Perspective

Structural Polymorphism in Transmembrane Channels

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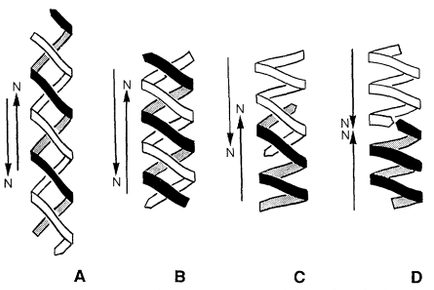

Fig. 1. Polymorphic transitions in gramicidin dimers. (**A**) The organization of the free gramicidin pore and the registration of the chains in the double-stranded antiparallel sheet that coils to form the pore. (**B**) The complex pore organization with the chains shifted. (**C**) Conjectures how continued chain shifting leads to hybrid structures with mixed single and double-stranded regions, to ultimately produce (**D**) the proposed channel structure.

HOW ARE CHARGED IONS TRANSPORTED ACROSS BIOLOGICAL membranes? This issue of *Science* contains two articles (*1, 2*) about the x-ray structures of gramicidin A, which provide the first atomic level views of the machinery responsible. Gramicidin A, which is produced by the bacteria *Bacillus brevis*, is an alternating D-L pentadecapeptide with a notably hydrophobic sequence:

Formyl-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-ethanolamine

The peptide has antibiotic activity arising from its ability to conduct monovalent cations across cell membranes. Owing to its relative structural simplicity, gramicidin A has been the subject of numerous experimental and modeling studies aimed at determining how it functions. The new crystallographic studies are particularly interesting both because they affirm some previous expectations and introduce some unexpected structural wrinkles.

Gramicidin A spontaneously forms dimers (*3*) that can interconvert among various different coiled forms, depending on solvent environment (*4*). Modeling studies have shown that, because of its alternating D-L peptide sequence, gramicidin can make left-handed coils that form hollow cylindrical structures. These structures have an exterior surface (normally buried in the surrounding lipid membrane) populated by hydrophobic amino acid side chains, and a central pore lined with polar peptide imino (NH) and carbonyl (CO) groups. The polar groups on the pore interior can facilitate ion transport by transiently stabilizing cations that diffuse through the membrane along the pore axis. However, within this common structural framework, there exists a surprising diversity of coiled dimer models that can potentially form pores long enough (about 25 to 30 Å) to span a membrane. Consequently, much work in recent years has been focused on trying to determine which model is functional in the biological environment.

The papers by Langs (*2*), and Wallace and Ravikumar (*1*) report the x-ray crystal structures of gramicidin both in the free form and as a complex with cesium chloride. In the free form structure, the gramicidin molecules are hydrogen-bonded together in the form of a double-stranded antiparallel beta sheet whose strands are in register (Fig. 1A). The double-stranded sheet is then tightly wound with a left-handed sense so that additional hydrogen bonds are formed between the adjacent edges of the coiled sheet.

Because the chains in the adjacent coils also run in antiparallel directions, the structure as a whole is hydrogen-bonded together like an extended, antiparallel beta sheet. The cylindrical pore formed by the free gramicidin is about 31 Å long and is characterized by pronounced bulges in the central channel that are large enough to incorporate isolated bound ions, although none are actually present in the x-ray structure. Since ions transiting the pore could intermittently twist the backbone peptide planes as they pass, the structure appears consistent with a mechanism of ion conduction that involves bulge propogation down the pore axis. It is consequently surprising that the structure of the CsCl complex differs in a fundamental way from the free form structure.

In the CsCl complex structure, the gramicidin dimer forms a shorter (about 28 Å) and somewhat fatter cylinder with Cs and Cl ions arranged in line down the pore axis. In this larger bore structure, it seems possible that ions could transfer without the propogation of the peristaltic bulge that seems necessary to accomodate passage through a cylinder with the free pore geometry. Indeed, the presence of both cations and counterions in the complex pore suggests that the structure may be "jammed" open, and so reflect the cumulative effect of local conformational changes associated with ion conduction down the pore axis.

In fact, the pore opening seen in the CsCl complex crystals produces a shift in hydrogen bonding registration between the strands of the coiled and antiparallel sheet (Fig. 1B). While similar shifts are characteristic of the alternative gramicidin models (*4–6*), the crystal structures make clear that the shorter, fatter, complex pore is produced from the free form by a relative translation of the antiparallel sheet strands in opposite directions. This shift in the pattern of interchain hydrogen bonds seems a logical consequence of trapping multiple peristaltic bulges, each of which causes transient disruption of backbone hydrogen bonds during ion passage through the pore. In this sense, the free and CsCl complex structures appear to represent metastable states of a structure whose polymorphism reflects its underlying dynamic mechanism.

Although the crystal structures basically resemble models for gramicidin A first proposed by Veatch (*5*), many studies indicate that this pore formed of antiparallel chains is, in fact, only a minor conformational component of functionally active gramicidin dimers in biological membranes. Instead, Urry has proposed a model "channel" arranged as a head-to-head dimer of left-coiled gramicidin chains (*6*) (Fig. 1D). This model, which is supported experimentally (*7, 8*), is composed of single chain coils whose successive loops are hydrogen-bonded together in a parallel beta sheet pattern, except for a short antiparallel region where the coiled monomers stack upon each other (*8*). The interesting point is that the channel form can be viewed as the ultimate result of progressively shifting the antiparallel chains of the pore structure past each other.

Interconversion of the pore and channel structures might then occur through the accretion of hydrogen bonding shifts, without ever necessitating simultaneous disruption of every hydrogen bond in the structure. This infers the transient existence of "mixed"

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15. Plasmid pMSKU16 (*30*) containing the cDNA for mPOMC was digested partially with Stu I and completely with Pst I to produce a fragment containing the entire mPOMC coding sequence preceded by 15 bp of its 5′ untranslated region. After incubation with T4 DNA polymerase to generate flush ends, the fragment was inserted by blunt-end ligation with T4 DNA ligase into the polylinker region (multiple cloning site) of the vector, pVV3, that was digested with Bam HI and Bgl II and converted to flush ends. This vector, pVV3, contains the vaccinia virus thymidine kinase (*tk*) gene interrupted by the constitutive vaccinia virus promoter, VV 7.5K, placed immediately proximal to the multiple cloning site (*14*). The resulting construct, pVV:mPOMC, was introduced by DNA-mediated transformation into mouse *tk*-deficient L cells that were infected with wild-type vaccinia virus (VV:WT), strain WR [0.05 plaque-forming units (PFU)/cell]. The mPOMC sequences were transferred into the vaccinia virus genome by homologous recombination. The recombinant virus, VV:mPOMC, was identified by plaque-filter hybridization techniques, and a stock of this recombinant was propagated as before (*14*).
15a. B. A. Thorne and G. Thomas, in preparation.
16. NG108-15 cells are a hybrid line (derived from a fusion of rat C6 glioma and mouse N18 neuroblastoma cells) and produce a low level of endogenous enkephalins [K. M. Brass and S. R. Childers, *J. Neurosci.* **3**, 1713 (1983)]. GH_4C_1 cells are a line of rat pituitary somato- or lactotrophs and secrete the large protein hormones, prolactin and growth hormone, via a well-characterized, regulated secretory pathway [A. H. Tashjian, F. C. Bancroft, L. Levire, *J. Cell Biol.* **47**, 61 (1970); J. G. Scammell, T. G. Burrage, P. S. Dannies, *Endocrinology* **119**, 1543 (1986)]. BSC-40 cells are a line of kidney epithelial cells from African green monkey that do not produce any endogenous prohormones and secrete other proteins via a constitutive secretory pathway (*15a*).
17. A 3.3-kb DNA fragment containing the entire yeast *KEX2* gene (*12*) was inserted into the Bam HI site of pVV3 and used to generate a recombinant vaccinia virus, VV:*KEX2*, as described above (*15*).
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28. Cell lines were grown at 37°C in an atmosphere of 5% CO_2 in the following media: AtT-20 cells, Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (HyClone); NG108-15 cells, DMEM with 10% serum and 100 μ*M* hypoxanthine, 0.4 μ*M* aminopterin, and 16 μ*M* thymidine; GH_4C_1 cells, Ham's F-10 (Gibco) with 10% serum; and BSC-40 cells and *Ltk*$^-$ cells (*14*).
29. M. A. K. Markwell, S. M. Haas, N. E. Tolbert, L. L. Bieber, *Methods Enzymol.* **72**, 296 (1981).
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31. In all the cell lines examined here, the only form of unmatured mPOMC found intracellularly was proopiomelanocortin, indicating that the NH_2-terminal hydrophobic leader (pre-sequence) has been removed from the primary translation product presumably by the action of signal peptidase during translocation of mPOMC into the lumen of the endoplasmic reticulum.
32. Analysis (by HPLC and radioimmunoassay) revealed that the cross-reacting material secreted into the medium (either with or without secretagogue stimulation) by cells infected with VV:mPOMC was almost exclusively uncleaved mPOMC, whereas the majority of the cross-reacting material released by cells coinfected with VV:mPOMC and VV:*KEX2* was processed peptides.
33. Antiserum 205–235 (Fig. 1) was used to measure mPOMC-related material. The prolactin radioimmunoassay utilized ^{125}I-labeled rat prolactin (New England Nuclear), synthetic rat prolactin (Peninsula Labs), and specific antibodies against prolactin (gift of P. Albert).
34. This report is dedicated to the memories of Professor Edward Herbert and Professor Choh Hao Li, two investigators whose pioneering studies established many of the fundamental principles of molecular endocrinology. Supported by a Damon Runyon–Walter Winchell Cancer Fund postdoctoral fellowship (DRG 797) and NIH research grant DK37274 (to G.T.), by NIH research grant HD18438 (to R.A.), by NIH research grant AI20563 (to D.E.H.), by a Helen Hay Whitney Foundation postdoctoral fellowship and a biomedical scholar award from the Lucille P. Markey Charitable Trust (to R.F.), and by NIH research grant GM21841 (to J.T.). The authors thank Lawrence W. Caton for expert technical assistance.

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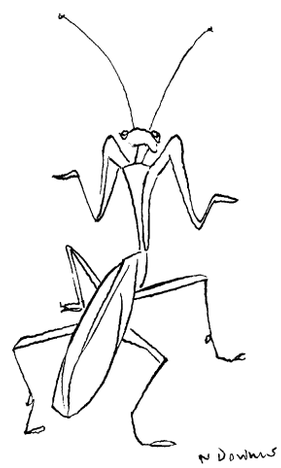
structures like Fig. 1C, which are at present only conjectural.

In summary, gramicidin A appears to be a structure whose polymorphism may underlie a dynamic mechanism of ion transfer. Although much more remains to be learned about gramicidin function, particularly regarding details of mechanism and the origins of ion transport specificity, the crystallographic studies finally provide a structural basis for definitive spectroscopic studies and computational simulations.



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