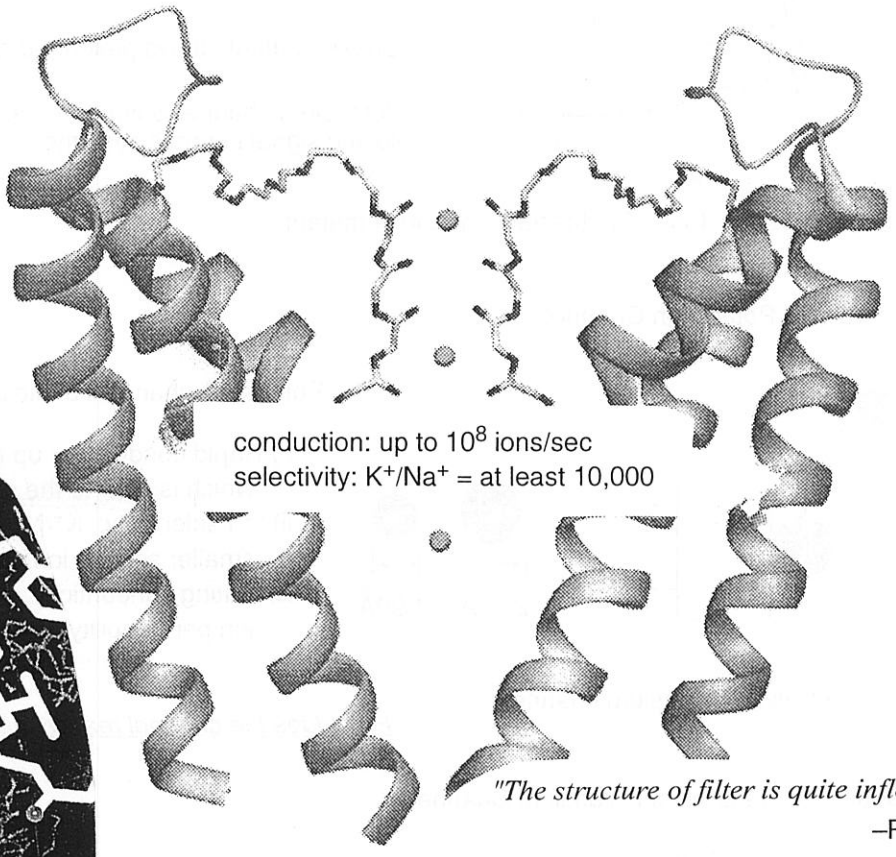
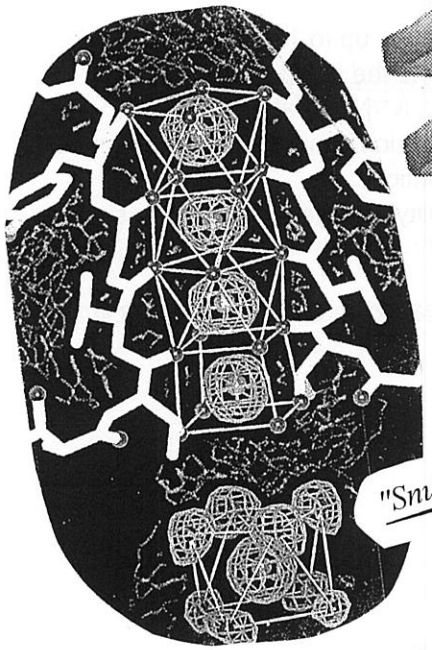
Potassium Channel

–*Selectivity in Dynamic Function*–



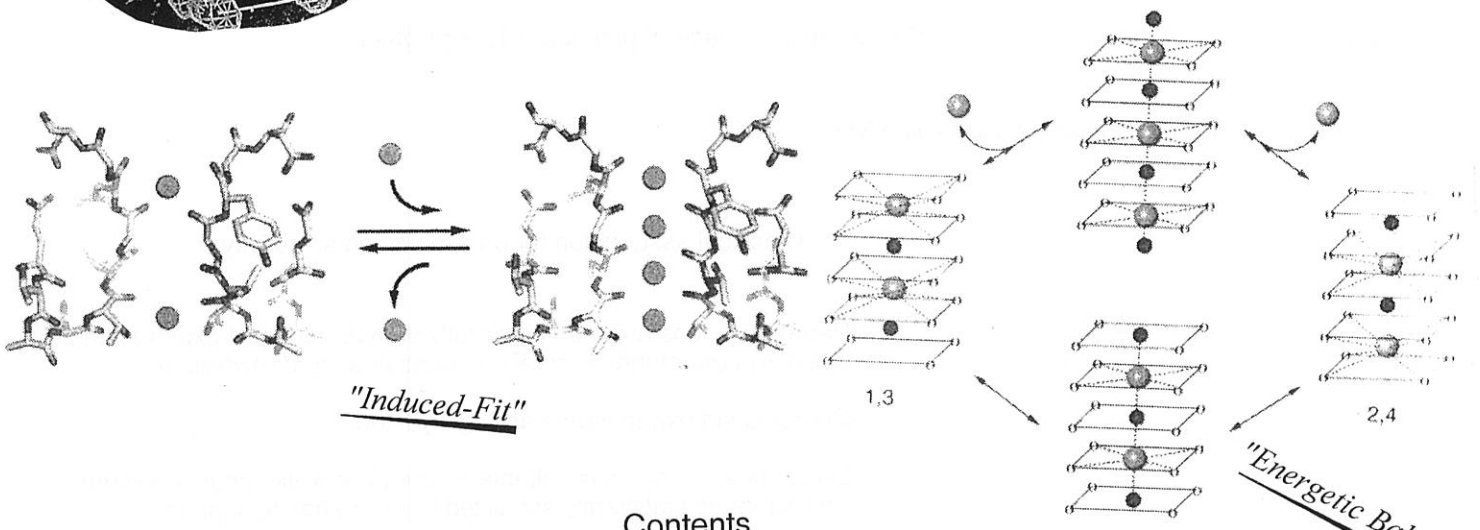
conduction: up to 10^8 ions/sec
selectivity: K^+/Na^+ = at least 10,000

"The structure of filter is quite inflexible."
–Roderick MacKinnon



"Snug-Fit"

"Even ion channel proteins appear to be inherently too flexible to satisfy the requirement of the traditional snug-fit mechanism."
–Benoit Roux



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2. Consideration Based on X-Ray Crystallography
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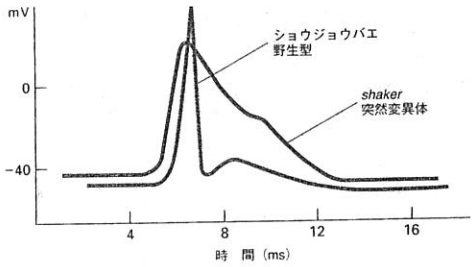
1. Basis of Potassium Channel

1. The Role of Potassium Channel

-Potassium selective channels are said to have various functions. Here, function in nerve cells is shown.

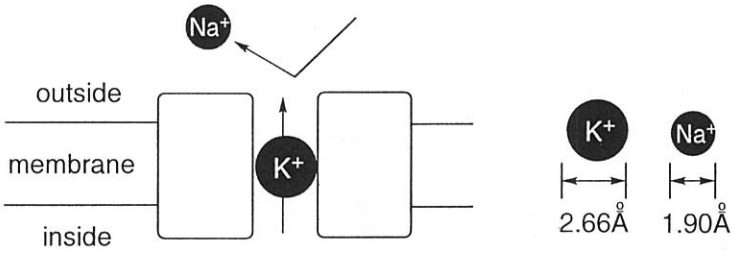


-*Shaker* mutant shows prolonged action potential.

-Potassium channel is important to make sharp electric signal in nerve systems.

Fig.1 Actoin potential in *shaker* (one of potassium channels) mutant.

2. Basic Properties of Potassium Channel



-Potassium channels exhibits three basic properties

i) Rapid conduction: up to 10^8 ions/sec
-which is near to the diffusion limit.
ii) Ion selectivity: K^+/Na^+ = at least 10,000
-smaller sodium ion cannot pass the channel.
iii) Gating condunction
-ion permeability changes according to signals.

Fig.2 Selectivity of potassium channel

How does the channel realize these properties at a time??

3. Classical Structural Features of Potassium Channel

i) Early picture of potassium channel

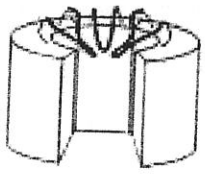
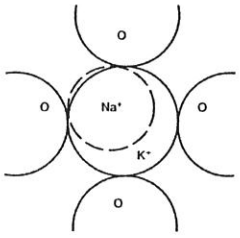


Fig.3

-Selectivity filter made of pore loops is highlighten.

ii) "Snug-Fit" model for potassium ion selectivity

-Classical assumption for potassium ion selectivity.



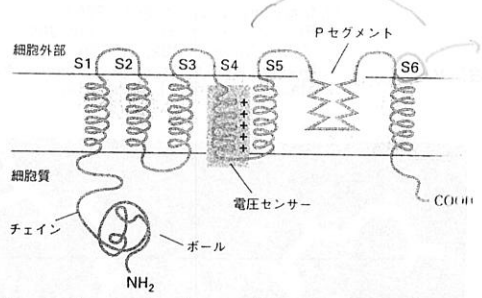
-Permeating ions are partially or fully dehydrated, and oxgen atom in a narrow pore of the channel compensates the dehydration.

-Coordinating oxgen atoms are rigidly fixed.

-Larger potassium ion is suitable for the pore while smaller sodium ion cannot be safficiently stabilized by coordinating ligands.

Fig. 4

iii) Structure of the protain



-Six membrane-spanning segments

-Tetramer

Fig.5 Secondary structure of potassium channel protein.

iv) Signature sequence in pore region

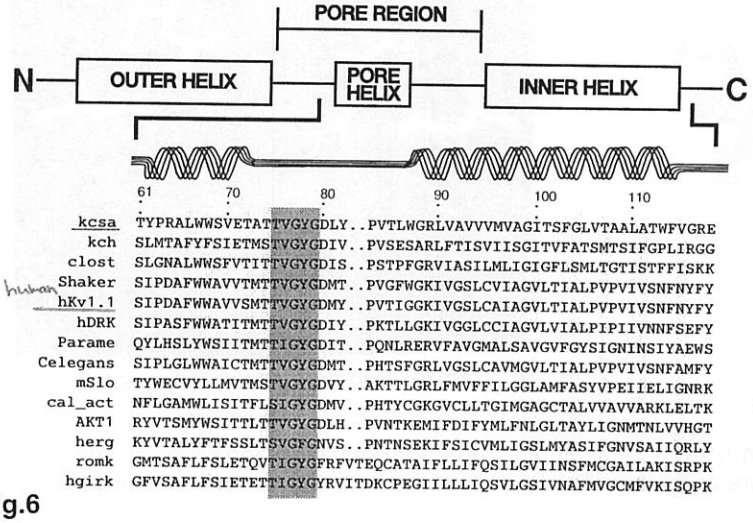


Fig.6

T:Thr ; R:

V:Val ; R:

I:Ile ; R:

G:Gly ; R: H

Y:Tyr ; R: 

-In all potassium channels, this signatuer sequence is conserved.

-This must be important in potassium ion selectivity.

2. Consideration Based on X-Ray Crystallography

In 1998, R. MacKinnon's monumental work on ion channel study was reported on *Science*.

-It reveals clearly the existance of selectivity filter and its structure.

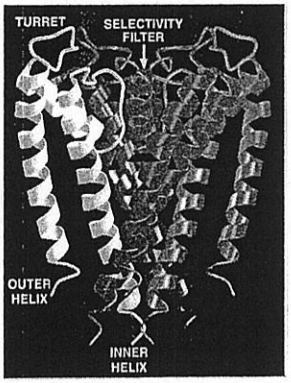


Fig.7 Ribbon view of K^+ channel structure

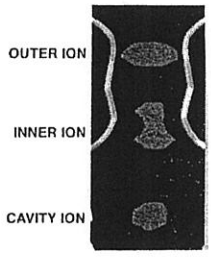


Fig.8 Ion positions in the pore

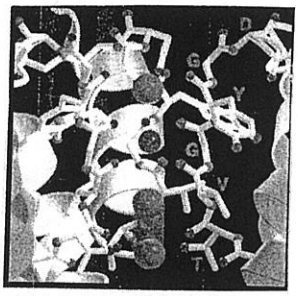


Fig.9 Structure of sellectivity filter

-They succeeded in x-ray crystallographic analysis of bacterial potassium channel "KcsA".

-The structure was very suggestive...

-Narrow selectivity filter

-composed by signature sequence

-sequential oxygen rings composed by main chain carbonyl group

-Large water filled cavity

-Two ions in the selectivity filter

~*Closer look*~

R. MacKinnon *et al. Science*, **1998,** *280*, 69
R. MacKinnon *et al. Nature*, **2001,** *414*, 37
R. MacKinnon *et al. Nature*, **2001,** *414*, 43
S. Berneche and B. Roux *Nature*, **2001,** *414*, 73
G. Yellen *Nature*, **2002,** *419*, 35
E. Gouaux and R. MacKinnon *Science*, **2005,** *310*, 1461
R. MacKinnon Nobel Lecture, 2003

1. Principle of Selectivity

~Mechanisms of potassium ion hydration and dehydration~

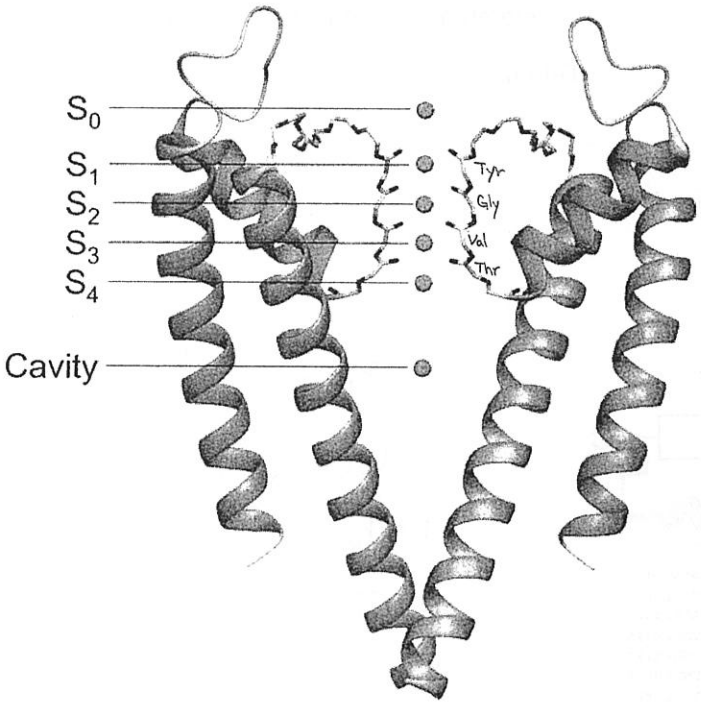


Fig. 10 Structure of K^+ channel

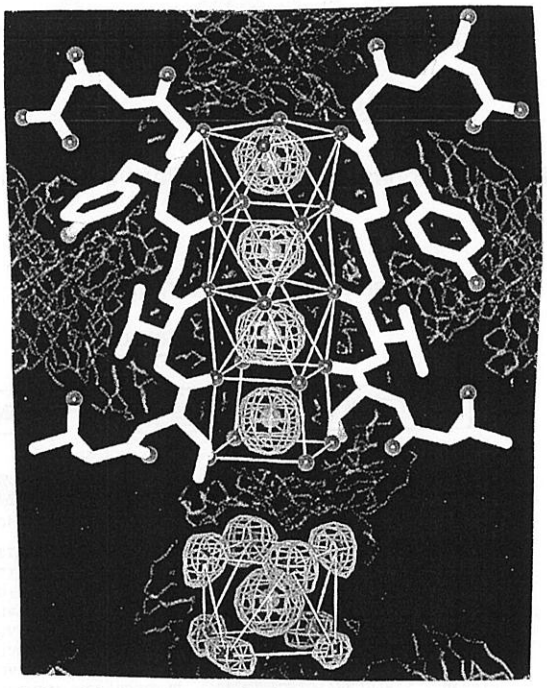


Fig. 11 Square antiprism binding site

~*Features*~ -Selectivity filter main chain forms sequential binding sites for potassium ion.
-Each binding site formes square antiprism structure, as if to mimic the waters to hydration.

~*Assumptions*~

-In each binding site, 8 carbonyl oxygens are placed on ideal possition to coordinate for potassium.
-In each binding site, potassium ion is held tightly, which conpensate the dehydration energy.
-Sodium ion, on the other hand, is too small to coordinate sufficiently, and to go through the channel *energetically.*

2. Maintein the Structure

–If the selectivity filter makes precise room for potassium ion, how is sustained it ??

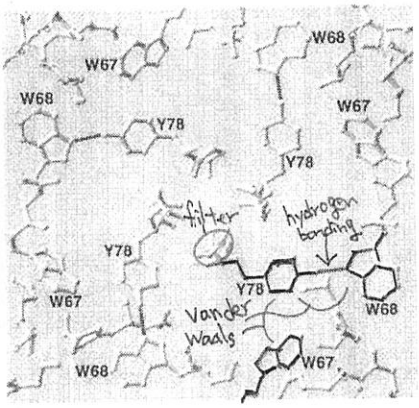


Fig. 12 Interaction of Y78(selectivity filter) and W67, W68(outer helix)

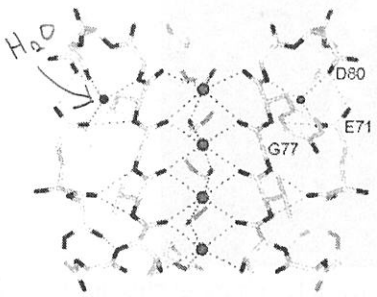


Fig. 13 Hydrogenbonding in selectivity filter

~*Features*~ -Tyrosine-78(selectivity filter) interacts through hydrogen bonding with tryptophan-68 and through van der Waals contacts with tryptphan-67,68 (outer helix)

~*Assumptions*~ -This structure behaves like a spring, and stretch the pore to hold it open at the diameter suitable for potasssium ion.

–If the precise tight binding is the mechanism for selectivity, how ions go through the filter ??

3. Two Ions in the Selectivity Filter

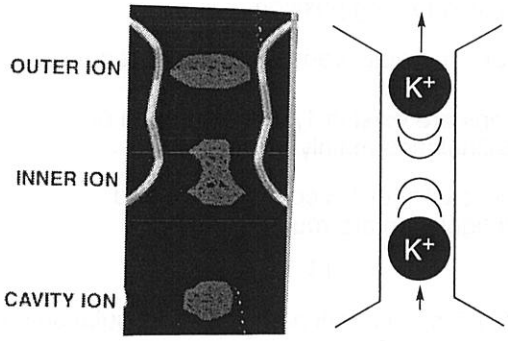


Fig.14 Two ions in selectivity filter

-In conductoin state, there are two ions in the selectivity filter, and electrostatic repulsion between them destabilize too tight interaction between ions and selectivity filter.

⟶ Rapid ion conduction

i) Detailed study using rubidium ion

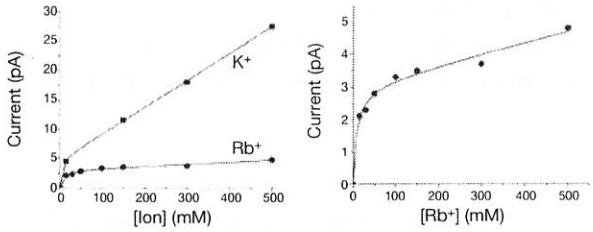


Fig.15 Ion conduction in various concentration

-In both K^+ and Rb^+, there is two phase according to concentration; steep regime (lower concentration) and linear regime (higher concentration).

-In the case of K^+, ion conduction continue to increase in linear regime.

–Are there structural difference between them ??

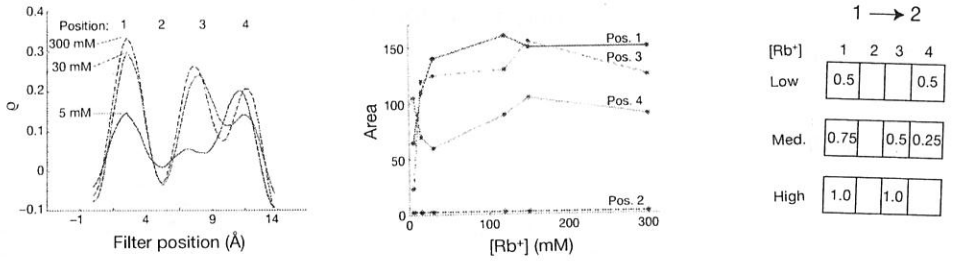
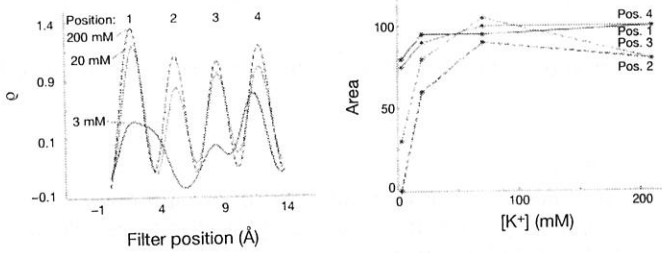


Fig.16 Crystallographic distribution of Rb^+ ions in the selectivity filter

-According to concentration, the number of ions in the selectivity filter and ion containing positions changed.

Lower: 1 ion in pos.1 and pos.4
Higher: 2 ions in pos.1 and pos.3



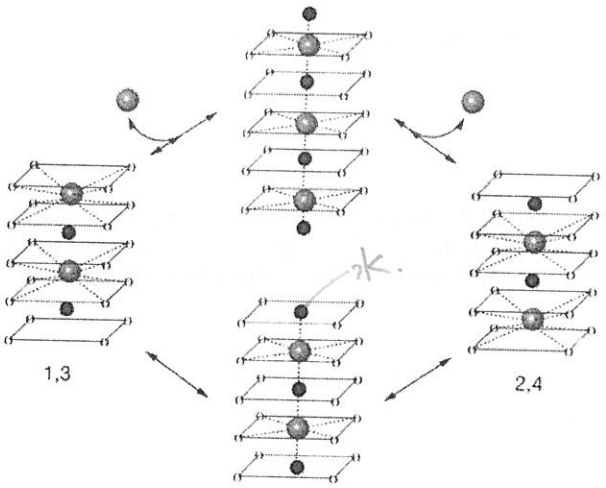
-At lower concentration, the profile is similar to Rb^+ case.

-In contrast with Rb^+, at higher concentration, two K^+ ions are delivered to 4 positions equally.

This result is in accordance with the two phase ion conduction depend on the ion concentration.

–What does "delivered equally" means ??

Fig.17 Crystallographic distribution of K^+ ions in the selectivity filter



-It is energetically very unfavorable that two ions exist in neighboring position.

-Two ions are delivered to 4 positions equally.

-Tow ions are exist in 1,3 configuration or 2,4 configulation mainly.

-The possibility of 1,3 configuration and 2,4 configulation are roughly equal.

⇓

The energy of 1,3 configuration and 2,4 configulation are equal.

Fig.18 Proposed throughput cycle for K^+ ions

~Proposed ion conduction mechanism~

-A queue of ions and water move in concerted manner.
-Exchange between the 1,3 configuration and 2,4 configulation occers when...
  -the ion pair jumps between configurations (concentration-independent path).
  -a third ion enters causing an ion to exit from the opposite side (concentration-dependent path).
-In transition state, K^+ ion is coordinated in octahedral manner, which seems to have small energy barrier.

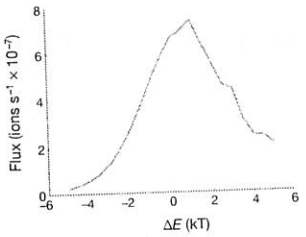


Fig.19 Simulated flux as a function of ΔE

ΔE : energy difference between 1,3 and 2,4 configuration

This simulation supports the assumption that energetic balance of 1,3 and 2,4 configuration is important to rapid flux.

ii) Queue of ions outside of selectivity filter

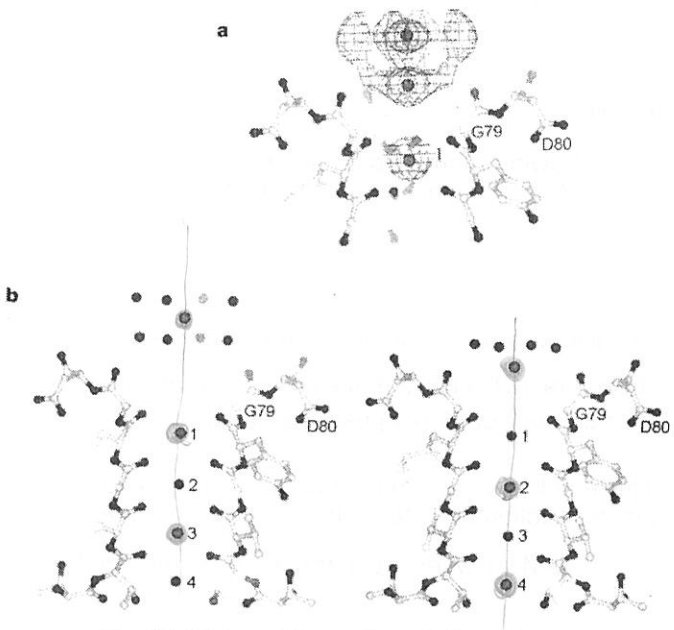


Fig.20 K^+ ion at the extracellular entryway

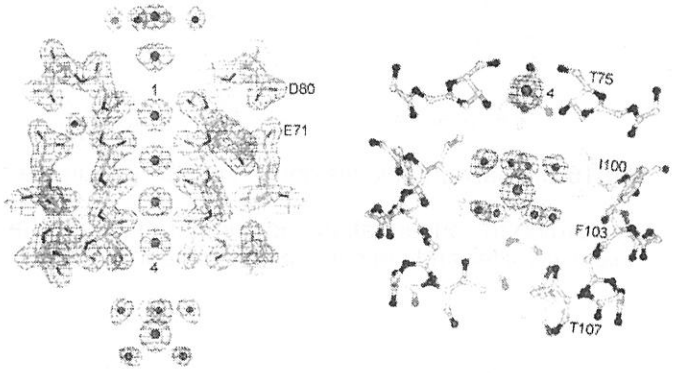


Fig.21 Hydrated K^+ ion in the central cavity

-Even at the outside of selectivity filter, ions are lined orderly.

↳ X線でわかる.

~Assumptions~

-It makes dehydration and hydration smoothly.
-Intuitively, it seems to support the concerted ion move supported by third ion.

iii) Energetic consideration using simulation

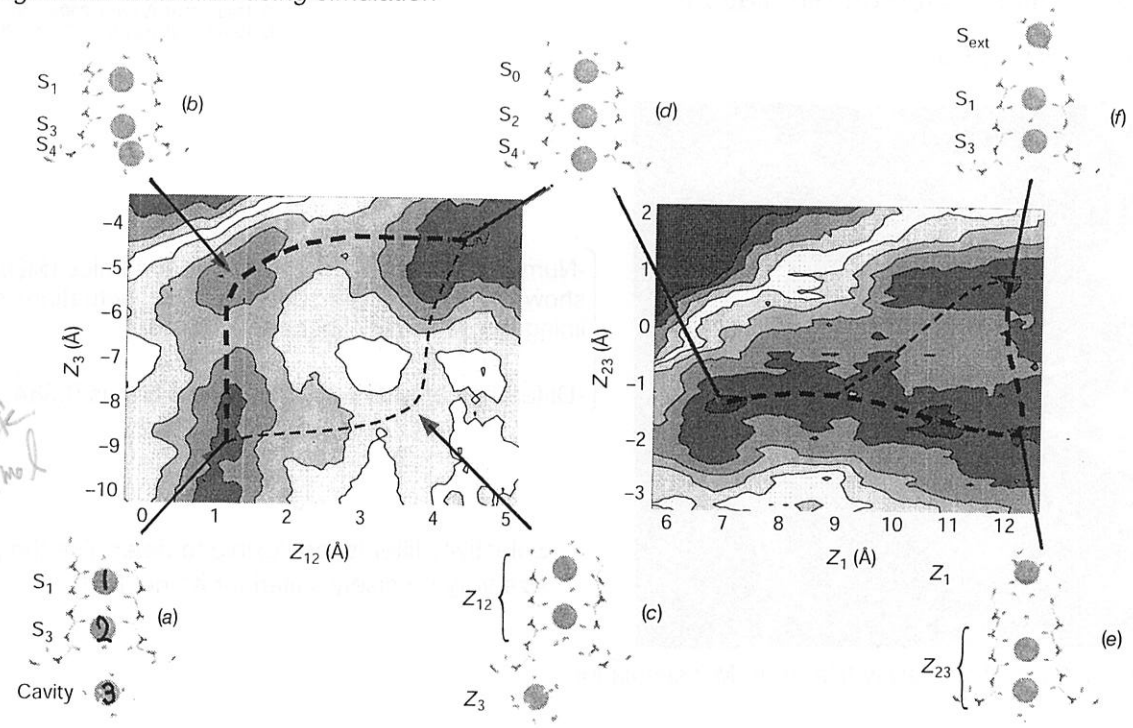


Fig.22 Topographic free energy maps of ion conduction

-Energetic calculation supports three ions participated concerted transition.
-*Remarkable*: Largest energy barrier for the process is on the order of 2-3 kcal/mol
; dehydration energy of K^+ is 80 kcal/mol

iv) Induced-Fit

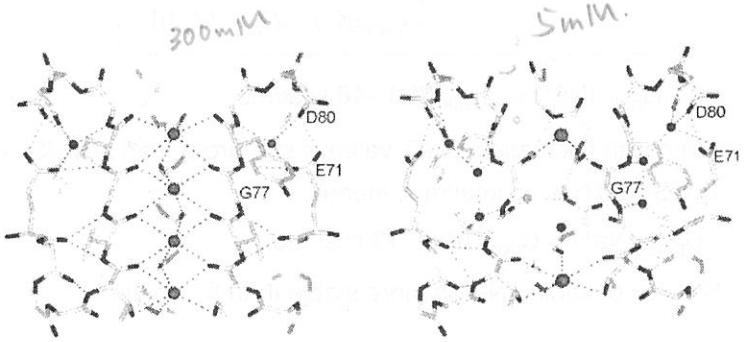


Fig.23 Induced-fit nature of selectivity filter

-The structure of selectivity filter differs dependent on the concentration.
-The structure of selectivity filter is made by the interaction of channel and ions, not channel alone.

~Assumptions~

-The low-K^+ structure explaines how the selectivity filter maintains its stability in a low-K^+ environment. (outside of the cell)
-Two ion conformation is under some tension, which will lower K^+ affinity. → high conduction rate

Though, potassium channel has induced-fit nature, Redrick MacKinnon mantioned as follows...

"Certainly, in high-K^+ concentration under whitch conduction occers, the structure of the filter is quite inflexible."
(R. MacKinnon, 2001, *nature*)

Utilizing free energy simulation as a tool, Benoit Roux proposed another principle for potassium ion selectivity, says...

"But proteins are relatively flexible structures that undergo rapid thermal atomic fluctuations larger than the small difference in ionic radius between K^+ and Na^+." (B. Roux, 2004, *nature*)

–Then, how is the selectivity explained ??

3. Consideration Based on Simulations

B. Roux *et al. Nature*, **2004,** *431*, 830
B. Roux *et al. Biophis. Chem.* , **2006,** *124*, 279

1. Flexible Selectivity Filter

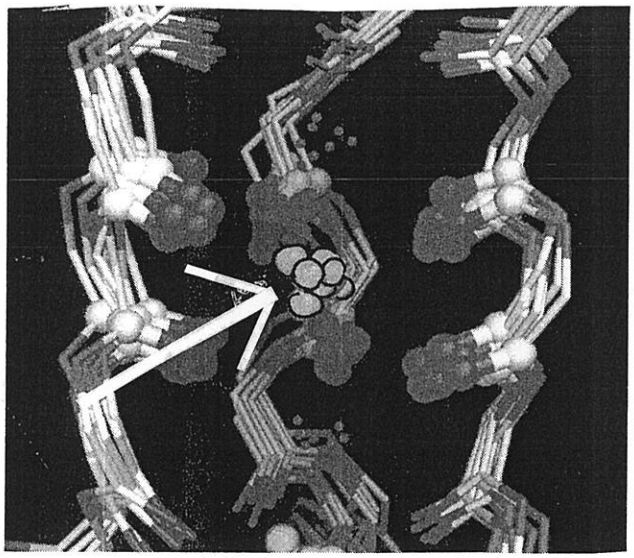


Fig.24 Fluctuations of selectivity filter from MD simulation

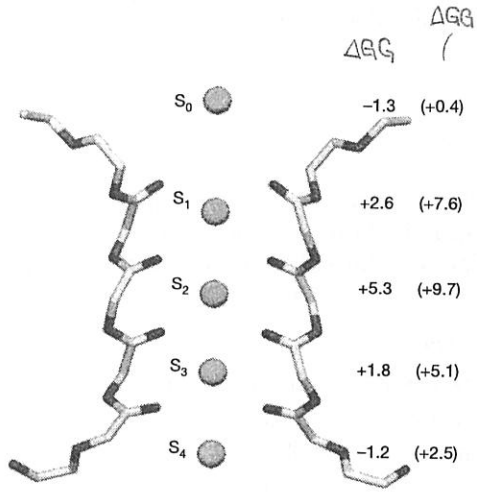
-Numerous indipendent molecular dynamics (MD) simulations shows that root-mean-square (RMS) fluctuations of atoms lining the selectivity filter are 0.75-1.0Å.

-Difference of radii between K^+ and Na^+ is 0.38Å.

⇓

Selectivity filter is too flexible to determine the pore size very precisely suited for K^+ not Na^+.

2. The Importance of Carbonyl-Carbonyl Repulsion



-Selectivity for K^+ can largely explained thermodynamically

⇓

-Free energy differnce $\Delta\Delta G$ is important.

$$\Delta\Delta G\,(K^+ \rightarrow Na^+) = [(G_{pore}(Na^+) - G_{bulk}\,(Na^+)) - (G_{pore}(K^+) - G_{bulk}\,(K^+))]$$

$G_{bulk}\,(Na^+) \fallingdotseq G_{bulk}\,(K^+)$ –18 kcal/mol

-Even with fluctuation, $\Delta\Delta G$ value is still larger than zero.(S_1 - S_3)

-For S_3, $\Delta\Delta G$ is ~5 kcal/mol, means...

$G_{pore}\,(Na^+) \fallingdotseq G_{pore}\,(K^+)$ –13 kcal/mol

S_2 site containing Na^+ is more stable than K^+ one !!

Fig.25 Free energy differnce in each binding site

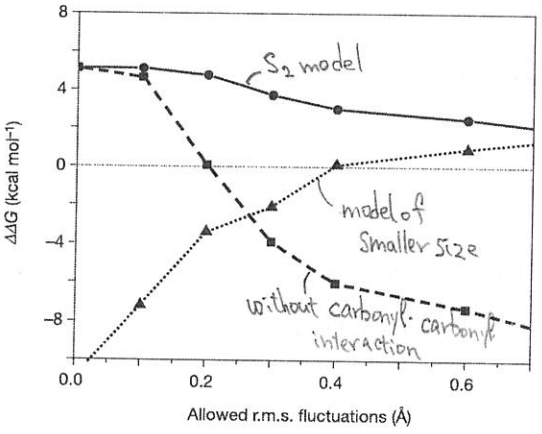


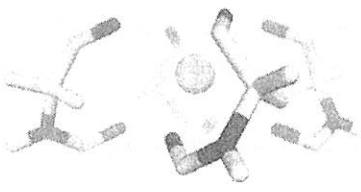
Fig.26 Selectivity of S_2 model as a function of flexibility

-With adequate flexibility, even the model with smaller pore (suitable for Na^+) shows K^+ ion selectivity.

-This implies the importance of the intrinsic local physical propaties of the ligands.

-Carbonyl-carbonyl interaction (repulsive) acts important role in selectivity obtained by flexible structure.

-Used model structure is



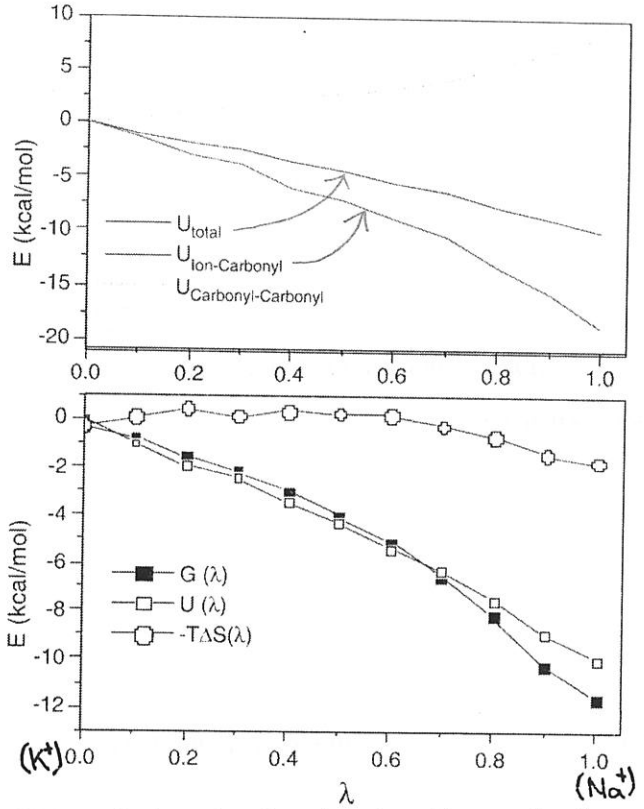


Fig.27 Contribution of carbonyl-carbonyl interaction for ΔG

$\Delta G\ (K^+ \rightarrow Na^+) = [G_{pore}(Na^+) - G_{pore}(K^+)]$

$G_{bulk}\ (Na^+) \doteqdot G_{bulk}\ (K^+) - 18$ kcal/mol

-Utilizing 1 ion and freely fluctuating 8 carbonyl like group model.

-Enthalpic contribution is major.

-Ion-carbonyl interaction favors smaller Na^+.

-Carbonyl-calbonyl interaction favors larger K^+.

Even in *freely* fluctuating model, K^+ selectivity appears !!

System	ΔΔG with all interactions (kcal mol $^{-1}$)	ΔΔG with no repulsion (kcal mol $^{-1}$)	Loss in ion selectivity (kcal mol $^{-1}$)
Fully flexible KcsA	5.3	−2.9	8.2
Fully frozen KcsA	9.7	9.7	0.0
Partly frozen KcsA	6.7	0.9	5.8
Liquid NMA	1.6	−8.9	10.5
Valinomycin	8.8	3.9	4.9

Table.1 Importance of carbonyl carbonyl interaction

-NMA: N-mathylacetamide

-Valinomycin: very rigid K^+ ionophore.

~Results~

-If the structure is flexible, corbonyl-carbonyl repulsion is important for selectivity.
-Even though the channel structure other than selectivity filter is flozen, still, corbonyl-carbonyl repulsion is important.

-In the case of NMA (*liquid*), carbon-carbon repulsion is *very important* for selectivity.
-In the case of valinomycin (robust), carbon-carbon repulsion is *not so important* for selectivity.

~Assumptions~

-In flexible system, carbonyl-carbonyl repulsive interaction is imprtant for selectivity.
-Potassium channel selectivity filter has "liquid-like" nature; means highly flexible.

3. Other Effects for Selectivities

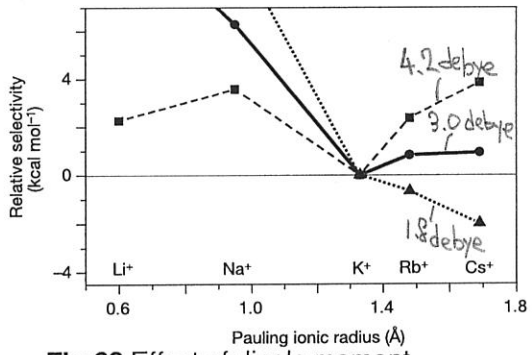


Fig.28 Effect of dipole moment

-Larger dipole moment prefers smaller cation.

-~3.0 debye (carbonyl group of main chain) seems suitable for K^+ selectivity.

-This result is in good accord with the previous knowledge that K^+ channel is very selective in K^+ *vs* Na^+, but not highly selective in K^+ *vs* Rb^+.

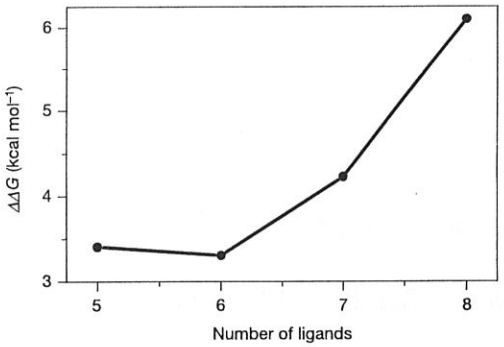


Fig.29 Effect of number of ligands

Table 2
The variation of $\Delta\Delta G$ as a function of a toy-model ligand composition

Number of carbonyls	Number of water molecules	$\Delta\Delta G$ (kcal/mol)
8	0	6.2
7	1	4.79
6	2	2.28
5	3	−0.69
4	4	−2.11
6	0	3.40
5	1	3.19
4	2	0.26

Table.2 Effect of number of ligands

-8 cordinating carbonyl group is advantageous for K^+ ion selectivity.

~Contrast between R. MacKinnon's view and B. Roux's view~

	R. MacKinnon	B. Roux
method	x-ray cryatallography	free energy simulation
nature of selectivity filter	inflexible	flexible
principle of selectivity	size of the pore defined by channel	intrinsic local physical propaties of ligands

~My private view~

Nature of selectivity filter: flexible

Principle of selectivity: complex interactions as a hole → defined by its structure

"minimum free energy" ⟸ Intrinsic propaty of the structure

-Flexible structure
-Complex interactions
-Many possibility

→ Converge on *one* structure, *one* selectivity

–Secret of life