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Ion Channels: Frozen Motion

Dispatch

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Our understanding of ion permeation through K^+ channels, and by extension through other channels, is advancing rapidly. New structural studies, together with computer simulations, have provided profound insights into ion conduction mechanisms.

When the crystal structure of the bacterial channel protein KcsA was first solved in 1998 [1], it provided us with our first view of an ion channel at atomic resolution. The structure, determined at the relatively modest resolution of 3.2 Å, revealed the basic architecture of the channel: an extracellular selectivity filter, a central cavity and an intracellular gate (Figure 1). The importance of KcsA lies in the conservation of this basic pore structure between KcsA and other classes of K^+ channels found in a wide range of organisms, including humans [2]. If we can understand ion permeation in KcsA, we will thus have grasped the fundamental mechanism of all K^+ channels. But, despite a large body of work stimulated by the earlier KcsA structure, fundamental permeation studies were hampered by its limited resolution.

New studies have extended the resolution of the KcsA structure to 2.0 Å, making ions and water molecules in the filter clearly visible [3]. Changes in structure in the presence of a low concentration of K^+ ions, and in the pattern of ion occupancy when Rb^+ ions are substituted for K^+ [4], have also been explored. These results have been combined with electrophysiological studies of the flux of K^+ versus Rb^+ ions through KcsA as a function of ionic concentration [4]. And detailed calculations have been made of the energetics of ion permeation through the KcsA filter [5]. Together, these new results provide a detailed and convincing picture of the mechanism of high throughput K^+ flux — about one ion every 10 nanoseconds — through K channels. Significantly, these higher-resolution structural studies confirm a number of predictions from earlier simulation studies, indicating that simulations really can tell us something new.

Attempts to understand the high-throughput permeation mechanism of KcsA have combined electrophysiological measurements on channels reconstituted in artificial lipid bilayers with X-ray studies of KcsA crystals in the presence of different concentrations of K^+ or Rb^+ ions. Fine details of ions and water molecules in the filter have been revealed by the high resolution (2.0 Å) structure of KcsA [3]. This was made possible by co-crystallisation of a channel–antibody Fab fragment complex, exploiting a technique for crystallisation previously used to study the membrane protein cytochrome c oxidase [6].

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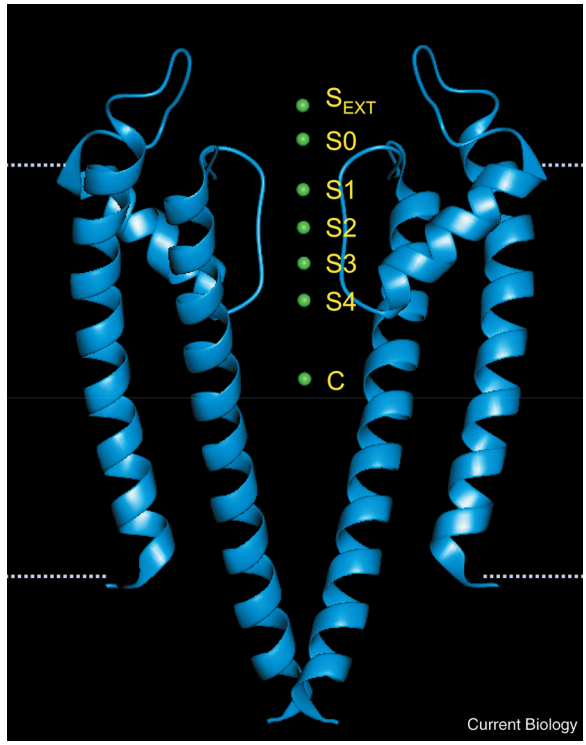

Figure 1. Structure of KcsA in the presence of high $[K^+]$.
Two subunits of the four are show (blue), plus the seven locations of K^+ ions revealed in the X-ray structure. These are the external mouth (S_{EXT}), the 5 sites (S0 to S4) in the filter, and the single site (C) in the central cavity. The approximate location of the bilayer is shown by the dotted grey lines.

In the earlier, 3.2 Å resolution X-ray study [1], four ion-binding sites within the filter were identified, S1 to S4 (see Figure 1). By comparing X-ray structures at low (3 mM) and high (200 mM) K^+, it can be shown that, at high K^+, all four sites are on average approximately equally occupied, consistent with a relatively flat permeation energy landscape and hence a high throughput of ions. A relatively flat energy landscape is also revealed by the energetics calculations [7]. The mechanism of rapid permeation is envisaged in terms of rapid shuttling between configurations 1,3 and 2,4 — those with K^+ ions at sites S1 and S3, and at sites S2 and S4, respectively.

The approximately equal probabilities of these two configurations results in equal average occupancies of sites S1 to S4 in the crystal structure. Such rapid switching between configurations 1,3 and 2,4 was predicted by simulation studies based on the earlier structure [8,9], and by earlier (more approximate) energetics studies [10,11]. Comparison of K^+ versus Rb^+ occupancies in the crystal structure provides

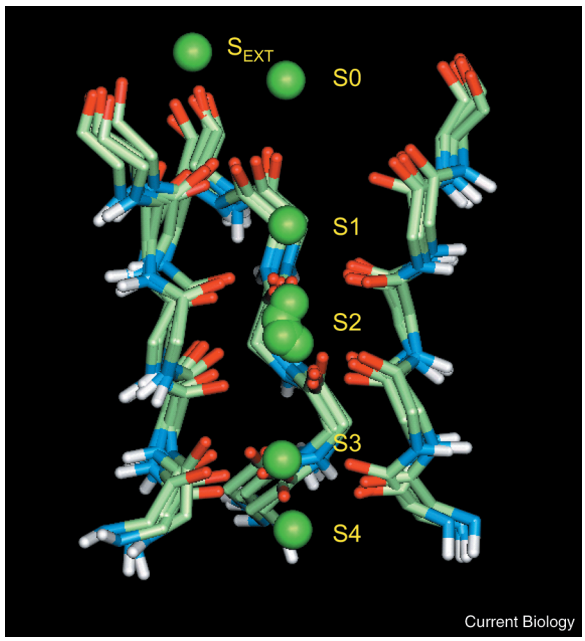

Figure 2. Potassium ions in the selectivity filter.

Four snapshots from a simulation are superimposed (our unpublished data). The filter region of three of the four subunits of KcsA are superimposed, and the K^+ ions (green spheres) are seen to occupy (at different times) sites S0 to S4.

further evidence of the evolutionary fine-tuning for K^+, in that the energy landscape is flatter for K^+ than for Rb^+. In particular, Rb^+ is not seen to occupy site S2 significantly.

Ion permeation through the narrow filter region of a K^+ channel requires the cation to be stripped of its hydration shell. Energetically, this implies that, as a K^+ ion enters the filter, its first hydration shell is replaced by ligands from the protein structure, namely oxygen atoms of the backbone carbonyl groups and (at site S4) of threonine sidechains. The 2.0 Å resolution crystal structure [3] reveals details of both hydration and coordination within the filter, at least as they exist in a crystal at a temperature of 100 K.

As observed at high K^+ concentrations, the coordination of K^+ ions within the filter at each of the four sites S1 to S4 is made up of eight oxygen atoms from the protein, arranged at the corners of a cube with a K^+ ion at its centre. Such coordination is seen in a number of simple complexes of K^+ ions, such as that formed by the antibiotic nonactin or by the synthetic molecule calix[4] tube [12], which exhibits remarkable potassium selectivity.

The high-resolution structure [3] reveals two further K^+ ion sites not observed in the earlier structure, which we may call S0 and S_{EXT}. S0 is formed by four oxygen atoms, from the carbonyls of residue glycine 79, with the remaining interactions provided by water molecules. The ion's hydration shell has thus been half-replaced by interactions with the protein. S_{EXT} is on the extracellular side of site S0, and an ion at this site still seems to be surrounded by waters. These new binding sites, observed at a temperature of about 100 K, are consistent with simulation studies (at 300 K) which revealed ions at S0 and close to the external mouth [7,13]. The results of such simulations are illustrated in Figure 2 (our unpublished data). Thus, both simulations and experimental data support the proposal that KcsA — and by extension, K^+ channels in general — is a multi-ion pore, with a succession of up to six ion-binding sites in a row, plus a binding site at the centre of the cavity.

The second 2.0 Å structure of KcsA, determined at a low concentration (3 mM) of K^+ ions, reveals that there is a degree of flexibility in the selectivity filter [3]. Under such conditions, there is a K^+ ion at S1 and S4, with some distortion of the polypeptide backbone, especially at residue valine 76 which contributes to site S3. Interestingly, distortion of the filter in the vicinity of valine 76 has been seen in a number of simulations ([9], and our unpublished data). So in this respect too simulations based on a single lower-resolution structure were able to predict aspects of the higher-resolution structures.

There are two respects in which distortions of the filter may be of importance. Small, short timescale (nanosecond) distortions may be necessary to render the filter sufficiently flexible to allow rapid ion permeation — to lower the activation energy barriers between adjacent sites, thus helping to flatten the permeation energy landscape. Larger, longer timescale (millisecond) distortions may be related to the electrophysiological observation of 'fast gating' — rapid closures of the filter that appear to be independent of activation gating of K^+ channels.

These new studies enable us to evaluate the relative merits of experimental and computational approaches to studying ion permeability. X-ray crystallographic studies have provided us with definitive images of the channel structure and its interactions with ions. It should be remembered, however, that these are time averages of snapshots obtained at 100 K of a dynamic process. In contrast, molecular simulations may be viewed as predictive, rather than definitive. But they do enable us to investigate the structural dynamics and energetics of ion permeation at 300 K — physiological temperature. What is particularly encouraging about the ongoing studies of KcsA is that the two methods are in good agreement, providing complementary views of the same biological process.

What does the future hold? The exact nature of ion selectivity in KcsA remains somewhat elusive, particularly given the observed flexibility of the filter. In addition, the much longer timescale (millisecond) process of channel gating will prove a challenge to both simulation and structure determination.

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