New and Notable

Occult Physiology: Electrical Cross-Talk between Membrane Lipid, Occluded Ions, and the Na-K ATPase

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It has long been known that electrogenic membraneous enzymes possess exquisite sophistications in their operation beyond merely acting as primary generators of, or being driven by, a transmembrane potential difference. My first appreciation of the intricacies these systems may possess or their response to otherwise hidden mechanisms arose during my Ph.D. studies when I read an article by McLaughlin et al. (1). This suggested how characteristic current-voltage relationships embodied in the Hodgkin-Huxley relations could undergo translational phase shifts along the voltage axis as a result of the presence of differing membrane electrostatic surface potentials. Since then, an incredible expansion of knowledge has taken place, ranging from atomic-level structural detail to systems and genomic information that have revealed many of the inner-workings of these molecular systems operating in a physiological context. Nevertheless, many outstanding questions remain regarding the explicit mechanistic reaction cycles of these systems. It is rather nice therefore, to see the recent article by Mares et al. (2) that reveals a possible mechanism and resolves a hitherto unexplained property of the Na/K-ATPase, perhaps the most heavily studied ion pump in existence.

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The analysis outlined in the article of Mares et al. (2) is both thoughtful and imaginative in addressing problems of the catalytic cycle of the Na/K-ATPase. The essence of their work is that they provide a mechanistic explanation of how ions that are in the process of being pumped across the membrane are prevented from returning to their original aqueous compartment (i.e., outside or inside the cell), underlying their vectorial movement across the membrane. The longstanding consensus among molecular physiologists (e.g., Hilgemann (3)) is that ions are considered to traverse intraprotein water-occupied channels to enter and leave binding sites on the extracellular face of the protein; they are thought to be free to migrate in this region and respond to the transmembrane field with the binding reaction, then exhibiting characteristic voltage-dependency. This is suggested to be possible due to an ion occlusion process, essentially isolating the ion within the protein.

Mares et al. (2) pose the question that if such ion binding is a voltage-dependent reaction and causes changes in the local electric-field strength within membrane, this could be observed using the fluorescence membrane probe RH421 (4). Interestingly, they saw no changes in fluorescence that could be attributed to voltage, and only occludable ions (K^+, Rb^+, or Cs^+) led to changes in fluorescence. This implied that the ion binding event is not a voltage-dependent reaction, whereas (only) ion occlusion is—a conclusion contradictory to some previous results (5). Mares et al. (2), however, nicely reconcile this inconsistency by deeper considerations of the nature of the electrical environment within the membrane and the protein and its influences on the kinetics of separate reaction steps of the Na/K-ATPase. Specifically, they suggest that electric fields influencing a particular reaction coordinate have different roles to play depending upon whether they arise from the transmembrane electrical potential difference or the membrane dipole potential. The latter is a more localized intramembrane electrical field arising from partial charges associated with lipids, water, and proteins.

Mares et al. (2) are not splitting hairs when they make this distinction, inasmuch as the differences in origins and behavior of both these membrane potentials are substantial. There is much scope for confusion related to membrane potentials, however, because three such electrical properties related to membranes are known to exist. Unfortunately, each has been referred to by this broad term. The physical origins of each are separately derived in a treatise on Bioelectrochemistry (6) illustrating their formal differences, and separately a short review outlines what this means in terms of possible biological effects (7). What Mares et al. (2) do with their elegant analysis is to offer a new avenue for considering the mechanistic roles that intramembrane dipole potentials may play in the catalytic mechanisms of membrane proteins.

Mares et al. (2) suggest that ions occluded within the Na/K-ATPase involve local deformations in the lipid membrane surrounding the protein. Their explanation suggests deformations in the lipid occurring simultaneously with conformational changes necessary for ion occlusion. Their use of a fluorescent probe detects a change in dipole potential arising from reorganization of lipids induced by the ATPase conformational change (not by binding of the ion). The membrane deformation thus results in changes in membrane dipole potential. Mares et al. (2) attempt to further characterize some of these effects using modeling approaches (e.g., using the software CHARMM) with some success.

The more general message arising from the work of Mares et al. (3) points to a way of considering how membrane dipole potentials may play roles in the

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catalytic mechanisms of the many complex membrane protein structures such as ion pumps. Thus, and despite the availability of high-resolution structural information (e.g., Shinoda et al. (8)), important details of many such mechanisms remain elusive. By using a fluorescent probe to complement the structural information, genuine progress seems to be possible. Such probes (e.g., Haldar and Chattopadhyay (9)) report more of the dynamic properties of molecular environments, and in conjunction with molecular structural techniques and computational modeling, allow better descriptions of the molecular processes. Mares et al. (2), utilizing all these approaches, illustrate that molecular geometry, particularly of partial charges associated with membrane components, can elicit effects on energetically minor events that lead to significant changes of the overall molecular behavior.

We had previously demonstrated (10) a complementary phenomenon by showing that the membrane dipole potential has significant effects on the structural geometry of intramembrane protein sequences (particularly α-helices) and related some functional effects (e.g., O'Shea (7)). Mares et al. (2), however, emphasize the local profiles of the dielectric interior and surfaces of the membrane and protein. Thus, for example, the distance-dependent profile of the dielectric constant of the membrane surface is complex and significantly different compared to that of bulk water (e.g., Robinson et al. (11)) with related complexities of the dielectric properties of a protein's interior (e.g., Haldar and Chattopadhyay (9)). Mares et al. (2) indicate how this, together with modification of the electric environment, plays a pivotal role in the catalytic mechanism of the Na/K-ATPase. This is already a very nice piece of work, but in addition what is quite exciting is that different lipid environments are known to lead to quite different dipole potentials with different surface dielectric constants (e.g., Robinson et al. (11)), and these may exert effects on the catalytic behavior of such membrane proteins along the lines described by Mares et al. (2). With this in mind, their work offers a route to understanding how the activity of a membrane protein may be modulated depending upon whether it is localized to a microdomain or not.

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