Photomodulated Blocking of Gramicidin Ion Channels

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Optical control of biomolecules can permit novel approaches for probing the chemistry of living systems.[1,2] Reversible control of ion transport systems, in particular, offers the possibility of investigating the importance of timing and rhythms in excitable cell systems. Several groups have designed ion transport systems that incorporate photochromic groups.[3] Photoisomerization has been designed to affect ion binding,[4] positioning of channel-forming molecules in the membrane,[5] creation of polar sites within the membrane,[6] or gramicidin channel dimerization.[7,8] We wish to report a novel approach in which photoisomerization can affect blocking of the gramicidin ion channel by a molecular "gate".

Ion channels formed by gramicidin are well-characterized both structurally and functionally.[9,10] We observed previously that attachment of ethylenediamine via a carbamate linkage to the C-terminal end of gramicidin resulted in significant blocking of cation flux.[11] The protonated alkylamine inhibited cation flux both electrostatically and sterically. Thermal cis/trans isomerization of the carbamate group resulted in different degrees of blocking; isomerization could be resolved as discrete steps in single-channel current recordings.[12]

Figure 1 shows the effect on the appearance of single-channel current recordings of lengthening the alkylamine.[13] The steps observed in the current amplitude are due to cis/trans isomerization of the carbamates.[11] Isomerization at the entrance (rather than the exit) of the channel has a dominant effect on ion flux; thus, two rather than four current levels are seen.[11] As the alkyl chain length increases, the size of the steps decreases. With a nine-carbon chain, the steps are barely discernible and the average current is nearly that of an unmodified gramicidin channel. The effectiveness of block by the protonated amino group thus depends on the length of the linker connecting it to the mouth of the channel.

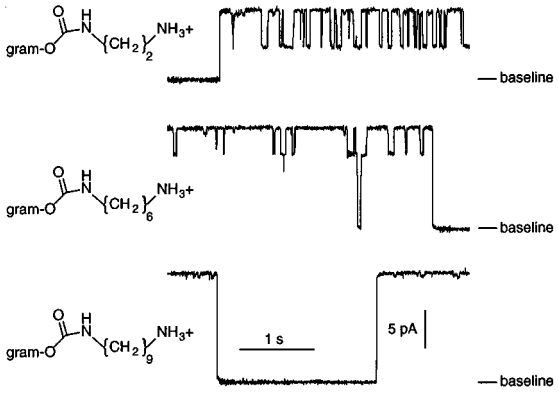

Figure 1. Single-channel behavior of gramicidin−alkylamine derivatives. The magnitude of the current increases with increasing alkyl chain length (highest current level: unmodified gramicidin 15.3 ± 0.9 pA (n = 59); $(CH_2)_2$ 9.8 ± 0.6 pA (n = 331); $(CH_2)_6$ 13.2 ± 0.5 pA (n = 100); $(CH_2)_9$ 14.3 ± 0.7 pA (n = 100)). Chains with three, four, and eight methylene units gave intermediate currents. Current steps observed (due to cis/trans isomerization of the carbamates) decrease with the length of the alkyl chain and are barely discernable when there are nine methylene units.

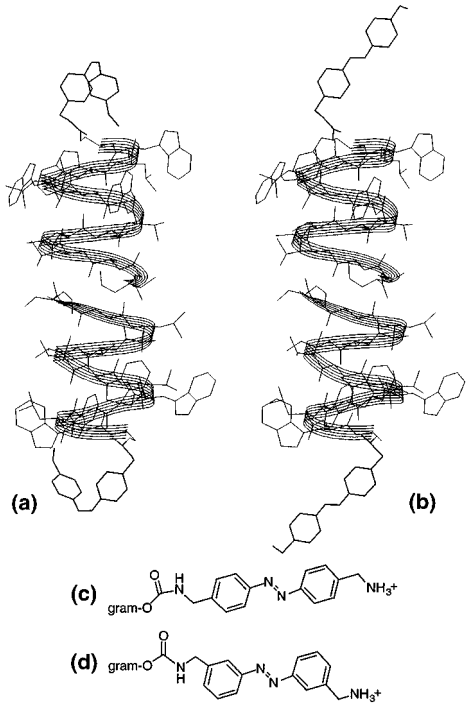

Figure 2. Structure of azobenzene-modified gramicidin channels. The model of the channel proper is based on the NMR data of Arseniev for gramicidin in lipid micelles.[17] The azobenzene-containing molecular "gates" are shown in cis/cis (a) and trans/trans (b) arrangements. The gates are flexible and only one of several possible conformations is shown. Covalent structure of the *para*-azobenzene (c) "gate" and the *meta*-azobenzene (d) "gate".

Photoisomerization of azobenzene changes its length.[14] We reasoned that replacement of the alkyl moiety in the above derivatives with an azobenzene group might permit photomodulation of channel blocking. Two compounds, 4,4′-bis(aminomethyl)azobenzene and 3,3′-bis(aminomethyl)azobenzene were therefore synthesized and attached to gramicidin via carbamate linkages[15] (Figure 2c and d, respectively). Figure 2 shows the gramicidin channel structure[16,17] with azobenzene groups at-

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(13) These derivatives were prepared exactly as described previously (Jaikaran, D. C. J.; Woolley, G. A. *Biochim. Biophys. Acta* **1995**, *1234*, 133−138). Peptides (∼10−50 nM in MeOH) were added to membranes formed from glycerol monooleate/hexadecane. Solutions were 1 M CsCl, 5 mM BES, pH 7.0, and all measurements were made at 24 ± 2 °C, 200 mV applied voltage. Currents were measured, and voltage was set using an Axopatch 1D patch-clamp amplifier (Axon Instruments). Data were filtered at either 100 or 200 Hz, sampled at 1 kHz, stored directly to disk, and analyzed using the program Synapse (Synergistic Research Systems).

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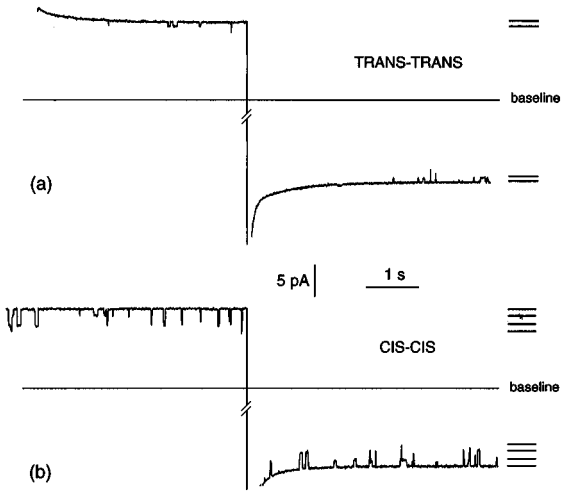

Figure 3. Single-channel behavior of *meta*-azobenzene-modified gramicidin channels. (a) Channel with both azobenzene groups in the trans conformation. Only small steps in the current are seen as indicated by the bars; highest current level 14.9 ± 0.4 pA ($n = 65$); step size 0.6 ± 0.1 pA ($n = 300$). (b) Channel with both azobenzene groups in cis conformations obtained after laser irradiation. Up to three steps are seen, and these are larger than in a (as indicated by the bars); highest current level, 12.8 ± 0.4 pA ($n = 72$); step 1, 1.1 ± 0.1 pA ($n = 700$); step 2, 2.6 ± 0.1 pA ($n = 700$); step 3, 3.5 ± 0.2 pA ($n = 100$). In both a and b, voltage reversal during the lifetime of the channel (the vertical line) changes only the sign, not the magnitude of the currents (the base lines (closed states at ± 200 mV) have been superimposed). Channel recordings of the *para*-azobenzene-gramicidin derivative were also obtained. The currents observed for the trans/trans (azo) photoisomer were 14.0 ± 0.4 pA ($n = 59$), 0.8 ± 0.1 pA ($n = 295$). For the cis/cis (azo) photoisomer: 14.4 ± 1.0 pA ($n = 61$), 1.2 ± 0.1 pA ($n = 600$), 2.9 ± 0.3 pA ($n = 600$), 4.0 ± 0.4 pA ($n = 10$).

tached at both ends in cis and trans conformations. The channel structure is unlikely to be greatly altered by the azobenzene groups since C-terminal modifications to gramicidin are generally well-tolerated.[10] Moreover, we observe that these azobenzene-modified peptides form heterodimeric channels with unmodified gramicidin, indicating that the channel architecture is intact.[18]

Figure 3a shows current passing through a representative single channel recorded after incorporation of *trans-meta*-azobenzene-modified gramicidin into a lipid bilayer[19] (structure Figure 2d). Very small steps in the current are observed on the time scale expected for thermal cis/trans isomerization of the carbamate linker. The size of these steps is similar to that of the steps observed with a nine-carbon alkylamine and is consistent with an extended structure for the azobenzene group that places the protonated amino group (on average) far from the mouth of the channel.

Irradiation of these gramicidin−azobenzene channels *in situ* with a nitrogen laser (337 nm) causes a significant change in the single-channel currents. Absorbance measurements in solution demonstrated that this treatment resulted in a photostationary state that was >85% cis. Figure 3b shows a representative single-channel record obtained after laser irradiation. Four distinct current levels are seen, whereas only two are discernible in Figure 3a. In addition, the size of these current steps is significantly larger than before irradiation. By analogy with the alkylamine case,[11] we assign the current levels in the *cis*-azobenzene record (in order of decreasing current) to trans/trans, trans(entrance)/cis(exit), cis(entrance)/trans(exit), and cis/cis conformations of the carbamate linkers. Importantly, both before and after irradiation, the absolute channel currents are unchanged when the voltage is reversed during the lifetime of a single channel (Figure 3). Thus the channels are behaving as symmetrical structures; the azobenzene groups at both the entrance and the exit of the channel are therefore likely to be in the same conformation (cis/cis or trans/trans). Channels that are not symmetrical with respect to voltage reversal were also observed (commonly after a brief irradiation, as expected) and these occurred in two complementary orientations. Brief exposure (10 min) of *cis*-azobenzene channels to light from a 100 W desk lamp resulted in conversion back to the behavior observed with *trans*-azobenzene channels (and hybrid trans/cis channels). Thus the photomodulated blocking observed is reversible *in situ*. Qualitatively similar single channel results were obtained with the *para* derivative.

To examine whether $N_{azo}-C_{phenyl}$ rotation was hindered under the conditions of these experiments, we synthesized both *para*- and *meta*-substituted azobenzene "gates". Different rotamers of the *meta*-substituted derivative would have the charged amino group at different positions relative to the channel mouth. If these conformations are not averaged on the time scale of the measurement a complex variety of channel conductance states should result. The internal symmetry of the *para*-substituted derivative should significantly reduce the number of possible conductance substates. For both the *meta* and *para* derivatives, however, only one distinct set of current steps could be detected for each photoisomeric state. Thus, $N_{azo}-C_{phenyl}$ rotation in all cases appears to be fast on the time scale of the channel measurements (>1 kHz).

The flexibility of the "gates" means that they will sample a variety of conformations, and the extent of blocking (*i.e.*, the residual conductance) observed will reflect an average of the conductance of each conformation weighted according to the stability of that conformation. The most distinct differences between the *cis*- and *trans*-azobenzene states of the channels occur when the carbamate groups are in cis conformations and the azobenzene group is *meta*-substituted (Figure 3). This arrangement presumably permits a subset of conformations of the "gate" to occur that block the channel particularly effectively.

The "double-headed" blocking concept presented here has an important advantage with respect to the completeness of the photoswitching. Since the photochromic blocking groups are arranged in series along the ion transport pathway, in principle, photoisomerization of either one could provide the desired gating. This design ameliorates one of the problems in using reversible photochromes for switching (*i.e.*, that light will catalyze isomerization in both the forward and reverse directions); the photostationary state achieved depends on the absorbance cross-sections of the two isomers, and for azobenzene, these overlap significantly.[14] A series arrangement of photochromes, however, means that 85% (photostationary) conversion to the cis state could result in a 98% conversion to the closed state of the channel. Efforts to optimize the geometry and rigidity of the connection between the azobenzene group and the channel mouth, so as to achieve complete blockage, are underway.

Supporting Information Available: Details of the synthesis and characterization of gramicidin azobenzene derivatives (1 page). See any current masthead page for ordering information.

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(19) Peptides bearing azobenzene groups in the trans form were obtained simply be storing peptide solutions in the dark for several hours. Irradiation with a desk lamp *in situ* also resulted in channels with predominantly trans form behavior. Laser irradiation (nitrogen laser, 337 nm, 150 mJ/pulse, 1.5 ns/pulse, 15 Hz pulse rate) via a light-tube produced *cis*-azobenzene channels. The half-life of the *cis*-azobenzene isomers appears to be at least an hour under the conditions of channel recording.
(20) This work has been supported by NSERC (Canada).