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Variable stoichiometry in reconstituted shark Na,K-ATPase engaged in uncoupled efflux

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In liposomes with reconstituted shark Na,K-ATPase produced to contain no internal K^+ or Na^+ addition of external Na^+ and ATP induce an uncoupled Na^+ efflux on inside-out oriented pumps which is electrogenic and accompanied by hydrolysis of ATP (Cornelius, F. (1989) Biochem. Biophys. Res. Commun. 160, 801–807). At saturating cytoplasmic Na^+ the net-charge translocated per ATP molecule split is compatible with a coupling ratio of Na_{cyt} transported per ATP split of 3:1 at pH ≥ 7.0. However, this ratio decreases to 1.5:1 below pH 7.0. At non-saturating cytoplasmic Na^+ the 3:1 stoichiometry is attained at pH 7.0–7.5, whereas outside this range of pH the net-charge translocated per ATP molecule split decreases.

Introduction

Under physiological conditions the Na,K-ATPase exchanges cytoplasmic Na^+ with extracellular K^+ deriving the chemical energy from the splitting of ATP with a stoichiometry which seems to be remarkably constant $3Na_{cyt}:2K_{ext}:1ATP$ in a variety of cells and tissues (for references see De Weer et al. [2]). Also in reconstitution studies this stoichiometry has been confirmed [3].

In the absence of extracellular Na^+ or K^+ the Na,K-ATPase catalyses an ATP supported Na^+ extrusion [1,4–7] which has been known as the uncoupled Na^+ efflux. Using the Albers-Post scheme [8,9] as a frame of reference the uncoupled Na^+ efflux takes place by a consecutive reaction in which three cytoplasmic Na^+ bind to an E_1A-form of the enzyme. By a phosphorylation the Na^+ ions become occluded and translocated across the membrane. The enzyme is then dephosphorylated and returns in a reaction with ATP with no cations bound to an E_2-form of the enzyme (Fig. 1). In the common Albers-Post scheme it is assumed that only enzyme forms saturated with cytoplasmic Na^+ become phosphorylated, occlude and translocate the three Na_{cyt} ions. Therefore, a coupling ratio of 3:1 results, i.e. three Na^+ ions are expelled per ATP molecule split, which is in concert with reconstitution experiments [1]. However, possible contribution to the over-all reaction from enzyme species with less than 3 Na^+ ions bound, especially at low Na_{cyt}, leading to a changed stoichiometry, may have to be considered (Fig. 1). Moreover, recent reports of Na^+/H^+- as well as

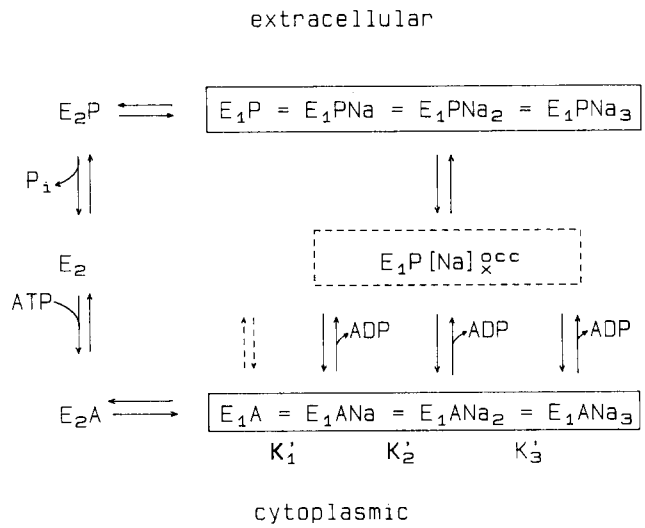

Fig. 1. Simplified scheme for uncoupled Na^+ efflux based on the Albers-Post model [8,9] as modified by Kalrish et al. [21] and Cornelius and Skou [13]. The solid lined boxes signify enzyme species in rapid equilibrium, and the apparent site dissociation constants are denoted K'. The dashed lined box indicates occluded forms (not in rapid equilibrium) with x being 1 to 3. The scheme depict the two enzyme conformations E_1 and E_2 and their phosphorylated- and occluded forms. All E_2A species with Na^+ bound is assumed to particpate in the overall reaction, be phosphorylated and occlude Na^+.

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H^+/K^+-exchange have appeared [10,11] which indicate that such exchange reactions may also play a role in the so-called uncoupled Na^+ efflux. It is therefore of interest to establish the stoichiometry during low cytoplasmic Na^+ concentration, and to examine it under different pH values to analyze if this so-called uncoupled mode under certain conditions of, e.g., low pH could be completed by a returning from the extracellular side of enzyme with bound H^+ instead of returning empty.

In the present paper reconstituted Na,K-ATPase from the spiny dogfish *Squalus achantias* is used to measure the initial rate of rise in transmembrane potential as well as the initial rate of ATP-hydrolysis accompanying Na^+ efflux in the absence of extracellular K^+ as a function of Na_{cyt} concentration and pH. By comparing the two measurements the number of net-charges translocated per molecule of ATP can be evaluated.

With this preparation it is shown that at saturating cytoplasmic Na^+ the stoichiometry decreases from 3 : 1 net-charges : ATP at pH ≥ 7.0 to 1.5 : 1 at pH < 7.0. Moreover, the net charge translocated per ATP-molecule split at non-saturating cytoplasmic Na^+ concentration is further decreased on either side of a pH optimum around pH 7.0.

Methods

In the experiments reported, liposomes with incorporated shark Na,K-ATPase were used. Only the fraction of enzyme molecules inserted as inside-out (i : o) was activated by addition of ATP, since right-side out oriented molecules have their ATP-site shielded from the external medium and enzyme molecules with both sides exposed (n-o oriented) are inhibited by preincubation with Mg^{2+} (5 mM), P_i (1 mM) and ouabain (1 mM) and tested in a medium also contained ouabain [12].

The preparation of proteoliposomes, determination of the orientation of inserted Na,K-ATPase and the recovery of specific hydrolytic activity was performed as previously described [12]. The proteoliposomes were produced in 260 mM sucrose, 2 mM $MgCl_2$, and 30 mM histidine for pH values ≤ 7.5, whereas at pH 8.0 Tris (5 mM) was used as buffer.

The expression efflux and influx refer to the cellular situation and is therefore equivalent to uptake and extrusion in the proteoliposomes due to the activation of exclusively i : o-oriented enzyme molecules.

The time intervals used to allow measurements of either hydrolysis or potential were kept small enough (less than 15 s) to prevent the increase in intravesicular (extracellular) Na^+ concentration to activate ATP-dependent Na^+/Na^+ exchange [1,13]. The rate of ATP-hydrolysis on i : o-oriented Na,K-ATPase was measured using [^{32}P]ATP employing the method of Lindberg and Ernster [14].

The initial rate of rise in membrane potential was assessed using the potential sensitive probe oxonol VI as previously reported [1] which is essentially as described by Apell and Berch [15]. Usually 100 μl proteoliposomes were added to 2.9 ml medium in a cuvette containing 530 nM oxonol VI, and the relative change in fluorescence upon addition of ATP measured using a Perkin-Elmer MPF 44A spectrofluorimeter with excitation wavelength 580 nm (slit width 20 nm) and emission wavelength 660 nm (slit 5 nm). Calibration of the fluorescence signal was achieved as follows: In the 3-ml fluorimeter cuvette containing 100 μl vesicles in 260 mM sucrose, 2 mM $MgCl_2$ and buffer (histidine or Tris) 2 μl valinomycin (1 mg/ml) and 2 μl CCCP (2 μg/ml) were added. These concentrations ensured rapid equilibration of K^+ without depressing the initial fluorescence signal due to imposed gradients of K^+. First, the proteoliposomes were equilibrated with 1 mM K^+ and the base-fluorescence was recorded. Then, additional K^+ was supplied in order to create known gradients of K^+ each new addition awaiting dissipation of the imposed gradient as controlled by a returning of the fluorescence signal to the base line, due to the combined presence of valinomycin and CCCP, before a new gradient of K^+ was established by readdition of K^+. In this way calibrations were performed using the actual proteoliposome batch employed in the experiments and for each new set of pH conditions. The calibration was identical using proteoliposomes produced to contain increasing concentrations of K^+ in order to achieve known gradients of K^+.

All fluorescence signals were collected and analyzed using a PS/2 computer by the aid of an RTI-800F A/D-board (Analog devices) and Snapshot storage scope data acquisition software (HEM Data Corp.).

Results and Discussion

At 22 °C and pH 7.0 the maximum hydrolytic activity accompanying uncoupled Na^+ efflux is found to be 45.7 ± 2.4 μmol/mg (i : o)-protein per hour (mean ± S.E., $n = 6$), corresponding to a turn-over rate of 3.43 ± 0.18 s^{-1} using a figure of 3.8 nmoles sites per mg protein [12]. This hydrolytic activity is the same as found previously [1], and corresponds to between 5 and 10% of optimum hydrolysis in Na^+/K^+ exchange [12].

In Fig. 2 typical experiments showing the development of the transmembrane potentials (inward positive) as indicated by the increase in fluorescence is depicted for three different pH values. The initial rate of increase in fluorescence is evaluated and transformed according to the initial calibration to initial rate of rise in transmembrane potential. Due to the low passive conductance (2.4 nS/cm^2) of these proteoliposomes the maximum turnover rate of charge translocation (ν_0) can be calculated from the maximum initial rate of increase in

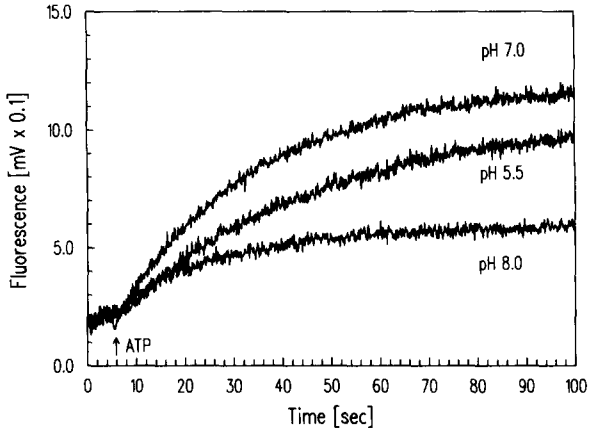

Fig. 2. Activation of uncoupled Na^+ efflux at different pH values by addition of ATP (100 μM) to proteoliposomes containing neither Na^+ nor K^+. The medium contains 130 mM Na^+, 2 mM Mg^{2+}, and 530 nM oxonol VI. The increase in fluorescence (mV output from fluorimeter) indicates the establishment of a transmembrane potential which is positive to the inside of the proteoliposomes, indicating a net inwardly directed positive current. The fluorescence signal is collected via a RTI 800F A/D-board with sweep time 120 s and a resolution of 100 ms. The initial slopes are calculated by fitting the data collected within the first 12 s after ATP addition to be 4.7 mV/s (pH 7.0), 1.95 mV/s (pH 5.5) and 1.54 mV/s (pH 8.0).

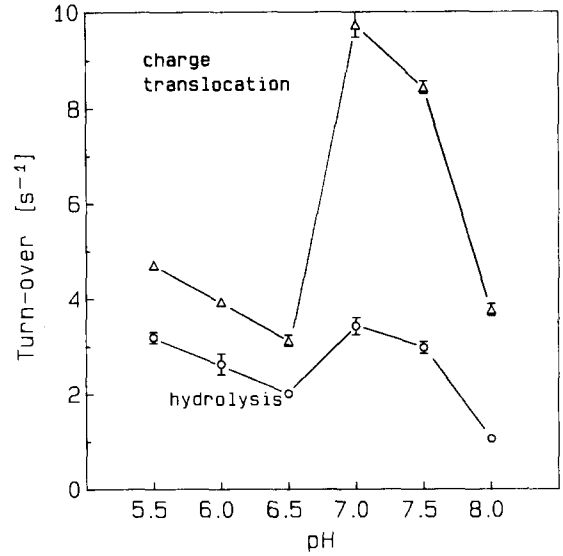

Fig. 3. Turn-over calculated measuring either rate of ATP hydrolysis (lower curve) or rate of charge translocation (upper curve) using proteoliposomes preincubated with ouabain (1 mM) in the presence of Mg^{2+} (5 mM) and P_i (1 mM) in order to inhibit n-o oriented enzyme. Proteoliposomes were prepared in 260 mM sucrose, 2 mM $MgCl_2$, 30 mM histidine (pH 7.5–5.5) or Tris (pH 8.0). The test medium contained: Na^+ 104 mM, ATP 25 μM, Mg^{2+} 1 mM, histidine 30 mM or Tris 5 mM and [^{32}P]ATP at a specific activity of $7.7 \cdot 10^{13}$ cpm/mol. In calculation of charge translocation $C_m = 1$ μF/cm^2 and $n = 5$.

membrane potential (dV/dt), the membrane capacitance C_m (1 μF/cm^2), and the number of exposed pumps, n as: $v_0 = AC_m(dV/dt)/ne$, in which A and e are the surface area (calculated using a mean radius of 100 nm) and elementary charge ($1.6 \cdot 10^{-19}$ C), respectively. n is calculated from measurements of protein and the symmetry of reconstitution. The fraction of pumps with inside-out (i : o) orientation is deduced from the fraction of protein which can be activated by intravesicular K^+, as previously described in detail [12]. In most preparations of proteoliposomes 50 pumps are found to be inserted per proteoliposome and with 10% i : o-orientation n becomes 5.

In Fig. 3 the rate of turn-over measured by initial rate of hydrolysis or initial rate of fluorescence increase are compared at different values of symmetric pH ($pH_{in} = pH_{out}$) at saturating cytoplasmic Na^+ (104 mM). A biphasic pH-profile for the hydrolytic activity is obtained with an optimum around pH 7.0 and some reactivation at low pH values, which is not observed in experiments where only the cytoplasmic pH is decreased (data not shown). In the upper curve the turn-over of net-charge calculated as described above is compared. In the whole range of pH investigated the uncoupled Na^+-efflux is found to be electrogenic in contrast to reports by Karlish et al. [16] who find Na^+ efflux electronetural at pH 7.0 but electrogenic at pH 8.5 using reconstituted renal Na,K-ATPase.

The principle shape of the curve of turn-over using net-charge translocation (Fig. 3, upper curve) resembles that calculated from hydrolytic activity (Fig. 3, lower curve), however, at pH < 7.0 the decrease in turn-over is more pronounced when calculated as net-charge than calculated from hydrolysis. Since the ratio of the two curves is a measure of the ratio of net-charge translocated per ATP molecule split, this ratio is decreased at pH < 7.0. The calculations show an almost constant stoichiometry of three net-charges translocated per ATP molecule split (3 : 1 stoichiometry) at pH values from 7.0 to 8.0, while it is decreased in the interval 7.0–6.5. From pH 6.5–5.5 it attains an also constant value of about 1.5 : 1, see Table I.

The results obtained at saturating Na_{cyt} at pH ≥ 7.0 is in concert with three cytoplasmic Na^+ translocated in exchange with no other ions per ATP split. At pH < 7.0 the results can be explained as a result either of, (i) an increased contribution from enzyme species with less than three Na^+ bound at low pH leading to less Na^+

TABLE I

Calculated stoichiometry of translocated net-charge per ATP molecule split at different pH values

pH	Stoichiometry (mean ± S.E., $n = 4$)
5.5	1.48 ± 0.06
6.0	1.50 ± 0.13
6.5	1.54 ± 0.06
7.0	2.84 ± 0.15
7.5	2.83 ± 0.12
8.0	3.50 ± 0.13

translocated per turn-over, or (ii) as an activation at low pH of some exchange reaction in which cytoplasmic Na^+ is countertransported for some other cations, or (iii) as Na_{cyt} cotransported for an anion [17], or (iv) as a 'partial' uncoupling in which some fraction of enzyme species hydrolyze ATP without net transport.

The first possibility is not considered likely since the Na_{cyt} concentration is so high that the fraction of enzyme specie swith less than three Na^+ bound in the rapid equilibrium segment at the cytoplasmic aspect must be very small according to the equilibrium constants estimated in a previous report [18] and from the present estimation of half-maximum dissociation constants ($K_{0.5}$), which only are doubled by changing pH from 7.0 to 5.5 (see Fig. 4). The second possibility is considered the most likely and since a slight activation in ATP hydrolysis is observed at increased low pH values a likely candidate to participate in such countertransport would be H^+. Such an explanation is in accord with previous results where increased activation by H^+ at some extracellular transport sites (K^+-sites) at low pH has been suggested [10,11]. At pH 5.5 the free concentration of H^+ corresponds to about 8 protons per vesicle (the majority being bound to the buffer anions), however, at pH 8.0 it corresponds to only 0.03 protons per vesicle, which could be taken to suggest the absence of the countertransport at the higher pH values since this would indicate a very high extracellular H^+ affinity of the sodium pump in order for H^+ to activate. The presence of a Na^+/H^+ exchange at the higher pH values would furthermore imply that more than three Na_{cyt} be translocated per ATP molecule split in order to attain the 3 : 1 net-charge : ATP stoichiometry. Therefore, taken together, an explanation where the reaction shifts from uncoupled Na^+ efflux (Na^+/O exchange) to Na^+/H^+ exchange at pH < 7.0 is favoured. This hypothesis is currently tested by measurements of H^+ transport in the proteoliposomes. A decrease in pump stoichiometry (Na^+ : ATP and Rb^+ : ATP) at low pH was also recently observed in inverted red blood cells by Polvani and Blostein [11], although they could not establish the physiological 3 : 2 : 1 Na^+ : K^+ : ATP ratio at normal pH. Variable stoichiometry has also been found for the Ca^{2+} pump where the Ca^{2+}/ATP ratio is found to be pH- and Ca^{2+}-dependent [19].

In Fig. 4 A–C the activation by cytoplasmic Na^+ of initial rate of hydrolysis (lower panels) and initial rate of rise in transmembrane potential (upper panels) is compared at pH 7.0 and at the two experimentally extreme pH values (5.5 and 8.0). The curves are sigmoid and can be fitted to the Hill equation, the fitting parameters being the half-maximum dissociation constant ($K_{0.5}$) and the Hill coefficient (n_H), both indicated on the figures. These fits are merely performed in order to compare quantitatively the curve forms and no specific models are assumed.

As indicated in Fig. 4 the activation curves of membrane potential are shifted to the right (towards higher Na^+ concentrations) compared to the activation curve of hydrolytic activity at pH values outside 7.0–7.5, i.e., the $K_{0.5}$ value for membrane potential is increased relative to that of hydrolytic activity. The resulting $K_{0.5}$

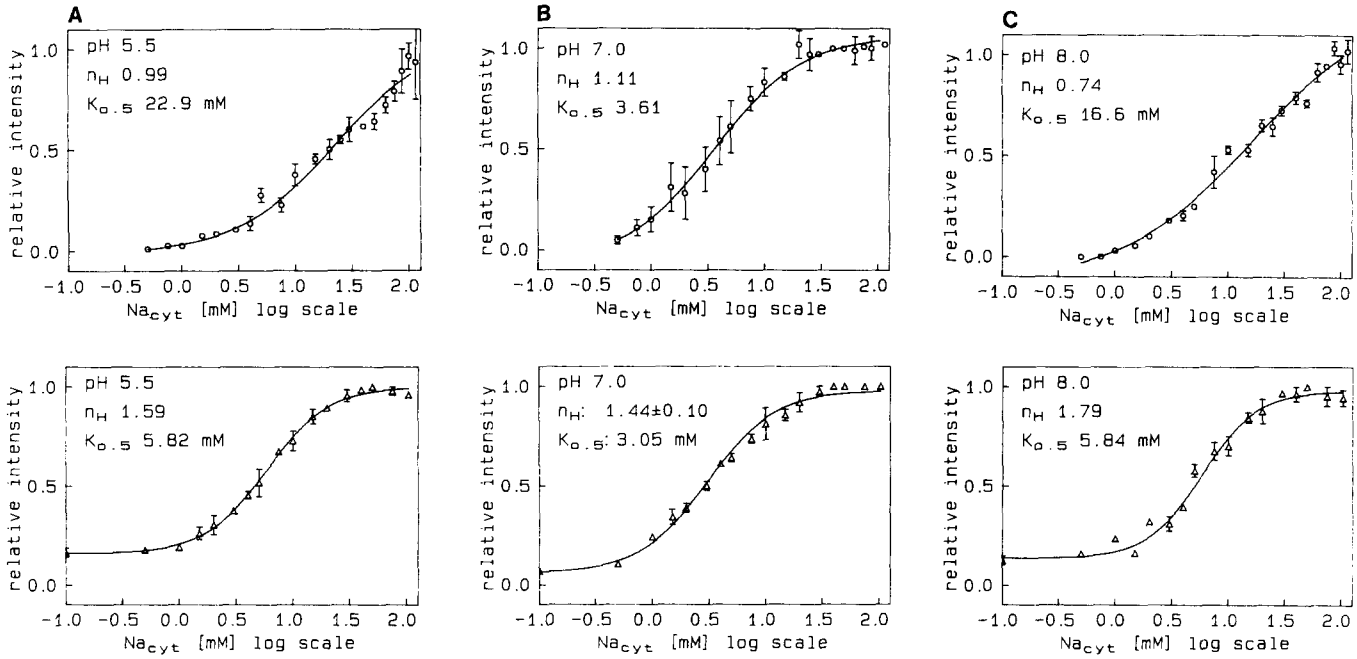

Fig. 4. The activation by cytoplasmic Na^+ of (lower panel, △) initial relative rate of hydrolysis and initial relative rate of rise in transmembrane potential (upper panel, ○) at pH 5.5, 7.0, and 8.0. The curves are scaled relative to each maximum turn-over, the absolute value of which can be seen in Fig. 3. The points have been fitted on computer to the Hill equation (the curves) and the fitting parameters (n_H and $K_{0.5}$) are indicated. Points represent means ± S.E. ($n = 4$).

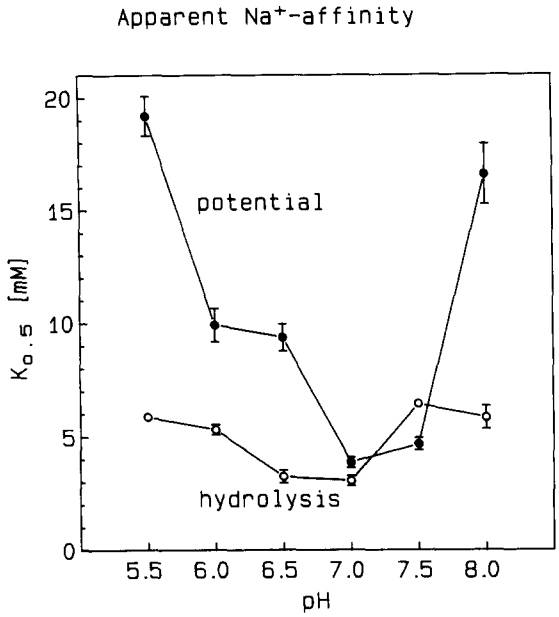

Fig. 5. Comparison of the fitting parameter ($K_{0.5}$) obtained for activation curves as depicted in Fig. 4 for hydrolysis and for potential as a function of all pH values investigated.

values calculated from such activation curves at pH values between 5.5 and 8.0 are given in Fig. 5. The Hill coefficients are found to be around 1.4 except at the two extremes of pH 5.5 and 8.0, where they decline to about 1.

The results that at any given non-saturating Na_{cyt}-concentration the net-charge translocated by the pump for each ATP molecule hydrolyzed is decreased on each side of the pH optimum around 7.0–7.5 (where a constant ratio of three net-charges translocated per ATP split is found at all Na_{cyt} concentrations tested) can plausibly be explained in the following way: The half-maximum activation of uncoupled Na^+ efflux by cytoplasmic Na^+ ($K_{0.5}$) is much the same as previously found for cytoplasmic Na^+ activation of ATP-dependent Na^+/Na^+ exchange [18], and there it was demonstrated by model simulation using the Adair-Pauling model for cooperative interaction [20] that such sigmoid activation curves are compatible with the assumption that all enzyme species with bound cytoplasmic Na^+ are able to translocate Na^+, their rates, expressed as rate per binding site, being equal. The constant stoichiometry of 3 Na_{cyt}:ATP found at pH 7.0 in the whole range of cytoplasmic Na^+ concentrations indicate that there it is the enzyme species with three Na^+ bound which is predominant in turn-over. Therefore a model in which a lowering in pH increases the fractional contribution to the overall reaction from enzyme species with less than three Na^+ bound giving rise to less charge translocated per turn-over could conceivably explain the present results of a shift in the Na_{cyt}-activation curves for turn-over measured as either hydrolysis or net-charge transfer. Increased recruitment of enzyme species with less than three Na_{cyt} bound in the over-all reaction could be caused by either a direct increase in rate constants leading from the enzyme species not saturated with Na^+ or by a decreases in the site affinity of E_1A for the third Na_{cyt} relative to the first two (e.g., a relative increase in K_3' compared to K_1' and K_2' in Fig. 1) shifting the equilibrium towards species with less Na^+ saturation, or both. Both explanations will lead to increased rate (fraction of enzyme species times the rate constant) from enzyme species with less Na^+ bound and therefore to translocation of less net-charge compared to hydrolysis at any given cytoplasmic Na^+ concentration.

Since the constant and low stoichiometry of 1.5 : 1 net-charges per ATP molecule split found at the low pH values and saturating Na_{cyt} is assumed to be due to the presence of a Na^+/H^+ exchange, this explanation implies the persistence of such a H^+ influx (Na^+/H^+ countertransport) also at non-saturating Na_{cyt} concentrations and low pH. At pH > 7.0 no decrease in stoichiometry of net-charge translocated per ATP molecule split is observed, so there no countertransport of H^+ need to be invoked in order to explain the results. This interpretation is substantiated further by the extremely high affinity for extracellular H^+ ions such an H^+ influx at the higher pH values would imply. If the suggested model of increased recruitment of enzyme species with less than three Na_{cyt} bound in the overall reaction is due to a shift between the three apparent site affinities (K_3' increases relative to K_1' and K_2') this could also explain the increase in apparent K_m ($K_{0.5}$) at both high and low pH values (Fig. 5) indicating the ionization of some residues near or at the cytoplasmic Na^+-binding sites with pK values in these ranges decreasing the Na^+ affinity. The transition from the Na^+-form to the K^+-form of the Na,K-ATPase is previously found to be accompanied by a protonation with two dissociation constants for H^+ with pK values about 5.5 in the presence of a high Na^+/K^+ ratio and 9 in the presence of a low Na^+/K^+ ratio [22].

In conclusion, a combined effect of pH on uncoupled Na^+ efflux is probably needed in order to explain the results: (1) At both saturating and non-saturating Na_{cyt} and pH 7.0–7.5 three cytoplasmic Na^+ are expelled per ATP split. (2) At saturating Na_{cyt} and pH below 7.0 activation of the sodium pump by extracellular H^+ takes place initiating Na^+/H^+ exchange resulting in a lower net-charge/ATP stoichiometry. However, at pH 7.0 and above the flux mode remains probably uncoupled due to the extremely low free H^+ concentration present. This hypothesis is currently tested by measurements of H^+ transport in the proteoliposomes. (3) At non-saturating Na_{cyt} and outside the pH optimum of 7.0 a further decrease in stoichiometry relative to saturating Na_{cyt} is observed and explained as a recruit-

ment of enzyme species with less than three Na^+ bound in the overall reaction probably caused by a shift in the relative distribution of apparent site affinities for Na_{cyt}. Of course these effects do not have to be mutually exclusive as indicated here, and a model with concomitant interplay of Na^+/H^+ exchange in combination with changes in apparent site affinities for Na_{cyt} could be conceived as well.

The suggested Na^+/H^+ exchange reaction probably has a low V_{max} value and at physiological pH values and concentrations of Na^+ and K^+, it is not likely to interfere with the Na^+/K^+ exchange reaction although the affinity for H^+ is apparently very high in order to activate the system at the very low concentrations of free H^+. A similar situation has been found to be the case for the Na^+-translocating ATPase in a bacterium [23] and could be an indication of the Na,K-ATPase being developed from an original proton pump in evolution.

The maximum hydrolytic activity accompanying uncoupled Na^+ efflux in the absence of extracellular Na^+ or K^+ amounts to about 70% of the maximum hydrolytic activity accompanying Na^+/Na^+ exchange in the presence of 130 mM extracellular Na^+ [18]. However, during conditions of Na^+/Na^+ exchange almost no uncoupled Na^+ efflux is likely to take place due to the high affinity of the enzyme for extracellular Na^+ or K^+: Taking the apparent site dissociation constants for extracellular Na^+ as found by Cornelius and Skou [18] to be 1 mM, 60 mM and 80 mM (successive addition of extracellular Na^+) it can be calculated that at 130 mM extracellular Na^+ less than 1‰ of the enzyme species of the E_2P pool is present as E_2P with no Na^+ bound ('empty' E_2P). Since the rate constant for this species out of the pool is probably lower than for the other species with bound Na^+, the rate of uncoupled Na^+ efflux which is supposed to proceed via this form must be extremely low. This consideration is pertinent to the discussion of consecutive (ping-pong) versus simultaneous (sequential) models for the action of the Na,K-ATPase reaction mechanism: Previous investigations on effects of ligands on V_{max} and their apparent K_m in red cells [24,25] as well as in experiments with reconstituted shark enzyme [18] supported simultaneous models rather than consecutive, however, in 1979 Sachs [26] explained the results from red cells as due to an appreciable contribution from uncoupled Na^+ efflux to the overall Na^+/K^+ reaction which made the kinetics compatible with a consecutive scheme. According to the calculations of the proportion of 'empty' E_2P given above for the reconstituted shark enzyme and since the affinity in red cells for extracellular K^+ or Na^+ is comparable for the two enzymes, it is a question if the relative proportion of 'empty' E_2P is so low that it cannot account for the finding that the kinetics of Na,K-ATPase apparently conforms to simultaneous kinetics and not to 'ping-pong' kinetics, as suggested by Sachs [26].

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References

1 Cornelius, F. (1989) Biochem. Biophys. Res. Commun. 160, 801–807.
2 De Weer, P. et al. (1988) Annu. Rev. Physiol. 50, 225–241.
3 Apell, H.-J. (1989) J. Membr. Biol. 110, 103–114.
4 Garrahan, P.J. and Glynn, I.M. (1967) J. Physiol. Lond. 192, 159–174.
5 Garrahan, P.J. and Glynn, I.M. (1967) J. Physiol. Lond. 192, 175–188.
6 Glynn, I.M. et al. (1974) Ann. N.Y. Acad. Sci. 242, 257–371.
7 Lew, V.L., Hardy, M.A. and Ellory, J.C. (1976) Biochim. Biophys. Acta 323, 251–266.
8 Fahn, S., Koval, G.J. and Albers, R.W. (1966) J. Biol. Chem. 241, 1882–1889.
9 Post, R.L., Kume, S., Tobin, T., Orcutt, B. and Sen, A.K. (1969) J. Gen. Physiol. 54, 306s–326s.
10 Hara, Y. and Nakao, M. (1986) J. Biol. Chem. 261, 12655–12658.
11 Polvani, C. and Biostein, R. (1988) J. Biol. Chem. 263, 16757–16763.
12 Cornelius, F. and Skou, J.C. (1984) Biochim. Biophys. Acta 772, 357–373.
13 Cornelius, F. and Skou, J.C. (1985) Biochim. Biophys. Acta 818, 211–221.
14 Lindberg, O. and Ernster, L. (1956) Methods Biochem. Anal. 3, 1–22.
15 Apell, H.-J. and Berch, B. (1987) Biochim. Biophys. Acta 903, 480–494.
16 Karlish, S.J.D., Goldschleger, R., Shahak, Y. and Raphaeli, A. (1988) in Progress in Clinical and Biological Research, Vol. 268A (Skou, J.C., Nørby, J.G., Maunsbach, A.B. and Esmann, M., eds.), pp. 519–524, Alan R. Liss, New York.
17 Dissing, S. and Hoffman, J.F. (1983) in Current Topics in Membranes and Transport, Vol. 19 (Hoffman, J.F. and Forbush, B., eds.), pp. 693–695, Academic Press, New York.
18 Cornelius, F. and Skou, J.C. (1988) Biochim. Biophys. Acta 944, 223–232.
19 Meltzer, S. and Berman, M.C. (1984) J. Mol. Biol. 259, 4244–4253.
20 Segel, I.H. (1975) Enzyme Kinetics, John Wiley & Sons, New York.
21 Karlish, S.J.D., Yates, D.W. and Glynn, I.M. (1978) Biochim. Biophys. Acta 525, 252–264.
22 Skou, J.C. and Esmann, M. (1980) Biochim. Biophys. Acta 601, 386–402.
23 Laubinger, W. and Dimroth, P. (1989) Biochemistry 28, 7194–7198.
24 Hoffman, P.G. and Tosteson, D.C. (1971) J. Gen. Physiol. 58, 438–466.
25 Sachs, J.R. (1977) J. Physiol. 273, 489–514.
26 Sachs, J. (1979) in Na,K-ATPase, Structure and Function (Skou, J.C. and Nørby, J.G., eds.), pp. 463–473, Academic Press, New York.