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A Molecular Theory of Ion-Conducting Channels: A Field-Dependent Transition Between Conducting and Nonconducting Conformations

(biological membranes/gramicidin A/dipole moments/electric field)

D. W. URRY

Division of Molecular Biophysics, Laboratory of Molecular Biology, University of Alabama Medical Center, Birmingham, Ala. 35233

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ABSTRACT **Structural and conformational requirements for an electric field-dependent transition between conducting and nonconducting macromolecular systems are: two kinetically interconvertible and energetically similar conformations, one conducting and the other nonconducting, which have axes spanning the lipid layer of biological membranes, but which have different net dipole moments along those axes. Two examples are described. A previously defined helix, the π^6_{LD}-helix now termed the $\beta^6_{3,3}$-helix, is proposed as the conducting species, and the linear peptide correlate of the cyclic hexapeptide conformation containing two β-turns and an inversion element of symmetry is proposed as a nonconducting species. The latter is termed an anti-β^6_2-spiral and contains little or no net dipole moment per turn, whereas the $\beta^6_{3,3}$-helix contains a net dipole moment along the helix axis of about 0.5 Debye per dipeptide unit. A related conducting and nonconducting pair with large net dipole moments of opposite sign, termed syn-β^6_2-spiral and $\beta^6_{2,4}$-helix, are also described. The spiral conformations are stabilized in a lipid layer by intermolecular hydrogen bonds, leading to a linear association of transmembrane structures. A conformational transition in one member of the array could lead to destabilization of an adjacent member of the array. The conformational analysis uses a concept of cyclic conformations with linear conformational correlates. The anti-β^6_2-spiral and $\beta^6_{3,3}$-helix are derivable from the conformations of the cyclic structure $\overline{\text{L-Gly})_3}$, whereas the syn-$\beta_2$-spiral and $\beta^6_{2,4}$-helix may be derived from the cyclic structure $\overline{\text{L-L-Gly})_2}$.**

The conformational analysis leads to the expectation that *N*-formyl-(L-Ala-L-Ala-Gly)$_n$ would form conducting channels.

If there should exist two kinetically interchangeable conformations that could span the lipid layer of biological membranes, but that would have substantially different net dipole moments oriented in transmembrane manner, then the application of an electric field across the membrane could lead to the interconversion from one species to the other. If one conformation is a conducting species, for example, a channel, and the other is nonconducting, then a field-dependent channel could form. It is the purpose of this manuscript to explicate two pairs of such conformations.

A conducting transmembrane channel has been described (refs 1–3 and Hladky, S. B. & Haydon, D. A., personal communication) that exhibits ion selectivity and a high specific conductance and for which a conformation has been proposed (4–6) and supported by spectroscopic data (6, 7). The proposed conformation is given in Fig. 1 for the π^6_{LD}-helix of gramicidin A. As will be discussed in greater detail below, the term $\beta^6_{3,3}$-helix is a more descriptive one. The fundamental requirement for this conformation is a specific aminoacid sequence. For gramicidin A, the requirement is an alternating (L-D)$_n$ optical isomeric sequence of hydrophobic amino acids with a glycine residue replacing one of the D-residues. Accordingly, if one were to adhere to the bias of no D-residues in mammalian systems, then the preferred sequence is (L-Gly)$_n$. The $\beta^6_{3,3}$-helix and a conformation labeled anti-β^6_2-spiral form a pair consisting of a conducting and nonconducting structure, respectively. A second pair of conformations use the sequence (L-L-Gly)$_n$. The corresponding conducting conformation is termed a $\beta^6_{2,4}$-helix and the nonconducting structure, a syn-β^6_2-spiral.

TRANSITION BETWEEN MOLECULAR CONFORMATIONS WITH DIFFERENT DIPOLE MOMENTS

Cyclic analogues

The two conformations of interest are most easily visualized as their cyclic hexapeptide analogues. The cyclic primary structure is $\overline{\text{L-Gly})_3}$ where the L-residue is hydrophobic. The two interconvertible conformations are given in Fig. 2*a*. In conformation *a*, the C–O peptide bond moments are perpendicular to the plane and they alternately point up out of the plane and down into the plane. It contains a 3-fold symmetry axis and an inversion element of symmetry. Conformation *a'* contains two β-turns related by a 2-fold symmetry axis and an inversion element of symmetry for the backbone atoms; four of the peptides are approximately in the plane and intramolecularly hydrogen bonded, whereas two peptides are in the same position as in conformation *a*. Conformation *a'* and *a* are easily related by rotation of four of the six planar peptide moieties. In Fig. 2*b*, the C–O groups of both end peptide moieties point upward out of the plane in conformation *b'*. While these are maintained in fixed position, the other four peptide moieties are rotated such that the peptide C–O groups point downward to give conformation *b*.

These conformations may have net dipole moments due to the large dipole moment of the peptide moiety, about 3.7 Debye (9). In conformations *a'* and *a*, the peptide moments cancel out giving no net dipole. Conformations *b'* and *b* have substantial net dipole moments of more than 6 Debye, but, significantly, in opposite directions. The controlling factor as to whether an end peptide points up or down lies in the aminoacid sequence (10, 11). The conformations of the cyclic hexapeptides indicated in Fig. 2 (conformations *a'* and *b'*) have been determined by x-ray diffraction (12, 13) and in

solution (14–17). Furthermore, β-turns have been defined in many naturally occurring polypeptides (18–21). Their presence has been subsequently supported with x-ray studies in oxytocin (22). The enniatin B–potassium ion complex, also determined by x-ray diffraction (23) and in solution studies (24), is analogous to conformation *a* of Fig. 2 with six C–O moieties alternately pointing on opposite sides of the ring.

The conformations in Fig. 2 provide easily visualized representations for the linear peptides. The conceptual transition from cyclic to linear is provided by gramicidin A and follows readily from the large number of residues per turn and low pitch of the helical and spiral conformations. Fig. 1*a* is the helix axis perspective of the $\beta^6_{3,3}$-helix. It contains 6.3 residues per turn, and the hydrogen bonding pattern between turns of the helix is the same as between chains of the parallel β-pleated sheet conformation, hence the nomenclature β-helix. The spectroscopic data (UV, circular dichroism, and nuclear magnetic resonance) for gramicidin A in trifluoroethanol and dimethyl sulfoxide argue for a left-handed β-helix (7, 8), and the black lipid membrane studies are nicely consistent with the $\beta^6_{3,3}$-helix (1– 3, 5, and Hladky, S. B. & Haydon, D. A., personal communication) as being the conducting transmembrane channel. Furthermore, the conformation of gramicidin A is readily varied by a change of solvents (7, 8), indicating two or more conformations of similar energies. Thus, it is reasonable to correlate cyclic and linear conformations when about six residues per turn are considered and, further, to consider transitions between two conformations, a closed, nonconducting structure and an open, conducting structure. This may be considered a concept of cyclic conformations with linear conformational correlates.

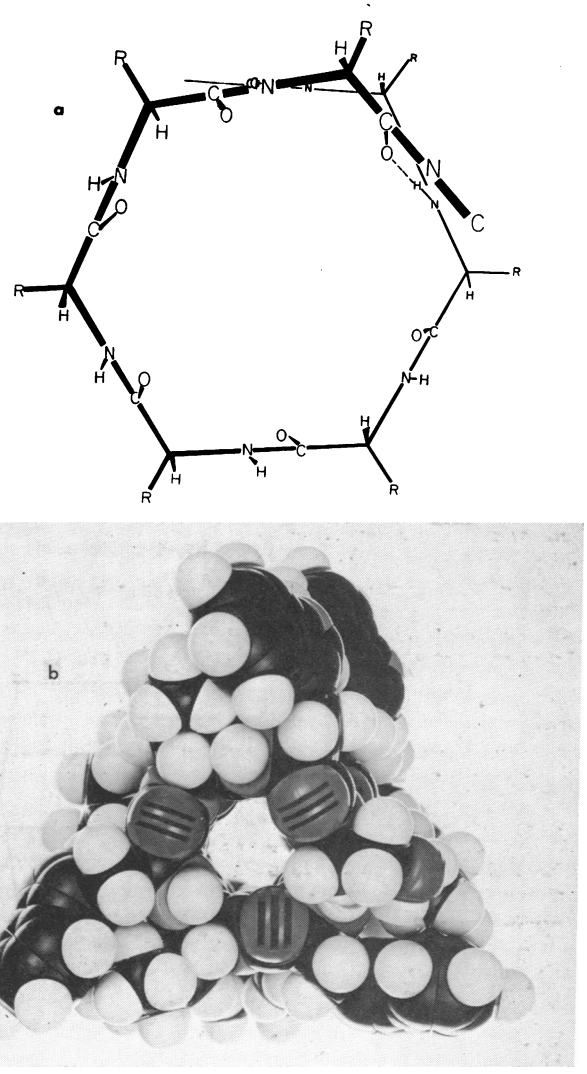

FIG. 1. (*a*) Helix axis perspective of the π^6_{LD}-helix (now termed a $\beta^6_{3,3}$-helix). Note that the peptide C–O moieties alternately point toward opposite ends of the helix. From Urry *et al.* (6).

(*b*) Channel view (helix axis perspective) of Corey-Pauling-Koltun molecular model of gramicidin A in the $\beta^6_{3,3}$-helical conformation. Note the triangular shape. (This will be diagrammatically represented in Figs. 2*c* and 5.)

Corresponding linear peptide structures

The linear peptide sequence corresponding to the cyclic structures in Fig. 2*a* would be $(\text{L-Gly})_n$, and that corresponding to those in Fig. 2*b* would be $(\text{L-LGly})_n$. For the linear conformation that corresponds to conformation *a'* in Fig 2*a*, the term spiral is used. Because it contains two β-turns, it is called a β_2-spiral, and since the cyclic representation contains six residues, the superscript six is used, i.e., a β^6_2-spiral*. The prefix anti- is used to indicate that the end peptides of the two β-turns are pointing in opposite directions parallel and antiparallel to the spiral axis, and the prefix syn- may be used when they are pointing in the same direction. A representation of the anti-β^6_2-spiral is given in Fig. 3*a* as conformation *a'*. When the hydrogen bonding of the end peptides of the β-turns is maintained and the side peptides are rotated appropriately, the $\beta^6_{3,3}$-helix (previously named the π^6_{LD}-helix) is formed. The superscript again refers to the number of residues in the cyclic representation, and the delineation β is used because the hydrogen bonding pattern between turns of the helix is that of the parallel-β-pleated sheet (4, 5). The subscripts indicate the number of peptide C–O moieties pointing parallel and antiparallel to the helix axis. Since the peptide orientation alternates, the numbers are 3 and 3.

In the anti-β^6_2-spiral, the C–O moieties of the end peptides point in opposite directions, resulting in a cancellation of the dipoles. Cancellation of peptide dipole moments also occurs with the cross-β peptides whose planes are nearly perpendicular to the spiral axis. Accordingly, the anti-β^6_2-spiral contains little or no net dipole moment. (See Fig. 3*a*, where the dipoles are of the same magnitude but opposite in sign.) In the $\beta^6_{3,3}$-helix, although an equal number of the peptide C–O bond moments point in opposite directions, the C–O bond moments that point toward the amino end of the polypeptide chains are at an angle to the helix axis (Fig. 3*a*). The result is a net dipole moment of more than 0.5 Debye per dipeptide unit. Thus, the equilibrium in Fig. 3*a* is between two conformations with different net dipole moments, and the application of an electric field along the axes (across the membrane)

* A nomenclature for the spiral structures containing β-turns has been chosen with the allowance for more such structures. Additional possible spiral structures would be a β^{10}_2-spiral in which there are two β-turns and 10 residues per turn with gramicidin S as the cyclic analogue, a β^{12}_3-spiral in which there are three β-turns and 12 residues per spiral with valinomycin (noncomplexed) as the cyclic analogue, etc.

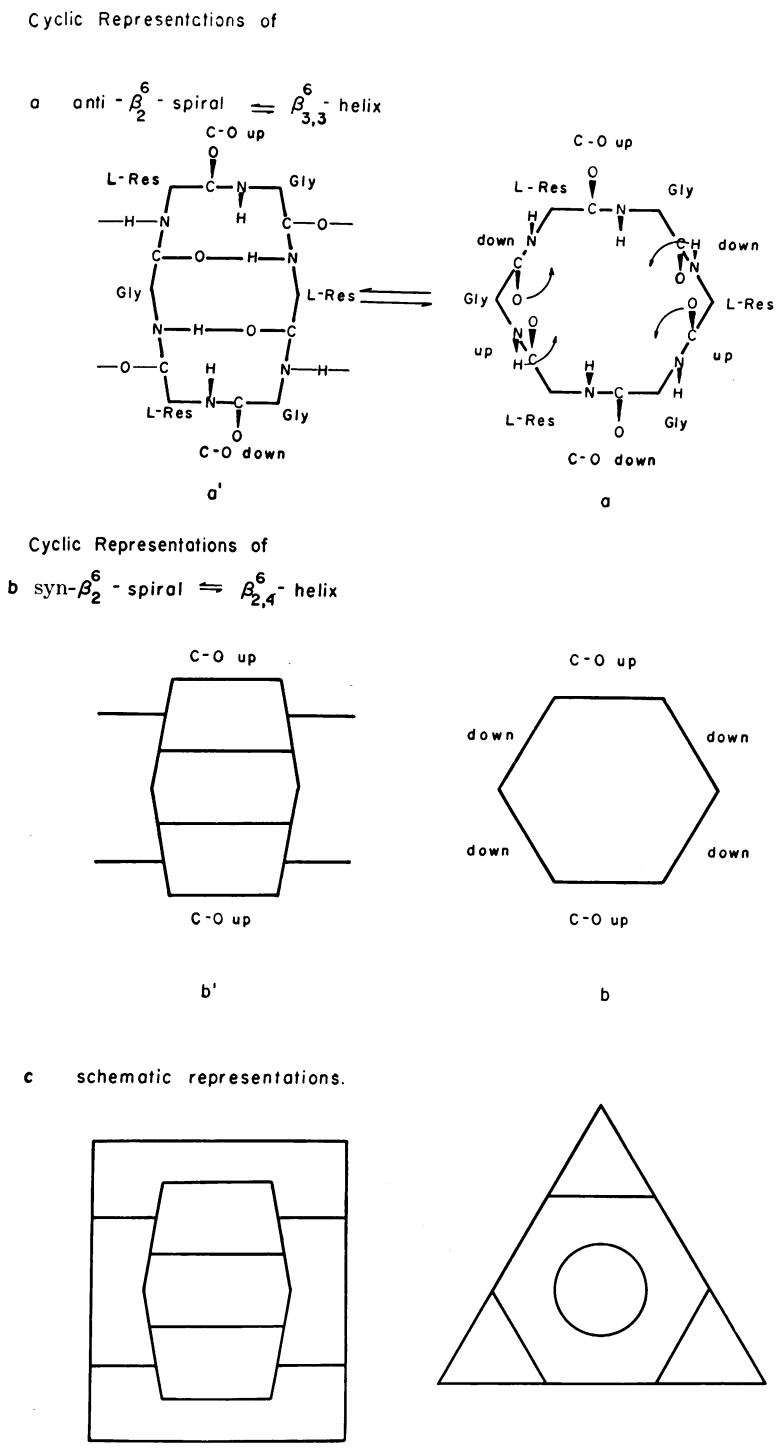

FIG. 2. Cyclic conformers that function as representations of corresponding spiral and helical structures. (*a*) Cyclic representation of the conformational change in going from an anti-β^6_2-spiral to a $\beta^6_{3,3}$-helix. In conformer *a'*, the C–O peptide at the top of the structure points up out of the plane and that at the bottom points down into the plane. These are called the end peptides of a β-turn. The peptides with C–O moieties pointing laterally, called the cross-β peptides, form two intramolecular hydrogen bonds resulting in a closed structure. Rotation of the cross-β peptides such that there is an alternation of the peptides pointing out of and into the plane (*up* and *down* as indicated) results in an open structure, as indicated in conformer *a*. The aminoacid sequence favorable for both conformers is $\overline{\text{L-(Gly)}_3}$. In going from the cyclic to the linear structures, left-handed spirals and helices are formed. The anti-β^6_2-spiral is a closed or nonconducting conformation, whereas the $\beta^6_{3,3}$-helix is a conducting conformation. (Gramicidin A in the $\beta^6_{3,3}$-helical conformation is given in Fig. 1*b*, and the anti-β^6_2-spiral is given in Fig. 4 as conformation *a'*.)

(*b*) The conformation of *b'* differs from that of *a'* in that both end peptides point up out of the plane. Rotation of the cross-β peptides such that their C–O moieties point down (into the plane of the paper) results in conformer *b*. The sequence compatible with both conformers is $\overline{\text{L-L-Gly})_2}$. (The syn-$\beta^6_2$-spiral is given in Fig. 4 as conformation *b'*, and the $\beta^6_{2,4}$-helix is also given in Fig. 4 as conformation *b*.

(*c*) Schematic representations of the interconversion from the spiral nonconducting conformation to the conducting conformation.

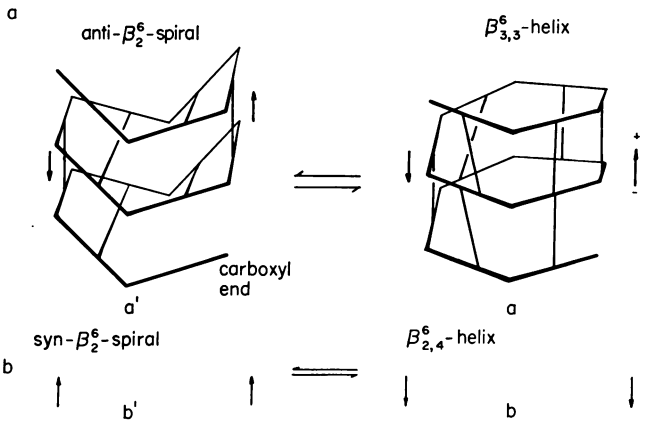

FIG. 3. Representation of the conversion from a spiral to helical conformation noting the effect on the dipole moments arising from the peptide moieties. (*a*) In the anti-β^6_2-spiral, the end peptide moieties point in opposite directions such that their dipole moments cancel out, as do those of the cross-β peptides in which the peptide plane is nearly perpendicular to the spiral axis. However, on conversion to the $\beta^6_{3,3}$-helix, the peptides point in opposite directions, but in one direction the peptide C–O bond vectors are parallel to the helix axis, whereas the C–O bond vectors of the peptide pointing in the opposite direction are at an appreciable angle to the helix axis. This results in a net dipole moment along the helix axis.

(*b*) The syn-β^6_2-spiral has both end peptide C–O moieties pointing in the same direction, resulting in a large net dipole moment for this conformation. On conversion to the $\beta^6_{2,4}$-helix, four peptide C–O moieties point in the opposite direction while the two that originated from the end peptides of the spiral structure maintain the same direction but skew slightly with respect to the helix axis. The result is that both conformations have large net dipole moments but in opposite directions.

would perturb the equilibrium toward the $\beta^6_{3,3}$-helix containing a 4.0-Å channel which, for gramicidin A, is selective for K^+.

The linear conformation that corresponds to conformation *b'* of Fig. 2*b* is termed a syn-β^6_2-spiral. The representation would be the same as for conformation *a'* in Fig. 3, but the end peptide C–O bond moments would be pointing in the same direction (*b'*, Fig. 3*b*). Since the dipole moments of the cross-β peptides with planes nearly perpendicular to the spiral axis cancel, this gives a net dipole of greater than 6 Debye per hexapeptide unit. When the cross-β peptide units are rotated such that the C–O moieties point downward (conformation *b* of Fig. 2*b*), the syn-$\beta^6_{2,4}$-helix is formed. The two peptides with C–O moieties pointing upward have bond vectors that are at a small angle to the helix axis, whereas those pointing downward are nearly parallel to the helix axis. The result is a net dipole moment of about 6 Debye per hexapeptide unit, but with a direction opposite to that of the conformation with which it is in equilibrium, i.e., the syn-β^6_2-spiral.

RELEVANCE TO MEMBRANE CHANNELS

In its development, the preceding argument began with the proposed (4, 5) and substantiated (2, 3, 5, 7, 8, and Hladky, S. B. & Haydon, D. A., personal communication) $\beta^6_{3,3}$-helix of gramicidin A, followed by the recognition, consistent with the conformational mobility observed as a function of solvent (7, 8), that there would be a facile transition to the anti-β^6_2-spiral. The next step was the realization that, by analogy to the cyclic hexapeptides, the syn-β^6_2-spiral could be constructed with an (L-L-Gly)$_n$ sequence and that the $\beta^6_{2,4}$-helix could be readily obtained from the syn-β^6_2-spiral. The Corey-Pauling-Koltun molecular models of *N*-formyl(L-Ala-L-Ala-Gly)$_n$ syn-β^6_2-spiral and $\beta^6_{2,4}$-helical conformations are given in Fig. 4

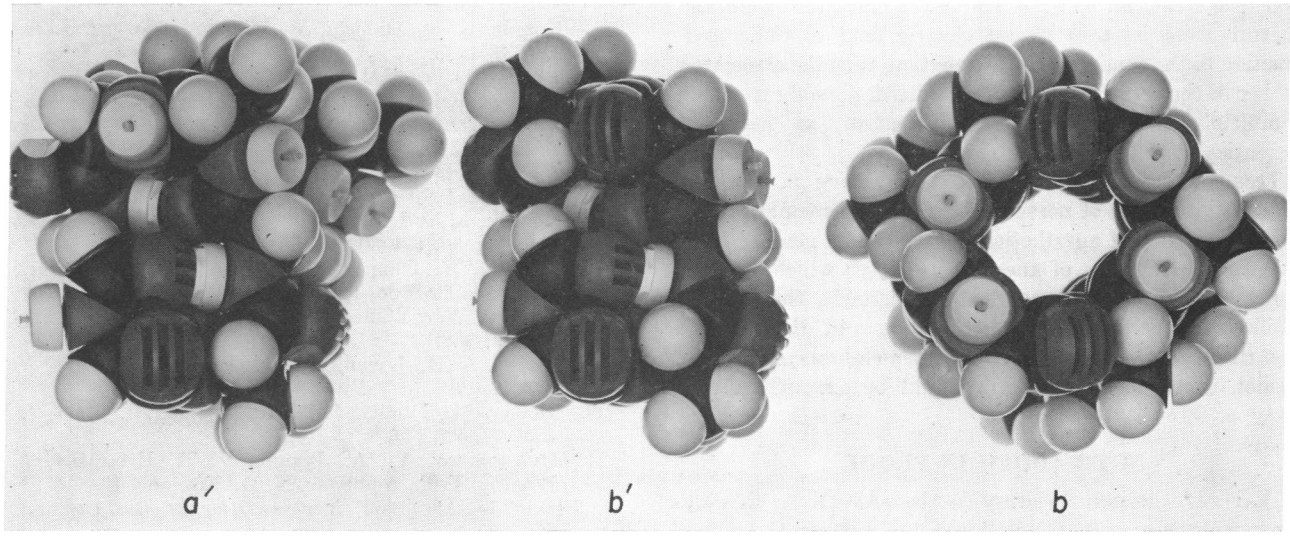

FIG. 4. Axis views of Corey-Pauling-Kolton models of the anti-β^6_2-spiral (conformation *a'*) with the sequence *N*-formyl$_2$(L-Ala-Gly)$_n$, the syn-β^6_2-spiral (conformation *b'*) with the sequence *N*-formyl(L-Ala-L-Ala-Gly)$_n$, and $\beta^6_{2,4}$-helix also with the sequence *N*-formyl(L-Ala-L-Ala-Gly)$_n$. In the anti-β^6_2-spiral (*a'*) the turns of the spiral skew, whereas in the syn-β^6_2-spiral(*b'*) the turns stack one directly above the other.

Conformation *b'* readily converts to *b* by rotation of the four cross-β peptides in each turn of *b'* and formation of hydrogen bonds between turns of the helix in *b* while the hydrogen bonding is maintained between the stacked β-turns of the spiral. Both *b'* and *b* have net dipole moments along their axes, but they point in opposite directions. Thus, these molecules, as transmembrane structures, could be converted from conformation *b'* to *b* by a rapid change in the direction of the electric field across the membrane.

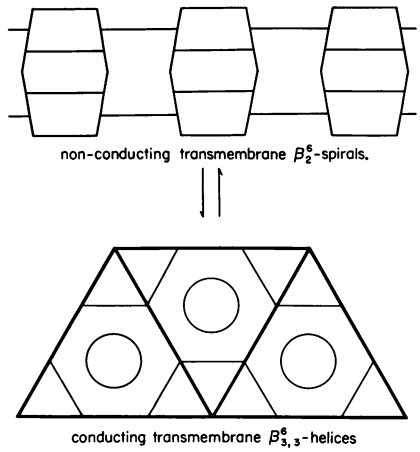

FIG. 5. Schematic representation of an array of spiral conformations (nonconducting structures) converting as a group to helical conformations (conducting structures).

as conformations b' and b, respectively, and the anti-$\beta^6{}_2$-spiral is given as conformation a'.

Electric field-dependent channel formation has been observed with stendomycin (Goodall, M. C., & Urry, D. W., unpublished results). This polypeptide antibiotic contains an L-D-L-D-L-D sequence cyclized by D-allo threonine lactone closure to the terminal carboxyl moiety. This sequence is analogous to a turn of a gramicidin A $\beta^6{}_{3,3}$-helix, which, as noted above, has a net dipole moment in the conducting conformation. As such, it provides an example of voltage-induced conductance formation.

The nonconducting structures (the $\beta^6{}_2$-spirals) are capable of hydrogen bonded association. Since the syn-$\beta^6{}_2$-spiral has exactly six residues per turn, each spiral sits directly above another, the N–H and C–O moieties, which are pointing laterally, allow four intermolecular hydrogen bonds per spiral, producing an array of spirals. If one transmembrane structure were induced by an electric field to undergo a transition to the conducting conformation, then the associated member of the array would be destabilized, possibly resulting in multiple associated channel formation, as indicated schematically in Fig. 5.

These conformations may be used to understand the conductance properties of nerve and cardiac cell membranes. A molecular theory of anesthetics, based on this model, would categorize three types of anesthetics—those which block or alter the efficacy of the conducting channels, those which stabilize the nonconducting structures, and those which effect dissociation of the arrays of transmembrane structures. Alcohol, ether, and chloroform would be examples of the last type.

NOTE ADDED IN PROOF

We have synthesized *N*-formyl(L-Ala-L-Ala-Gly)$_5$; this was subsequently tested by Goodall in a lipid bilayer system and found to give discrete conductance increments indicative of channels. These appeared to be of two sizes (about 10^{-9} and about 10^{-10} Ω^{-1} at 150 mEq K^+). The smaller channels appear first and remain on, followed by the larger channels that appear to turn on and off as a function of voltage and exhibit a weak K^+ selectivity.

Perhaps it should also be noted that in addition to being the first wholly synthetic transmembrane channel, it is also the first characterized channel that does not contain D-aminoacid residues.

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