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How shortening a channel may lower its conductance. The case of des-Val^7-DVal^8-gramicidin A

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A shortened analog of gramicidin A has been shown by Urry et al. (Biochim. Biophys. Acta 775, 115–119) to have lower conductance than native gramicidin A. They argue this suggests that the major current carrier is the doubly occupied channel. A different perspective is presented here. Channel formation does not alter bilayer width. In a shortened channel an ion approaching the binding site moves further toward the center of the lipid-pore system. The electrostatic contribution to the energy barrier near the constriction mouth is greater for the shorter channel. As long as entry to the channel is rate limiting singly occupied short channels should exhibit lower conductance. The data are not inconsistent with singly occupied channels being the major current carriers. Experiments on other gramicidin analogs are suggested to more clearly distinguish between singly and doubly occupied channels as the dominant conducting species.

Urry and his co-workers [1] have recently reported the synthesis and characterization of a shortened analog of gramicidin A. This new polypeptide is des-Val^7-DVal^8-gramicidin A, a compound lacking residues 7 and 8 of its parent which has the primary structure (HCO-Val^1-Gly^2-Ala^3-DLeu^4-Ala^5-DVal^6-Val^7-DVal^8-Trp^9-DLeu^{10}-Trp^{11}-DLeu^{12}-Trp^{13}-DLeu^{14}-Trp^{15}-NHCH$_2$CH$_2$OH) [2]. The native molecule, when introduced into lipid bilayer membranes, forms ionophoric channels by head to head dimerization utilizing the amine ends of each monomer [3,4]. Dimerized gramicidin A forms a channel of physical radius of approx. 0.21 nm [5] with cationic binding sites approx. 0.25 nm from the entrance to the channel [6]. The regions involved in junction formation are residues 1 through 5; those utilized in forming the binding site are residues 9 through 16 [1]. These parts of the shortened molecule and its parent are structurally identical.

When dimerization occurs and channels form, the potential energy profile of a permeant ion in the channel is the sum of two terms [7–9]. The first represents short-range interaction between the ion and nearby polar moieties forming its inner solvation shell. The other is a long-range interaction with the more distant charge distributions, whether in the channel forming protein or at the water-lipid interface. This term is significantly affected by the fact that the lipid bilayer is only slightly polarizible; it specifically accounts for image forces due to the dielectric discontinuity. Since the chemically significant parts of both gramicidin A and its shorter analog are identical, the solvation energy profile for the two dimers should be the same (except that the distance between the binding sites is approx. 0.3 nm less in the shorter channel [1]). The difference between the potential energy profiles for the two dimers is thus attributed to the electrostatic term, which is repulsive for cation permeation through both gramicidin A and its analog. At its peak (the channel midpoint) this is

greater for the longer channel, at least as long as the surrounding lipid bilayers have the same electrical and physical structure [9–12].

Conductance measurements on both native and shortened gramicidin A, in bilayers formed from the same lipid, diphytanoylphosphatidylcholine (DPhPC) in *n*-decane, yielded a counterintuitive observation [1]; the conductance was smaller by nearly 50% in the shorter channel. The authors argued that this finding was inconsistent with singly occupied channels being the major conducting state. It is consistent with the current carrier being a doubly occupied channel, a state that probably occurs for gramicidin A [13,14]. Electrostatic repulsion between ions in the doubly occupied channel is somewhat greater for the shorter gramicidin than for the native form. Double occupation would be a less probable occurrence for the shortened gramicidin A. There would be fewer current carriers and the conductance would be less. Numerical estimates of the difference in the electrostatic repulsion energy for the doubly occupied short and long channels are consistent with the observed decrease in conductance (Ref. 1 and Jordan, P.C., unpublished calculations).

This report is devoted to demonstrating that under the reported experimental conditions the observation of lower conductance in the shorter channel is consistent with the major current carrier being the singly occupied channel. The only caveat is that the rate limiting step for ion motion through a singly occupied channel is the process of ion entry, a condition that is generally accepted [8,13,16]. While this does not rule out the possibility that the major conducting state is doubly occupied, it does provide an alternate interpretation. Finally, an experiment involving other gramicidin analogs will be suggested to eliminate the uncertainty.

The basic observation is that both the short and the native channels are inserted into membranes formed in the same way. The bilayer thickness does not depend upon whether the dimer being studied is that of gramicidin A or that of its shorter analog. When the transmembrane channel forms the bilayer water interface must deform to create a mouth. Different size mouth regions must occur when the two channels, which are only about 2.8 nm and about 2.5 nm long [1], span a membrane which is most probably approx. 5 nm wide, typical of bilayers formed from C_{18}-lipid, *n*-decane solutions [17]. As illustrated in Fig. 1a, the shorter channel has a slightly larger mouth region where the bilayer deforms to accomodate the channel protein. The binding site and position of the peak in the hydration-dehydration barrier occur in the regions of residues 9 through 15. This is closer to the center of the channel for the shorter system (the points marked × in Fig. 1a). Qualitative potential profiles are illustrated in Fig. 1b. As long as membranes of the same thickness are being compared, an ion moving toward the binding site is more affected by the presence of lipid when the channel is shorter. In contrast, at the channel center, the longer channel is more affected by the surrounding lipid.

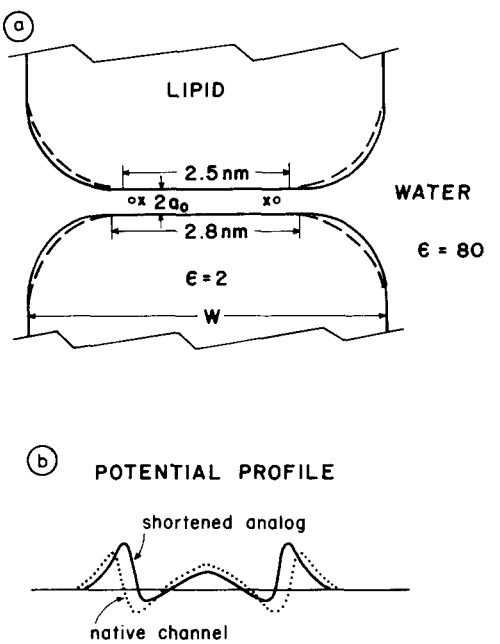

Fig. 1. (a) Diagram illustrating gramicidin-water-membrane geometry used for electrostatic calculations. Native gramicidin A is assumed 2.8 nm long [1]; its binding sites are at points marked O. The shortened gramicidin is only 2.5 nm long; the binding sites are at points marked ×. The membrane width, *W*, is constant. The mouth region is delinated by the dashed line for the shorter gramicidin. The dielectric constant in the polar regions is 80; that in the non-polar regions is 2. The electrical radius, a_0 is 0.25 nm [9,18]. (b) Qualitative potential energy profiles for shortened and native gramicidin channels in membranes of same overall width and composition.

This argument can be made quantitative. Before presenting the analysis it is important to contrast the Urry experiments [1] with those of Hladky and Haydon [18] and Kolb and Bamberg [19]. The latter groups have investigated the effect that varying membrane width and composition has on the conductance of channel formers of constant length. They observed that channel conductance is quite insensitive to bilayer width variation. The contrast between these results and those of Urry is both striking and puzzling; if the experimental conditions were truly similar one might expect, on electrostatic grounds, a sharp conductance decrease with increasing membrane width. However, the Urry experiments compare systems in which gramicidins of slightly different length are inserted into identical membranes; the perturbations of the bilayer structure may be assumed to be quite similar. The older experiments [18,19] are not so felicitous; the membrane widths vary from 4 to 6 nm. Gramicidin certainly perturbs the lipid structure in the immediate vicinity of where it is incorporated. The effect upon phospholipid packing could depend strongly on membrane width and solvent composition; i.e. phospholipid head group orientation in the vicinity of the channel could be very sensitive to differences between channel length and membrane width. The membrane dipole potential in the vicinity of the channel could decrease with increasing membrane width thus compensating for increases in the polarization energy. This suggestion, while speculative, conflicts with no experimental data. The observations of Pickar and Benz [20], which demonstrate that the membrane dipole potential is not influenced by membrane width, use lipophilic ion probes which are unlikely to signficantly affect phospholipid packing. What happens to local structure when channels are incorporated is unknown; however, substantial changes must be expected.

A quantitative study of the effect of small changes in channel length in membranes of constant width can be carried out by treating the lipid-water-pore former-ion system in the two dielectric approximation [9]. The lipid and the lipophilic exterior of the gramicidin are assigned a dielectric constant of 2; the bulk water, the water within the pore and the polar interior of the gramicidin are assigned a dielectric constant of 80. The pore's electrical radius is a bit greater than its physical radius, to accomodate pore former polarity [21]; the value used is 0.25 nm [9]. There are three contributions to the total electrostatic energy, E_{elec},

$$E_{elec} = E_{im} + E_{pore} + E_{lw} \quad (1)$$

E_{im} accounts for the permeant ion's repulsion by the surface charges it induces at the electrical phase boundary [10,12,22,23]; E_{pore} is the interaction (attractive) between the cation and the mean charge distribution in the polypeptide [9]; E_{lw} is the repulsion due to the fact that the lipid membrane is at a higher electrical potential than the aqueous surroundings [20,24]. Table I lists the differences in the electrostatic energy between the shorter and longer channels for a monovalent cation at selected points in the channel; the calculations use electrostatic modeling methods [10–12]. For this purpose the lipid-water potential difference is +390 mV, reasonable for a saturated C_{18} phosphatidylcholine [20]; the mean charge distribution in this polypeptide, determined from computer modeling of the structure [25], is equivalent to a radial dipole potential of −460 mV [9]. In addition to listing the total energy difference, calculated on the basis of Eqn. 1, the differences in the image energy alone are also included.

The results are unambiguous. At the binding site and in the region where hydration/

TABLE I

ELECTROSTATIC ENERGY DIFFERENCES, IN kJ·mol^{-1}, BETWEEN SHORTENED AND NATIVE GRAMICIDIN CHANNELS CONTAINING MONOVALENT CATIONS

The energies quoted are for differences (shortened channel) minus (native channel). Different lipid widths are contrasted.

Ionic position	Image energy lipid width		Energy from Eqn. 1 lipid width	
	4.5 nm	5.5 nm	4.5 nm	5.5 nm
Channel center	−1.00	−1.03	−0.56	−0.35
Binding site (B)	0.62	0.39	0.97	1.01
B+0.125 nm	0.89	0.52	1.16	1.17
Constriction entrance (B+0.25 nm)	0.93	0.77	1.28	1.27
B+0.375 nm	1.11	0.83	1.38	1.39

dehydration occurs, the electrostatic contribution to monovalent cation energy is greater for the shorter channel. Depending upon precisely where the rate determining step is presumed to take place and whether one used Eqn. 1 or the image energy alone as the discriminant, the energy difference is between 0.4 and 1.4 $kJ \cdot mol^{-1}$. Assuming the peak in the entrance barrier occurs at the mouth of the constriction (B + 0.25 nm), which is consistent with one interpretation of molecular dynamics simulations [26], and using Eqn. 1, the energy barrier is 1.28 $kJ \cdot mol^{-1}$, equivalent to a Boltzmann factor of 0.60. Because the ion must penetrate more deeply into the lipid when the channel is shorter, a conductance reduction is expected. A small correction must be applied to the factor just calculated. Since the ion moves closer to the channel center as it approaches the binding site in the shorter channel, it senses a larger fraction of the applied voltage drop. Electrostatic modeling calculations [10,12] indicate the difference is about 2.5%. As the measurements were made at 100 mV [1], the extra driving force is 2.5 mV; this would increase conductance in the shorter channel by a factor of approx. 1.10. Even with this correction it is still only 66% of that of the longer one, very close to the value of 58% found experimentally [1]. The conclusion is clear. As long as the rate-determining step occurs near the channel mouth, the shorter channel should exhibit the lower conductance even when the current carrier is the singly occupied pore.

The data presented in Table I suggest one further observation. If the rate-determining step were passage over the central barrier the shorter channel should exhibit greater conductance if singly occupied channels are the major current carriers. On energetic grounds alone the increase should be at least a factor of approx. 1.2 if experiments analogous to those described by Urry et al. [1] could be carried out. In fact it would probably be more because the shorter channel accomodates fewer water molecules. The translocation step requires less correlation of water and ion motion. If there is one fewer water molecule in the channel, the translocation rate should roughly double [9]; the shorter channel might well conduct 2- to 3-times as much current as the longer one. If the current carrier were the doubly occupied channel, the presence of a high central barrier would be irrelevant. Conductance characteristics would be governed by the population of doubly occupied channels which would be lower for the shorter system [1]. The shorter channel would then have the lower conductance.

The experiment just described may well be possible. Gramicidin M^- is an analog in which all tryptophan residues are replaced by phenylalanines [27]. Conductance studies indicate that the translocation rate in this analog is about the same as in gramicidin A while the rate constant for exiting the channel is higher for gramicidin M^- than for gramicidin A [28]. This suggests that, in gramicidin M^-, the central barrier may be rate limiting. By making a shortened analog of gramicidin M^- with residues 7 and 8 removed, one could carry out the suggested test. If the shorter channel has greater conductivity the singly occupied state is the dominant current carrier. If the conductance characteristics are reversed, the doubly occupied state must account for most of the conductance.

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