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Article *in* Physiological chemistry and physics and medical NMR · February 2001

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A Note

How to Explain Established Relationships between Ion Fluxes across Cell Membranes and Na,K-ATPase Activities under the Assumption that the Na,K-ATPase is no Ion Pump

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ACCORDING TO THE MEMBRANE PUMP THEORY (MPT)—described in virtually all textbooks—the Na,K-ATPase is the Na,K pump which is responsible for an active transport of the Na^+ ion from the cytoplasm of a living cell into the extracellular liquid and for the simultaneous active transport of K^+ from the outside solution into the cytoplasm. According to the MPT it is assumed that most of the cellular alkali-metal ions K^+ and Na^+ are freely dissolved in free cellular water. Hence the maintenance of the steady state between extracellular high Na^+, low K^+ concentrations and intracellular low Na^+, high K^+ concentrations by means of the ion pump must be an energy consuming process. Energy that is stored in the ATP molecule is liberated during hydrolysis of ATP by means of the ATPase (Skou, 1957) and is used for fueling the pump. The idea that the Na,K-ATPase is the pump has been deduced from the following experimental findings (Post *et al.,* 1960; Dunham and Glynn, 1961):

- The Na,K-ATPase and the postulated ion pump are present in the cell membrane.
- Both systems utilize ATP but not inosine triphosphate.
- Both systems require the presence of Na^+ and K^+.
- Both systems require the same concentration of cations for half-maximal activity.
- Both systems are inhibited by cardiac glycosides (e.g., ouabain).

Taken together, the enzyme system and the transport system are qualitatively and quantitatively the same. Further support for this conclusion was provided by the demonstration of a significant correlation between the Na,K-ATPase activity and the active cation flux in six tissues (Bonting and Caravaggio, 1963).

However numerous experimental findings—almost completely ignored by the scientific establishment—show that the idea of ion pumps situated in the cell membrane of unifacial cell systems is a misconception (Ling, 2001, chapter 12). In 1962 Ling presented the results of a three year long inquiry into the feasibility of the postulated Na^+ pump from a thermodynamic standpoint. He compared the minimum energy need of the postulated Na^+ pump in frog muscle cells for a recorded period of time with the maximum energy available to these cells during the same period of time. The experiments—carried out at 0°C—showed that the minimum energy need of the postulated Na^+ pump is from 1500% to 3000% of the maximum available energy (Ling, 1962, chapter 8). Disparities of this magnitude are decisive by any standard and until now there has been no challenge in print against the experiments, nor the conclusions of Ling. In addition, experiments designed to prove directly the existence of pumps failed. The perfused squid axon contains functioning Na,K-ATPase and is able to increase Na^+ efflux by addition of ATP, and the efflux is sensitive to ouabain; however, a net Na^+ efflux against an electrochemical gradient could not be observed (for review see Ling, 1984, p. 127). Similar results were obtained with nonleaky "white ghosts" obtained from red blood cells. These ghosts are not able to accumulate K^+ or extrude Na^+ despite the fact that they contain Na,K-ATPase functioning in a normal manner (Ling and Tucker, 1983). In addition, Ling and Negendank (1980) analyzed the claim that phospholipid-ATPase vesicles pump Na^+. They came to the conclusion that ATP did not actually cause a net gain of Na^+ by these vesicles. The criticism of Ling and Negendank has not been refuted in print. (Note that the active transport across bifacial cell systems like different epithelia or frog skin has not been disputed, rather, models explaining this active transport have been published by Ling 1965, 1981, 1990).

Based on the finding that the pump idea is not tenable and by using statistical mechanics the association-induction hypothesis (AIH) has been developed (Ling, 1962, 1984, 1992, 2001). The AIH is an equilibrium theory that describes physiological phenomena of living cells without membrane pumps by considering the molecular interactions between the main components of living cells. "The source of energy for biological work performance is the *potential energy sustained in the high-energy resting living state*. The cooperative interaction among the major components of the cell—including proteins, water, and ions—and cardinal adsorbents like ATP maintains a high state of potential energy which is transformed into work when a *key ingredient, ATP*, is removed or otherwise destroyed (Ling, 1984, p. 314)." (According to Ling, 1992, p. 15, the idea that a high energy stored in ATP can be used for work performance is a misconception: Podolsky and Morales, 1956 and George and Rutman, 1960 have clearly shown that *the so called "high-energy phosphate bond" of ATP does not contain high energy*). It is noteworthy that the AIH is able to describe the four physiological manifestations of living cells, namely solute distribution, solute permeability, volume regulation, and cellular electrical potentials by rigorously derived equations whose parameters have clearly defined physical significance (Ling, 2001, p. 282 ff.).

In the following experimentally verified postulations incorporated in the AIH are sketched what are used to explain the physiological role of the Na,K-ATPase during ion movements into and out of living cells.

(1) The cell surface (membrane) region and the cytoplasm are areas endowed with similar but not necessarily identical protein-ion-water-cardinal sites systems in which monovalent cations (K^+, Na^+) are adsorbed with a high selectivity to β- and γ-carboxyl groups car-

ried respectively on the aspartic and glutamic residues of proteins. In the resting state *most adsorption sites are occupied by K^+ ions* (Ling and Ochsenfeld, 1966; Ling, 2001, chapter 10; Edelmann, 1988, 2001). The water in these systems is not normal free water but organized hydration water (polarized multilayers, Ling, 1965; Ling & Negendank, 1970). Water polarized in multilayers suffers both translational and rotational restriction and it has *reduced solubility for the highly hydrated Na^+ ion* as well as for large and complex molecules (Ling, 2001, chapter 11).

(2) For maintaining subunits of these systems in the metastable living state binding of ATP at cardinal sites is essential. A metastable subunit controlled by an ATP molecule is changing its structure as soon as ATP is hydrolyzed with the result of liberation of adsorbed ions and of a break down of the organized water structure (Ling, 2001, chapter 14).

(3) Movements of ions into and out of cells may happen on two paths: either *via* water domains or *via* the adsorption-desorption route (Ling and Ochsenfeld, 1965).

We now consider possible events that may be caused by different activities of the Na,K-ATPase present in the cell membrane. At a very low ATPase activity, e.g., at 0°C, extra- and intracellular mobile ions (Na^+, K^+) of frog muscle cells are continuously exchanging by using the above mentioned paths. The cation adsorbing sites of the surface area and of the cytoplasm prefer K^+ ions with a high selectivity—even at this low temperature. The asymmetric distribution of Na^+ and K^+ between the cell and the external solution will be maintained for a rather long period of time (Ling, 1962, chapter 8). With increasing ATPase activity at higher temperatures more and more ATP molecules of the membrane system are hydrolyzed. As a consequence, certain proteins of the above mentioned metastable subunits that are no longer under the control of ATP change structure and loose their capabilities of polarizing water molecules and of adsorbing alkali-metal ions. (Ling, 2001, p. 184, Figure 56). Liberated ions diffuse into the free surrounding water and diffuse simultaneously into the extracellular solution and towards the cytoplasm. Those ions reaching the adjacent cytoplasm will compete for the cytoplasmic anionic sites that are still available with their normal high selectivity. The disturbed subunits of the cell membrane will be brought back to their metastable living state as soon as newly formed ATP is bound to their cardinal sites.

According to this model the ATPase is not a pump but only a protein that hydrolyzes ATP. This event leads to electronic changes in certain proteins with the result of local water structure changes and liberation of adsorbed ions. By means of this mechanism the cytoplasm is transiently and locally exposed to a higher K^+ concentration (or to different Na^+ and K^+ concentrations) than is found in the extracellular solution. The action of the ATPase is thus leading to an accelerated exchange of extra- and intracellular cations and in general to local higher membrane permeabilities of different kinds of solutes.

The described model can explain why the maintenance of asymmetric distributions of Na^+ and K^+ between muscle cells—kept at a low temperature—and the extracellular solution does not require much energy. It can explain why perfused squid axons and white ghosts with perfect functioning ATPase are not pumping Na^+ or K^+ and it can also explain why there is usually a good correlation between the ATPase activity and flux rates—even in the perfused squid axon. Understanding of the simultaneous effect of ouabain on the protein ATPase and on ion fluxes poses no difficulties. The general effect of ouabain is a selectivity change of the anionic sites of cellular proteins towards alkali-metal ions (Ling, 2001, chapter 15). Hence, not only ATPase activities and flux rates but also the ratio of different adsorbed cellular cations (K^+, Na^+) change in predictable directions upon ouabain treatment.

Finally, the coupling of transmembrane movements of many solutes (e.g., sugars) to different Na^+ fluxes at different ATPase activities is also readily explained.

One may ask whether there is experimental support for the view that the surface region of cells is a specific ion adsorbing system different from the cytoplasm. Indeed, by measuring the efflux of the alkali-metal ions K^+, Rb^+, and Cs^+ from ion loaded guinea pig papillary muscle cells—kept at 37°C—it was found that a small fraction of the total cellular ions exchanged rapidly with extracellular ions (Edelmann *et al.*, 1974). This fraction was localized in the cell surface region and could be related to the height of the resting potential which is a function of the amount of surface adsorbed ions (Edelmann, 1973). The exchange of the bulk of cytoplasmic ions took place at much lower rates. Such a phenomenon could not be detected with frog muscle experiments carried out at 20°C or lower temperatures. The ATPase activity is certainly much higher in mammalian heart muscle cells at 37°C than in the investigated frog muscle cells and detection of the amount of surface adsorbed ions by flux studies at low ATPase activities is probably very difficult.

Consideration of postulated and experimentally verified molecular mechanisms of the AIH led to the described model of ion transport across cell membranes with activated Na,K-ATPase. The model appears to be simple and convincing. In my opinion it would be worthwhile to reconsider generally the role of ATPases during physiological work performance according to the AIH as it has already been done explicitly for the work performance during muscle contraction (Ling, 1984, pp. 566–584).

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Received September 13, 2001;
accepted October 13, 2001.