

Two-bit anion channel really shapes up

David C. Gadsby

Channels that conduct anions or cations across cell membranes are of immense physiological importance. But what do they look like? A chloride channel turns out to have a double-barrelled structure quite unlike that of cation channels.

LIKE choosy border guards, ion channels select exactly which kind of ions they will allow to cross cell membranes, and when, and where. They open and close their gates in response to precise orders received in local or broadcast messages. The resulting orchestrated flows of the cations Na^+ , K^+ , Ca^{2+} , and the anion Cl^- , generate nearly all the electrical activity in our brains and hearts, in every nerve and muscle fibre — indeed, in every single cell in our body.

Almost two decades of recording currents flowing through microscopic patches of membrane have probably given us more detailed kinetic information about how ion channels work¹ than for any other class of protein. And yet we don't have much of a clue as to what they look like. Like other integral membrane proteins, they're prohibitively difficult to crystallize — a prerequisite for determining their three-dimensional structure.

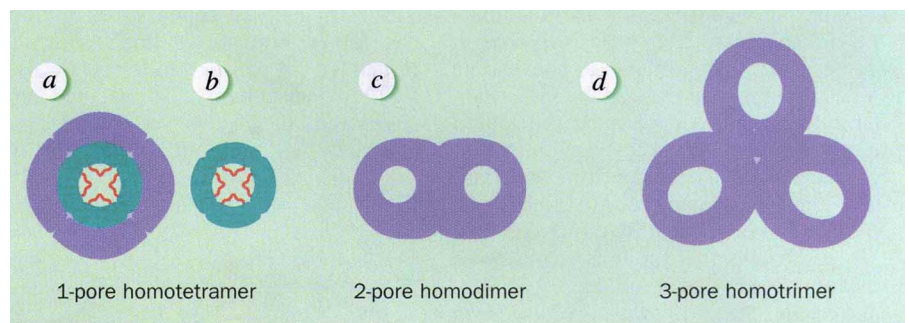
That's the bad news. The good news is that comparisons of wild-type and mutated channels have begun to provide glimpses of their structural features. Much of this work has focused on the reigning stars of the field, voltage-gated cation channels. (All channels, whether selective for a cation or an anion, need energy to swing their gates; common energy sources are the membrane potential and ligand binding.)

Voltage-gated cation channels and their close relatives adopt a pseudo-symmetric tetrameric architecture: a central ion pore lies at the axis of fourfold symmetry and part of its lining is formed by four loops², each contributed by one of the subunits (*a* and *b* in the figure). Now, work from the Miller and Jentsch laboratories (by Middleton *et al.*³ and Ludewig *et al.*⁴; pages 337 and 340 of this issue) turns the spotlight on the CIC family of anion channels, which are prevalent in skeletal muscle, neurons and epithelia. The new studies establish that the founding member of this family, the CIC-0 channel cloned from the electric organ of the ray *Torpedo*, exists as a homodimer containing two identical and independent, off-axis pores each contributed solely by one or other of the subunits (*c* in the figure). This is a channel structure that has never been encountered before. Indeed, until now, the only structures even remotely resembling it — that is, having a pore in each subunit — were porins, poorly selective, triple-barrelled

channels from bacteria (*d* in the figure).

In terms of structure, function and public relations, anion channels have been the poor cousins of cation channels — with two exceptions. One is the cystic fibrosis transmembrane conductance regulator (CFTR), an epithelial Cl^- channel encoded by the gene defective in cystic fibrosis patients. The other is the family of Cl^- channels typified by CIC-0, a polypeptide of relative molecular mass 90K that, like CFTR, includes 12 hydrophobic, possibly

What's more, the probabilities of observing the three different levels are accurately predicted by the binomial theorem. And the measured 'dwell' times (which are exponentially distributed) at each of the three levels quantitatively match estimates based on just one of those dwell times and the observed probabilities. This set of results prompted the postulate that CIC-0 might be a double-barrelled channel made up of two identical ion-conducting pores, each with its own independent



Schematic representations (not to scale) of quaternary structures of ion channels viewed from above, emphasizing the multimeric architecture and disposition of the ion-conducting pore. *a*, Voltage-gated cation channel, with a single axial pore at the centre of a homo- or heterotetramer in the case of K^+ channels, and at the centre of a single polypeptide in a tandem, pseudotetrameric arrangement in the case of Na^+ or Ca^{2+} channels. The four loops (red) are sequences of <20 residues known to protrude into, and define, the pore², and they link two (the fifth and sixth) membrane-spanning regions of each subunit; the first to fourth membrane-spanning regions (purple) include the sequence containing several basic residues that contribute to the voltage sensitivity of gating. *b*, Inwardly rectifying K^+ channel, with a single axial pore at the centre of a homo- or heterotetramer. *c*, CIC-0 Cl^- channel, with two off-axis pores, each contributed by one of the subunits of the homodimer. *d*, Porin from the outer membrane of Gram-negative bacteria: here there are three large off-axis pores, each contributed by one of the subunits of the homotrimer.

membrane-spanning segments. Gating of CIC-0 is voltage-sensitive and, as in voltage-dependent cation channels, depolarizing the membrane (reducing the amplitude of the internal negative potential) rapidly opens the channels, while prolonged depolarization slowly inactivates them. But when unitary currents are recorded in single CIC-0 channels, they reveal an extraordinarily rich gating behaviour.

Between electrically silent periods that reflect the inactivated state, the CIC-0 unitary currents vacillate between three equally spaced current levels. The lowest corresponds to the fully closed channel, the middle to a 10-picosiemens channel and the upper to a 20-picosiemens channel. These bursts of unitary currents invariably include both middle and upper levels, never one without the other.

activation gate, but with a slow inactivation gate in common⁵.

Middleton *et al.*³ and Ludewig *et al.*⁴ take this view of CIC-0 channel structure from supposition to certainty. Their experiments exploit hybrid channels composed of wild-type subunits associated with subunits bearing functional tags in the form of point mutations (at the lysine at residue 519 or the serine at residue 123). The mutations alter the ease with which anions negotiate the pore (measured as conductance), and the pore's ability to select among different anions, as well as its pattern of opening and closing. The new measurements show that two pores, with wild-type and mutant signatures, coexist in a hybrid complex (isolated by an affinity tag or by fusing the subunits), and thus that the two pores in a CIC-0 channel

are functionally distinct and independent.

But how many CIC-0 subunits make up one double-barrelled complex? Sedimentation experiments suggested⁶ that the purified, active channel is a homodimer of relative molecular mass about 200K. When expressed alone, CIC-0 mutants, such as those with a cysteine at residue 519 and threonine at 123, show a uniformly reduced conductance of both pores in a channel complex; and they recapitulate that same conductance in one of the pores when coexpressed as hybrids with wild-type CIC-0 subunits (also when expressed as fused wild-type-mutant or mutant-wild-type complexes⁴). Given the absence of intermediate conductance levels, it seems that each pore-affecting residue exerts its effect only once per pore, and that CIC-0 channel complexes must therefore be made of just two subunits.

In an elegant, real-time application of the cysteine accessibility method, Middleton *et al.*³ are able to show that precisely two cysteine-modification events, by a cytoplasmic (but not extracellular) reagent that introduces a positive charge, are necessary and sufficient to convert the low conductance of the cysteine-519 channel to the higher conductance anticipated upon restoring the wild-type charge at residue 519. This both confirms the channel's dimeric structure, and places the lysine at 519 on the cytoplasmic side.

But do both subunits contribute residues to each pore, or does each subunit circumscribe its own pore? Ludewig *et al.*⁴ addressed this question by expressing a doubly mutated tandem channel (with glutamic acid at residue 519 in one subunit, and threonine at 123 in the other), incorporating one mutation per subunit, at opposite ends of the linear sequence. The double mutant showed two low-conductance pores as might be expected if each mutation affected just one pore, supporting a model (c in the figure) in which each subunit contributes residues to one pore only. Nonetheless, more detailed analysis of additional double mutants covering the rest of the sequence may be required to settle the matter.

There are, of course, further outstanding issues. If each CIC-0 subunit does make a pore, presumably several parts of the molecule contribute pore-lining residues. But which parts? And what holds the two subunits together? Are the

pores in lone subunits inactivated? Are all CIC family members dimers^{3,4,6,7}

Mutational studies can take us only so far, and full answers must await a high-resolution structure of one of these channels. That entails preparing large quantities of pure protein for growing two- or three-dimensional crystals, and perhaps here too the advantage has passed from cation to anion channels—Miller and colleagues have already exploited a powerful expression system, the *Torpedo* electric organ, to achieve the analytical-scale purification of fully functional CIC-0 channel protein⁶. With progress accelerating in the preparation of protein samples for crystallization, and

so many channelologists lusting for any sign of success, perhaps it won't be too long before we see the first atomic structure of these all-important ion channels.

Until then, the two new studies give us plenty to ponder. They simultaneously close a chapter, by ending debate over the multimeric nature of CIC-0 channels, and open a whole new book on the molecular structure, and mechanisms of gating and permeation, in the family to which they belong. □

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SUPRAMOLECULAR CHEMISTRY

The medium is the message

Achim Müller and Christian Beugholt

THE complexity of appearances in nature has forced humans to think about the principles of order and organization. Werner Heisenberg, for instance, contemplated the 'order of reality', and especially its prebiotic origin: "In the

beginning was symmetry; that is certainly more correct than the Democritean thesis, in the beginning was the particle"¹. Modern science looks for symmetries and symmetry breaking everywhere². So why do we find such high symmetries

in simple and complex molecular systems, and even in viruses? Beissel and co-workers have reported³ on a principle of symmetry-driven self-assembly deduced from natural products—a principle that they have used to design the formation of highly symmetrical metal clusters.

We could read only a couple of years ago that cluster self-assembly was "... a hit-or-miss affair, with only limited rational planning"⁴. This problem is of general importance, not only for pure academic chemistry but also for the construction of molecular materials and for the purpose of mimicking some of nature's tricks. Beissel *et al.* ask what it is exactly that leads to the high symmetry of non-covalently bonded molecular clusters, like that of virus coats and some special proteins in nature.

Indeed, nature has brought forth molecular clusters of stunning symmetry and beauty. The poliovirus, for instance, has an icosahedral shell with fivefold and threefold symmetry axes (Fig. 1a), con-

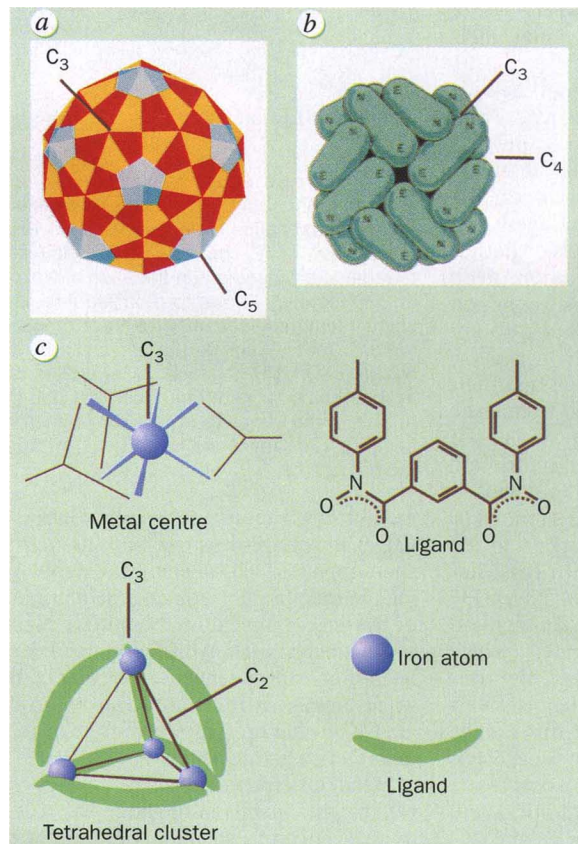


FIG. 1 a, Model of the poliovirus 60-mer (from ref. 5) and b, the apoferritin 24-mer (from ref. 3), showing their symmetry axes. c, The formation of the tetrahedral cluster $Fe_4(ligand)_6$ from the designed ingredients: metal centres with their characteristic property of octahedral coordination, and double-bidentate ligands with a twofold axis. This yields a metal tetrahedron with both twofold and threefold symmetry axes.

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