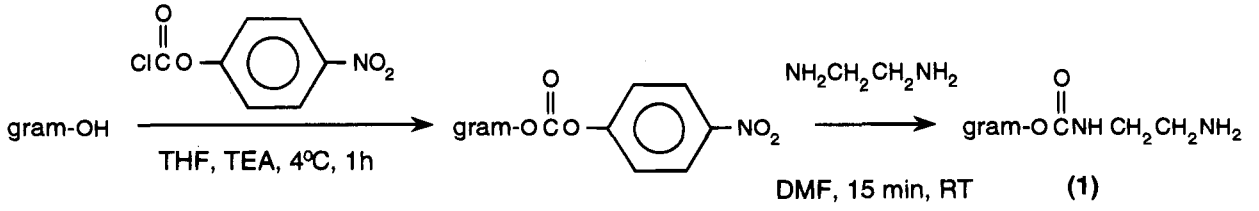


Figure 1. Single-channel currents of gramicidin derivatives. The structures of the C-terminal extensions are shown. (A) Gramicidin−ethylenediamine channels show distinct current levels during the lifetime of the dimer. The highest and lowest current levels are 9.8 pA (±0.6 pA; $n = 331$) and 4.8 pA (±0.6 pA; $n = 348$). (B) Gramicidin A channels under the same conditions have one predominant current level of 15.3 pA (±0.9 pA; $n = 59$). (C) Gramicidin propylamine channels (14.3 ± 0.9 pA; $n = 108$). (D) Gramicidin−piperazine channels (9.7 ± 0.5 pA; $n = 528$).

Scheme 1



gram-OH = HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-CONHCH$_2$CH$_2$OH

0.001 cm pathlength. Concentrations of the peptides were based on the absorbance measured at 280 nm ($\epsilon_m = 22\,600$). The spectropolarimeter was calibrated using (+)-10-camphorsulphonic acid, and all measurements were made at room temperature (22 ± 2 °C).

Single Channel Measurements. Peptides (~10 nM in MeOH) were added to membranes formed from glycerol monooleate/hexadecane using established techniques.[9] Solutions were unbuffered 1 M CsCl (pH = 5.8) (except where noted), and all measurements were made at

23 °C (±1 °C), 200 mV applied voltage. Currents were measured, and voltage was set using an Axopatch 1D patch-clamp amplifier (Axon Instruments). Data was filtered at either 100 or 500 Hz, sampled at 1 kHz, stored directly to disk, and analyzed using the program Synapse (Synergistic Research Systems). State lifetimes were estimated by maximum likelihood fitting of histograms of log(channel duration) using MacTac (Skalar Instruments) as described.[10]

Results and Discussion

The peptide gramicidin−ethylenediamine (**1**) was synthesized as outlined in Scheme 1; it bears an amino group connected by a carbamate linkage to the C-terminal end of gramicidin. Extensions to the C-terminal end of gramicidin are expected to have relatively minor effects on the overall structure of the channel. Consistent with this expectation, the circular dichroism spectrum of membrane-bound gramicidin−ethylenediamine has a shape characteristic of the normal channel form of gramicidin.[11] When small amounts of gramicidin−ethylenediamine are incorporated into lipid bilayers, single channels form (Figure 1A). Compared to that of the parent compound, gramicidin A (Figure 1B), the single-channel current is significantly reduced. The most striking difference, however, is the presence of different current levels during the lifetime of a gramicidin−ethylenediamine dimer.

These different conducting states are clearly related to the presence of the charged amino group since gramicidin−propylamine, the corresponding derivative with CH_3 in place of NH_3^+, shows only one level with a conductance similar to that of gram A (Figure 1C). The different levels cannot be due to different states of protonation (*i.e*, NH_2 vs NH_3^+) since, at the pH of the measurements (pH = 5.8), protonation of the amino group should be complete. Furthermore, the fraction of time spent in the different states is not sensitive to pH (when pH < 10; not shown).

Instead, these states appear to be due to cis−trans isomerization of the carbamate linkages in the single channels. Molecular models indicate that cis−trans isomerization would alter the proximity of the positively-charged amino group to the mouth of the channel (Figure 2). This, in turn, could alter the flux of Cs^+ ions (the measurements are made in CsCl) through a combination of electrostatic and steric effects (*vide infra*).[12] Carbamate bonds are known to have barriers to rotation on the order of 55−70 kJ/mol.[13,14] Cis and trans isomers should then have lifetimes on the order of 1−300 ms at 25 °C. Dynamic NMR measurements of isomerization of *N*-methyl-*N*-propylethylcarbamate (a closely related model compound for the present case) gave a Gibbs energy of activation of 63.2 kJ/mol (a lifetime of about 25 ms) in $CDCl_3$ solution.[14] The lifetime of the lowest current state of gramicidin-ethylenediamine is 30 ms; that of the upper state is 72 ms (Figure 3A). These lifetimes are remarkably similar to that of the model compound given the difference in environment.

Further evidence that the states we measure are due to cis−trans isomerization in single channels is provided by the behavior of a another derivative, gramicidin−piperazine. In this derivative, cis and trans isomers are degenerate, so only one state should be observed, despite the presence of a charged amino group. Only one conducting state is found (Figure 1D).

The gramicidin−ethylenediamine dimer should, in principle, exhibit four states: cis or trans at the exit and at the entrance of the channel (Figure 2). The single-channel records show three resolved states (Figure 3A), but the difference between states is nonuniform. A fourth state could thus simply be indistinguishable from one of the other three in terms of conductance. Nonuniform differences between current levels are understandable if cation conductance is more sensitive to the presence of an amino group at the entrance of the channel than the exit (or *vice versa*).

To address the relative importance of a positive charge at the entrance or exit of the channel, hybrid channels were formed in which one monomer was gramicidin A and the other was gramicidin−ethylenediamine (Figure 3B). First, gramicidin A was added to both sides of the membrane, and then, gramicidin−ethylenediamine was added to *one side only* of the preformed membrane. In this way, hybrids form (in addition to gramicidin homodimers) and the orientation of the hybrid channels is known.[15] Figure 3B shows a single-channel record of such a hybrid channel. When the voltage is positive, the positively charged arm is at the exit of the channel. Only two states are observed which differ little in the current they pass (13 pA; 13.7 pA). If the voltage is abruptly switched during the lifetime of one of these hybrids, the side with the positively-charged arm now becomes the channel entrance. Again, only two states are observed, but this time, they differ significantly in the current they pass (4.8 pA; 10 pA). Both the latter states pass less current than the two states observed with positive voltages. A positive charge at the channel entrance thus inhibits the flow of cations more than a positive charge at the channel exit. In the homodimer, which has a positive charge at both the entrance and the exit, both small and large current steps are observed (Figure 3A).

It remains to assign the observed conducting states to the corresponding conformational states. A preference for trans geometry is well-known for monosubstituted amides,[16] and *trans*-carbamates are calculated to be similarly more stable than *cis*-carbamates.[17] At the single molecule level, the mean lifetime of the trans state should thus be longer than that of the cis state. For the gramicidin−ethylenediamine homodimer (Figure 3A), we therefore assign the highest current level to the state with trans bonds at both the entrance and the exit of the channel. The next highest level has a trans bond at the channel entrance and a cis bond at the exit (since isomerization at the exit has a smaller effect on the current than isomerization at the entrance). These states combined have a mean lifetime of 72 ms (Figure 3A). The lowest level has a cis bond at the channel entrance. It may have either a cis or trans bond at the exit, but these states presumably differ little in the current they pass. The lowest level has a mean lifetime of 30 ms (Figure 3A), significantly shorter than the higher levels.

The lower conductance for a channel in a cis state might result from an electrostatic barrier created by the proximity of the charged amino group to the channel entrance or from a steric

(9) Busath, D.; Szabo, G. *Biophys. J.* **1988**, *53*, 689−695.
(10) Sigworth, F. J.; Sine, S. M. *Biophys. J.* **1987**, *52*, 1047−1054.
(11) The CD spectra (recorded as described in the Experimental Section) were the same as those reported previously for the channel form of gramicidin (ref 6a and references therein). Further evidence for an unaltered channel conformation is provided by the observation of hybrid formation between gramicidin−ethylenediamine and gramicidin A (Figure 3B, ref 15b).
(12) The permeability of gramicidin−ethylenediamine to Cl^- ions was checked by looking for currents with choline Cl^- as the electrolyte. No current was observed until KCl was added, indicating that the channel is impermeable to chloride.
(13) (a) Muraoka, O.; Minematsu, T.; Tsuruzawa, J.; Momose, T. *Heterocycles* **1985**, *23*, 853−857. (b) Kornberg, N.; Kost, D. *J. Chem. Soc., Perkin Trans.* **1979**, *2*, 1661−1664. (c) Martin, M. L.; Mabon, F.; Trierweiler, M. *J. Phys. Chem.* **1981**, *85*, 76−78.
(14) Kleinpeter, E.; Kretschmer, M.; Borsdorf, R.; Widera, R.; Muehlstaedt, M. *J. Prakt. Chem.* **1980**, *322*, 793−797.
(15) (a) O'Connell, A. M.; Koeppe, I., R. E.; Andersen, O. S. *Science* **1990**, *250*, 1256−1259. (b) Durkin, J. T.; Koeppe, I., R. E. *J. Mol. Biol.* **1990**, *211*, 221−234.
(16) Stewart, W. E.; Siddall, I., T. H. *Chem. Rev.* **1970**, *70*, 517−551.
(17) Remko, M.; Scheiner, S. *J. Mol. Struct. (THEOCHEM)* **1988**, *180*, 175−188.

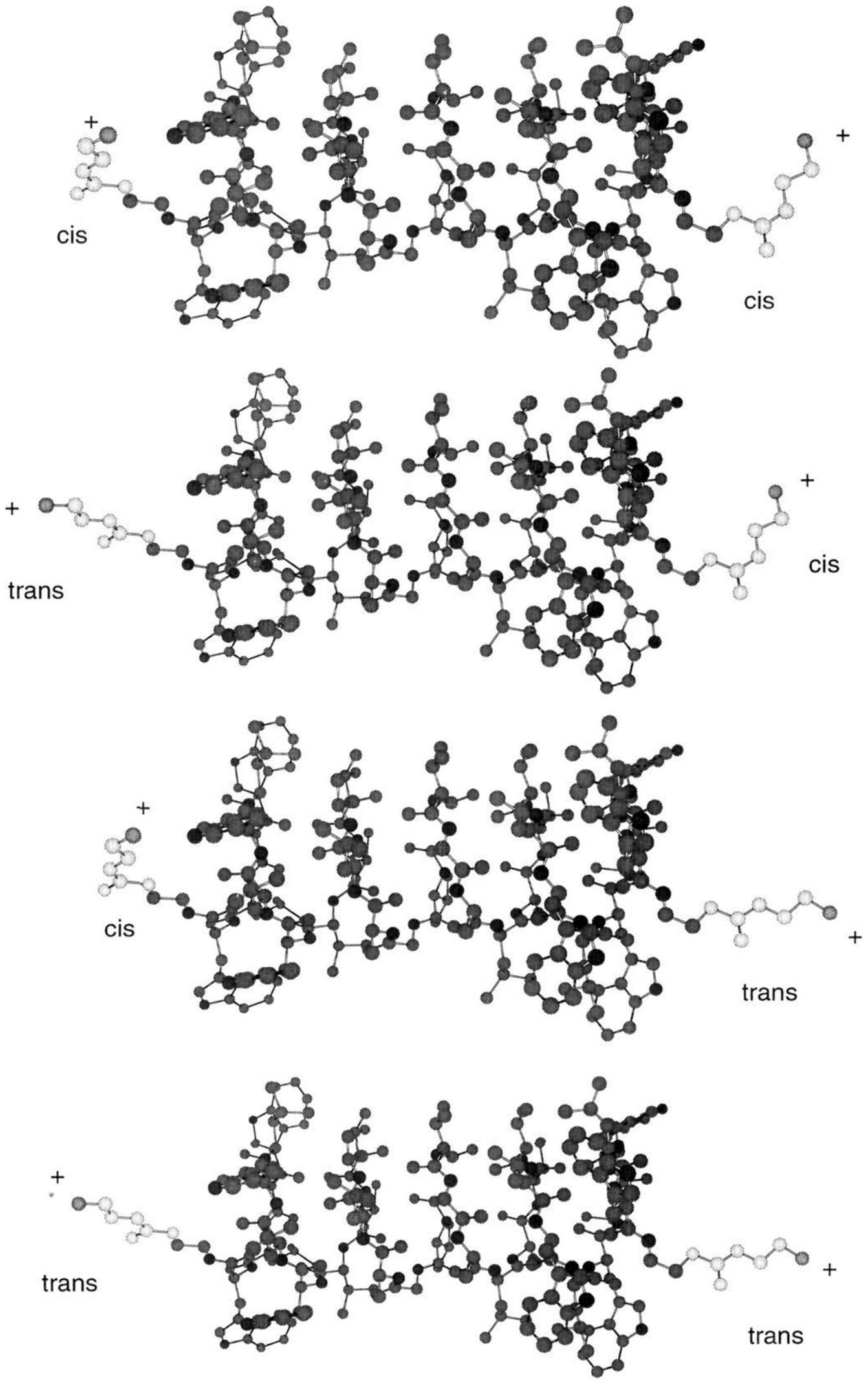


Figure 2. Molecular models of the four conformational states assumed by the gramicidin−ethylenediamine channel which differ by rotation about the C−N carbamate bond. Models were built using coordinates derived from the NMR data of Arseniev[20] for gramicidin in lipid micelles. The carbamate-linked extension is light gray and the positively-charged terminal nitrogen atom is darker gray. These are static representations of a dynamic system. On average, however, a *cis*-carbamate is less extended than a trans linkage.

block of the entrance, or both. To distinguish these effects, we examined the behavior of a further derivative, gramicidin−β-alanine. This derivative has a carboxylate group in place of the amino group of gramicidin−ethylenediamine. Under the conditions of the experiment, this group will be negatively charged. If electrostatic effects predominate, this derivative should show reversed behavior (*i.e.*, the cis state should have a *higher* conductance than the trans); if steric effects predominate, then a behavior similar to that of gramicidin−ethylenediamine should be observed. Figure 4 shows a comparison of single-channel currents for gramicidin−ethylenediamine (A) and gramicidin−β-alanine (B) in 0.2 M CsCl. The gramicidin−β-

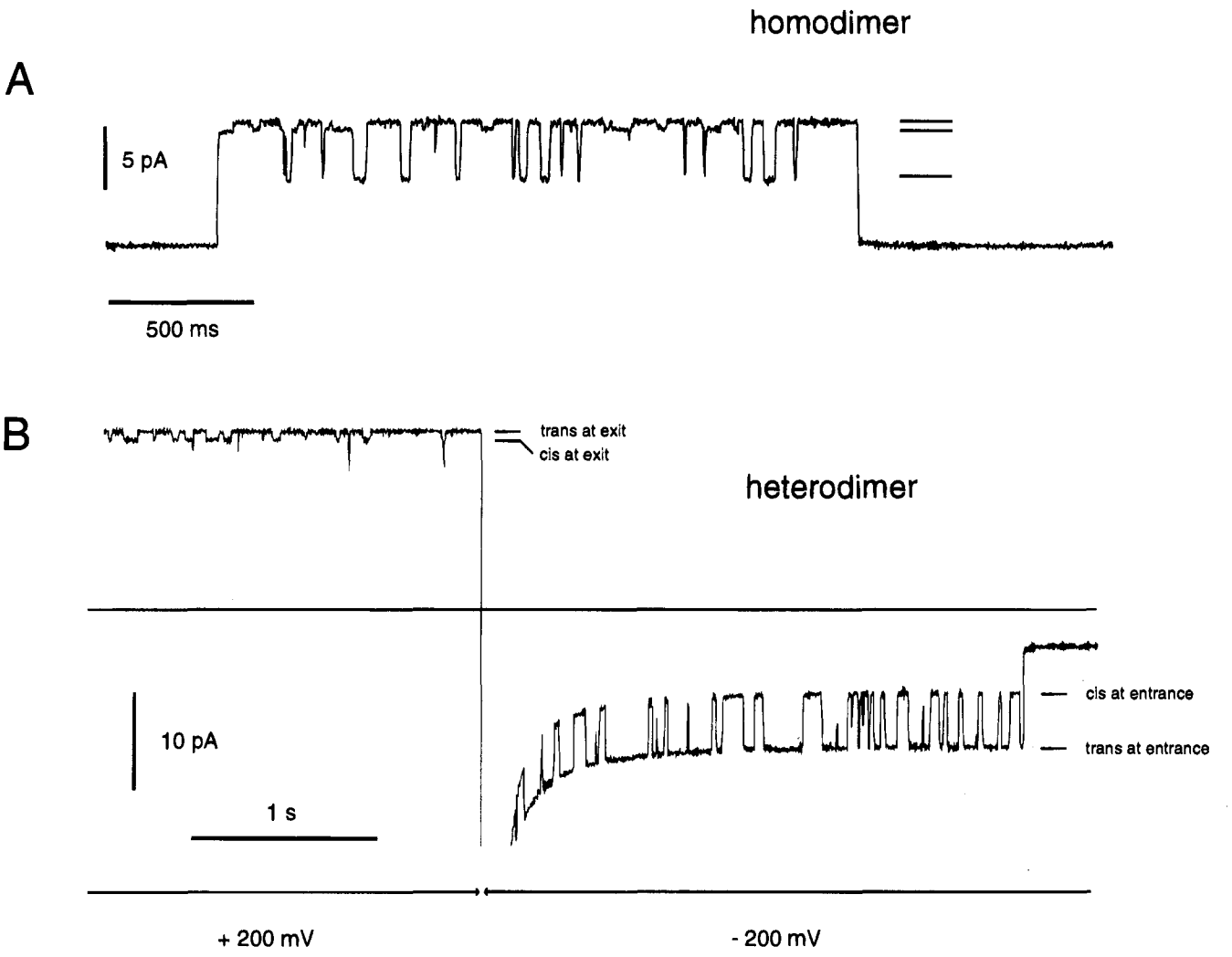


Figure 3. (A) Expanded view of a gramicidin−ethylenediamine (homodimer) single-channel current trace (1 M CsCl; +200 mV; filtered at 200 Hz). Three levels are evident (as indicated by the horizontal bars). The highest and lowest levels are 9.8 pA (±0.6 pA; $n = 331$) and 4.8 pA (±0.6 pA; $n = 348$), respectively. Small variations between channels obscure the intermediate level when many transitions are averaged. Within any one channel, the intermediate level can be resolved clearly and passes about 0.9 pA less current than the highest level. The lifetime of the lowest current level is 30 ms (estimated by maximum likelihood;[10] $n = 586$). The lifetime of the top level and the intermediate level combined (*i.e*, states with a *trans*-carbamate at the channel entrance (see text)) is 72 ms ($n = 640$). (B) Reversal of the direction of ion flow during the lifetime of a gramicidin−ethylenediamine/gramicidin A hybrid. Gramicidin A was present at both sides of the membrane. Gramicidin−ethylenediamine was added to one side of the membrane only (the side opposite the patch-clamp headstage). Hybrid channels of the of two types formed depending on the sign of the applied voltage (asymmetry was >90%). At +200 mV (left half of figure), the carbamate extension is at the channel exit and two current levels are seen: 13.7 and 13 pA (±0.5 pA, $n = 33$). When the voltage is abruptly switched to −200 mV, Cs^+ ions flow in the opposite direction so that the carbamate extension is at the channel entrance. After the capacitive transient decays, two new current levels are seen for the same channel (4.6 and 10.0 pA, ±0.5 pA, $n = 62$). For the assignment of cis and trans states, see text.

alanine channels show two current levels, but now it is the *lower* level which has the longer average lifetime, *i.e.* the reverse of the gramicidin−ethylenediamine case. In addition, gramicidin−β-alanine displays a significantly higher conductance in both cis and trans states than does gramicidin−ethylenediamine. Electrostatic effects thus appear to dominate magnitudes of the observed conductances.

It is possible, however, that steric effects also play a role. Whereas the lower conductance state of gramicidin−β-alanine passes about 90% of the current of the higher state, in the case of gramicidin−ethylenediamine, the lower state passes only about 35% of the current of the higher state under the conditions of Figure 4. This difference may be due to an additional effect which could arise with positively-charged gramicidin derivatives. Gramicidin is a cation-selective channel, and ions such as ammonium and methylammonium are permeant.[18] Selection for cations over anions is believed to occur as a consequence of interactions of the ions with carbonyl groups at the mouth of the channel.[19] The charged primary amino group of gramicidin−ethylenediamine may thus find a weak binding site at the mouth of the channel. Modeling indicates that there is sufficient flexibility in the C-terminal extension of gramicidin−ethylenediamine for such an interaction to occur, particularly with a *cis*-carbamate linkage. Such binding would completely block the channel but only transiently, if the interaction were weak. This blocking, in turn, would result in a lowering of the average observed conductances.

These observations of cis−trans isomerization within single molecules provide a framework for the design of novel channel activities. To be useful for design, however, a system must behave in a predictable manner. If this system is predictable, we should be able to alter the relative stability of cis and trans

(18) Seoh, S.; Busath, D. *Biophys. J.* **1993**, *64*, 1017−1028.

(19) Sung, S.; Jordan, P. C. *Biophys. Chem.* **1987**, *27*, 1−6.

(20) Arseniev, A. S.; Barsukov, I. L.; Bystrov, V. F.; Lomize, A. L.; Ovchinnikov, Y. A. *FEBS Lett.* **1985**, *186*, 168−174.

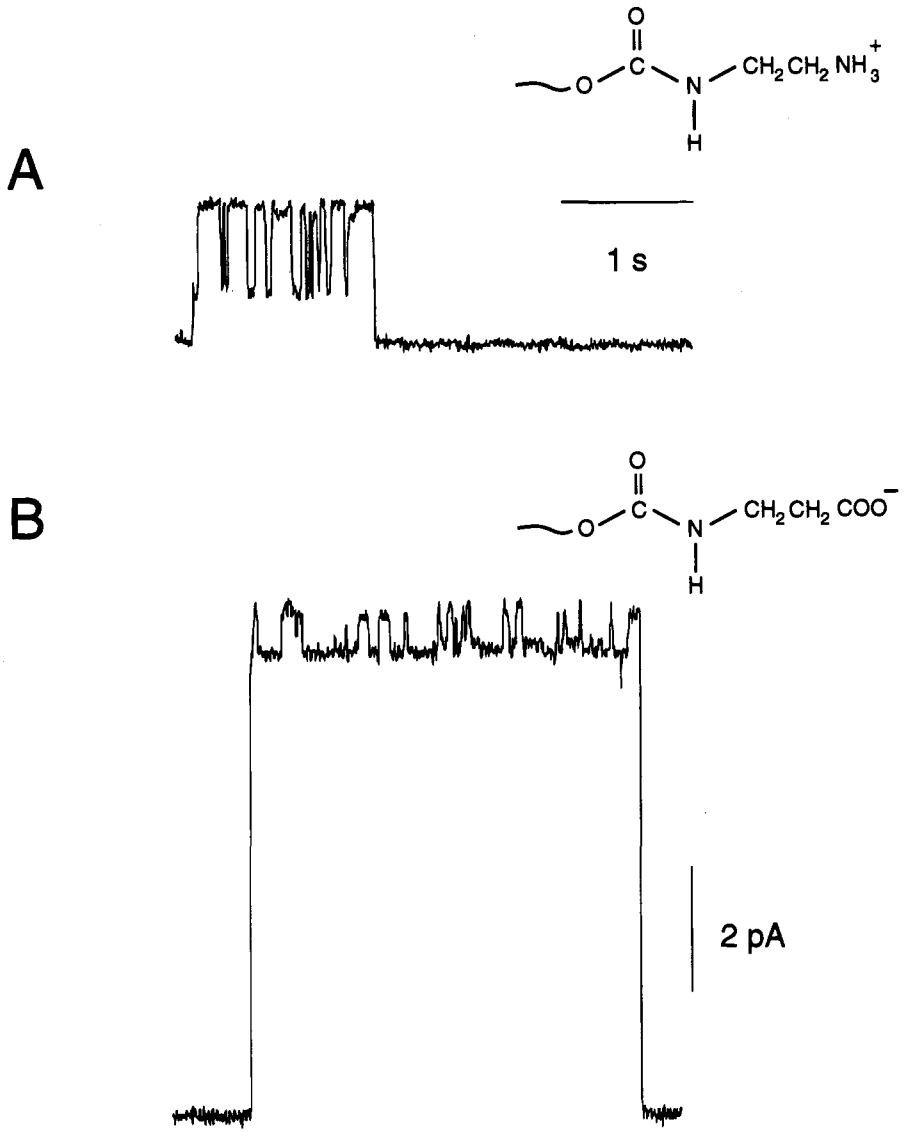


Figure 4. Single-channel currents of gramicidin−ethylenediamine (A) and gramicidin-β-alanine (B) in 0.2 M CsCl (+200 mV, filtered at 50 Hz). Two current levels are seen in each case (A: 2.4 pA, 0.9 pA, $n = 19$; B: 8.2 pA, 7.4 pA, $n = 147$). In B, however, it is the *lower* level which has the longer average lifetime. At 1 M CsCl, the two states of gramicidin−β-alanine are difficult to resolve because the channel current is nearly saturated.

isomers by chemical means and see a corresponding change in channel activity.

The stability of cis and trans isomers of gramicidin−ethylenediamine are unequal since the two substituents on nitrogen (−H and $-CH_2CH_2NH_3^+$) are different. By replacing the proton with a methyl group, the two substituents become stereoelectronically more alike. The time spent in the cis and trans states should thus become more equal. The peptide gramicidin−*N,N,N′* trimethylethylenediamine was, therefore, prepared and its channel activity compared with that of gramicidin-*N,N* dimethylethylenediamine (the extra methyl groups on the terminal nitrogen were required by the synthetic procedure). A closely-related model compound, ethyl *N*-methyl-*N*-isobutylcarbamate has been studied by dynamic NMR.[14] Two equally-populated states were observed with a barrier to interconversion of 64.5 kJ/mol (state lifetimes of about 40 ms).

Two main conducting states are now resolved (Figure 5); by analogy with gramicidin−ethylenediamine, these presumably result from isomerization at the channel entrance. The ratio of time spent in the two states is about 1:1 for gramicidin−*N,N,N′* trimethylethylenediamine and 1:4 for the dimethyl derivative. The single-channel behavior has thus changed as predicted. The lifetime of the cis state of gramicidin−*N,N,N′* trimethylethylenediamine (76 ms) is equal (within experimental error) to the lifetime of the trans state (76 ms). For gramicidin−*N,N* dimethylethylenediamine, the lifetimes are 23 ms (cis) and 84 ms (trans).

It is interesting to note, as an aside, that the difference in conductance between the cis and trans states of gramicidin−*N,N* dimethylethylenediamine is considerably smaller than the difference observed with gramicidin−ethylenediamine itself, under the same conditions (compare Figure 5B and Figure 1A). This finding is consistent with the proposal that an interaction of the primary amino group of gramicidin−ethylenediamine with a site at the mouth of the channel may contribute to the observed conductances (*vide supra*).

We are thus able to regulate the behavior of this "nanoscale device" by the placement of charge and by altering the stability of different conformations within a well-defined structural framework. Single-channel measurements permit detailed evaluation of the design. The predictable nature of this system suggests the possibility of designing channels that could be regulated in more complex ways (*e.g.*, by light or by the

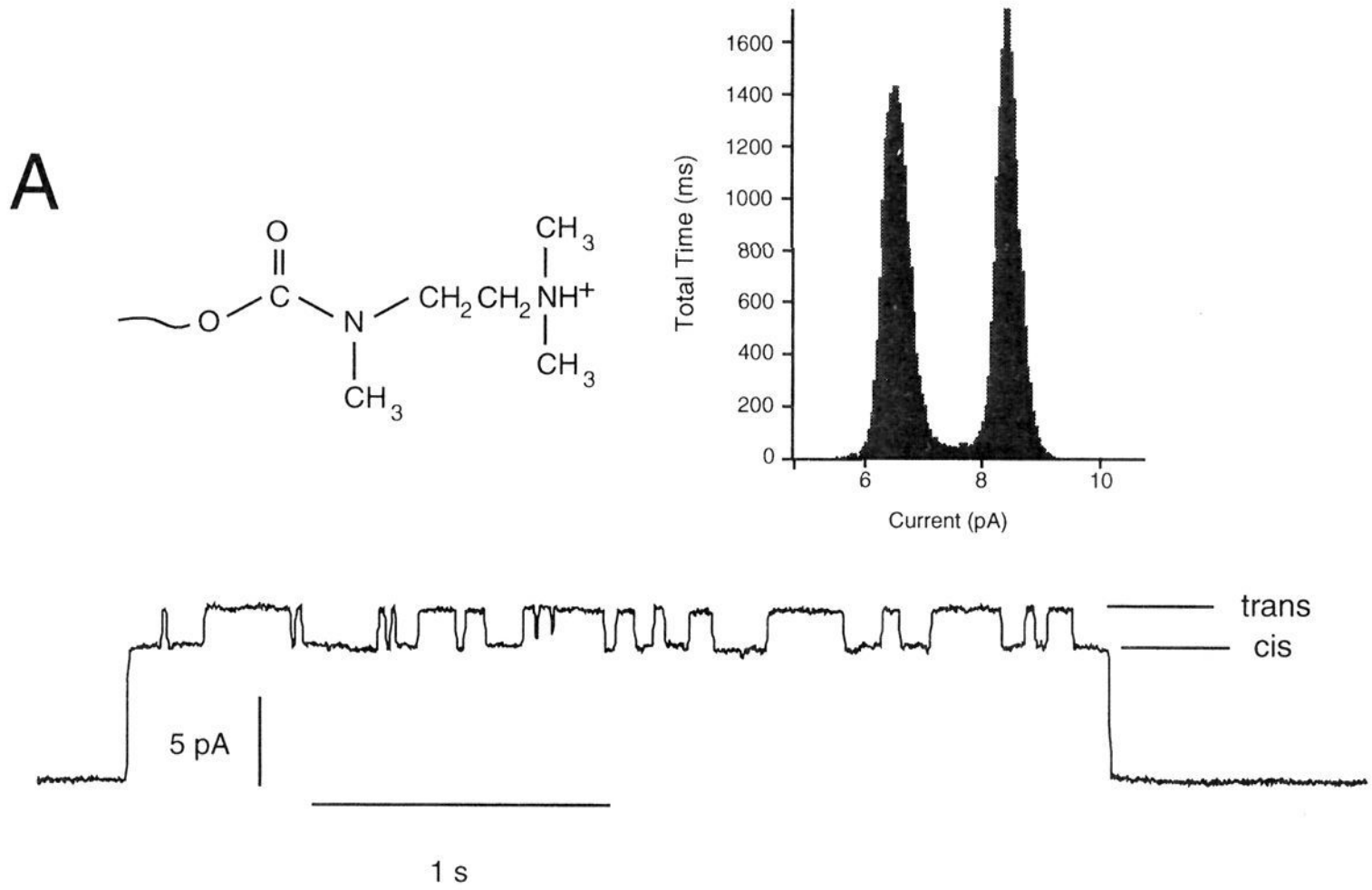


Figure 5. (A) Single-channel record of gramicidin−*N*,*N*,*N*′-trimethylethylenediamine (1 M CsCl; +200 mV; filtered at 100 Hz). Two current levels (9.3 ± 0.5 pA, $n = 155$ (lifetime = 76 ms, $n = 505$) and 7.4 ± 0.5 pA, $n = 155$ (lifetime = 76 ms, $n = 503$)) are observed. The histogram shows the total (integrated) time spent in the two conducting states. Currents from several channels were binned until the ratio of the area of the two peaks became constant. The ratio is 1:1. (B) Single-channel record of gramicidin−*N*,*N*-dimethylethylenediamine (1 M CsCl; +200 mV; filtered at 200 Hz). Two current levels (10.2 ± 0.4 pA, $n = 85$ (lifetime = 84 ms, $n = 363$) and 8.0 ± 0.4 pA, $n = 85$ (lifetime = 23 ms, $n = 339$)) are observed. The histogram shows the total (integrated) time spent in the two conducting states. The ratio of areas is 1:4.

presence of another molecule). Such channels might interact with living cells in novel and useful ways.

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