Supplemental data to:

TRANSPORT MECHANISM AND PH REGULATION OF THE NA^+/H^+ ANTIPORTER NhaA FROM *ESCHERICHIA COLI*. AN ELECTROPHYSIOLOGICAL STUDY

Thomas Mager, Abraham Rimon, Etana Padan, and Klaus Fendler

1) Reconstruction of the pump current from the measured current in a capacitively coupled system.

The pump current generated by the transporter $I_p(t)$ is distorted by capacitive coupling via the network of the compound membrane formed by the solid-supported membrane (SSM) and the adsorbed liposomes. The resulting measured current is I(t). The compound membrane can be described by an equivalent circuit (Fig. 1) where Cp and Cm are the capacitances of the liposomes and that of their contact areas with the SSM and G_p is the conductance of the proteoliposomes (1,2). Using the parameters of the equivalent circuit the original pump current of the transporter can be reconstructed according to an algorithm suggested by Läuger and coworkers(1). However, this algorithm is restricted to small steady state turnover of the transporter because it neglects the voltage dependence of the transporter activity. During extended activity the transporter builds up a voltage V_p across the liposomal membrane. In the following we present a modification of the algorithm for reconstruction of the original current of the voltage dependent transporter.

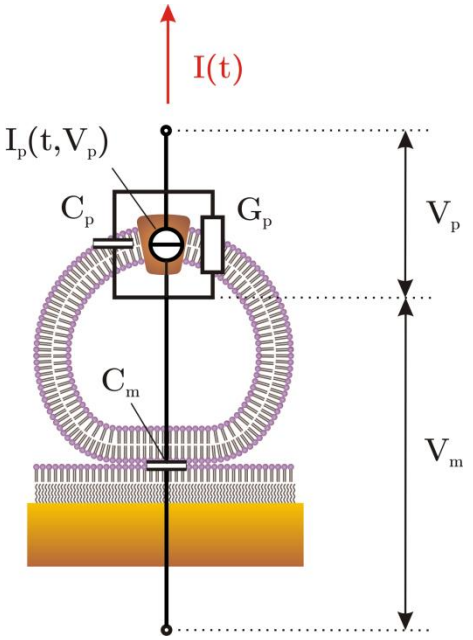

Figure 1: Liposomes adsorbed to the SSM and the equivalent circuit describing the electrical properties of the compound membrane formed from the liposomes and the underlying SSM. The transporter generates the pump current $I_p(t,V_p)$ which charges the liposome membrane to the voltage V_p. Capacitive coupling via the SSM-liposome contact region C_m yields the measured current $I(t)$.

A linear voltage dependence of the transporter is assumed yielding a pump current:

$$I_p\,(t,V) = I_p^T(t) + I_p^\infty(V)$$

Here, the current is dissected in a voltage independent transient current $I_p^T(t)$ and a voltage dependent steady state current $I_p^\infty(V)$. The rationale behind that is that the relaxation in the steady state represented by the transient current consists of a single turnover during which so little charge is translocated that the activity of the transporter is hardly affected. During the steady state activity, on the other hand, a voltage is built up across the liposomal membrane which reduces the current. For simplicity we limit the analysis to a time range where the voltage dependence of the transporter is approximately linear(3):

$$I_p^\infty(V) = I_p^\infty(0)(1 - \tfrac{|V_p|}{V_*}).$$

$I_p^\infty(0)$ is the steady state current at zero voltage, V_* is the reversal potential and V_p is the voltage across the liposomal membrane (Fig. 1). Under these conditions the reconstructed pump current at zero voltage is given by(1):

$$I_p(t,0) = (1 + \frac{C_p}{C_m})\left\{I(t) + (k_0 + k_\infty)\int_0^t I(t)dt\right\}$$

With:

$$k_0 = \frac{G_p}{C_m + C_p}$$

$$k_\infty = \frac{I_p^\infty(0)}{V_*(C_m + C_p)}$$

This is completely analogous to the equation given by ref. (1) if the reciprocal time constant $1/\tau_I$ in ref. (1) is replaced by $k_0 + k_\infty$. For simplicity we used the abbreviation $k_0 + k_\infty = 1/\tau$ in the main manuscript. Note that τ is the so called system time constant (2,3) which in the case of the voltage dependent transporter depends, as expected, on the charging pump current $I_p^\infty(0)$. The reciprocal system time constant is introduced into the measured current (2) as an additional component and can, therefore, be determined from the experimental current traces. The factor $1+C_p/C_m$ depends exclusively on the properties of the compound membrane and is constant throughout the measurement. It was, therefore, arbitrarily set to 1. Since τ can be determined from the measured transient current using a least square fit, the reconstructed transporter current at zero voltage $I_{rec} = I_p(t,0)$ can be calculated with the above equation.

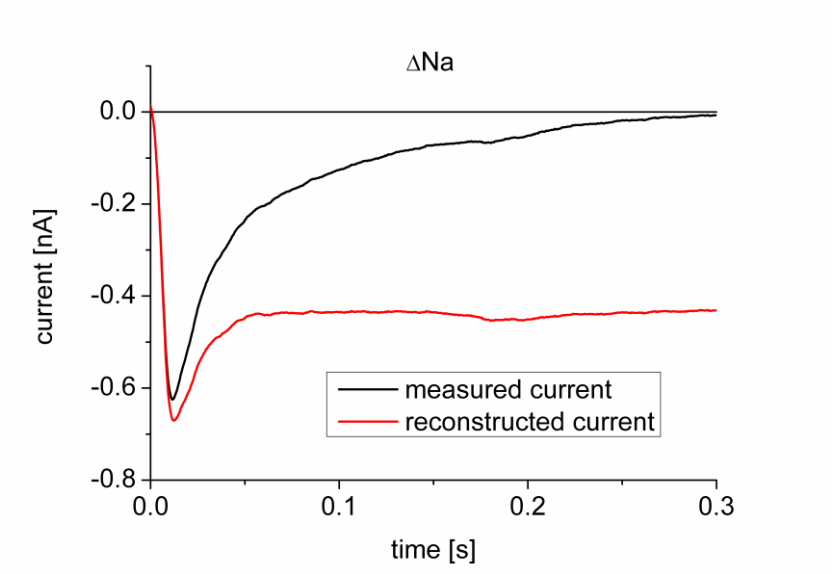

Figure 2: Measured current (black) and reconstructed current (red) using the algorithm described above. The current was measured after a 10 mM Na^+ concentration jump at pH 8.5 with G338S NhaA RSO proteoliposomes (LPR 10). Detailed conditions see Fig. 2 of main manuscript. The parameter $k_0 + k_\infty = 11.4\ s^{-1}$ was determined from the measured current by fitting the decay in the range of 0.06 – 0.35 s with a single exponential.

Fig. 2 shows a comparison of the measured and reconstructed transporter currents. As an example we have taken a transient current generated by the Na^+/H^+ exchanger NhaA from *Escherichia coli* after a 10 mM Na^+ concentration jump. The black line is the current *I(t)* as measured using SSM-based electrophysiology. The red line is the reconstructed current $I_p(t,0)$ as generated by NhaA in the proteoliposomal membrane at zero voltage. Reconstruction was performed using the procedure described above and setting the scaling factor *1 + Cp/Cm* arbitrarily to 1. The algorithm which has been proposed by Läuger and coworkers (1) was modified to account for a voltage dependent transporter (see above). The currents are shown on a reduced time scale (< 30 ms) because the reconstruction algorithm fails at larger times. The reconstructed current clearly shows the presence of a steady state current after a rapid initial charge displacement.

2) Kinetic solution to the minimal kinetic model

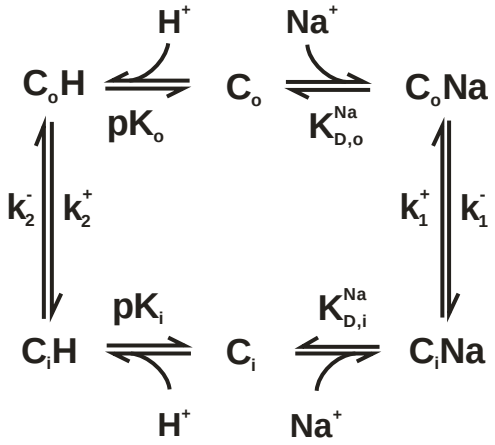

Figure 3: Kinetic model for Na^+/H^+ antiport. In the "forward mode" the outside directed transporter C_o binds H^+ (This could be one or two H^+ ions, for simplicity only one is considered in the model) from the periplasm, performs a conformational transition to the inward directed form C_i and releases H^+ to the cytoplasm. Subsequently, Na^+ is bound from the cytoplasm and released to the periplasm.

The relevant conditions are the concentrations of Na^+ outside (periplasmic) and inside (cytoplasmic), Na_o and Na_i, as well as the pH outside and inside, pH_o and pH_i. The model is defined by 8 kinetic parameters as given in Fig. 3. One of the kinetic parameters can be calculated using the principle of detailed balance:

$$\frac{10^{-pK_o} K_{D,i}^{Na} k_1^+ k_2^+}{10^{-pK_i} K_{D,o}^{Na} k_1^- k_2^-} = 1$$

Since H^+ and Na^+ binding is assumed to be in equilibrium the fractions of Na-loaded and H-loaded carrier in inside/outside facing conformation is (x = o, i):

$$F_x^{Na} = \frac{C_x Na}{T_x} = \frac{Na_x}{Na_x + K_{D,x}^{Na}(1 + 10^{(pK_x - pH_x)})}$$

$$F_x^{H} = \frac{C_x H}{T_x} = \frac{10^{-pH_x}}{10^{-pH_x} + 10^{-pK_x}(1 + \frac{Na_x}{K_{D,x}^{Na}})}$$

Where T_x is the total inside/outside facing enzyme concentration:

$$T_x = C_x H + C_x + C_x Na$$

The two pools T_o and T_i are related by the kinetic equation:

$$T_i \underset{\tilde{k}_2}{\overset{\tilde{k}_1}{\rightleftarrows}} T_o$$

With the effective rate constants:

$$\tilde{k}_1 = F_i^{Na} k_1^+ + F_i^H k_2^- \text{ and } \tilde{k}_2 = F_o^{Na} k_1^- + F_o^H k_2^+.$$

And the effective equilibrium constant:

$$K = \frac{\tilde{k}_1}{\tilde{k}_2}$$

This yields the concentration of the two pools:

$$T_o = T\frac{K}{1+K} \quad \text{and} \quad T_i = T\frac{1}{1+K}$$

The turnover is the flux from e.g. C_iNa to C_oNa divided by the total enzyme concentration T:

$$turnover = \frac{1}{T}(T_i F_i^{Na} k_1^+ - T_o F_o^{Na} k_1^-)$$

Using the expressions calculated above for T_o and T_i one obtains:

$$turnover = \frac{1}{1+K} F_i^{Na} k_1^+ - \frac{K}{1+K} F_o^{Na} k_1^-$$

To account for a potential dependent transporter function the transitions $C_oNa \leftrightharpoons C_iNa$ and $C_oH \leftrightharpoons C_iH$ are assumed to be voltage dependent translocating charges z_1 and z_2 across the membrane. Their rate constants are then given by (4):

$$k_1^+ = k_1^+(0) e^{z_1 \frac{U}{V}}$$

$$k_1^- = k_1^-(0) e^{-z_1 \frac{U}{V}}$$

$$k_2^+ = k_2^+(0) e^{-z_2 \frac{U}{V}}$$

$$k_2^- = k_2^-(0) e^{z_2 \frac{U}{V}}$$

With $V = \frac{2k_B T}{e_0}$, the Boltzmann constant k_B, temperature T, elementary charge e_0 and $k_i^{\mp}(0)$ the rate constant at zero voltage U.

References

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