Three in a row—how sodium ions cross the channel

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Sodium channels are central to a host of fundamental cellular processes, including sensory perception, pain, and muscle contraction. In order to understand any of these processes in detail, it is necessary to know the atomic structure of the channel proteins both with and without bound sodium ions. In this issue, Naylor *et al* (2016) present the structure of a bacterial sodium channel tetramer. The three bound, partially hydrated sodium ions line up neatly in a row inside the selectivity filter, providing us with the first detailed insights into ion conduction in sodium channels, and the mechanisms by which sodium and potassium ions are discriminated.

See also: **CE Naylor *et al*** (April 2016)

Ion channels that conduct sodium, potassium or calcium ions through the lipid bilayer of the membrane play a central role in cellular physiology. Sodium channels are key components of electrically excitable cells, where they contribute to the neuronal action potential and sensory perception, including vision, hearing and pain. These channels have been subject to countless investigations ever since Hodgkin and Huxley first published their classical studies of sodium and potassium conductance in the squid giant axon (Hodgkin & Huxley, 1952). Famously, sodium channels are blocked by spider and scorpion toxins that paralyse or kill prey. They are of great pharmacological importance as key targets for local anaesthetics, anti-epileptic drugs and compounds to treat cardiac arrhythmia.

The lipid bilayer of biological membranes is electrically insulating. Most ions cannot

pass through it unaided, or can do so only very slowly. Ion channels are passively conducting protein pores that lower the free energy barrier to ion permeation. Most channels specifically conduct one, and only one type of ion. The difference in ion concentration within and outside a cell creates the membrane potential as one of the defining features of life. Permanently open channels will break down the membrane potential and result in cell death (this is what pore-forming bacterial toxins do). It is therefore critical that the opening and closing of ion channels in the membrane, also called gating, is tightly controlled. Not surprisingly, biophysicists, cell physiologists and structural biologists have long been fascinated by these sophisticated macromolecular devices.

Many well-known ion channels, such as the *Drosophila* shaker K channel, voltage-gated calcium channels or the sodium channel from electroplax of electric eel share a basic, common architecture, whereby four equal membrane protein modules form an assembly of exact or nearly exact fourfold symmetry, creating a narrow aqueous pore on the fourfold axis. Potassium channels are homo-tetramers of four identical subunits, whereas mammalian sodium channels are large single-chain proteins with four homologous, concatenated, pore-forming repeats. The pore can open and close by subtle iris-like or tilting movements of the pore-lining alpha helices (see Fig 1). A critical part of ion channels that bestows them with their specificity is the selectivity filter, consisting of four short parallel polypeptide stretches along the fourfold axis on the extracellular side of the pore. The precise arrangement of the polypeptide chain in the selectivity filter

determines which ions can pass through, and which not.

Any understanding of how these channels work and how exactly they achieve their superb ion specificity therefore requires detailed, atomic structures. While there are several excellent high-resolution crystal structures of K⁺ channels, eukaryotic Na⁺ channels are large, complex and fragile membrane protein assemblies that have resisted any crystallization attempts. Fortunately however, it was discovered that some bacteria also have sodium channels (Ren *et al*, 2001). Their biological function is not understood—a role in chemotaxis or cell motility has been suggested—but for structural biologists, they are a blessing. As with all other bacterial homologues or ancestors of membrane proteins, they are simpler, more robust and more tractable for structural studies than their eukaryotic counterparts. Yet they function in the same way, and some are even blocked by substances that also inhibit human channels. Bacterial ion channels therefore offer excellent model systems for studying the structure and mechanisms of the vital mammalian Na⁺ and K⁺ channels.

In recent years, the structures of a few bacterial Na⁺ channels have been determined. However, until now, none of them have shown details of bound sodium ions, which is an essential prerequisite for understanding ion selectivity and conduction. The team that determined the present structure solved the channel from this particular bacterium first without (McCusker *et al*, 2012) and now, 4 years later, with bound ions (Naylor *et al*, 2016). This is how long it took to improve the crystals, which is not untypical even for bacterial membrane

Figure 1. Structure of a bacterial sodium channel tetramer (Na_vMs) with three bound sodium ions. The partially hydrated sodium ions line up in a row inside the selectivity filter (call-out), providing detailed insights into ion selection and conduction by sodium channels. Negatively charged side chains in the selectivity filter attract sodium ions and allow them to pass, while the larger hydrated potassium ions are excluded. Negatively charged glutamate residues in the filter partially compensate the positive charge of the sodium ions passing through. For clarity, only two of the protomers (red and green) in the sodium channel tetramer are shown.

proteins. Evidently the ions stabilize the channel, which helps to enhance the visible detail.

For crystallization and structure determination, the protein construct was stripped to the bare essentials, consisting only of the pore-forming helices and the selectivity filter. Soaking the isolated tetramers in salt solution trapped sodium ions and water in the selectivity filter. In the X-ray structure, three Na⁺ ions and one water molecule neatly line up in a row along the fourfold axis of the channel.

The lack of direct contacts between the three metal ions and the protein indicates that Na⁺ ions bind and pass through the channel in a hydrated state. Some water molecules are resolved, including one that is suspended between the second and third Na⁺ in the row. Since Na⁺ ions and water molecules have the same number of electrons—10—and X-rays are scattered by electrons, it is difficult to be sure that this density is indeed water, and not a fourth sodium ion with lower occupancy.

This distinction, which would require considerably higher resolution, will be crucial for unravelling the ion conduction mechanism of Na⁺ channels at the atomic level. The distance between sodium ions 1 and 2 is too close to accommodate one, let alone two complete hydration shells; therefore, the ions cannot be fully hydrated. The high positive charge density at this position of close Na⁺ contact seems to be partly compensated by the negatively charged carboxyl groups of four glutamate residues arranged in a ring, a hallmark of Na⁺-channel selectivity filters. Gratifyingly, the selectivity filter contains only two Na⁺ ions when this glutamate is replaced by an aspartate, which is shorter by one CH₂ group. As a result, the negative charge of the carboxyl group cannot get close enough to the Na⁺ ions, charge compensation is compromised, and the third Na⁺ ion fails to bind due to charge repulsion.

It is most instructive to compare the present structure to that of a—likewise bacterial—K⁺ channel (Doyle *et al*, 1998;

Zhou *et al*, 2001). Overall, the pore and selectivity filter architectures are similar. However, the filter of the K⁺ channel has four equally spaced ion interaction sites, each with four main-chain carbonyls arranged in a ring. The ring diameter matches the ionic radius of a dehydrated K⁺ ion. When a hydrated K⁺ ion binds to the selectivity filter, the carbonyls displace the bound water, and the K⁺ ions pass through the filter without their hydration shells. Paradoxically, Na⁺ ions cannot pass, even though they are smaller. Most likely sodium ions prefer the solvated state in bulk solution to the neutral environment of the K⁺ channel filter, whereas they would be attracted by the ring of negative charges in the Na⁺ selectivity filter. Moreover, the dehydration energy for Na⁺ is significantly higher than for K⁺ (on account of the smaller ion radius and hence, higher charge density of Na⁺) and would have to be compensated by the protein, which it cannot do. Conversely, as the new structure of Naylor *et al* (2016) shows, the disposition of main-chain carbonyls and negatively charged side chains in the Na⁺ selectivity filter is unsuitable for dehydrating K⁺ ions, and hydrated K⁺ ions would be too large to fit through. Along with other, more subtle effects of ion solvation and protein conformational dynamics, these considerations go a long way to explain the exquisite ion selectivity of sodium and potassium channels, and ultimately the sensation of pain and propagation of neuronal action potentials.

What comes next? Although excellent, the 2.7 Å resolution of the current Na-bound sodium channel structure leaves room for improvement. Perhaps higher resolution can be achieved by devising multivalent antibody fragments that tie the four protein subunits in the Na⁺ channel tetramer more firmly together. This strategy has proven exceptionally successful with bacterial K⁺ channels (Zhou *et al*, 2001). But even if the resolution of the bacterial Na⁺ channel can be increased to, say, 1.5 Å (which is by no means certain), we still need detailed maps of the pharmacologically important mammalian, ideally human Na⁺ channels, to understand how they work. The formidable difficulties in expressing and isolating these fragile membrane protein complexes in a pure and stable form make it unlikely that their structures will be solved by protein crystallography. However, the recent spectacular progress in electron cryomicroscopy

(Subramaniam *et al*, 2016) shows that for large complexes crystals are no longer needed. Before too long, a eukaryotic Na⁺ channel may thus join the growing retinue of other previously intractable membrane protein complexes that have recently been solved by cryoEM: the mammalian transient receptor potential channel TRPV1 (Liao *et al*, 2013), human gamma secretase (Bai *et al*, 2015) and the Ca_v1.1 channel from rabbit skeletal muscle (Wu *et al*, 2015). The—still astonishing—resolution of 3.4–4.2 Å achieved in these three instances may be sufficient to facilitate robust molecular dynamics simulations (Köpfer *et al*, 2014) and drug development, but would not in itself reveal details of metal ion hydration and coordination, which are crucial for a full mechanistic understanding. In the long term, we need both: cryoEM to give us the bigger picture and high-resolution X-ray structures to furnish the fine detail.

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