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SINGLE CHANNEL PROPERTIES OF D-LEU^2-GRAMICIDIN A

SIDE CHAIN MODULATION OF CHANNEL LIFETIME

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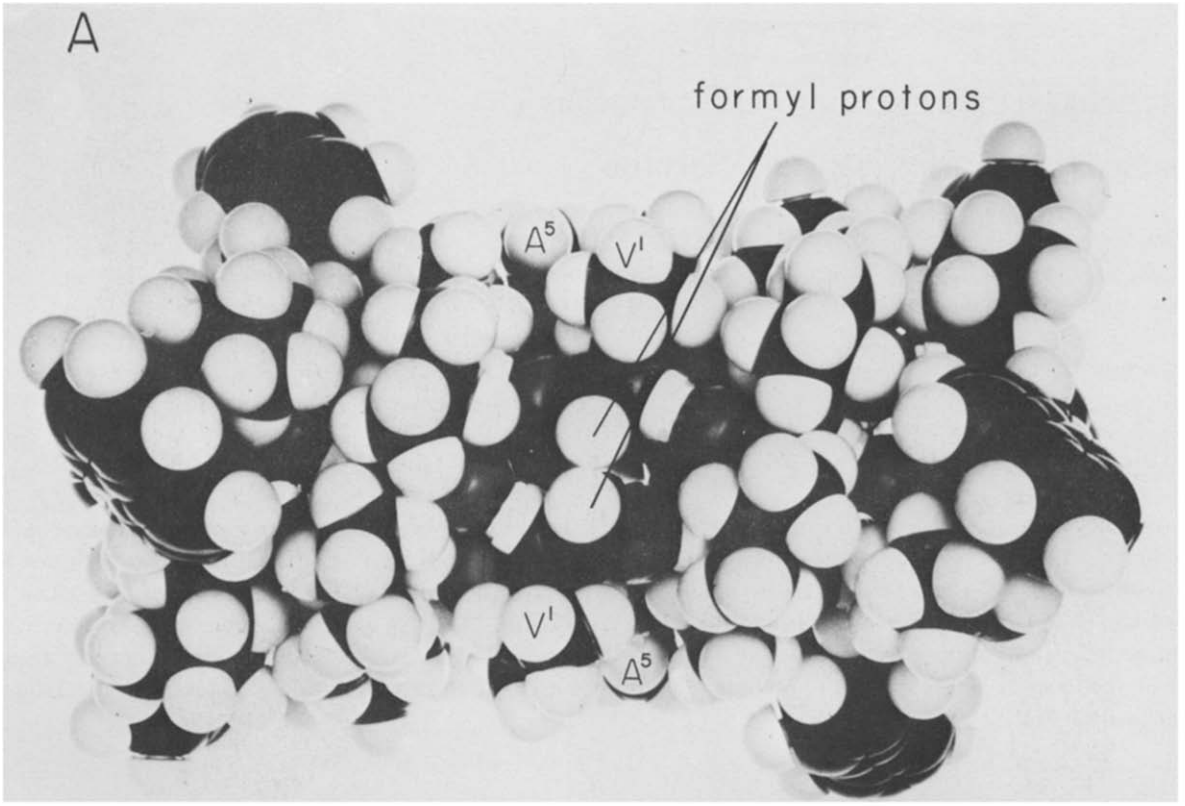
The channel forming properties of synthetic gramicidin A and DLeu^2-gramicidin A were compared in black lipid membranes. The most probable single channel conductance was identical for both derivatives but in each case a distribution of smaller channel sizes was observed. However, the lifetime of the channel formed by DLeu^2-gramicidin A was considerably shorter than for gramicidin A. The DLeu^2 substitution is considered to interfere with the head to head hydrogen bonding which forms the conducting dimer, thus destabilizing the dimeric structure of the channel and reducing the lifetime. This represents the first demonstration of side-chain modulation of channel lifetime.

The primary sequence of gramicidin A is. HCO-LVal^1-Gly^2-LAla^3-DLeu^4-LAla^5-DVal^6-LVal^7-DVal^8-LTrp^9-DLeu^{10}-LTrp^{11}-DLeu^{12}-LTrp^{13}-DLeu^{14}-LTrp^{15}-NHCH$_2$CH$_2$OH [1]. Two gramicidin A molecules associate head to head (formyl end to formyl end) by means of six intermolecular hydrogen bonds to form a dimeric transmembrane channel [2–9], as shown in Fig. 1. Each monomer is comprised of a $\beta^6_3;^3_3$-helix of 6.3 residues/turn [10,11], and the hydrogen bonding pattern between turns is that of the parallel chain β-pleated sheet and that between monomers is that of the antiparallel chain β-pleated sheet.

Naturally occurring gramicidin is a mixture of gramicidin A, B and C which differ in only one amino acid. Gramicidin B has an LPhe at position 11 and gramicidin C has an LTyr at position 11 [12]. In black lipid membranes the single channel conductances of gramicidin A and gramicidin C are similar but gramicidin B has a much reduced conductance [13]. The channel lifetimes for all three, however, are similar [13]. On the other hand synthetic changes in the amino terminus of the gramicidin peptide either block channel formation as in the case of *N*-pyromellityl-desformyl gramicidin A [5] or dramatically alter the channel mean lifetime as in the cases of *N*-succinyl-desformyl gramicidin A [5] and *N*-acetyl-desformyl gramicidin A [8] by interfering with the hydrogen bonded dimerization of channel formation. These are all changes in the amino blocking group. The question of direct concern here is whether an appropriate change of amino acid in the sequence involved in the head to head hydrogen bonding can alter channel mean lifetime.

It is of interest to note that the amino terminal sequence involved in the head to head hydrogen bonding, residues 1 through 5, uniquely contains several amino acids with small side chains whereas the remainder of the sequence is comprised of amino acids with bulky side chains. In this regard it may be noted further that oligopeptides with small side chains, (e.g. oligomers of LAla) form antiparallel chain β-pleated sheets whereas oligopeptides with bulky side chains (e.g. oligomers of LVal) form parallel chain β-pleated sheet structures [14]. Thus amino acids with small side chains are what would be


A
formyl protons
A5
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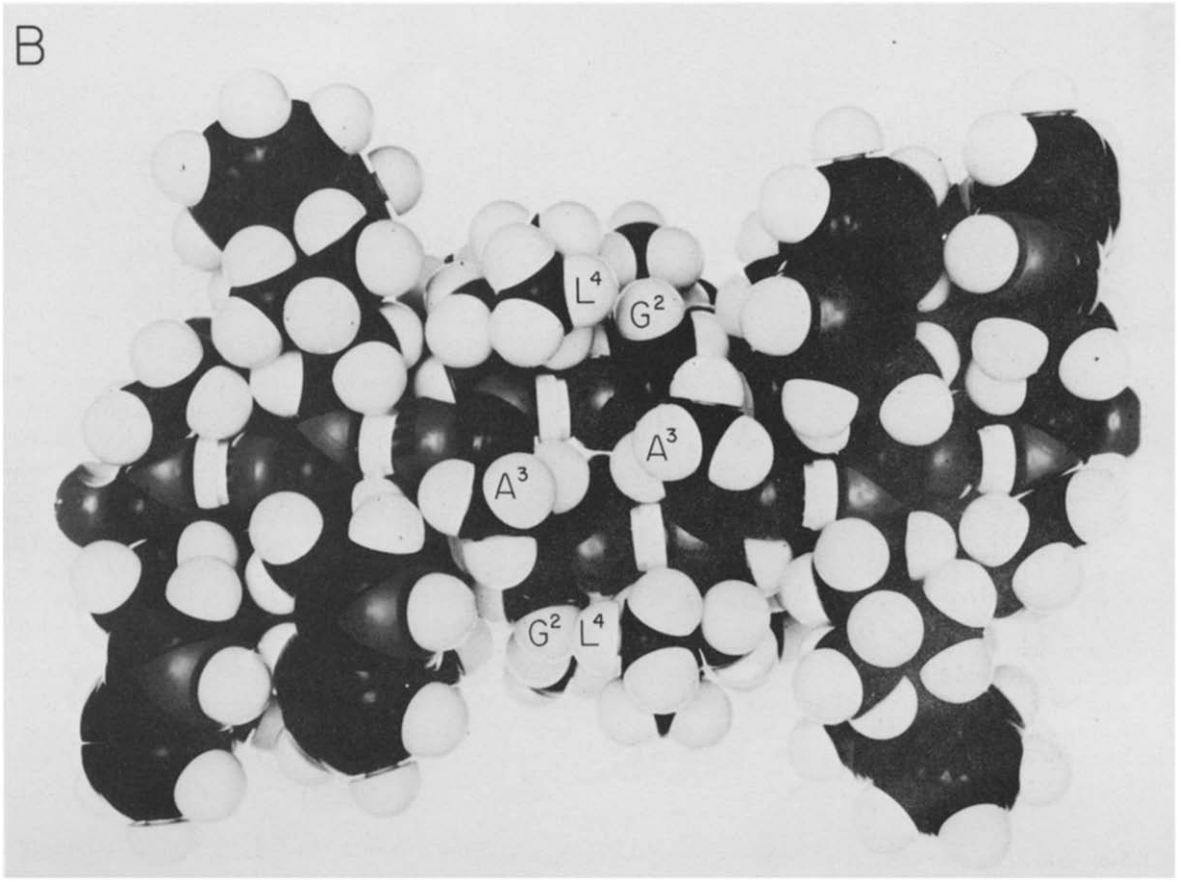

Fig. 1. B. Showing the LAla^3...LAla^3 and Gly^2...DLeu^4 side chain pairs. Note the steric effect of replacing the Gly proton with a Leu side chain, (A = Ala, G = Gly, L = Leu, V = Val).

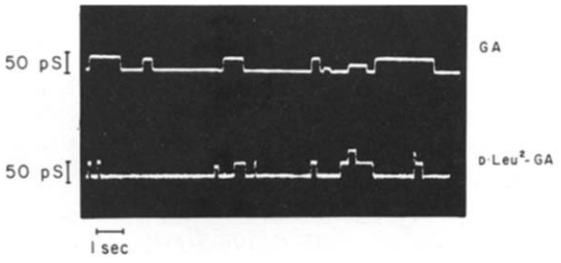

Fig. 2. Single channel recordings for gramicidin A and DLeu^2-gramicidin A in black lipid membranes. Potential was clamped at 100 mV with silver chloride electrodes and membrane current was amplified by a Keithley 427 current amplifier. The bilayer was bathed in 2 M potassium chloride containing picomolar concentrations of peptide. The conductance of the most commonly observed event was 28.4 pS for both gramicidin A and DLeu^2-gramicidin A.

28.4 pS. However, both peptides show a distribution of smaller channels which are seen less frequently (Fig. 3). There was evidence from the distribution that some discrete sizes were more common than others.

From the single channel events, as depicted in Fig. 2, it is seen that the channel lifetime for DLeu^2 is considerably shorter than for gramicidin A. Spectral analysis of the current noise for gramicidin A gave an average τ value of 800 ms whereas for DLeu^2-gramicidin A τ was 210 ms (Fig. 4). Therefore DLeu^2 substitution reduces the channel lifetime by 75% indicating that the barrier for dissociation of the DLeu^2-gramicidin A dimer is lower than for gramicidin A.

As may be seen in Fig. 1B, replacing the Gly^2 α-proton by a bulky Leu side chain would result in

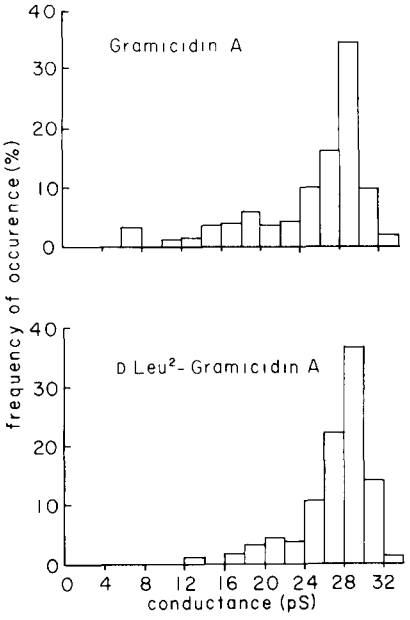

Fig. 3. Percentage distribution of single channel conductances for gramicidin A and DLeu^2-gramicidin A. Consecutive single channel events (N = 500) were measured from records where no more than two events occurred simultaneously, as in Fig. 2. The membrane potential was 100 mV and the bathing solution was 2 M KCl.

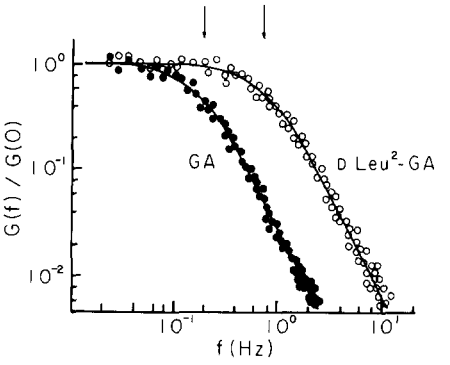

Fig. 4. Normalized power spectra for gramicidin A and DLeu^2-gramicidin A computed from the fast Fourier transform of conductance fluctuations due to many channel events. After low-pass filtering (3000 Hz) the analog records were digitized at a rate of 1024 points for each 2 s epoch. The power spectra were averaged over 128 epochs. The recording conditions were as in Fig. 1 but using a 5-fold higher concentration of peptide. Approximately 100 channels were active in the bilayer during data acquisition. The solid lines show the best fitting Lorentzian functions for each spectra and the arrows depict the half-power frequencies (fc). The relaxation time τ was calculated from the formula $\tau = 1/2\pi fc$. From the average of 15 experiments carried out as above τ = 800 μs for gramicidin A and 210 ms for DLeu^2-gramicidin A.

steric crowding and a decrease in the entropy due to freedom of side chain motion. This can be expected to raise the free energy of the dimeric state making the channel state less probable. As the barrier for dissociation occurs when the monomers of the dimer are at a greater displacement from each other, the free energy barrier for dissociation is not expected to be raised by as much, and therefore the rate of dissociation can be expected to be increased. This is what has been found. Since the amount of transport is directly affected by the channel lifetime, increasing the rate of dissociation is an effective means of decreasing channel efficacy. This is, of course, an easily quantitated element of the related decreased probability of channel formation. For the first time, therefore, side chain modulation of transport has been demonstrated by fine tuning channel mean lifetime.

An additional element of side-chain modulation of transport relates to the data in Fig. 3. It was noted above that gramicidin B has a lowered most probable single channel conductance [13] due to indole side-chain replacement by phenyl. This has been proposed to arise from the effects of side chain on the energetics of the peptide carbonyl motions (librations) necessary for effective lateral coordination of ions in the channel [11,15]. Somewhat analogously it has been proposed [16] that the occurrence of less probable states of lower conductance shown in Fig. 3 and also previously observed for gramicidin B [13] and commercial gramicidin [17] are due to distributions of side-chain rotamer states leading to altered energetics of peptide libration required for ion interaction.

As the highest barrier for transport of ions through the channel is at the entrance and exit [18], the most influential side-chain rotamer states in altering conductance would be those involving the side chains of residues 10 through 15. As previously reported [16], the primary restrictions to motions of side chains are due to side chain interaction of residue i with residue $i+1$ and of residue i with residue $i+6$. The latter is the side chain interaction

between turns of helix. In the case of the head to head junction, the side-chain interactions between helical turns of the channel were noted above. The comparison made in this report constitutes a change from Gly^2...$DLeu^4$ to $DLeu^2$...$DLeu^4$ and the experimental results reported herein provide the first experimental evidence of the effect of side chain interactions between turns of the helix on modulation of channel transport by β-helices.

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