tle attention has been given to developing improved models of tightly packed lipid assemblies. The recent works by Sun et al. and Snyder et al. suggest that further work on such "simple" systems is clearly justified.

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Revisiting the Ionic Selectivity of Na^+ Channels

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Voltage-dependent Na^+ channels are responsible for the generation and propagation of the action potential in many types of excitable cells. Na^+ channels discriminate effectively between the two most common monovalent cations in biological systems, Na^+ and K^+, preferring Na^+ by a ratio of 30:1 (Favre et al., 1996). Rapid membrane depolarization is effected by Na^+ channels through simple positive feedback: the channels open in response to small membrane depolarizations, and as a result the membrane potential further depolarizes approaching the Na^+ reversal potential. This selectivity for Na^+ over K^+ is vital to Na^+ channel function.

The question of how channels differentiate among the cations present in physiological solutions is not trivial. Na^+ channels are faced with a dual problem. First, they must distinguish between Na^+ and K^+, two ions with identical charge and only slightly different size. Second, they must allow Na^+ permeation while excluding Ca^{2+}, an ion of similar size but different charge. In this issue of *Biophysical Journal*, Favre et al. (1996) address in detail the molecular mechanisms underlying these selectivities, and suggest that in Na^+ channels, a single residue serves a "sentry" role to prevent Ca^{2+} permeation and is also crucial in the discrimination between Na^+ and K^+.

At the molecular level, Na^+ channels belong to the S4 family of voltage-gated cation channels (Noda et al., 1984). These ion channels are formed by four homologous domains arranged around a central conduction pore. Although in some cation channels the four domains are actually identical subunits, in Na^+ and Ca^{2+} channels the domains are homologous repeats within a single protein. The ion conduction pathway is lined by the four P-region segments located between the fifth and sixth putative transmembrane regions in each domain (MacKinnon, 1995). In both Na^+ and Ca^{2+} channels, the P-regions contain numerous charged residues. The pseudosymmetry of these channels dictates that when a charge appears at the same position in all four domains, the pore will contain a "ring" of charge. The initial illustration of the importance of these rings came from work localizing the tetrodotoxin and saxitoxin binding sites (Noda et al., 1989; Terlau et al., 1991). Neutralization of charged residues in either of two rings had large effects on both toxin affinity and single-channel conductance.

Subsequently, Heinemann et al. (1992) showed that one of these rings is critical in determining the differences in permeation properties between Na^+ and Ca^{2+} channels. As expected of residues that influence ionic selectivity, the residues in this ring differ between channels: in Na^+ channels, they are DEKA (one residue contributed each by domains I-IV), whereas in Ca^{2+} channels they are EEEE. Heinemann et al. mutated the Na^+ channel to make it similar to the Ca^{2+} channel by altering the residues in domains III and IV. The resulting mutant Na^+ channels displayed conduction properties that resembled those of Ca^{2+} channels. The DEEA and DEKE channels were permeable to both Na^+ and K^+. Furthermore, the double mutant DEEE channel was blocked by low Ca^{2+} concentrations, whereas it conducted current at high Ca^{2+} concentrations. Although the Heinemann et al. work stimulated substantial research into the question of permeation in Ca^{2+} channels (Yang et al., 1993; Ellinor et al., 1995), the problem of selectivity in Na^+ channels has only been addressed recently (Chiamvimonvat et al., 1996; Favre et al., 1996).

In the current study, Favre et al. use site-directed mutagenesis to examine the contribution of each charged DEKA residue in terms of its effect on two different permeation characteristics: exclusion of Ca^{2+} and discrimination between monovalent cations. They expressed the mutant channels in *Xenopus* oocytes and assessed the relative permeabilities to different ions by measuring the change in reversal potential with exchanges of the bath solution.

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Favre et al. used two approaches to address the issue of Ca^{2+} permeability. First they neutralized each of the charged DEKA residues to examine its contribution in the context of a normal channel. Mutant channels in which either the D(I) or E(II) residue was neutralized retained the basic property of excluding Ca^{2+}. In contrast, mutations at K(III) dramatically altered Ca^{2+} permeability. The effect of a K(III) mutation depends simply on the charge of the side chain: channels with positively charged side chains at K(III) do not conduct Ca^{2+}, whereas channels with neutral or negatively charged substitutions are permeable to Ca^{2+}. The authors concluded that the presence of a positively charged residue at the K(III) position acts as a "sentry" guarding against Ca^{2+} conduction.

Next Favre et al. began with a mutant channel that was uncharged (AAAA) at the DEKA positions and added charges back in domains I-III. The mutant channel became permeable to Ca^{2+} with the introduction of even a single negative charge in any one of domains I-III. Thus in the native Na^+ channel, the negative charges of the DEKA locus allow Ca^{2+} conduction in the absence of K(III), while the presence of K(III) is sufficient to inhibit Ca^{2+} permeation.

Although these mutant Na^+ channels conduct Ca^{2+}, they differ markedly from the L-type Ca^{2+} channel. Monovalent current through the Ca^{2+} channel is blocked with high affinity by Ca^{2+}, with an apparent K_i of ~3 μM (Lansman et al., 1986). Although Ca^{2+} blockade was not addressed in this work, the earlier Heinemann et al. study of mutant Na^+ channels showed micromolar affinity for Ca^{2+} blockade only when all four DEKA sites contain negatively charged residues (Heinemann et al., 1992). Furthermore, in the presence of Ca^{2+}, the mutant Na^+ channels show only a modest selectivity for Ca^{2+} over monovalent cations (Favre et al., 1996). The mutant Na^+ channels are actually more reminiscent of mutant Ca^{2+} channels, in which several of the EEEE charges are neutralized (Ellinor et al., 1995).

Although the ability of the Na^+ channel to prevent Ca^{2+} entry is determined simply on the basis of the charge at position K(III), the means by which the channel distinguishes among monovalent cations is more complex. Previous studies have shown that mutations at several positions near and within the DEKA tetrad disrupt Na^+ selectivity (Heinemann et al., 1992; Chiamvimonvat et al., 1996). By mutating each of the DEKA charged residues, Favre et al. confirmed that neutralization of either E(II) or K(III) affects the relative permeability of Na^+ to K^+. The precise nature of the K(III) side chain is crucial in this case, in sharp contrast to the role of K(III) in Ca^{2+} exclusion described above. Even the K(III)R mutation, which conserves positive charge, completely eliminates selectivity among monovalent cations.

How might K(III) be situated, such that other positively charged side chains can substitute for it in selecting against Ca^{2+}, but not in discriminating Na^+ from K^+? Favre et al. suggest several possibilities. In one model, K(III) prevents Ca^{2+} entry by Coulombic repulsion between the charged side chain and the Ca^{2+} ion. A second possibility is motivated by a consideration of ion binding sites in proteins of known structure. In this model, the authors propose that K(III) exerts its effects on ion permeation indirectly, by interacting with one of the DEKA carboxylate groups. With the neutralization of K(III), both negatively charged DEKA residues would be available to facilitate the conduction of Ca^{2+}. Favre et al. envision that the K(III) side chain acts as a "tethered ion" within the pore. As such K(III) could orient and stabilize groups that coordinate permeant ions. The precise mechanism for determining the selectivity of Na^+ over K^+ is obscure, but could involve an intimate coordination of the permeant ion with various carboxylate and carbonyl oxygen atoms. Then even slight perturbations could disrupt selectivity, and other positively charged residues would not necessarily substitute for K(III). It is easy to imagine how even conservative point mutations could perturb the precise geometry of such a coordination site if one of the carboxylates interacts directly with K(III).

The results and models presented here raise many questions that are amenable to experimentation. Is the "sentry" role of K(III) a simple result of Coulombic repulsion? If cysteine-replacement and sulfhydryl reagents are used at K(III) to change the chemical nature, but not the length, of the side chain, can the Na^+/K^+ selectivity be preserved? These results can also be used to investigate the symmetry of the Na^+ conduction pore. Can a positively charged residue prevent Ca^{2+} entry from any of the four DEKA sites or only from the third domain? How important is the relative positioning of the two negative and one positive residues at DEKA in preventing Ca^{2+} entry? Favre et al.'s confirmation of the crucial role that K(III) plays in Na^+ selectivity will provide a valuable basis for further work on Na^+ channel permeation.

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Want to Exchange Your Virus? Try Microdialysis and Raman

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Three years ago I presented a New and Notable review on the use of a microdialysis cell to observe the time-dependent effects of deuterium-hydrogen exchange on the Raman spectrum of bean pod mottle virus (Carey, 1993). In the source paper, Li et al. (1993) showed that as a solution of virus particles in H_2O is exposed to D_2O, it is possible via the Raman spectrum to follow the replacement of hydrogens by deuterium atoms in protein capsids and separately in the RNA bases, as a function of time. From this observation of exchange dynamics, the authors were able to reach structural information on the RNA-protein contacts, concluding, for example, that the 4CO-3NH-2CO network of uracil and the 6CO-1NH-2CNH_2 network of guanine form extensive and rigid contacts with the protein capsids. These results from Li et al. highlighted a unique advantage of Raman spectroscopy in that the technique is able to follow the details of $H \leftrightarrow D$ exchange in complex assemblies such as virus particles. They used a "homemade" microdialysis flow cell to bring about the exchange, which, at the same time, served as the Raman cell. Although the initial results from the use of this cell were never in question, it is necessary to define quantitatively the cell's isotope exchange characteristics in both spatial and temporal terms and to probe its limitations. These are the goals of Tuma and Thomas's (1996) latest publication, "Theory, design, and characterization of a microdialysis flow cell for Raman spectroscopy."

As any old-time Raman spectroscopist (tell me about mercury lamps, Grandpa!) will confirm, one of the delights of the Raman technique is its experimental flexibility. The laser, the spectrometer, and the photon detector may, of necessity, be "high tech," but the sample arrangement could still almost be back in the era of string and sealing wax science. As long as you can focus the laser beam in or on the sample and can collect the scattered light (not forgetting the direction of the incident **E** vector), there is complete flexibility over the nature of the sample. Thus, the sample arrangements can be fiendishly complex or, as in the case of Thomas's dialysis cell, simple and elegant. The latter is also easy to describe to the nonspecialist: imagine a standard glass capillary tube, 1 mm in internal diameter and 2 cm in length, in the horizontal plane and containing a drop of the macromolecular solution for examination. A laser beam entering the capillary in the vertical direction is focused into the drop, and the scattered light is collected by a lens and analyzed by a spectrometer to provide the Raman spectrum of the drop. Tens of thousands of such experiments have been performed, but Thomas and coworkers realized that if they threaded a narrow ($\cong$200 μm) dialysis tube through the glass capillary and the droplet, and sealed the capillary ends with wax, they could flow D_2O through the dialysis tubeand slowly (on the time scale of a few minutes) exchange D_2O into the H_2O solution in the drop. They could then observe the effect of this exchange on any macromolecules contained in the drop. Thomas's group has now used this approach with considerable effect in studying the exchange characteristics of the P22 virion (Reilly and Thomas, 1994) and the PRD1 virus II (Tuma et al., 1996), as well as the bean pod mottle virus, alluded to above. There are very interesting differences in the hydrogen exchange properties of the constituents in these three systems, but here the emphasis will be on the biophysical/chemical experiments undertaken to characterize the microdialysis cell.

The underlying assumption governing the operation of the microdialysis cell is that diffusion governs the transport of small molecules between the membrane boundary of the microdialysis tubing and the glass capillary containing the macromolecular solution. The applicability of a diffusion model was justified by agreement between calculated and measured efflux rates for a number of solutes. Thus, the efflux of D_2O, calcium ions, and EGTA were measured via the appearance of their characteristic Raman features and found to be consonant with calculated values. Applicability of the microdialysis technique to pH titrations was explored by measuring the kinetics of glutamate protonation in the Raman spectrum of a peptide copolymer [poly-(Glu,Lys,Tyr)]. The results showed that pH-titration rates of up to 3.3 pH units/min can be monitored. The kinetics of deuterium exchanges in single and double stranded nucleic acids and with basic pancreatic trypsin inhibitor were all in agreement with the values obtained by other techniques. The message from all this is that the Raman microdialysis cell is a reliable means of measuring isotope exchange kinetics and that it appears to be free of unpleasant surprises for the unwary investigator. Tuma and Thomas also discuss modifications to the design of the cell to improve the present time resolution of about 2 min; they discuss

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