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ARTICLE

Simo Salminen · Aarne Ekman · Jussi Rastas

Distributions of Li^+, Na^+, K^+, Rb^+, and Cs^+ tracer ions in erythrocytes at 38 °C in relation to entry rates of these ions into cells at 0 °C

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Abstract Forces that are able to transport Na^+ and K^+ into two compartments were investigated. A modified Nernst-Planck equation for coupled flows of electric current, water, and ions was integrated. The result shows that if alkali ions in the ion channel of the cell membrane are separated by their electric-current-induced inward flows against an electro-osmotic outward flow of water, the logarithms of the stationary cell/medium distributions of these ions should be proportional to the inverse of their diffusion mobilities. The relationship was tested in human erythrocytes. From inward and outward movements of tracer alkali ions, calculations were made to obtain their stationary distributions at infinite time. The cell/medium distributions determined in this way at 38 °C are $Li^+ = 0.59$, $^{22}Na^+ = 0.044$, $^{42}K^+ = 10.0$, $^{86}Rb^+ = 11.9$, and $^{137}Cs^+ = 3.07$. The entry rates of ions into the cell at 0 °C are understood to represent their diffusion mobilities in the pump channel. The entry rates are $Li^+ = 1.44$, $^{22}Na^+ = 1$, $^{42}K^+ = 2.22$, $^{86}Rb^+ = 2.39$, and $^{137}Cs^+ = 1.72$ relative to that of $^{22}Na^+$. There is an expected negative correlation between the logarithms of the stationary cell/medium distributions at 38 °C and the inverse of the entry rates into the cell at 0 °C for the five ions. It is suggested that the proposed physical forces cause the separation of alkali ions in the channel of Na,K-ATPase.

Key words Nernst-Planck equation · Ion mobility · Electric current · Pump channel · Na,K-ATPase

Introduction

There is an abundance of experiments showing that the Na,K-ATPase enzyme is involved in the separation of Na^+ out of and K^+ into the cell. However, despite decades of investigations, the basic transport mechanism of these ions has remained a major unresolved issue (Blostein 1999). The Na,K-ATPase is a kind of ''black box''; there is a lot of data available on *what* this box is doing, but hardly any idea of *how* it is done (Apell et al. 1998).

It may be useful to look at the forces that are able to separate Na^+ and K^+ into two compartments. We attempt to explain the function of the Na,K pump in human erythrocytes utilizing concepts of physical chemistry and electrochemistry. Multitracer experiments are designed to test whether the theoretically expected relationship of the metabolism-induced stationary cell/medium distributions of different alkali ions to their metabolism-independent diffusion mobilities in the ion channel exists.

S. Salminen (✉)
Wihuri Research Institute, Helsinki, Finland
E-mail: simo.sal@fimnet.fi

A. Ekman · J. Rastas
Laboratory of Physical Chemistry and Electrochemistry, Helsinki University of Technology, Espoo, Finland

Movements and distributions of ions across the membrane

Previous model systems

In a hydrostatic pressure-driven two-compartment system, the logarithm of the stationary selection ratio between Na^+ and K^+ across a porous membrane is a linear function of the rate of water flow through the membrane (Teorell 1951; Nims and Thurber 1961; Ekman et al. 1963; Rapoport 1965). Because of the different interactions of Na^+ and K^+ with water, the flow of water is an effective ion-selective carrier for these ions. High selectivity has been demonstrated, with the K^+ to Na^+ ratio increasing in the compartment from which water is flowing out (Rastas et al. 1966). The concentrations of both ions, however, decrease in this compartment. To explain the high concentration of K^+ in the cell, an additional force that drags cations against the flow of water is required. Such a system has been accomplished in a model driven by simultaneous flows of an acid and water. Owing to these flows, and the

mobility differences between the ions involved, Na^+ and K^+ are transported in opposite directions in the membrane (Ekman et al. 1963; Salminen 1963). Teorell (1964) discusses a complicated system driven by diffusion, electric force, and convection. The author points out that fixed charges in the membrane may introduce an electro-osmotic contribution to the convection. Ekman et al. (1978) show that very high concentration gradients and selectivities in the distributions of cations are achieved when an electric current is added to an aqueous multicomponent diffusion system.

Forces that move ions in the membrane

The movement of an ion in relation to water depends on its concentration gradient and the concentration gradients of other components and on the electric current. The movement of an ion in relation to the membrane is expressed in a somewhat simplified form by:

$$J_i = -D_i \mathrm{d}c_i/\mathrm{d}x + c_i v + D_i c_i \varepsilon = -D_i \mathrm{d}c_i/\mathrm{d}x + c_i \alpha_i \qquad (1)$$

where D_i is the "diffusion coefficient" of ion i, v is the velocity of water flow, and α_i is the sum $v + D_i\epsilon$. In essence, the Nernst-Planck equation is modified to include the term $c_i v$ that takes into account the flow of water.

Equation (1) does not explicitly contain the gradient of electric potential. The electric force ϵ is the current density divided by the electrical conductivity, plus a small contribution from the interaction with other ions. The dimension of the electric force ϵ is $(\text{length})^{-1}$; D_i is proportional to the mobility of the ion i.

The measured quantities were relations involving the amounts of tracers and time. A similar method has been used in diffusion and self-diffusion experiments (Rastas 1966). For this reason, and for some formal simplicity, we have used the quantity diffusion coefficient rather than mobility in our formulas.

The relationship between stationary distributions and mobilities of ions in the cell

The variables in Eq. (1) can be separated and the equation integrated from the outside of the cell, where $x = 0$ and $c_i = c_i'$, to the inside, where $x = l$ (the pore length) and $c_i = c_i''$. The result, where the index i is dropped for simplicity, is:

$$(D/\alpha)\ln[(\alpha c'' - J)/(\alpha c' - J)] = l \qquad (2)$$

In the stationary state when $J = 0$, Eq. (2) gives:

$$\ln(c''/c') = \alpha l/D \qquad (3)$$

The flow per cell volume $(A/V)J$ (A is the pore area and V is the cell volume) can be determined by the tracer rate constants k_{in} and k_{out} for inflow and outflow of the tracer:

$$(A/V)J = k_{\text{in}}c' - k_{\text{out}}c'' \qquad (4)$$

In the stationary state, this with Eq. (3) gives:

$$1\mathrm{n}(k_{\text{in}}/k_{\text{out}}) = 1\mathrm{n}(c''/c') = \alpha l/D = vl/D + \text{constant} \qquad (5)$$

Note that $\alpha = v + D\epsilon$ is constant. The ionic "diffusion coefficient" can be estimated from experiments where diffusion prevails. From experiments of Thurber and Thompson (1967) [see also Nims (1968)] we conclude that this is the case at 0 °C, when the rate of metabolism is low, $v \to 0$, and α is small. In this case:

$$J = (D/l)(c' - c'') \qquad (6)$$

On the other hand, the flow per cell volume measured with the aid of tracer rate constants at 0 °C is given by Eq. (4), and from this and from Eq. (6) we obtain:

$$(A/V)(D/l)(c' - c'') = k_{\text{in}}c' - k_{\text{out}}c'' \qquad (7)$$

At 0 °C the system approximates the state of equilibrium where flows stop and $J = 0$. The diffusion coefficient D is directly proportional to the tracer rate constants k_{in} and k_{out} measured at this temperature. The entry rates of tracer ions into the cells at 0 °C were taken to represent their relative diffusion coefficients (mobilities) in the pump channel.

Equations (5) and (7) show that if the metabolism-induced compartmentalizations of ions comply with the differential Eq. (1), the logarithms of the stationary $k_{\text{in}}/k_{\text{out}} = c_{\text{cell}}/c_{\text{medium}}$ distribution ratios at 38 °C should be negatively correlated to the inverse of the entry rates of these ions into the cell at 0 °C. To test the proposed relationship, experiments with more than two different alkali ion species have to be carried out.

Calculation of stationary cell/medium distributions and rate constants of tracer ions

The flow of the tracer into the cell from the outside is:

$$J = \mathrm{d}c''/\mathrm{d}t = k_{\text{in}}c' - k_{\text{out}}c'' \qquad (8)$$

where c'' and c' are the inside and outside concentrations and k_{in} and k_{out} the rate constants, respectively. The volumes of the inside and outside compartments were adjusted to be equal. The cell/medium ratio of the concentrations is:

$$R = c''/c' \qquad (9)$$

Differentiated with respect to time it gives:

$$\mathrm{d}R/\mathrm{d}t = [\mathrm{d}c''/\mathrm{d}t - (c''/c')\mathrm{d}c'/\mathrm{d}t]/c' \qquad (10)$$

The total amount of the tracer in the system is constant, and the volumes of the compartments are equal. Thus $\mathrm{d}c'' = -\mathrm{d}c'$, and:

$$dR/dt = (1/c')(1+R)dc''/dt = (R/c'')(1+R)dc''/dt \quad (11)$$

From Eqs. (8), (9), and (11) we obtain:

$$dR/dt = R(1+R)(k_{in}/R - k_{out}) = -k_{out}R^2 + (k_{in} - k_{out})R + k_{in} \quad (12)$$

When the stationary state is reached, the net flow of the tracer ceases, and the constant c_{cell}/c_{medium} distribution ratio R_∞ of tracer concentrations exists:

$$R_\infty = c''_\infty/c'_\infty = k_{in}/k_{out} \quad (13)$$

With this, Eq. (12) can be rewritten as:

$$(1/k_{out})dR/dt = -R^2 + (R_\infty - 1)R + R_\infty \quad (14)$$

Integrating this differential equation from $t=0$ and $R=0$ to $t=t$ and $R=R$, we obtain:

$$\ln[(1+R)/(|R_\infty - R|)] + \ln R_\infty = (k_{in} + k_{out})t \quad (15)$$

and further:

$$\ln[(1+1/R)/(|1/R - 1/R_\infty|)] = \ln[(1+\tilde{R})/(|\tilde{R} - 1/\tilde{R}_\infty|)] = (k_{in}+k_{out})t \quad (16)$$

where $\tilde{R}_\infty = 1/R_\infty = c'_\infty/c''_\infty = k_{out}/k_{in}$.
When $R \to 0$, from Eq. (14) we obtain:

$$dR/dt \to R_\infty k_{out} = k_{in} \quad (17)$$

and correspondingly when $\tilde{R} \to 0$:

$$d\tilde{R}/dt \to k_{out} = k_{in}\tilde{R}_\infty \quad (18)$$

The experiments were started with the tracer in the medium $R=0$ for inflow, or in the cells $\tilde{R} = 1/R = 0$ for outflow. The ratio $R_\infty = 1/\tilde{R}_\infty$ for infinite time was determined by searching the values of R_∞ and $\tilde{R}_\infty$ which gives the same constant $(k_{in}+k_{out})$ in the inflow and outflow experiments according to Eqs. (15) and (16). The values for k_{in} and k_{out} were then derived individually from Eqs. (17) and (18).

The stationary tracer distribution ratio $R_\infty = k_{in}/k_{out}$ could thus be determined within a reasonable time in vitro for ion species that in vivo do not exist in the system.

Materials and methods

Fresh heparinized human blood was centrifuged, then the plasma and buffy coat were discarded. Erythrocytes were washed with buffer solution with the composition (mmol/L): NaCl 112, $MgCl_2$ 1.0, $CaCl_2$ 0.5, $NaHCO_3$ 30, Na_2HPO_4 1.7, KCl 4.3, KH_2PO_4 0.4, and glucose 13.9. The pH was 7.4. The cells were resuspended in the buffer, incubated in a water bath at 38 °C or at 0 °C and mixed gently. When the cell suspension had reached the desired temperature, the inflow experiment was started by adding a mixture of Li^+, $^{42}K^+$, $^{22}Na^+$, $^{86}Rb^+$, and $^{137}Cs^+$ tracers dissolved in sufficient buffer solution to give a hematocrit of 50%.

For outflow experiments, a separate set of cells was incubated at 38 °C for 120 min in a medium containing all the five alkali ion tracers. The loaded cells were washed and resuspended at 38 °C to a hematocrit of 50% in a tracer-free buffer solution.

Aliquots of cell suspension were centrifuged at time intervals, a sample of the supernatant was taken for analysis, and the remaining supernatant was carefully removed. The cells were washed rapidly with cold buffer. In long-duration outflow experiments, a slight hemolysis was visible at the end; the experiment in which the least hemolysis occurred was accepted.

The radioactivity of the cell and medium samples was measured with a high-resolution NaI crystal detector connected with a multichannel analyzer. The net counts in the energy region of each nuclide were added together, and the contribution of the other nuclides was eliminated by solving the system of four linear equations using coefficients obtained by determining the relative contribution of each nuclide in the regions of the others. The samples were measured immediately, and the results were used to calculate the amount of the short-living $^{42}K^+$, which has the highest energy. A second measurement after a week was made to determine the three long-living nuclides.

To account for Li^+ as a tracer, a method to measure low concentrations of Li^+ is necessary. The concentration of added LiCl has to be low enough not to change the properties of the system that is to be "traced". Addition of 2.5 mmol of LiCl per liter of the cell suspension was found to be sufficient for measurements of Li^+ with an atomic absorption spectrophotometer.

Figure 1 shows the procedure for obtaining the stationary cell/medium distribution at 38 °C for Li^+ and for radioactive tracer ions.

Results

Table 1 shows the rate constants and stationary $k_{in}/k_{out} = c_{cell}/c_{medium}$ ratios of the five tracer ions at 38 °C. Figure 2 shows the entry rates of ions into the cell at 0 °C. The entry rates relative to that for Na^+ were $Li^+ = 1.44$, $Na^+ = 1$, $K^+ = 2.22$, $Rb^+ = 2.39$, and $Cs^+ = 1.72$. The bulk ion concentrations in the cell were $Na^+ = 7$ mmol/l and $K^+ = 95$ mmol/l. The outside concentrations were $Na^+ = 145$ mmol/l and $K^+ = 5.1$ mmol/l. If ideal experimental conditions were possible, the in vitro k_{in}/k_{out} ratios of tracer ions should equal the in vivo c_{cell}/c_{medium} ratios of the corresponding bulk ions. For bulk Na^+ the in vivo c_{cell}/c_{medium} ratio was $7/145 = 0.048$, and for tracer Na^+ the in vitro k_{in}/k_{out} ratio was 0.044. For bulk K^+ the in vivo $c_{cell}/c_{medium\ ratio}$ was $95/5.1 = 19$, and for tracer K^+ the in vitro k_{in}/k_{out} ratio was 10.

Unidirectional flux, calculated as a product of the concentration of bulk ion and the rate constant of the corresponding tracer, was for Na^+ 3.6 mmol/h inward and 4.0 mmol/h outward in a liter of cells. For K^+, the unidirectional flux was 1.3 mmol/h inward and 2.4 mmol/h outward in a liter of cells.

The difference of unidirectional fluxes gives the net flow of the ion. At 38 °C, the net outflow for Na^+ was −0.37 mmol/h, and the net outflow for K^+ was −1.1 mmol/h in a liter of cells. It was thus not possible to keep cells strictly at the stationary state. In vitro experimental conditions, including some hemolytic breakdown of cells, may explain this. The same reasons may explain the result that the in vitro k_{in}/k_{out} ratio of tracer K^+ did not reach the high in vivo c_{cell}/c_{medium} ratio of bulk K^+.

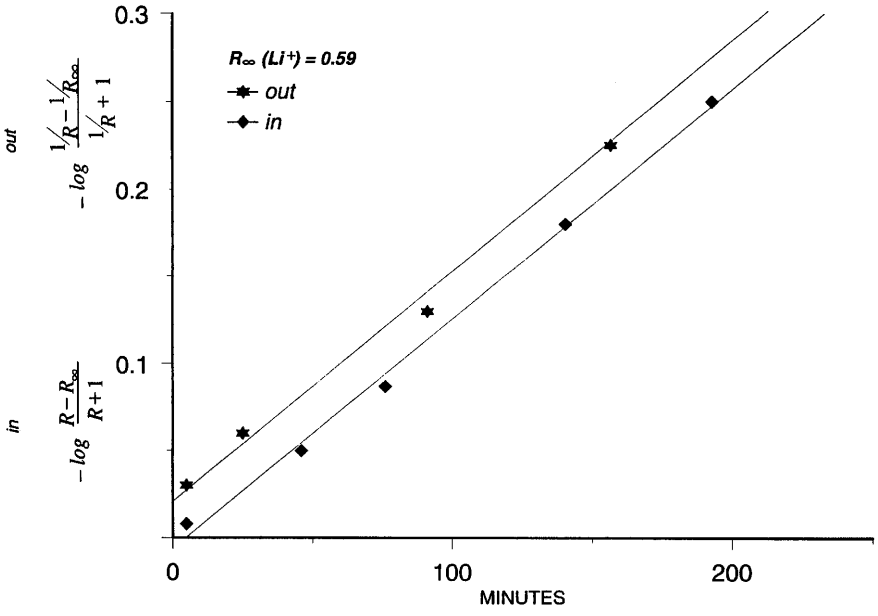

Fig. 1 Procedure to obtain the stationary cell/medium distribution at 38 °C for Li^+ and for radioactive tracer ions. R is the cell/medium concentration ratio of Li^+ measured at 38 °C in inflow and outflow experiments as a function of time, and, R_∞ is the stationary-state c_{cell}/c_{medium} distribution ratio of Li^+ at infinite time. The value of $R_\infty = k_{in}/k_{out} = 0.59$, found through trial and error, makes the inflow and outflow slopes parallel. A similar graph was plotted from the inflow and outflow measurements of the other tracer ions to obtain their k_{in}/k_{out} ratios at 38 °C

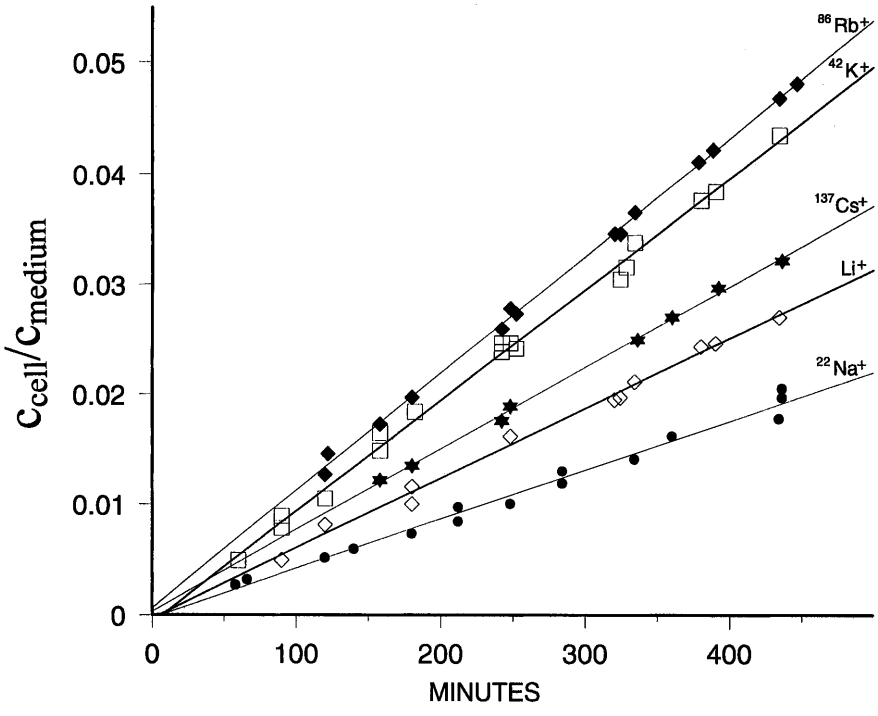

Fig. 2 The entry rates of tracer ions into the erythrocytes at 0 °C. The cell/medium distributions of ions after their addition to the medium are shown as a function of time

Table 1 Rate constants and stationary $k_{in}/k_{out} = c_{cell}/c_{medium}$ distribution ratios of tracer alkali ions in human erythrocytes at 38 °C

	k_{in} (h^{-1})	k_{out} (h^{-1})	k_{in}/k_{out}
Li^+	0.065	0.11	0.59
$^{22}Na^+$	0.025	0.57	0.044
$^{42}K^+$	0.250	0.025	10.00
$^{86}Rb^+$	0.250	0.021	11.90
$^{137}Cs^+$	0.046	0.015	3.07

Figure 3 shows a graph of the logarithms of the stationary c_{cell}/c_{medium} ratios of tracer ions at 38 °C plotted against the inverse of their entry rates into the cell at 0 °C.

Discussion

The mobility-dependent separation of alkali ions

For Na^+ and K^+, the tracer rate constants and unidirectional fluxes obtained here do not differ markedly

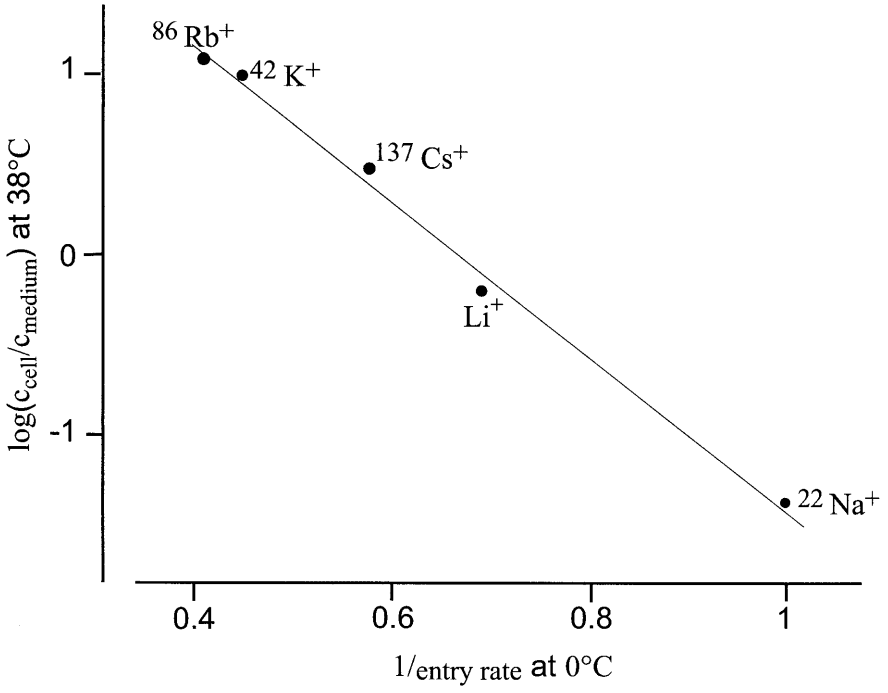

Fig. 3 The logarithms of the stationary c_{cell}/c_{medium} distributions of tracer ions at 38 °C plotted against the inverse of their entry rates into the cells at 0 °C. The inverse of the entry rates of ions at 0 °C is given relative to that of Na^+

from those given in the literature. In a liter of erythrocytes, Funder and Wieth (1967) reported unidirectional fluxes to be 2.5–3.1 mmol/h for Na^+ and 1.6–2.1 mmol/h for K^+.

For rare Li^+, Rb^+ and Cs^+, the tracer rate constants and the cell/medium distributions are previously not well known. Experiments carried out in both directions at 38 °C enabled us to obtain the metabolism-induced rate constants and cell/medium distribution ratios of all the five tracer ions (Table 1). The entry rates of ions into the cell at 0 °C (Fig. 2) are taken to represent their metabolism-independent relative diffusion mobilities in the ion channel. Figure 3 shows our main result, the relationship between the metabolism-induced stationary cell/medium distributions of ions and the inverse of their mobilities.

In stationary-state cells, the only net flow across the membrane is that of nutrients and end-products. They are destroyed or created by chemical reactions in the cell. Respectively, they enter and leave the cell in the downhill direction. In a liter of human erythrocytes, 1–1.5 mmol of glucose per hour is converted to 2–3 mmol of lactic acid. Nims (1961) predicted that there should be a linear correlation between the rate of lactic acid outflow and the logarithm of the stationary selection ratio of Na^+ and K^+. Thurber and Thompson (1967) investigated this relationship in stationary-state erythrocytes at different temperatures. The decrease of the logarithm of the selection ratio of these ions was indeed the expected linear function of the decreased outflow rate of lactic acid in the range of 37 °C to 4 °C.

Nims (1968) constructed an artificial ion transport system where the enzymatic hydrolysis of urea represented metabolism. The flow rate of the end-products, ammonia and carbon dioxide, was altered by changing the amount of urease enzyme in the compartment where the chemical reaction occurred. The Cellophane membrane was permeable to all the other species in the system except the enzyme. The logarithm of the selection ratio between Li^+ and K^+ across the membrane was again a linear function of the flow rate of the end-products through the membrane. The experiment is a unique model of the coupling between metabolism and ion transport. The physical chemical nature of the coupling was, however, not understood. This was due to the complexity of the system and the unknown structural properties of the Cellophane membrane. The author pointed out that if the membrane has greatly different structural properties, such as high densities of fixed charges in certain parts of the membrane, the efficacy of a cycle of given chemical reactions to separate Li^+ and K^+ could be enhanced.

In chemical reaction-driven open systems, including erythrocytes, the response of the selection ratio of Na^+ (Li^+) and K^+ to the flow of end-products is thus similar to the response of the selection ratio of Na^+ and K^+ to the flow of water in hydrostatic pressure-driven systems (see section on Previous model systems, above).

The finding of Thurber and Thompson (1967) that the stationary-state distributions of Na^+ and K^+ in erythrocytes are related to the outflow rate of lactic acid deserves consideration. The dissociation constant of lactic acid is $K_a = 1.39 \times 10^{-4}$ (25 °C). Lactic acid is thus almost completely dissociated at the pH of blood. In aqueous channels of the cell membrane (e.g., Weiss 1996), H^+ is able to jump through OH_3^+ and $O_2H_5^+$, and H^+ is easily and rapidly permeated along a chain of water in narrow channels in which the permeation of other small ions is restricted (Hille 1992). For other

small cations, the cell membrane permeability is much lower than for small anions, despite the fact that the difference in their mobilities in water is low. This suggests that the channels through which small ions (except H^+) pass contain high densities of fixed positive charges, so that only a very minute amount of free cations is present among the anions. With these structural membrane properties, the exit pathway of H^+ differs from that of lactate ions. This means that the lactic acid outflow-induced circuit of electric current is generated. The lactate ion-carried part of the current in the channel with fixed positive charges leads to a concurrent electro-osmotic outward flow of water. In such a pump channel, the few free cations are flowing inward by the electric current against the outward flow of water. Then the cations are accumulated into the cell according to their mobilities in the channel.

Figure 3 shows that rapidly moving K^+ and Rb^+ are accumulated in the intracellular compartment from which lactic acid is coming, while slowly moving Na^+ remains in the extracellular compartment. Li^+ and Cs^+ are intermediate, Li^+ being closer to Na^+, and Cs^+ closer to K^+ and Rb^+ in their mobilities and stationary-state cell/medium distributions. The linear negative correlation of the logarithms of the stationary c_{cell}/c_{medium} distributions of ions at 38 °C to the inverse of their entry rates into the cells at 0 °C conforms to the physical chemical system described in Eqs. (1)–(7).

The relationship of the present model to the structure and function of the Na,K-ATPase

Post-Albers reaction schemes (Läuger 1991; Suzuki and Post 1997) are generally accepted as a model of the action of the Na,K-ATPase. Reversible conformational changes in the Na,K-ATPase lead first to an occlusion of three Na^+ that are carried from an inside compartment out of the cell, and then to an occlusion of two K^+ that are carried from an outside compartment into the cell. The pumped ions leak passively back through another pathway.

Skou (1989), the discoverer of the Na,K-ATPase, writes: "The (Post-Albers) schemes say nothing about the molecular events. What determines the specificity for the cations? What is the nature of molecular events that lead to occlusion-deocclusion? How is the effect of ATP conveyed to a change in affinities and to occlusion-deocclusion? How are the cations translocated? Our knowledge about these questions is sparse." Skou (1989) concludes: "It may be disappointing that despite thirty years of extensive work by a large number of investigators from many countries, it has not yet been possible to come to a better understanding of the transport process." Repke and Schön (1992) present similar criticism. Nevertheless, the Post-Albers schemes prevail (De Weer 1997), and other possibilities have not been seriously discussed.

Experimental findings in cells, such as non-equilibrium selective distributions of alkali ions, bidirectional tracer ion movements, and unidirectional fluxes, could be demonstrated in non-living stationary-state systems where electric current, water, and ions are coupled to flow in a single channel through the membrane. Then the pump and leak concept is not reasonable, and the unidirectional flux ratio of two alkali ions equals the ratio of the concentrations of these ions in the system. It is of interest that in erythrocytes the unidirectional flux ratio of 3:2 for Na^+ to K^+ corresponds to the ratio of the concentrations of these ions in blood.

At the wall of the ion channel of the Na,K-ATPase (Gadsby et al. 1993; Scheiner-Bobis 1998), enzyme-bound ATP may expect to be hydrolyzed, depending on Na^+ and K^+ in the channel. Hydrolysis of bound ATP results in fixed positive charges at the wall of the channel, providing a structural basis for the suggested electric current-driven function of the Na,K-ATPase pump.

An explanation should be found not only for the separation of Na^+ and K^+, but also for that of Li^+, Rb^+, and Cs^+. Schless et al. (1975) found that in erythrocytes of two genetic variants of sheep, the low K^+ erythrocytes had a higher concentration of Li^+ than the high K^+ erythrocytes. This suggests that the same factors that influence the cell/medium distribution of Na^+ and K^+ influence also the distribution of Li^+. It is tempting to think that the same mobility-dependent mechanism separates all the five alkali ions in the pump channel of the Na,K-ATPase.

"Active transport" from the viewpoint of physical chemistry and electrochemistry

Na^+ is thought to act as a substrate in Na,K-ATPase-mediated chemical reactions which are believed to lead directly to the movement of Na^+ in the uphill direction against its electrochemical potential gradient (e.g., Glynn 1993). The system is termed active transport. The decisive steps involve a transfer of free energy from the energy donor ATP to the transported ion (Tanford 1982).

Because of the strict law of electroneutrality, the concentrations of ions are not independent variables. Consequently, electrochemical potentials of ions cannot be unambiguously defined in rigorous thermodynamics (e.g., Ekman et al. 1978; Førland and Ratjke 1980). For the same reason, the terms active transport, electrogenic pump, and electric potential or voltage across the membrane are difficult to interpret. We avoid the concepts belonging to electrostatistics, and believe that instead of electric charge and electric potential, the concepts electric current and electromotive force should be used.

Unfortunately, there is some confusion in understanding the meaning of electromotive force, potential difference, and voltage (Page 1977). This confusion may contribute to the difficulties in explaining electrochemical phenomena in biology. For example, Na^+ flows through the skin of a frog despite the voltage being

clamped to zero by the current from an external battery (Ussing 1994). The finding is interpreted as demonstrating an active transport of Na^+. However, Kirchoff's mesh laws shows that the ohmic potential drop in the skin compensates for the contribution of the electromotive force of the skin in the voltage-measuring circuit in a zero-voltage state. Accordingly, the flow of Na^+ carries the electric current that in short-circuit experiments is taken from the external battery and is added to the flow through the frog skin.

Equation (1) describes how electric current, diffusion, and water flow are involved in coupled movements of ions across the membrane. Undefined mechanisms are not needed to explain the uphill movement. Different ions contribute according to their transport numbers to the electric current. The mobility of the ion is the decisive property. In electroneutral systems, such as in erythrocytes, where the Debye length is in the order of one nanometer, there can be no accumulation of net charge. The electric current vector must be solenoidal, and the current flows in circuits. The quotient of the converted chemical free energy to the electric current, the electromotive force, is a property of the entire circuit. The electromotive force cannot be localized to a certain part of the circuit or to a certain part of the total chemical change of the circuit.

Analogously, Hill and Eisenberg (1981) emphasized that small-molecule free energies cannot be separated from enzyme contributions or from each other except at the complete cycle level. At the intermediate stages of the cycle, the free energy of the system is inextricably mixed up among the enzyme and the ligands and cannot be assigned to the separate ligands. Regarding the setting of the problem, Hill (1983) does not agree with Tanford (1982). According to Hill (1983), the question of where in the cycle the free energy is transferred to an uphill movement of the ion is meaningless.

In agreement with Nims (1961) and Hill (1983), we believe that the complete cycle of coupled chemical reactions, rather than any intermediate reactions within the cycle, delivers energy in the cell. To quote Nicholls and Ferguson (1992), the frequently supposed capability of the "high-energy phosphate" bond of ATP to store energy and to drive reactions in otherwise unfavorable directions is a myth. Hydrolysis of ATP and conformational changes of the Na,K-ATPase enzyme have their place; the Na,K-sensitive hydrolysis of ATP adjusts the structure of the pump to regulate water and alkali ions in the cell, while the irreversible cycle of the entire metabolism results in the primary force, the electric current circuit for the function of this pump.

Nims (1967) in his essay on ion transport mechanisms writes: "The application of precise physical concepts to the description of the molecular traffic in membranes is in its infancy, and as in the case of all new developments, may take a variety of awkward positions before full maturity is reached." Our work was initiated from the assumption that the well-known electrical and mechanical forces move ions also in the Na,K-ATPase channel of the living cell. This assumption led us to discover a new, at first sight curious, correlation between the logarithms of the stationary cell/medium distributions of Li^+, Na^+, K^+, Rb^+, and Cs^+ tracer ions in human erythrocytes at 38 °C and the inverse of their entry rates into the cells at 0 °C.

Conclusions

A model is discussed where alkali ions are separated by their electric current-driven inward flows against the electro-osmotic outward flow of water in the pump channel of Na,K-ATPase. In such a system, the logarithms of the metabolism-induced stationary cell/medium distributions of alkali ions should be linearly proportional to the inverse of their diffusion mobilities in the pump channel. Experiments with five ion species enabled us to test the existence of this ion separation relationship.

The expected mathematical relation of the stationary cell/medium distributions of ions at 38 °C to their diffusion mobilities at 0 °C does not necessarily mean a cause and effect relationship. It should not be ruled out that some property of alkali ions other than mobility explains the result. However, little work has previously been carried out to study the mechanism of the cell/medium distributions of rare alkali ions. It is difficult for us to discuss alternative possibilities to the mobility-dependent separation.

Theoretical considerations and experimental methods described here for erythrocytes are applicable to other cells. Further work is required to test whether a correlation exits between the logarithms of the stationary metabolism-induced cell/medium distributions of alkali ions and the inverse of their diffusion mobilities in the ion channel of other cells.

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