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Photo-modulated ion channels based on covalently linked gramicidins

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The covalent coupling of two gramicidin A monomers proved to be a useful tool for the rational design of ion channels with predictable electrophysiological properties (Stankovic, C.J., Heinemann, S.H., Delfino, J.M., Sigworth, F.J. and Schreiber, S.L. (1989) Science 244, 813–817; Stankovic, C.J., Heinemann, S.H. and Schreiber, S.L. (1990) J. Am. Chem. Soc. 112, 3702–3704). Herein we report on our first efforts to equip such channels with an artificial gating mechanism. Gramicidin monomers were covalently linked with 3,3′-azobis(benzeneacetic acid). Based on computer modeling of the β-helix channel motif, this linker in its dark-adapted (*trans*) form does not allow for the formation of unimolecular ion channels, while the photo-activated (*cis*) form was expected to provide this possibility. The electrophysiological assays showed that (A) the *trans*-isomer does form characteristic ion channels, and (B) irradiation transforms these channels into a new distinct, flickering channel type in a reversible manner. The results are discussed in the framework of intermolecular gramicidin aggregates.

Introduction

Gramicidin A [1,2] is a naturally occurring pentadecapeptide produced by *Bacillus brevis* (ATCC-8185) and consists of an alternating sequence of D- and L-amino acids [3]. The structure of gramicidin A varies depending on its environment [4]; in organic solvents it adopts a double-helical structure [5] and the X-ray structure of crystals grown from these solvents confirms this [6,7]. However, in an ordered amphiphilic environment (i.e., micelles, lipid vesicles or lipid bilayers) gramicidin A adopts the single helical dimer structure originally proposed by Urry in 1971 [8]. This structure is thought to be formed by the head-to-head (N-terminus to N-terminus) dimerization of two gramicidin A monomers, stabilized by six intermolecular hydrogen bonds, to form a continuous pore through a lipid bilayer. This dimer is approx. 26Å in length and has a pore diameter of approx. 4Å. These dimensions are just sufficient to accommodate a column of water molecules hydrogen bonded together, through which monovalent cations may pass in single file.

As part of an ongoing effort to design and synthesize novel ion channels, we recently reported results on tartaric acid-gramicidin A hybrids (Stankovic, C.J., Heinemann, S.H. and Schreiber, S.L., unpublished work, and see Ref. 9). These dimers form long-lived conducting channels due to the covalent linkage between the two monomers. We have recently extended these investigations with the goal of producing ion channels with controllable gating properties. Although a variety of artificial stimuli could be used to control ion channel function, we chose to design a photon-operated channel gate. The ability to produce artificial photo-gated or modulated ion channels offers the possibility of a better understanding of the structural basis for channel formation and of photoreactions in a defined environment, and may have applications in the area of micro-electronics.

Shinkai and Manabe [10] and Irie and Kato [11] have previously created photo-modulated ion carriers (ionophores) by linking ion chelating groups to a photoisomerizable function. These compounds were able to transport ions from one side of a lipid membrane to the

Abbreviations: DPPA, diphenylphosphoryl azide; ^{1}H-NMR, proton nuclear magnetic resonance; CD, circular dichroism; DMF, dimethylformamide; DMSO-d_6, hexadeuterodimethyl sulfoxide; UV, ultraviolet light; DMPC, dimyristoylphosphatidylcholine; GMO, glycerol monooleate.

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other. For an alternative strategy for the photocontrol of ion flux across a lipid bilayer that uses ion carriers, see Ref. 12. In analogy to the work of Shinkai, we have chosen 3,3′-azobis(benzeneacetic acid) to serve as the photoisomerizable linker in the design and synthesis of a new ion channel molecule. Spectroscopic investigations of the absorbance characteristics of the free linker and the linked gramicidins in solution and lipid vesicles indicate that they undergo photo-induced conformational changes. This photo-active linker couples the two half-barrels of the channel in a manner that, because of its rigidity, imposes geometric constraints that prevent a unimolecular pore from being formed when the linker is in the dark-adapted (*trans*) form. A photoisomerization to the *cis* isomer was expected to manifest an alteration of the electrophysiological properties, i.e., to form functional ion channels. A modulation of the synthesized channels by photons was indeed observed. For the interpretation of the results on the molecular level, however, consideration of the formation of intermolecular aggregates, in addition to unimolecular helices, was required.

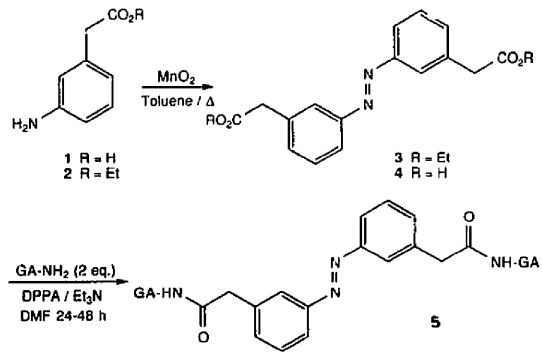
Methods

Computer-aided channel design

Molecular structures of the covalently linked gramicidin A channel molecules were generated with the MACROMODEL V2.0 molecular modeling software package. Minimizations were performed with the OPLS-A force field with an electrostatic cutoff of 8 Å [13], and continued until the first-derivative root-mean-square gradient was 0.01 kJ/Å or less using the Polak-Ribiere conjugate gradient algorithm [14]. The coordinates for the left-handed helix were provided by Karplus and Roux [15]. (Recently, a number of studies have pointed toward the right-handed helix as the correct structure for gramicidin A in lipid bilayers [16–18]; however, since the handedness is not important in our studies (see Ref. 9), we chose to use the standard left-handed model.) The structures generated represent minimized versions of two conformations that we considered important in our model. Given the conformational polymorphism of gramicidin A, an extensive search of the conformational space of these molecules was not practical. The structures used here are illustrative of the key features of our model. These computer-generated models are useful for judging the relative steric accessibility of these structures.

Synthesis and sample preparation

The linker 3,3′-azobis(benzeneacetic acid) (or *meta*-azobis(benzeneacetic acid, MABA), **4**, was synthesized according to the method of Wheeler [19]. The commercially available 4-aminophenylacetic acid, **1**, was converted to the ethyl ester, **2**, by Fischer esterification (see



GA-NH$_2$ = H$_2$N-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp
HOCH$_2$CH$_2$NHCO-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu

Fig. 1. Synthesis of the photo-dimer.

Fig. 1). This ester was then oxidatively dimerized to the azobenzene, **3**, by refluxing in toluene with excess manganese dioxide in 10% yield. Saponification of this diester provided the diacid, **4**, in 87% yield after recrystallization from methanol/water. The dimer was prepared by coupling this diacid with desformyl gramicidin A (prepared by the method of Sarges and Witkop [20]) with diphenylphosphoryl azide in DMF to yield the dimer, **5**, in 40% yield.

Vesicles were prepared according to the method of Killian et al. [21]. Samples of the dimer and about 2 mg of DMPC (1 : 50 mole ratio) were dissolved in trifluoroethanol and concentrated to a thin film on a rotary evaporator. This film was then suspended in 1.5 ml of pH 7.0 phosphate buffer and heated (with vortexing 3 ×) at 65 °C for 1 h, followed by sonication at 65 °C for an additional hour. The resulting solutions were transferred to vials and centrifuged at 16000 × *g* for 10 min. The supernatant was then filtered through a 0.45 μm syringe filter to provide the final solutions. These samples were used within 48 h for CD and UV studies, but were stable for up to 2 weeks.

Photolysis experiments

Light sources: For > 320 nm light, a Hanovia 400 W Hg-lamp (ACE Glass) with a uranium glass filter was used. For 337 nm light an N_2 pulse laser (Photochemical Research Associates, Ontario, Canada) operating at approx. 1.2 mJ/pulse with a 1 ns pulse at approx. 5 pulses/s gave rise to an average energy output of approx. 6 mW. Irradiations were carried out for 0.5 to 2 h (11–45 J total energy), depending on the sample, to ensure complete conversion to the *cis* isomer. For light at > 450 nm, an argon ion laser (Spectra-Physics 171), lasing at 458 nm (5%), 476 nm (12%), 488 nm (32%), 501 nm (5%), and 514 nm (37%), was used to give an average total energy output of approx 0.3 W. Samples

were irradiated for 2–10 min (36–180 J total energy) to ensure that the photostationary state had been reached. Note that these energy calculations are approximate numbers for the total energy beam and are not corrected for the portion of the beam not hitting the sample or other such factors.

Spectroscopic assays: NMR and UV analyses

Proton magnetic resonance spectra (^{1}H-NMR) were recorded on a Bruker AM-500 (500 MHz) instrument. Samples were recorded in DMSO-d_6, and chemical shifts are reported in parts per million (ppm) downfield from residual DMSO-d_5-H ($n = 2.49$) as an internal standard; a line broadening of 5 Hz was routinely used for data processing.

UV spectra of solutions of the dimer in DMPC vesicles (see above) were recorded in 1 ml quartz cuvettes (1 cm pathlength) on a Hewlett-Packard 8452A diode array UV-visible spectrophotometer and were corrected for scattering due to the vesicles by extrapolation of the scattering from 600–500 nm.

Electrophysiological recordings

Artificial bilayers were formed on the tips of patch clamp pipette tips (3–5 μm diameter) and the currents were recorded as described by Sigworth et al. [22]. Glycerol monooleate (GMO, Nu Check Prep., Elysian, MN, U.S.A.) 40 mg/ml in squalene (Sigma, St. Louis, MO, U.S.A.) was used as the lipid. Experiments were carried out in symmetrical unbuffered salines of 40 mM HCl (pH 1.9), prepared with Millipore-filtered water.

Current data were recorded with a commercial patch clamp amplifier (EPC7, List Medical, Darmstadt, F.R.G.) and stored in digital form on video tape. Further data processing was performed on a VME bus-based laboratory computer system (Motorola MVME147, Tempe, AZ, U.S.A.).

The gramicidin samples were stored in methanolic stock solutions at −20 °C. Approx. 1 μl of a 10 ng/ml gramicidin solution was added to 1.5 ml of the lipid/solvent mixture before membranes were formed. In order to obtain a high yield of *trans* versus *cis* configurations, samples were incubated for 24 h at 45 °C in the absence of light. During recordings from dark-adapted samples, the laboratory was illuminated with a dim red-light source.

Upon measurements of typical dark-form channels, the entire assembly (consisting of saline, lipid-gramicidin mixture, and patch pipette) was irradiated with light from a 75 W xenon lamp (XBO 75W/2, Osram, Munich, F.R.G.) from a distance of 20 cm focussed to approx. 2 cm^2. The light was passed through glass filters UG1 (3 mm) and Duran 8330 (5 mm) both from Schott (Mainz, F.R.G.), which yield a spectral window of 320–400 nm. The lower cutoff served to protect the tryptophans of the gramicidin A from photolytic degradation that occurs at wavelengths lower than 290 nm, see Refs. 23, 24.

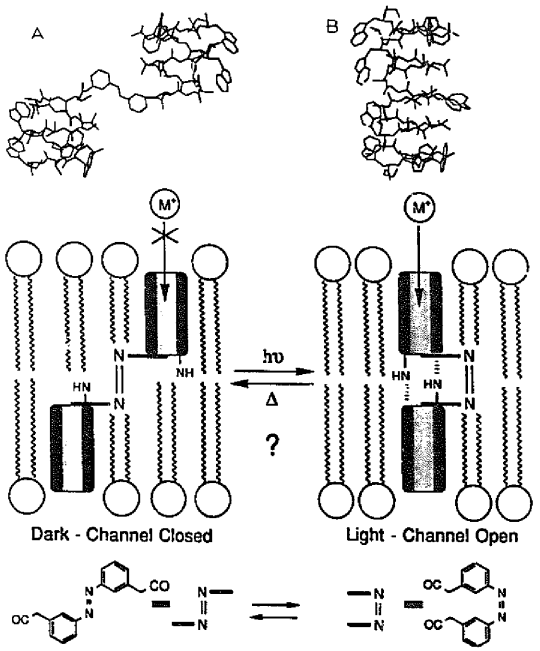


Fig. 2. Proposed model for the design of a photo-gated ion channel. The top panel shows computer generated structures for the dark (*trans*) channel (A) and the light (*cis*) channel (B). The diagram below shows how these dimers were expected to respond to light and heat in a lipid bilayer to form open (B) and closed (A) channel states. In the diagram the linker diacid is replaced by a simplified azo-linker.

Results

Applying the results reported in earlier studies by Shinkai for ionophoric substances, a simple model of the functional components of a photo-gated transmembrane ion channel was developed. Fig. 1 shows the synthesis of the linker and the corresponding dimer, and Fig. 2 illustrates conceptually how these dimers were expected to behave when exposed to light. The linker diacid is readily available, albeit in modest yields, from the commercially available amine as shown. This diacid is then coupled to desformyl gramicidin A with DPPA to provide the dimer in 40% yield. Purification of the linker dimer by flash chromatography [25], and then by reverse-phase HPLC yielded a sample with ^{1}H-NMR, UV, CD and mass spectral data that were fully consistent with the assigned dimer structure.

The diagram in Fig. 2 illustrates schematically one possible reaction pathway for this dimer consistent with the current models for the conducting conformation of gramicidin A. For simplicity the linker is replaced with

a nondescript azo-linker and each gramicidin A monomer is represented as a simple cylinder, where the outer dark bands represent the hydrophobic side-chains, and the lighter center represents the conducting pore. In the thermodynamically more stable *trans* isomer the dimer was expected to be nonconducting since the two 'monomer' halves are geometrically constrained in a manner that prevents the formation of a single conducting pore. However, upon exposure to light of approx. 330 nm wavelength the linker was expected to isomerize to the *cis* isomer. This would allow for the possibility of the two halves coming together to form a single continuous pore. To test this hypothesis, a number of photolysis experiments were performed.

Photo-isomerization of the linker

Initial studies (data not shown) of either the biscarboxylic acid, **4**, or diethyl ester, **3**, showed that indeed this azobenzene derivative behaved much like other related azobenzenes, with an absorbance maximum at approx. 330 nm and another much weaker band at approx. 440 nm for the *trans* compound. The *cis* isomer (produced by irradiation at 337 nm > 20:1 *cis*:*trans* as monitored by ^{1}H-NMR) had a weaker (50%) absorption maximum at shorter wavelengths of approx. 300 nm and a stronger (50%) absorption maximum at 440 nm. (A more quantitative analysis of the UV absorption spectra was hampered by the inability to isolate a pure sample of the *cis* isomer.) These studies also demonstrated that this photoisomerization could be completely reversed, producing the *trans* isomer > 20:1 (^{1}H-NMR), by simply heating the samples in the dark at about 45°C for at least 24 h (dark-adapted). This isomerization could also be partially reversed by exposure to white light (> 320 nm) to reach the photostationary state (3:1 *trans*:*cis*) in a few minutes. With these results in hand we continued this study on the linked dimer. Previous studies had demonstrated that photolysis of natural gramicidin A with light at less than 300 nm causes irreversible photodegradation of the tryptophans, which leads to changes in the ion conductance of channels formed with such samples [23,24]. To avoid these problems we used light with wavelength above 320 nm.

Photoisomerization in DMSO

Photolyses were conducted on samples on the dimer in DMSO-d_6 and monitored by ^{1}H-NMR. Simple irradiation of the dimer with white light (> 320 nm), in analogy to the linker, produced a photostationary state of 1:3 *cis*:*trans*. However, selective irradiation at 337 nm (N_2 laser) provided ratios of > 10:1 *cis*:*trans*. This ratio could be returned to > 20:1 *trans*:*cis* by simply heating in the dark, or could quickly be returned to the photostationary state by irradiation with white light for a few minutes. For the conductance experiments it was desirable to be able to quickly change from all *trans* to all *cis* and back rapidly. Irradiation at 337 nm accomplishes the forward reaction, but a solution to the reverse reaction remains elusive. In an attempt to find conditions to achieve this latter reaction we tried to selectively irradiate at > 450 nm (Argon ion laser); however, this failed to offer any significant improvement over the results obtained with white light. Presumably this failure results from the less favorable ratio of extinction coefficients at the 440 nm maxima (ignoring possible differences in quantum yields).

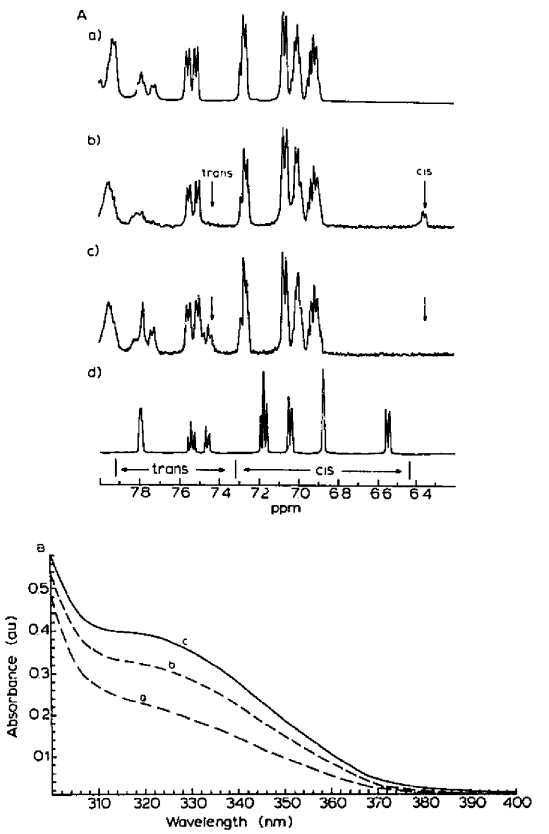


Fig. 3. (A) NMR evidence of photoisomerization in solution. ^{1}H-NMR of the aromatic and amide region of: (a) of natural GA for comparison, (b) of the light (*cis*) isomer of the linked dimer **5**, (c) of the dark-adapted (*trans*) isomer of the linked dimer **5**, (d) and of a 2:1 mixture (*cis*:*trans*) of the linker diacid, **4**, for comparison. Most of the linker signals overlap with signals from the tryptophans in gramicidin A: however, signals for the *cis* at δ 6.4 ppm, and the *trans* at δ 7.45 ppm are clearly discernable in the *cis* and *trans* isomers respectively, but the minor isomer in each case is almost unobservable. The signals for the linker in the dimer are slightly shifted from the free acid, presumably reflecting the different environment in the peptide. (B) UV spectra of the dimer **5** in DMPC vesicles. (a) Sample after irradiation at 337 nm representing the *cis* isomer, (b) of a mixture of *cis* and *trans* isomers, and (c) of the dark adapted all-*trans* isomer.

Fig. 3 illustrates a sampling of these results. The ^{1}H-NMR of the aromatic region of the dimer in DMSO-d_6 is shown in Fig. 3A for the all-*trans* isomer (c) and for the *cis* isomer from irradiation at 337 nm (b). The same region of gramicidin A (a) and of the linker diacid as a 2 : 1 mixture of *cis* : *trans* isomers (d) are shown for comparison. Signal overlap, even at 500 MHz, complicates the analysis, but one non-overlapped peak from the linker can be seen at 6.4 ppm for the *cis* and at 7.45 ppm for the *trans* isomer (the linker signals are slightly shifted in the dimer relative to the diacid). Integration of this peak relative to one of the tryptophan peaks which is also not overlapped allows for an estimation of the *cis* to *trans* ratios.

Photo-isomerization in vesicles

The aforementioned photoisomerization results illustrated that in principle our plan was feasible, but the photoisomerization of the dimers in DMSO is not a very good model for the dimer in a lipid bilayer. Despite earlier reports that gramicidin A adopts the $\beta^{6.3}$ helix conformation in DMSO solutions based on NMR analysis [26,27], more recent studies indicate that gramicidin A has no ordered secondary structure in DMSO (Roux, B., personal communication). To mimic the lipid bilayer used for our conductance studies we decided to study the photoisomerization of this dimer in DMPC vesicles. The structure of gramicidin A in DMPC vesicles and bilayers has been studied extensively by CD [28,29,21] and NMR [30,31,17], respectively, and is believed to be the same as the active channel-forming conformation. Incorporation of our dimer into DMPC vesicles provided a sample which had a CD spectrum that was virtually identical to that of natural gramicidin A prepared by a similar procedure (data not shown) and to that reported as the channel form of gramicidin A [20]. To study the photoisomerization of this dimer in DMPC vesicles we used UV spectroscopy to avoid the problems associated with the NMR analysis of such systems (long relaxation times lead to line broadening and loss of long-range NOE's). Fig. 3B shows the UV spectrum of the dark-adapted all-*trans* isomer (c), of an intermediate mixture of *trans* and *cis* (b), and of the presumably all-*cis* isomer (a) from irradiation at 337 nm (further irradiation does not change the spectrum). It is more difficult to establish the amount of *cis* isomer in this case due to the difficulty in measuring the extinction coefficient for this isomer, since it cannot be obtained as a pure sample by an unambiguous route. However, in analogy to the solution experiments, we assumed a ratio of approx. 10 : 1 *cis* : *trans*. Clearly, these experiments show that the dimer in DMPC vesicles can be photoisomerized to yield a significant concentration of the *cis* isomer. This knowledge allows us to extrapolate this result to the dimer in lipid bilayers formed from GMO used for the conductance studies described below.

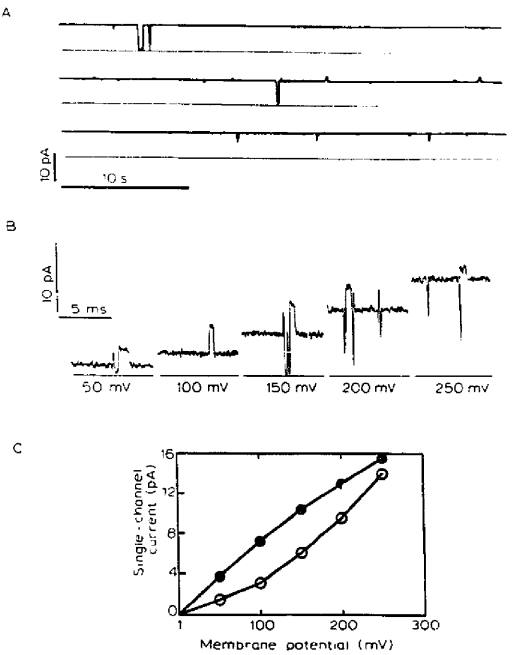


Fig. 4. Conductance properties of 'dark channels'. (A) Single-channel current of dark-adapted MABA in GMO/squalene membranes in symmetrical 40 mM HCl solution and at 200 mV membrane potential. Characteristic of these ion channels are brief current events exceeding the main level. (B) Examples of these hyperlevels at various membrane potentials. (C) Current–voltage relationships of hyperlevels (filled circles) and main current levels (open circles).

Observation of functional ion channels

Fig. 4 shows representative recordings of dark-adapted MABA channels in symmetrical solutions of 40 mM HCl and 200 mV membrane potential. The readily apparent ion conducting properties for the dark-adapted (presumably all-*trans*) dimers was quite surprising. According to our original hypothesis (Fig. 2) we had anticipated that the *trans* isomer could not form conducting channels. This, of course, assumed that only unimolecular channels could form (vide infra). The observed channels have a lifetime that is longer than that of regular gramicidin A and shorter than that observed for gramicidins covalently coupled with short linkers (see Ref. 9). The single-channel current under these particular experimental conditions is approx. 40% smaller than the control with gramicidin A. A remarkable feature of these traces is the appearance of current events that exceed the main current level. These 'hyperlevels' have an average duration of 260 ($\pm$16) μs and occur with a frequency of 10 ($\pm$1) s^{-1}, mostly accompanied by downward current fluctuations (see Fig. 4B).

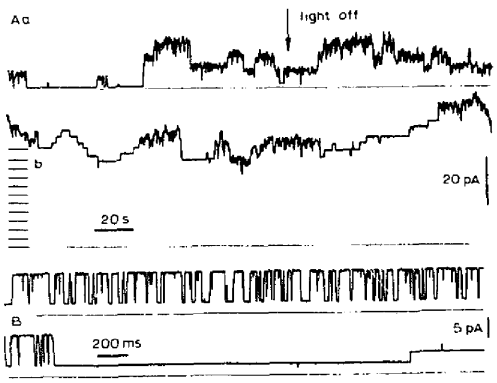


Fig. 5. Conductance properties of 'light channels'. (Aa) Single-channel current events of MABA in GMO/squalene membranes in symmetrical 40 mM HCl solutions at 100 mV membrane potential. The membrane patch and the surrounding solution and lipid/MABA mixture were irradiated with light in the band 320–400 nm for approx. 1 min before the beginning of this trace, until the time indicated by the arrow. During and after irradiation two types of ion channel were observed: the 'dark' channel as shown in Fig. 4 and a new 'light channel' with a marked flickering not fully resolved at this time resolution. (Ab) This trace was recorded from the same membrane patch as the one shown in (Aa) but 35 min after the light was turned off. From the total number of 10–15 channels subsequently incorporated into the membrane patch only one or two are of the 'light' type, indicating the reversibility of the photo-induced effect. (B, above and below) Expanded traces showing the flickering behavior of the 'light' channel type on top of a 'dark' channel (above). At the end of the trace (below) a second 'dark' channel opens.

The current–voltage relationship (i–V) of the hyperlevels is saturating in contrast to the slightly hyperlinear i–V of the main level (Fig. 4C). This was the only characteristic channel type observed in dark-adapted MABA samples.

Upon irradiation with light in the band between 320 and 400 nm another type of channel was observed (Fig. 5Aa) having a single-channel current similar to that of gramicidin A (in 40 mM HCl, 14 pA at 200 mV; 8.5 pA at 100 mV) and displaying a marked flickering behavior. Under these experimental conditions a mean open time within a burst of 40 (±3) ms and a mean closed time of 13 (±2) ms was measured. After the light was turned off again, the frequency of occurrence of this new channel type slowly decreased. Fig. 5Ab shows a section of a trace recorded 35 min after the light was turned off, showing only one 'light channel' out of about 15 'dark channels' as compared with the ratio of greater than 1 : 1 during irradiation.

The reversibility of the light-induced functional channel modulation was shown in other experiments where a sample was irradiated in a vial leading to a mixture of 'dark' and 'light' channels. Upon another dark-adaption of the same sample, only 'dark' channels were observed.

At this point it could be argued that some effect other than the proposed photoisomerization of the linker was responsible for the observed phenomena. To test this hypothesis we examined conductance behavior of a gramicidin A-dimer that is covalently linked with a tartaric acid derived moiety ($SS\text{-}CH_2$, see Ref. 9). This dimer differs from the dimers reported here only in the nature of its linker, which is not photosensitive. Irradiation of this dimer under the same conditions as used above did not cause a change in single-channel behavior, indicating that the azo-group of the investigated compound has been affected by the UV light used.

Discussion

The results reported herein illustrate that the conductance of the ion channels formed by these new azo-linked dimers can be modulated by irradiation with light. The effects are reversed by thermal relaxation. However, these channels display a number of unexpected and interesting characteristics. Creating a unified model that can explain all of these results at a molecular level has proven quite difficult. The first surprising

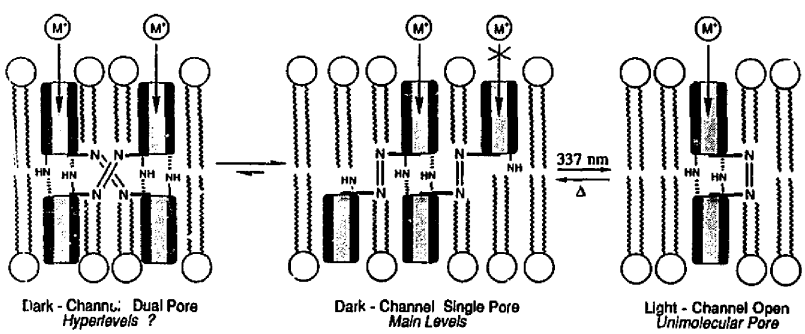


Fig. 6. Proposed model for observed photo-modulation. The diagrams illustrate possible pore structures for the observed channel states. (left) The dual pore structure represents a possible explanation for the observed hyperlevels, while the single pore 'tetramer' (center) represents the main conductance state and forms in analogy to natural gramicidin A dimers. When irradiated with light the linker isomerizes to the *cis* isomer allowing a unimolecular single pore channel to form (right).

outcome was the unexpected conductance observed for the dark-adapted channels. A priori it could be argued that this conductance is caused by residual amounts of the *cis* isomer in the dark-adapted equilibrium. However, these dark channels clearly do not belong to the same conducting species as that observed upon irradiation (compare Figs. 4 and 5). One simple explanation could be that two linked dimers form an intermolecular dimer of dimers (tetramer) [33,34]. In principle such a 'tetramer' can form a single pore or a dual pore channel. The structures in Fig. 6 illustrate one possible mechanism based on aggregates of dimers. Although this model is probably over-simplified, and other possible models cannot yet be excluded, we describe this model since it offers logical explanations for the following observations.

This hypothetical model offers an explanation for the hyperlevels observed in the dark-adapted channels. If the main conductance is caused by the formation of the first pore, then the hyperlevels could represent temporary formations of the second pore. The short lifetime of these hyperlevels would reflect a relative instability of the dual pore channel, perhaps due to entropic factors.

Irradiation is expected to isomerize one of the linked dimers in this 'tetramer'; in principle this can occur without disruption of the intermolecular dimer; but it is unlikely to leave the channel pore intact. This would explain the lack of direct transitions from the dark to the light channel forms in these experiments. This mixed *cis/trans* 'tetramer' could then dissociate and the *cis*-linked dimer would be free to form a unimolecular pore, the so-called 'light channel.' Our molecular modeling indicates (as do simple physical models) that this *cis* dimer is stable but significantly strained. This strain should manifest itself in at least two ways. First, it should increase the rate of thermal isomerization back to the *trans* isomer as is observed. The half-life of the dimer in solution is about 1 h at 45°C (data not shown), as compared to the apparently shorter half-life at 20°C as observed in the conductance measurements. Second, this strain should destabilize the channel structure leading to a separation of the monomers and causing the closure of the functional channel. The covalent linkage, however, does not allow the two monomers to separate far away from one another leading to a high probability of re-formation of the channel pore. The resultant of these effects would be brief channel closing events or flickers, as were observed for these 'light channels'.

In summary, we have used the dimer interface of the β-helix model of the conducting gramicidin A channel to design linker elements that were expected to modify the ion conducting properties of gramicidin A [9]. In the present study, we designed and synthesized linked dimers that were expected to photomodulate the conductance of the transmembrane channel, due to the presence of the azo-chromophore. The conductance of these novel transmembrane channel molecules are indeed photomodulated. The unusual properties of these compounds can be understood by consideration of a model that embraces aggregates of the linked dimers. Although details of the underlying mechanisms still remain elusive and are under investigation, these studies represent one significant step towards the goal of artificial channel molecules with controllable gating properties.

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