SEMINARS IN
NEPHROLOGY

Structure-Function Relationships in the Na^+,K^+-Pump

Dwight W. Martin

The Na,K-pump was discovered about 50 years ago. Since then there has been a methodic investigation of its structure and functional characteristics. The development of the Albers-Post model for the transport cycle was a milestone that provided the framework for detailed understanding of the transport process. The pump is composed of 2 subunits that exist in the membrane as an $\alpha\beta$ heterodimer. All known enzymatic functions of the pump occur through the α subunit. Although necessary for activity, the complete role of the β subunit is not understood fully. Numerous studies have established that the $\alpha\beta$ protomer is the minimal functional unit needed to perform the Albers-Post reaction cycle. However, higher orders of aggregation [$(\alpha\beta)_n$] are commonly detected. There is little evidence that oligomerization has functional consequence for ion transport. The Na^+,K^+-adenosine triphosphatase (ATPase) is a member of the P-type ATPase family of transporters. Proteins within this family have common amino acid sequence motifs that share functional characteristics and structure. Low-resolution 3-dimensional reconstruction of 2-dimensional crystal diffractions provide evidence for the similarity in tertiary structure of the α subunit and the Ca^{2+}ATPase (a closely related P-type ATPase). The spatial location of the β subunit also is obvious in these reconstructions. Recent high-resolution reconstructions from 3-dimensional crystals of the Ca^{2+}ATPase provide structural details at the atomic level. It now is possible to interpret structurally some of the key steps in the Albers-Post reaction. Some of these high-resolution interpretations are translatable to the Na^+,K^+-ATPase, but a high-resolution structure of the Na,K-pump is needed for the necessary details of those aspects that are unique to this transporter.
Semin Nephrol 25:282-291

KEYWORDS Na^+,K^+-ATPase, Na, K pump, structure, Na(+)-K(+)–exchanging ATPase

Essentially all animal cells maintain a cytoplasmic environment that is relatively high in potassium and low in sodium compared with extracellular space. The mystery of how this ionic gradient is maintained began to be unraveled in the early 1950s as the red cell developed into a valuable system for studying sodium and potassium transport.[1] In 1957, Skou[2] discovered a membrane-associated enzyme in nerve tissue that hydrolyzes adenosine triphosphate (ATP) in the presence of Na and K. Subsequent studies established that the Na^+- and K^+-dependent ATPase and the Na,K-pump studied in red cells were the same enzyme.[3] (In 1997, Skou[4,5] shared the Nobel Prize in Chemistry for his classic work in the discovery and characterization of the Na^+,K^+-ATPase.)

Division of Hematology, Stony Brook University, Stony Brook, NY.
Supported in part by National Institutes of Health grant DK-19185.
Address reprint requests to Dwight W. Martin, PhD, Division of Hematology, Stony Brook University, Stony Brook, NY 11794-8151. E-mail: dwight.martin@sunysb.edu

Since its initial identification, a tremendous scientific effort has been devoted to understanding the details of the structure and function of this enzyme. The need for this understanding is made clear when one considers the important physiologic roles of the Na^+,K^+-ATPase (also known as *Na^+,K^+pump*, *Na^+ pump*). The ionic gradient created by the Na^+,K^+-ATPase is responsible for maintaining the membrane potential in vertebrate cells that is essential in excitable cells, making action potentials and neuronal transmission possible.[6] A major role of the Na^+,K^+-ATPase is that of an energy transducer converting the chemical energy from the hydrolysis of ATP to the chemical potential energy created by an ionic concentration gradient. This gradient is coupled to and provides the energy for other transport processes that are responsible for cell volume regulation; for secretory processes in epithelia; for intracellular transport of vital solutes such as glucose, amino acids, and neurotransmitters within the organism; and for the absorption of metabolites from the intestine.[3,7,8] The electrochemical gradient generated by the

 0270-9295/05/$-see front matter
doi:10.1016/j.semnephrol.2005.03.003

Na^+,K^+pump provides the driving force for reabsorption of sodium and many other vital solutes in the kidney where the pump exists in its greatest concentration in the outer renal medulla.[9] In a resting animal, greater than 20% of cellular ATP is consumed by the Na^+,K^+-ATPase to maintain these and other functions.[3] One easily could characterize the Na^+,K^+-ATPase as a sine qua non of animal life. This review provides an overview of what is known currently about the structure-function relationship for the Na^+,K^+-ATPase. I have tried to avoid most speculative issues although certainly some aspects remain open-ended. There is a vast pool of biochemical data relating to the issues discussed later and most of the details of that data are beyond the limits of this review. However, for those interested in more biochemical and molecular biological details, I refer the reader to these reviews and the references found therein for classic background[3] and more recent developments.[10,11]

The Albers-Post Reaction Model

Before structural details about the pump were obtained, there was an accumulating body of data concerning the functional aspects and mechanistic requirements for the active transport process. In the mid-1950s, evidence collected, in the main, from studies using red blood cells established some fundamental properties of the transport process. Ouabain, a plant-derived steroid, was shown to be a potent inhibitor of the transporter and proved to be a valuable tool in the dissection of Na^+,K^+-ATPase function.[12] Pioneering work by Post and Jolly[13] established that the transport stoichiometry was 3 Na^+ transported out of the cell for 2 K^+ transported into the cell. The 3 Na/2 K–exchange ratio of the Na^+,K^+-pump remains a unique feature of this ATPase. Another characteristic feature of the Na^+,K^+-ATPase is that during the course of ion transport the pump goes through a cycle that involves, in the presence of Na^+, the transfer of the high-energy phosphate of ATP to form a covalently bound phosphorylated intermediate of the transport enzyme.[14] This phosphorylated intermediate represents a key step in the transfer of the energy needed for active transport. Further along the reaction cycle, the phosphate group is released from the enzyme (promoted by K^+) as a free phosphate (P_i), and the pump returns to a state ready to bind another ATP. It was observed that the pump reaction cycle is reversible and under the proper conditions the ATPase generates ATP from adenosine diphosphate and P_i.[15-22] These observations prompted proposals in the mid- to late 1960s of a reaction that now is referred to commonly as the Albers-Post model.

The Albers-Post model has provided a framework for the investigation of the mechanism of the Na^+,K^+-ATPase for more than 30 years and has been shown to be applicable to other transport proteins that belong to the general class of P-type ATPases discussed later. As more information has been obtained the model has been revised with greater detail. A simplified version of it appears in Fig. 1. A key feature of the reaction model is that the ATPase must exist in at least 2 major conformational states, characterized as E_1 and E_2. Simply stated, in the E_1 conformation the ion binding sites face the cytosol and have a high affinity for Na^+ and a low affinity for K^+, in the E_2 state the binding sites face the extracellular space and have a lower affinity for Na^+ ($K_D > 0.3$ mol/L) and a higher affinity for K^+ ($K_D < 1.3$ mmol/L). Another feature of the reaction model is that at particular stages of the transport cycle the ions are occluded and presumably inaccessible to either the cytoplasmic or extracellular surfaces of the cell. Additionally, 1 of the 3 Na^+ ions is bound with a lower affinity than the other 2 and comes off the transporter in a distinct step.[23-25] There has been extensive kinetic investigation of this reaction scheme and controversy still exists about the details of some steps. ATP can bind to either the E_1 or E_2 conformation of the ATPase. In the E_1 conformation, ATP binds with high affinity ($K_D \sim 50$ nmol/L) whereas it binds with a reduced affinity to the E_2 state ($K_D \sim 300$ μmol/L). The binding of ATP to the E_2(2K) form accelerates the conformational transition from E_2 to E_1 and the release of K^+ into the cytosol. Under optimal conditions the overall reaction can proceed at a rate approaching 10,000 cycles/min.[26]

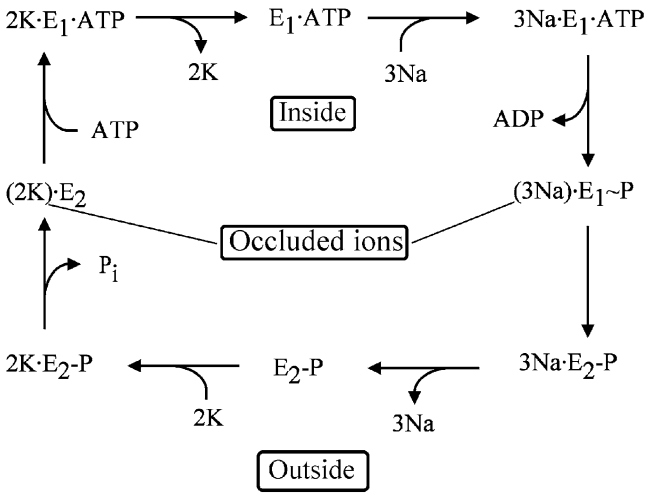

Figure 1 The Albers-Post reaction cycle. This is a simplified version of the reaction cycle as it is currently understood. Na release to the outside, which is shown as a single step in this figure, actually occurs in 2 steps with 1 ion being released as a step separate from the release of the other 2 ions. The arrows indicate the normal direction of the reaction cycle, however, all steps are reversible. Adapted from Glynn.[129]

Na^+,K^+-ATPase Structure

Subunits

The Na^+,K^+-ATPase is composed of 2 protein subunits termed α and β. The α subunit has a molecular weight of approximately 112,000, based on amino acid composition. The β subunit has an amino acid mass of approximately 35,000, but it is highly glycosylated. On a typical electrophoretic gel the α subunit migrates as a well-defined band with a M_r of approximately 100,000 whereas the β subunit presents itself as a diffuse band that ranges from an M_r of approximately 45,000 to 58,000. Virtually all that is known about the function of the Na^+,K^+-ATPase pertains to opera-