The Gramicidin A Transmembrane Channel: Characteristics of Head-to-Head Dimerized \( \tau_{(L,D)} \) Helices

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ABSTRACT A series of helical structures for gramicidin A, with alternating L and D residues, are characterized as to number of residues per turn, atoms in hydrogen-bonded rings, and dihedral angles. Because of alternating peptide C-O directions, these helices are capable of forming head-to-head hydrogen-bonded dimers with the capacity of functioning as transmembrane channels. The dimers are characterized as to channel length, pore size, and expected ion selectivity.

In a test of the proposed head-to-head association for channel formation, the malonyl dimer \([N_{l}N_{d}l-dideformyl gramicidin A]-malonamide\) was synthesized. The chemical and conformational integrity of the product was verified by nuclear magnetic resonance in lipid bilayer studies, the dimer was found to be a potent mediator of ion conductance with the predicted concentration dependence.

Thus, the results on malonyl gramicidin A prove head-to-head association in formation of the transmembrane channel, and the results are consistent with the specific geometrical configuration involved in head-to-head dimerization of \( \tau_{(L,D)} \) helices. At this stage, the action of gramicidin A on membranes with lipid-layer thicknesses of 30 Å or less can best be understood in terms of the \( \tau_{(L,D)} \) helix with 6.3 residues per turn.

On the basis of the Pauling–Corey–Donohue postulates for polypeptide structure (1–4), the conformational energy diagrams for backbone dihedral angles of polypeptides (5, 6), and the characteristics of gramicidin A–mediated ion conductance across lipid bilayers (7, 8, 9), coupled with the significant fact that the amino acids in this peptide are in alternating L-D sequence (9, 10), a left-handed \( \tau_{(L,D)} \) helix has been proposed for the gramicidin A transmembrane channel (11), which has the unusual property of being capable of head-to-head dimerization. The head-to-head hydrogen-bonded association of two helices is possible because the peptide C-O bonds alternate in direction, with orientations primarily parallel and antiparallel to the helix axis. In this paper we generalize the possibilities of head-to-head association of left-handed gramicidin A helices of the \( \tau_{(L,D)} \) type and demonstrate, by examining the lipid bilayer activity and NMR spectrum of a derivative obtained by chemically coupling the \( \alpha \)-amino moieties of two deformyl gramicidin A molecules (i.e., by forming the malonamide dimer), that the transmembrane channel is formed by head-to-head association.

THE SET OF \( \tau_{(L,D)} \) HELICES

The sequence of gramicidin A, 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-NHCH2CH2OH, with its alternating L and D residues, makes possible a set of helices that differ from all previously described helices in that the peptide C-O bond vectors alternate with components parallel and antiparallel to the axis of the helix. If we define the direction of the helix axis vector from the amino to the carboxyl terminus, the C-O moieties of the L residues in \( \tau_{(L,D)} \) helices of gramicidin A have components parallel to this vector, whereas for the D residues, they are antiparallel to it. In the L-helix, on the other hand, all C-O bond vectors have components parallel to the helix axis vector.

The alternating C-O directions require, in schematic representation, that the number of sides of the helical structures be even. This is illustrated in Fig. 1, where two structures are represented with four and six sides. The C-O directions are the same for a given side of the helix. The alternation of one side with C-O moieties directed upward and the next side with the C-O moieties directed downward (necessary for intramolecular hydrogen bonding) requires an even number of sides. When these structures are built in accordance with the Pauling–Corey postulates, they are found to be allowed and of low energy in the dihedral angle conformational energy diagrams (5, 6). The structure in Fig. 1a has already been described (11); it is properly characterized as 4.4 residues per turn, with two different hydrogen-bonded rings of 14 and 16 atoms. The perspective along the helix axis of the structure in Fig. 1b is given in Fig. 2. To distinguish these conformations we will use a superscript that indicates the number of sides for the schematic representations in Fig. 1. These helices are characterized in Table 1, which also includes the pore size and the length of two helices associating head to head.

The pore size of \( \tau_{(L,D)} \) is of interest with respect to the ion selectivity exhibited by the transmembrane channel, \( \text{NH}_4^+ > K^+ > \text{Na}^+ \). The ammonium ion could pass through the channel with the least perturbation of the structure. \( K^+ \), with its 2.66 Å diameter, would require that the majority of its coordinations be supplied by the channel, necessitating an induced relaxation of conformation in which the directions of the C-O vectors are rotated toward the center of the channel. \( \text{Na}^+ \), with its 1.92 Å diameter, would require the greater perturbation of structure. The possibilities of the \( \tau_{(L,D)} \) helix have been discussed (11); the \( \tau_{(L,D)} \) helix, and helices with larger pore size, would exhibit virtually no ion selectivity. If we assume conformational energy to increase with deviation from the optimal helix coordinates, and relaxation of conformation to be the limiting factor energetically, it would be expected that the ion selectivity would be \( \text{Cs}^+ > \)
Rb⁺ > K⁺ > Na⁺ for τ(L,D) and the reverse for τ(L,D). Although these assumptions are not unreasonable, they must be checked with calculations involving the ion. All helices except τ(L,D) would be permeable to water. Steric crowding of the bulky R groups disfavors the helices with larger pore size.

The length of the channel formed by the dimers can best be given in terms of a range of values. The C–O of the terminal Trp-15 residue is directed into solution, giving the longest length of the channel as the distance between the peptide (tryptophyl) oxygen atoms at each end of the double helical axis.

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**Fig. 1.** Schematic representation of hydrogen-bonding patterns for helices in which peptide C–O moieties alternately point toward the amino and the carboxyl ends of the helix. The alternating directions of the C–O moieties require that these schematic representations have an even number of sides, i.e., 4, 6, 8, etc. A stereochemically correct representation, along the axis of the helix, of the structure represented in Fig. 1b is given in Fig. 2; that of Fig. 1a has already been described (11). a, C–O toward amino end, 14 atoms in H-bonded ring; C–O toward carboxyl end, 16 atoms in H-bonded ring. b, C–O toward amino end, 20 atoms in an H-bonded ring. C–O toward carboxyl end, 22 atoms in an H-bonded ring.

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**Fig. 2.** Helix axis perspective of τ(L,D)-type helices, showing approximately 6.3 residues per turn with 20- and 22-membered hydrogen-bonded rings.

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**Fig. 3.** An unrolled perspective of the τ(L,D)-type helices, showing the head-to-head junction. The turn of helix that involves head-to-head association of two helices has the chains running antiparallel, and exhibits the hydrogen-bonding pattern of the antiparallel β-pleated sheet (see regions b). The intramolecular hydrogen-bonded turns exhibit a hydrogen-bonding pattern characteristic of parallel β-pleated sheets. The junction site of two formyl moieties at the center of the diagram facilitates the transition between the two hydrogen-bonding patterns. When deformyl gramicidin A' molecules are chemically coupled, malonyl provides a satisfactory intermediate structure.
Head-to-Head Dimerized Gramicidin A

Fig. 4. 220-MHz PMR spectra of gramicidin A' and its derivative. a, gramicidin A'; b, malonyl gramicidin A'. Spectra were obtained using 10% (w/v) solutions in Me2SO-d6 at 23°C. Gramicidin A' is a commercial preparation (Nutritional Biochemicals) containing approximately 72% gramicidin A, 9% gramicidin B, and 19% gramicidin C; the latter two congeners differ from gramicidin A only in the replacement of Trp-11 by a phenylalanine and a tyrosine, respectively† (13).

Table 1. Characteristics of ρ(L,D) helices

<table>
<thead>
<tr>
<th>Helix</th>
<th>Residues/turn*</th>
<th>H-bonded rings</th>
<th>Dihedral angles‡</th>
<th>Length of dimer‡ (Å)</th>
<th>Pore size (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ρ4(L,D)</td>
<td>4.4</td>
<td>16, 14</td>
<td>φ = -125, ψ = +85</td>
<td>35-39</td>
<td>1.4</td>
</tr>
<tr>
<td>ρ6(L,D)</td>
<td>6.3</td>
<td>22, 20</td>
<td>φ = -120, ψ = +105</td>
<td>25-30</td>
<td>4</td>
</tr>
<tr>
<td>ρ8(L,D)</td>
<td>8.4</td>
<td>28, 26</td>
<td>φ = -145, ψ = +135</td>
<td>18-24</td>
<td>6</td>
</tr>
<tr>
<td>ρ10(L,D)</td>
<td>10.4</td>
<td>34, 32</td>
<td>φ = -135, ψ = +150</td>
<td>14-21</td>
<td>8</td>
</tr>
</tbody>
</table>

* Approximate number of residues per turn obtained from molecular models. These values will be improved by carrying out the conformational energy calculations and obtaining coordinates for the low-energy conformation.
† Approximate dihedral angles obtained from the molecular models. These values will be refined by carrying out the conformational energy calculations. The values for the L residues correspond directly to the dihedral-angle conformational energy diagrams. The values for the D residues, in parentheses, are also to be related to the conformational energy diagrams for L residues. They are energetically equivalent positions. The positions on a conformational energy diagram for D residues are related by the following expressions: φD = φL + 240°; ψD = ψL - 240°. All these values fall within the broad low-energy basin on the dihedral-angle conformational energy charts (5, 6).
‡ The higher value is the distance between the outermost end-peptide (tryptophyl) oxygens, which are directed into solutions (including the oxygen van der Waals radii), and the shorter distance is from the innermost pair of tryptophyl oxygens that are directed into solution. The shorter distance would probably be close to the preferred lipid-layer thickness.
parallel association, whereas the regions indicated by $b$ are those characteristic of antiparallel association. The formyl ends, at the center of the figure, allow the flexibility for the transition. Accordingly, if one wishes to chemically attach the head, or amino, ends with the purpose of obtaining a functioning and complete transmembrane channel, this change in hydrogen-bonding pattern should be considered. The malonyl moiety, $-\text{CO-COCH}_2\text{CO}-$, replacing two formyl moieties, provides an intermediate situation of 4 atoms in the polypeptide backbone between hydrogen-bond associations, as compared to 5 and 3 (see Fig. 3). In the parallel pleated sheet, 3 backbone atoms are paired with 5, whereas in the antiparallel pleated sheet the backbone atoms are paired 3 with 5 and 5 with 5. The pairing of 3 with 4, as in the case of malonyl coupling, allows a satisfactory structural transition between 5 with 3 for continuing the parallel association and 3 with 3 as the next pattern expected for the antiparallel association.

**NMR of Gramicidin A' Derivatives**

For the purposes of this communication the NMR of gramicidin A' (see legend of Fig. 4 for definition of gramicidin A') and its derivatives serves to verify the formation of the derivative, to determine purity, and to assess the effect of the chemical modification on conformation in perdeutero-dimethyl sulfoxide (Me$_2$SO-d$_6$). The assignment of resonances is given in Fig. 4a.† The formyl proton peak near 8 ppm in Fig. 4a is missing in 4b, in which a malonyl CH$_2$ proton peak is observed near 3.5 ppm. The spectra are otherwise nearly identical as can be expected for subsequent NMR scans, with the exception of small changes in the peptide N-H region. Thus, it can be concluded that the Me$_2$SO-gramicidin A' conformation can exist with chemical coupling of the amino ends of the molecule. The results are consistent with a head-to-head association involving some peptide N-H protons. On the basis of aCH-NH coupling constants and correlation of absorption, circular dichroism, optical rotatory dispersion, and infrared data, the conformation in Me$_2$SO is in agreement with what is to be expected with $\pi_{(L,D)}$ helices.

**Lipid Bilayer Studies**

Previous studies (8) on gramicidin A' have demonstrated a second-order dependence of conductance on concentration. On the basis of the proposed association for channel formation, it was predicted that malonyl gramicidin A' would constitute an active channel with a first-order concentration dependence. This is confirmed by the results in Fig. 5. At concentrations above $10^{-10}$ M, there is a marked falling off in conductance increment, probably due to aggregation, which is known to occur also with gramicidin A', though at higher concentrations ($>10^{-7}$ M); this would account for the apparent order being somewhat less than 1 over the range indicated. The absence of autocatalytic behavior with erythrocyte lipid (12) (see Fig. 5) is noteworthy, since gramicidin A' itself shows it. For lecinthin–cholesterol, on the other hand, the rate constant $k \sim 2 \times 10^{-3}$ sec$^{-1}$ is approximately one order of magnitude lower than with the unmodified gramicidin A' under similar conditions.

At the lowest concentration ($10^{-12}$ M), quantal increments of conductance (13) were observed having an average value

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for $\Delta g(K^+)$ of $7 \times 10^{-11} \Omega^{-1}$, the same for both lipids, and approximately one-third of those seen with unmodified gramicidin A' under the same conditions. The K$^+$-Na$^+$ bionic potential was $17 \pm 2$ mV at 100 mM electrolyte concentrations, compared to 42 mV for gramicidin A', which indicates a reduced ion selectivity for malonyl gramicidin A'. The NH$_4^+$-K$^+$ potential was $+7$ mV. None of the above results changed as the mole ratio of cholesterol to phospholipid was varied from 0 to 1 to 2.

Another feature is the very high specific conductance, $10^{7}$ $\Omega^{-1}$ cm$^{-2}$ mol$^{-1}$; a comparable figure for valinomycin (14) at the same electrolyte concentration (1 mM potassium phosphate, pH 7) would be $10^{4}$ $\Omega^{-1}$ cm$^{-2}$ mol$^{-1}$.

The kinetics of malonyl gramicidin A' are essentially what would be predicted if dimerization is absent or not rate-limiting (8)*. That the rate constant is lower is also to be expected if it represents the entropy of conformational change required to pass from the aqueous to the lipid phase.

From the value of channel conductance $\Delta g(K^+)$ and an assumed thickness for the hydrophobic region of 40 Å, one obtains a partition coefficient $\beta = 430$ for the lecithin. From the above value of $\Delta g$ one can also calculate the mobility $u(K^+) \sim 7 \times 10^{-4}$ cm$^{2}$ sec$^{-1}$ V$^{-1}$ of K$^+$ in a channel, which turns out to be the same as in water. Since the partition coefficient of valinomycin (15) is somewhat greater, $\beta = 3400$, this high mobility is the principal cause of the high specific conductance with malonyl gramicidin A'. On the other hand, the relative constancy of $\Delta g$ with varying lipid composition indicates that lipid specificity operates here principally by changes of $\beta$. This explains the markedly lower lipid specificity for malonyl gramicidin A' as against gramicidin A', since it depends here on $\beta$ rather than $\beta$. For the lipids in Fig. 5, malonyl gramicidin A' gave one, whereas, gramicidin A' gave two orders of magnitude difference. The somewhat lower value of $\Delta g(K^+)$, together with the reduced K$^+$/Na$^+$ selectivity, shows that $\Delta g(Na^+)$ remains unchanged. That there is a change for K$^+$, however, seems to indicate that not all the ion selectivity is exercised at the polar–nonpolar interface.

That cholesterol has no effect with malonyl gramicidin A' shows that its action with gramicidin A'—a reduction in specific conductance (8)—is most probably on the intramembrane dimerization.

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**Correction.** In the article "Molecular Characteristics of Some Carcinogenic Hydrocarbons," by Ercole Cavalieri and Melvin Calvin, which appeared in the June 1971 issue of Proc. Nat. Acad. Sci. USA, 68, 1251–1253, the protons identified as No. 1 and No. 3 in Table 1 and Fig. 2, should be reversed, i.e., what is labeled proton No. 1 should be proton No. 3 and what is labeled No. 3 should be proton No. 1, everywhere in the article.