Commentary

Ion channels and D-amino acids

Maintenance of appropriate ion balance across a biological membrane is crucial for cellular integrity and function. Ion channels are important cellular components that are involved in the maintenance of such an ion balance. They represent an important class of transmembrane proteins, and serve as key elements in signaling and sensing pathways and to connect the inside of the cell to its outside in a selective fashion. They are crucial for normal functioning of cells since a defective ion channel can lead to diseases (Cooper and Jan 1999) such as cystic fibrosis (Stutts *et al* 1995). In addition, ion channels are known to be specific targets for neuroactive toxins (Garcia 2004). The recent success in crystallographic analyses of ion channels, starting with the *Streptomyces lividans* K^+ channel (KcsA) (Doyle *et al* 1998), constitutes an exciting development in contemporary membrane biology (Rees *et al* 2000).

The linear gramicidins are a family of prototypical channel formers and are extensively used to study organization, dynamics and function of membrane-spanning channels (Koeppe and Andersen 1996; Miloshevsky and Jordan 2004; Rawat *et al* 2004). Due to their small size, ready availability and the relative ease with which chemical modifications can be performed, gramicidins serve as excellent models for transmembrane channels. Gramicidins are linear pentadecapeptide antibiotics (the first antibiotic used clinically!) produced by the soil bacterium *Bacillus brevis* during sporulation and believed to play a functional role in gene regulation during the shift from vegetative growth to sporulation (Killian 1992). It has one of the most hydrophobic sequences known (see figure 1). Interestingly, the amino acid sequence of gramicidin consists of alternating L- and D-amino acids (Sarges and Witkop 1965), in sharp contrast to most proteins which contain exclusively L-amino acids (Mitchell and Smith 2003). Gramicidin is synthesized by non-ribosomal multienzyme complexes, and the conversion of L- to D-amino acids takes place during biosynthesis. Yet, it is interesting to note that gramicidin represents a useful model for realistic determination of conformational preference of proteins in a membrane environment, in spite of the alternating sequence of L-D chirality generally not encountered in naturally occurring peptides and proteins. This is due to the fact that the dihedral angle combinations generated in the conformation space by various gramicidin conformations are 'allowed' according to the Ramachandran plot (Andersen *et al* 1996).

The molecular design of ion channels generally consists of a hydrophobic outer surface (which faces the nonpolar membrane interior) and a comparatively hydrophilic interior (through which polar water molecules and ions are allowed to pass). This, in turn, demands that the amino acid composition of ion channels contain suitably placed hydrophobic and hydrophilic residues which would allow the molecule to adopt such an amphiphilic conformation in the membrane. In spite of the lack of charged or polar residues and the hydrophobic nature of gramicidin, how does it perform the role of an ion channel? The alternating L-D chirality plays a subtle yet crucial role here. In contrast to other channels that are formed by the lateral association of multiple transmembrane helices, the gramicidin channel is formed by the transverse, transmembrane, head-to-head dimerization of $b^{6.3}$ helices. The aqueous pore of the channel is therefore formed by the inner lumen of a single helix. The inner wall of the gramicidin channel is lined by the polar groups of the peptide backbone, a structural feature possible due to the unique alternating L-D chirality found in gramicidin. The alternating L-D arrangement allows the amino acid side chains to project from only one side of an extended peptide strand (Andersen *et al* 1996). This would not be possible with all L- or all D-gramicidin.

The structural features that make gramicidin channels so unique raise the question whether gramicidin channels are appropriate models for "real" ion channels. Fortunately, the elucidation of the crystal structure of the *S. lividans* K^+ channel (KcsA) (Doyle *et al* 1998) in molecular detail (which

subsequently resulted in the award of a Nobel prize) has provided an opportunity to evaluate the features of the gramicidin channel that are shared with other channels from natural sources. This has led to the rather interesting finding that gramicidin channels share important structural features with "real" ion channels. An essential feature of ion channels is the ability to select for specific ions using a selectivity filter. Remarkably, both the gramicidin channel and the selectivity filter of the KcsA K^+ channel are lined by the polar carbonyls of the peptide backbone and ion selectivity in both cases arises due to backbone interactions with ions (Wallace 2000). While in the gramicidin channel such interactions are possible due to the alternating L-D chirality, KcsA utilizes two highly conserved glycine residues in the selectivity filter (see figure 1).

The choice of glycine is obvious since it is achiral, and has a larger allowed region in the Ramachandran plot, and so effectively it can behave as a D-amino acid. A very recent report (Valiyaveetil *et al* 2004) confirms and extends this notion by pointing out that the two absolutely conserved glycine

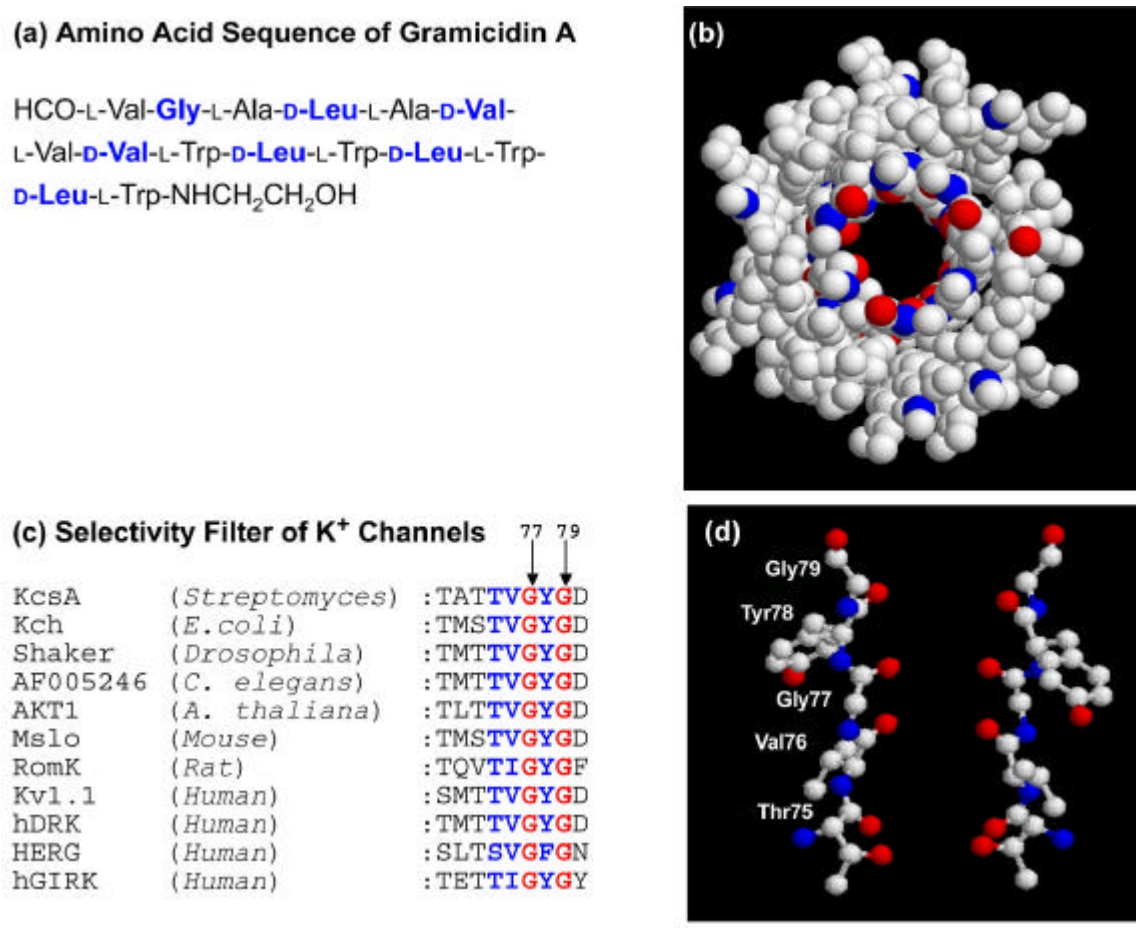

Figure 1. A comparison of the gramicidin channel and the selectivity filter of potassium channels. (**a**) Amino acid sequence of gramicidin A. Note that all amino acid side chains are either hydrophobic (Ala, Leu, Val) or amphipathic (Trp). In addition, the $-NH_2$ and –COOH termini are blocked making the sequence unusually hydrophobic. Alternating D-amino acid residues are shown in blue. (**b**) Top view of the gramicidin channel as a space-filling model (colour code: white, carbon atoms; blue, nitrogen atoms; red, oxygen atoms) made using RASMOL ver. 2·7·2·1 (Sayle and Milner-White 1995) and using coordinates from (PDB code 1MAG). Note how the alternating L-D arrangement allows all amino acid side chains to project outward from the channel lumen. (**c**) Sequence alignment of the selectivity filter sequence of several K^+ channels from organisms across the evolutionary spectrum. The conserved glycines, Gly-77 and –79, are shown in red and the conserved sequence around them in blue. Adapted from Valiyaveetil *et al* (2004). (**d**) Structure of the selectivity filter (residues 75–79) of the KcsA K^+ channel. Two subunits are shown in ball-and-stick representation made using RASMOL ver. 2·7·2·1 (Sayle and Milner-White 1995) and using coordinates from (PDB code 1BL8). [Colour code: same as in (**b**)].

residues in the KcsA K^+ channel, which are essential in the K^+-selectivity filter, serve as *surrogate* D-amino acids, reminiscent of the D-amino acids found in the gramicidin channel. These glycine residues (at positions 77 and 79) are conserved in the selectivity filter of K^+ channels in a wide range of species across the evolutionary spectrum which include bacteria, plants, worms, insects, rodents, and humans. More importantly, Valiyaveetil *et al* (2004) showed that the functional characteristics of the KcsA K^+ channel could be recovered by replacement of Gly-77 in the selectivity filter region with D-Ala while replacement with L-Ala resulted in a nonfunctional channel. Since it is not common to have a protein containing D-amino acids incorporated using the ribosomal machinery, these authors used a novel semi-synthetic approach (Valiyaveetil *et al* 2002) to incorporate the unnatural D-residue. Essentially, a C-terminal peptide containing the D-residue was synthesized using solid phase synthesis and an N-terminal peptide was expressed in *Escherichia coli* and targeted to inclusion bodies. These peptides were then chemically ligated *in vitro* and refolded in a membrane environment to recover functional KcsA.

The use of glycine as a surrogate D-amino acid in the selectivity filter could be a general mechanism employed by ion channels. Direct experimental verification of this design could be challenging and time consuming since generating high resolution structures of ion channels continues to be a formidable challenge (Rees *et al* 2000). However, searching for conservation of glycine residues in selectivity filters of other ion channels using bioinformatic approaches could prove to be useful.

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