

Is a β -barrel model of the K^+ channel energetically feasible?

Stephen Bogusz and David Busath

Section of Physiology, Brown University, Providence, Rhode Island 02912

INTRODUCTION

A mutation of Shaker D431 or T449 strongly affects external tetraethylammonium (TEA) binding (1) whereas a mutation of Shaker T441 (2) or NGK2/DRK1 chimera L374 (equivalent to Shaker V443) and chimera V369 (3) affect internal TEA blocking in the potassium channel. Changes in Shaker T449 (1), F433, T441, T442 (4), and chimera L374 and V369 (3) affect ion conductance in the K^+ channel. These facts suggest that SS1 (~430–440 in Shaker) and SS2 (~440–450 in Shaker) line the channel, passing through the cell membrane and back. Mutations of several residues just outside of the SS1–SS2 region affect external charybdotoxin block (5). Mutations of chimera residues M379 and V368 (3), and Shaker D431 (1) have little or no effect on channel conductance. The K^+ channel is comprised of four equivalent monomers (6). Using molecular mechanics, we addressed the following questions: (a) Could four pairs of SS1 and SS2 hydrogen bond together to form a β -barrel which could span the bilayer and form the pore-lining region of the channel? (b) Is there a model consistent with the assumption that site directed mutagenesis of residues with side chains projecting into the barrel would be most likely to affect permeability properties? (c) Would there be room in the β -barrel for the inward projecting side chains? (d) Would water and K^+ fit in the channel?

RESULTS AND DISCUSSION

A family of eight different β -barrels was evaluated in terms of the inward-projecting side-chains.¹ The most densely packed case, T439 CCW, is considered here. The hydrogen bonding pattern for two of the four strands, each comprised of 21 amino acids, is shown in Fig. 1. This configuration qualitatively satisfies 11 of the

14 criteria established by the site-directed mutagenesis results stated above.

The 3-dimensional coordinates for the NGK2 equivalent² of the structure in Fig. 1 were constructed using Quanta (Polygen Corporation, Waltham, MA). An end view of the barrel is shown in Fig. 2. The peptide barrel is ~26 Å long and consists of 792 atoms. After energy minimization of the entire structure from an arbitrary starting configuration, the potential energy of the 36 inward-projecting side chains was computed (Table 1). To determine if the interactions between the bulky inward-projecting side chains are prohibitive, this was compared to the potential energy calculated after separation of the four monomers. In addition, the side chains were further minimized after separation (keeping the backbone fixed), to explore the degree of intramonomer crowding. The van der Waals and electrostatic energies between side chains are more favorable in the barrel than in the separated configuration, even after relaxation. The bond, angle, and dihedral energies are reduced after separation and relaxation indicating that the side chains had been somewhat stressed in the packed structure. Yet, the total side-chain energy of the relaxed structure is similar to that of the packed structure, demonstrating the energetic feasibility of the barrel structure.

To determine whether water could fit in the barrel, 108 water molecules (Quanta type TIP3) were randomly packed in and near each end of the barrel. The system was relaxed with 10,000 steps of molecular dynamics and afterwards energy minimized to RMS force <0.1 kcal/mol-Å. After the minimization, 13 waters were grouped in the first 5 Å of the barrel, (measuring from the intracellular end); three in single-file boxed by the W435 tetrad in the next 7 Å; and 22 were grouped in the final 14 Å. The remaining 70 waters were grouped outside the barrel at either end. The packing did not stress the walls of the barrel, judging by the number of hydrogen bonds in the β -sheet (unchanged at ~80) and by the barrel-

¹Bogusz, S., A. Boxer, and D. Busath. An SS1–SS2 β -barrel structure for the voltage-activated potassium channel. Submitted for publication.

²We continue to use Shaker numbering in the text even though we refer to this NGK2 model.

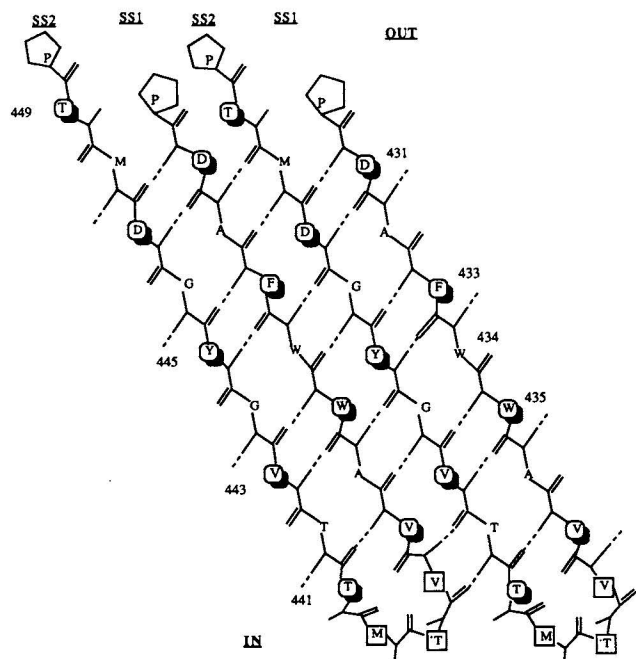


FIGURE 1. Hydrogen bonding pattern for two of the four monomers comprising an antiparallel right-tilted β -barrel with four-fold symmetry. α -carbons are marked with the single letter code for the amino acid. Those which project into the barrel are circles. Ambiguous cases (in the β -turn) which tend to point more inward than outward are in squares. To form the complete barrel, this pattern is duplicated to one side and the sides of the pattern are lifted to form a cylinder so that the hydrogen bonds on the right are aligned with those on the left.

water interaction energy which is always favorable (see Table 2 below).

The potassium transport energy profile was estimated by sequential replacement of the water molecules along the channel axis with K^+ and system energy minimiza-

TABLE 1

	TOTE	VDW	ELEC	BOND	ANGLE	DIHE	IMP
Before separation	-105	-73	-310	26	124	100	28
After separation	36	14	-257	26	124	100	28
Separated and relaxed	-110	-45	-275	25	82	80	23

Energy component abbreviations are: TOTE = Total potential energy, VDW = van der Waals, ELEC = Electrostatic, DIHE = Dihedral, IMP = Improper dihedral. The side-chains potential energy include the energy of interaction of the side-chains with the backbone.

tion (Fig. 3). A variation of adiabatic mapping, this approach neglects the effects of thermal vibrations and uses only a coarse grid for ion positions, but gives a reasonable approximation to the free energy profile (7) when system entropy changes are modest as expected here. There is a 26 kcal/mol barrier in the neighborhood of the W435 tetrad (position 3) and a -20 kcal/mol well near the D447 tetrad (position 8). At position 5 the ion is surrounded by the Y445 tetrad. The energy decomposition at these key points in the ion trajectory appears in Table 2. At position 3, the ion-water interaction energy is significantly increased relative to "ref" indicating that ion dehydration is the main component of the barrier. At position 5, tyrosine hydroxyls and multiple waters coordinate the ion. The energy well at position 8 is due to interactions with the D431 tetrad, acidic side chains coordinating the ion more than waters.

Ion permeability would be undetectably small for an energy profile with a 26 kcal/mol barrier and a -20 kcal/mol well. However, there have been several factors neglected in this computation which may exaggerate these values. The dehydration barrier occurs as the ion



FIGURE 2 Stereo pair end view of the β -barrel with the outward-projecting side chains deleted for clarity.

TABLE 2

Position	Potential energy (kcal/mol)			
	ref*	3	5	8
Barrel only	-1619	-1611	-1619	-1607
Water only	-922	-961	-937	-986
Barrel-water	-902	-892	-880	-843
Ion-water	-136	-68	-106	-28
Barrel-ion	-10	-31	-40	-147
Total system P.E.	-3590	-3564	-3582	-3610

*K⁺ located at $x = 0$ Å.

passes four tryptophans, the W435 tetrad. All of the eight models that we have considered have either a W435 tetrad or a W434 tetrad. The tryptophan carbon parameters did not represent the aromatic π cloud

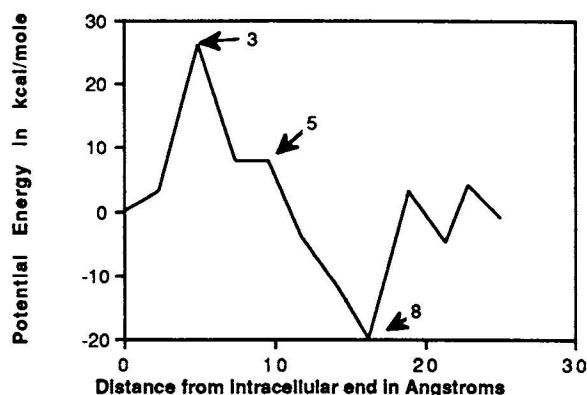


FIGURE 3. Relative system potential energy for K⁺ in the hydrated barrel vs axial distance from the intracellular end of the barrel. As a reference, we subtracted the system energy with the ion at $x = 0$ Å where the ion is surrounded by waters as it would be in the bath. At each position, the energy minimization (steepest descents, 25 steps, and adopted basis Newton-Raphson, 250 steps) was performed with the following constraints: the ion and a few backbone atoms at each end of the barrel were immobilized and the β -sheet hydrogen bonds in the barrel were fortified with distance constraints. Numbered positions are referred to in the text.

which has been shown to interact strongly with alkali ions (8). This factor would reduce the energy barrier at position 3. Counterions at position 8 would reduce the well depth. It is premature to question whether Na⁺ would be excluded by these model barrels. Such questions must await improved parameterization of the amino acid chains, of the water molecules, and more importantly, improved knowledge of the channel structure. But the calculations shown here demonstrate the feasibility of the β -barrel packing and K⁺ permeability.

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