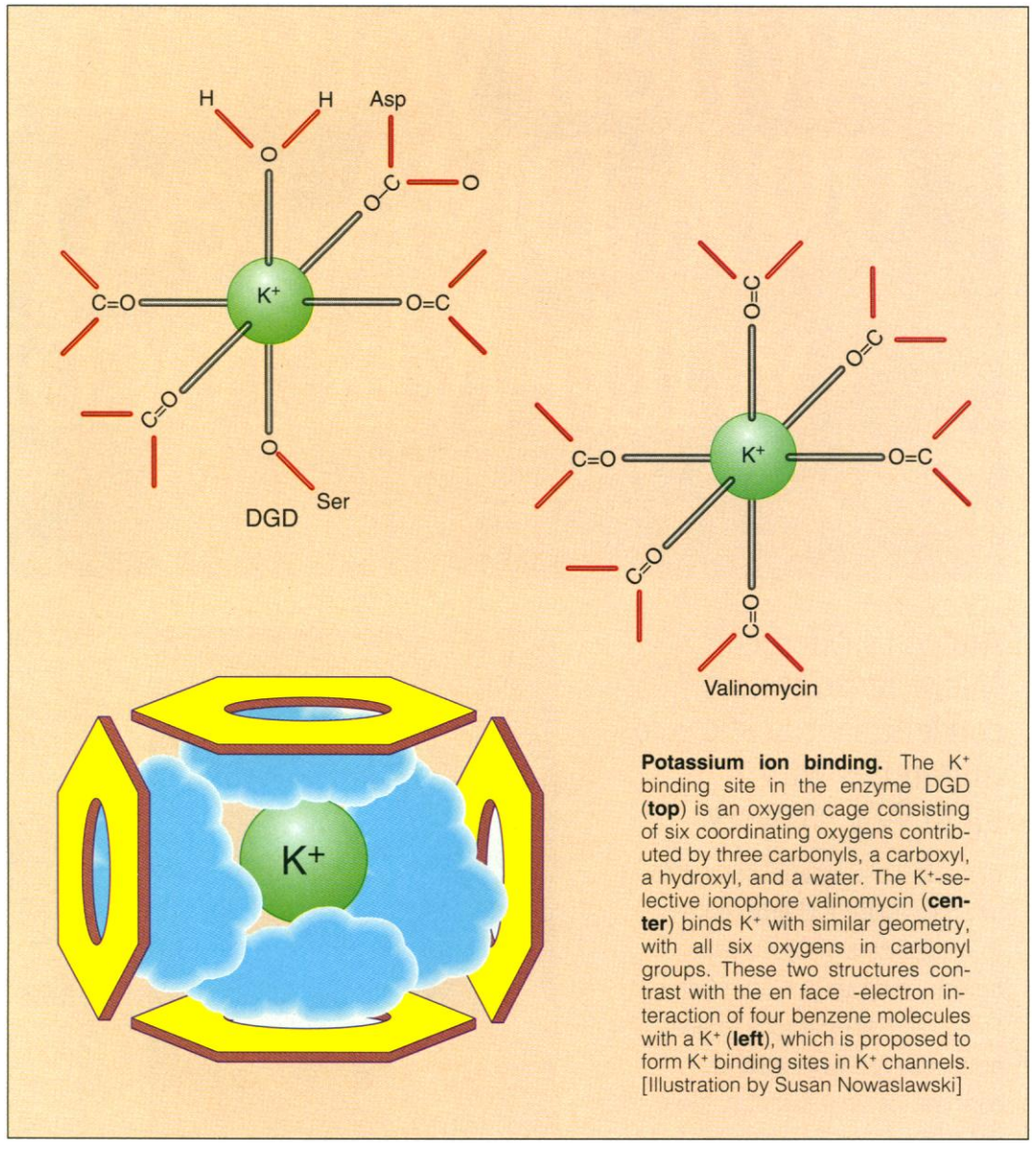
Potassium Selectivity in Proteins: Oxygen Cage or in the F ace?

Christopher Miller

In high-school chemistry, one of the first things most of us deduced from the periodic table was that K^+ is just like Na^+, only different. In the biological world, a great deal rides on this difference: Virtually all cells accumulate K^+ from the environment and exclude Na^+ from the cytoplasm in order to accomplish essential physiological tasks. The cell's ability to discriminate between these two monovalent cations underlies many basic processes, such as the heart's generation of rhythmic electrical signals, the kidney's unceasing sifting of vital and toxic solutes in the blood, or the eye's precise control of the lens' refractive index. For these kinds of purposes, biological membranes carry many ion-transporting proteins that handle Na^+ and K^+ with high selectivity. For instance, the Na^+/K^+–adenosine triphosphatase literally "pumps" K^+ into and Na^+ out of the cell at the expense of adenosine triphosphate hydrolysis. (Most of the 10 W put out by a human brain—awake or asleep—is heat-generated as this enzyme labors to maintain the Na^+ and K^+ gradients drawn upon by neural activity.) Likewise, K^+-channel proteins form aqueous pores that allow K^+, but not Na^+, to "leak" passively across the plasma membrane. Two recent papers in *Science* (*1*, *2*), one in this issue, offer sharply differing views of the chemical nature of K^+-selective binding sites that must reside within such proteins.

What is the molecular basis of K^+ selectivity in proteins? What sort of binding site can distinguish by 100- to 1000-fold between structureless cations as similar as Na^+ and K^+, with crystal diameters of 1.9 and 2.7 Å, respectively? Since nearly all processes employing K^+ over Na^+ discrimination rely on membrane proteins, we have had no high-resolution structures of aqueous, crystallizable proteins to guide us in our speculations about what K^+-selective sites might look like. [This situation is very different from the analogous problem of Ca^{2+}-selective binding sites, for which many high-resolution structures are known and the energetics of selectivity is currently clarifying (*3*).] But now, following crystallization of a water-soluble K^+-requiring protein, Toney and colleagues (*1*) have given us our first look at a macromolecular, K^+-specific binding site. The protein is dialkylglycine decarboxylase (DGD), a pyridoxal phosphate-requiring enzyme that carries out some admirable synthetic chemistry. But what concerns us here is the fact that K^+ in the 10 mM range is required for enzyme activity, which is antagonized by Na^+ at higher concentrations. We would conclude, therefore, that the enzyme must contain some sort of K^+-selective site, the occupancy of which is linked to an active conformation.

This site reveals itself beautifully in the structure of DGD. A K^+ ion is snugly buried in a cavity formed near a dimer interface and is coordinated to six oxygen ligands in octahedral geometry (as if the K^+ ion floats in the center of a cube, and each oxygen sits at the center of a face). Three of these oxygens are donated by main chain carbonyls, and one each by an aspartate side chain carboxyl, a serine hydroxyl, and a bound water molecule. This "oxygen cage" (see



Potassium ion binding. The K^+ binding site in the enzyme DGD (**top**) is an oxygen cage consisting of six coordinating oxygens contributed by three carbonyls, a carboxyl, a hydroxyl, and a water. The K^+-selective ionophore valinomycin (**center**) binds K^+ with similar geometry, with all six oxygens in carbonyl groups. These two structures contrast with the en face -electron interaction of four benzene molecules with a K^+ (**left**), which is proposed to form K^+ binding sites in K^+ channels. [Illustration by Susan Nowaslawski]

The author is in the Howard Hughes Medical Institute and the Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254.

figure) is exactly the sort of binding site anticipated for a K^+-binding protein, from analogy to the K^+-selective ionophores of known structure that are always trotted out hopefully as models for K^+-transporting proteins: valinomycin, the macrotetralides, and the huge collection of synthetic cryptands (*4*). These small, flexible compounds bind cations by encapsulating them, replacing their waters of hydration with electronegative oxygen ligands (carbonyls, ethers, and in some cases carboxylates), often with octahedral geometry.

The site in DGD is clearly K^+-selective, since the crystals were grown in solutions containing 7.5 mM K^+ and 75 mM Na^+. What happens if K^+ is removed from the crystallization solution? With no K^+ to occupy the cavity, a remarkable change in structure occurs. The cavity's diameter shrinks by about 0.8 Å so that a smaller Na^+ ion can now fit tightly. But the cage can collapse around the cation in this way only if the number of coordinating oxygens is reduced. In this structure, only five oxygens abut the Na^+, in a trigonal-bipyramidal geometry, a change accomplished mainly by expulsion of the serine hydroxyl from the cation coordination sphere. This crucial alteration forces secondary adjustments of the orientations of close-by tyrosine and glutamate residues that contribute directly to the catalytic site. Presumably, this is what shuts the enzyme down in the Na^+-liganded state.

For several decades, the mechanism by which K^+ and Na^+ channel proteins select their respective cations for permeation has been a subject of speculation, often with overtones of desperation, given the structural inaccessibility of these proteins and the central role ion selectivity plays in their function. The canonical view of channel selectivity, articulated by Eisenman, Hille, and others (*5*, *6*), pictures electronegative oxygen ligands projecting into the transmembrane pore, fixed in a rigid geometry to mimic the waters of ionic hydration. For the fourfold symmetric K^+ channels, the oxygen ligands are pictured in a square-planar configuration, which would produce octahedral coordination in a narrow, water-filled pore. The DGD structure is in satisfying harmony with such speculations.

But last year, Heginbotham and MacKinnon (*7*) concluded that in K^+ channels at least, the π electrons at the face of aromatic side chains might serve as the electronegative source for coordination to cations (see figure). This proposal, although idiosyncratic among ion channel biophysicists, was not radical; favorable en face interaction between primary amines and aromatic π rings in proteins had been noted by Burley and Petsko (*8*), and an extensive series of aromatic "cages" for quaternary amines had been synthesized in Dougherty's laboratory (*9*). Moreover, Kebarle's group (*10*) had demonstrated experimentally that in the gas phase, the K^+ ion interacts more favorably with benzene, through quadrupole and induced-dipole effects, than with the water molecule. Heginbotham and MacKinnon's proposal was provocative because it offered a possible rationale for the puzzling preponderance of aromatic residues (over the oxygen-rich residues expected from the canonical view) found in the pore-forming regions of K^+-channel sequences. Might it be possible that the strongly K^+-selective binding sites known to reside in the pores of these tetrameric proteins (*11*) actually arise from K^+ interactions at the face of aromatic rings arranged in some sort of square-planar cage?

This question has now been taken up by Kumpf and Dougherty (*2*), who have calculated interaction free energies for cations with benzene in aqueous solution, using optimized liquid simulation potentials and Monte Carlo methods. Ab initio calculations show that in the gas phase, monovalent cations interact with benzene en face, and that the smaller ion always wins; Na^+ is preferred over K^+. However, in liquid water, the situation changes. Now, K^+ is always preferred over Na^+ in ternary complexes in which two benzene molecules sandwich a cation between them. This K^+ preference, which arises largely from the difficulty of water molecules to intrude into the thinner Na^+ sandwich, can be substantial, up to 15 kcal per mole, depending upon the geometries assumed for the complexes. These calculations, while accurate for liquid phase, are not meant to apply realistically inside K^+ channels. For instance, only a twofold sandwich complex was treated, not a fourfold cage, as envisioned in a K^+ channel. But they do serve to establish cation-π interactions as plausible alternatives to oxygen cage structures in models of channel selectivity. We should soon hear of specific tests of these alternatives, as mutagenesis efforts aimed at the aromatic residues in the pore domains of K^+ channels ripen in several laboratories. Of course, the possibility remains that K^+-selective pores are lined not with side chains at all, but with main chain carbonyls, which no mutagenesis experiments can touch.

The structure of a binding site does not by itself reveal the energetic reasons for K^+ selectivity. A clear message from all known metal-ion binding sites, one illustrated nicely in the K^+ and Na^+ forms of DGD, is that the ion is always close-packed in the cavity to maximize close-range dispersion interactions. The protein adjusts to a "misfit" ion to avoid empty space in the cage, and the distortions are paid for by loss of binding affinity. Moreover, K^+ has a built-in advantage over Na^+ for binding: The difference in energy of dehydration, which is 20 kcal per mole more unfavorable for Na^+, because the higher electric field at the surface of the smaller cation orients water more strongly. In the thermodynamic tug-of-war between the binding site and the aqueous phase, water pulls more strongly on Na^+ than on K^+ (*12*). Perhaps that is why Na^+ channels are less selective for Na^+ than K^+ channels are for K^+ (*5*). But in order to make coherent mechanistic proposals for K^+ selectivity in channels or other types of membrane proteins, we will need to have the kind of detailed structures under consideration here; they show us that it all happens in the last half-angstrom!

References

1. M. D. Toney, E. Hohenester, S. W. Cowan, J. N. Jansonius, *Science* **261**, 756 (1993).
2. R. A. Kumpf and D. A. Dougherty, *ibid.*, p. 1708.
3. E. E. Snyder, B. W. Buoscio, J. J. Falke, *Biochemistry* **292**, 3937 (1990); M. Renner, M. A. Danielson, J. J. Falke, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6493 (1993).
4. M. Dobler, *Ionophores and Their Structures* (Wiley, New York, 1981).
5. B. Hille, *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA, ed. 2, 1991).
6. G. Eisenman and R. Horn, *J. Membr. Biol.* **76**, 197 (1983); G. Eisenman and J. A. Dani, *Annu. Rev. Biophys. Biophys. Chem.* **16**, 205 (1987).
7. L. Heginbotham and R. MacKinnon, *Neuron* **8**, 483 (1992).
8. S. K. Burley and G. A. Petsko, *FEBS Lett.* **203**, 139 (1986).
9. T. J. Shepodd, M. A. Petti, D. A. Dougherty, *J. Am. Chem. Soc.* **110**, 1983 (1988); D. A. Stauffer and D. A. Dougherty, *Tetrahedron Lett.* **29**, 6039 (1988); D. A. Dougherty and D. A. Stauffer, *Science* **250**, 1558 (1990).
10. J. Sunner, K. Nishizawa, P. Kebarle, *J. Phys. Chem.* **85**, 1814 (1981).
11. J. Neyton and C. Miller, *J. Gen. Physiol.* **92**, 569 (1988).
12. G. Eisenman, *Biophys. J.* **2**, 259 (1962).