

Adiabatic Pumping Mechanism for Ion Motive ATPases

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An ion motive ATPase is a membrane protein that pumps ions across the membrane at the expense of the chemical energy of adenosine triphosphate (ATP) hydrolysis. Here we describe how an external electric field, by inducing transitions between several protein configurations, can also power this pump. The underlying mechanism may be very similar to that of a recently constructed adiabatic electron pump [M. Switkes *et al.*, *Science* **283**, 1905 (1999)].

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Ion motive ATPases are proteins that span a cell or organelle membrane and use energy from adenosine triphosphate (ATP) hydrolysis to pump ions across the membrane, thus generating and maintaining the ion electrochemical gradients essential for life [1]. Because the times between the individual chemical steps of ATP hydrolysis are random, it has long been held that strictly regulated coupling between the chemical events of ATP hydrolysis and mechanical events of transport is essential for the function of a molecular pump [2]. The rigid requirement for such lockstep coupling has been challenged by recent experiments carried out by Tsong and colleagues [3–5] who applied a fluctuating external electric field to a suspension of red blood cells. Amazingly, the zero average applied fields were able to drive thermodynamically uphill transport via the ion pump sodium, potassium ATPase (Na,K ATPase) even under conditions where ATP hydrolysis could not occur.

Here we show how externally driven protein structural changes can lead to net ion pumping by a mechanism similar to that of a recently constructed adiabatic electron pump [6], and we relate this mechanism to pumping driven by ATP hydrolysis. In the present context adiabatic means that the ion binding process can be at equilibrium at every instant. The energy transfer between the applied electric field and the protein drives two internal conformational processes to oscillate out of phase with one another. The resulting cycling through conformational states results in ion pumping. A significant feature is that at low frequency the current driven by an adiabatic pumping mechanism is linearly proportional to the frequency. This is in contrast to previously proposed non-adiabatic mechanisms [7] for ac electric field induced pumping where the external ion binding process itself is driven out of equilibrium. At low frequency these mechanisms give rise to a current that is proportional to the square of the frequency.

In a simple picture of an ion pump, the protein structure presents energy barriers (gates) for ion permeation at the two entrances, one on either side of the membrane, surrounding an energy well (binding site) in the middle. As ATP is bound to the protein, hydrolyzed, and product

released to complete a catalytic cycle, the protein undergoes shape changes that in turn change the relative gate and binding energies for the ion. Figure 1 shows an energy diagram for this two-barrier, one-site model of an ion transporter.

The two dashed lines represent the surface of the membrane separating two reservoirs (e.g., the inside and the outside of a cell) each having constant chemical potential of the transported ion. This is an excellent approximation so long as the number of ions in the reservoirs is large compared to the number of pump proteins.

Because different conformations have different dipole moments an oscillating electric field can drive structural changes of a pump protein and hence cause time dependent modulation of a kinetic parameter, the relative barrier height $u(t)$, and of a thermodynamic parameter, the well energy $\epsilon(t)$. This causes ions to flow back and forth between the reservoirs and well as the binding energy periodically increases and decreases. The differential barrier height $u(t)$ and well energy $\epsilon(t)$ are internal parameters [8] controlled by the conformation of the protein

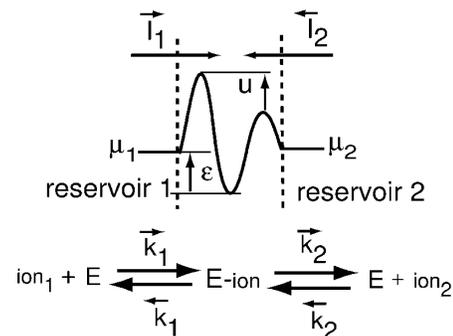


FIG. 1. Energy diagram for the two-barrier, one-site model described in the text. The relevant energy levels necessary for determining the rate coefficients are u and ϵ , the differential barrier height and the well energy, respectively, and the overall ion electrochemical potential difference $\Delta\mu = \mu_2 - \mu_1$ in units of the thermal energy $k_B T$. Below the diagram is the kinetic scheme on which our calculations are based.

and do not influence the overall transport equilibrium. Here we show that externally driven fluctuations of $u(t)$ and $\epsilon(t)$ can nonetheless drive ion pumping.

The rate of change of the binding probability, Q , is the sum of the currents from the two reservoirs to the well

$$dQ/dt = I_1(t) + I_2(t). \quad (1)$$

The fraction F of the current coming from reservoir 1 is

$$F(t) = \frac{I_1(t)}{I_1(t) + I_2(t)}. \quad (2)$$

Once any transients have decayed, the net current is the time average of FdQ/dt . If, e.g., during a period of the applied oscillating field $F > 1/2$ when Q is increasing, and $F < 1/2$ when Q is decreasing there is net transport from reservoir 1 to 2 even if the reservoirs have equal chemical potentials. This is very similar to a flashing ratchet mechanism for molecular motors [9]. To better understand the mechanism, consider a specific kinetic model (Fig. 1) to describe the transitions of ions between the reservoirs and the well. This is based on a Kramer's description of the thermally activated barrier crossing [10], a good approximation so long as any changes in the system constraints are carried out slowly compared to the intrawell relaxation time [$\approx kTL^2/(DE)$] [11]. With a length scale governed by the width of the membrane ($L \approx 10^{-8}$ m), energy scale dictated by the barrier height (order $E \approx 10$ kT), and an ion diffusion coefficient of $D = 10^{-10}$ cm²/sec this time is around 10^{-7} s.

For simplicity, we take the case that either 0 or 1 ion can be bound at the binding site of the protein. Then the currents from the reservoirs to the well $I_{1,2}$ are

$$I_1 = \vec{k}_1(1 - Q) - \overset{\leftarrow}{k}_1Q, \quad I_2 = \overset{\leftarrow}{k}_2(1 - Q) - \vec{k}_2Q. \quad (3)$$

With these relations, Eq. (1) can be integrated to obtain $Q(t)$ for arbitrary time dependence of the rate constants with any value of the chemical potential difference. The result can then be inserted into either of the relations (3) and averaged over a period of oscillation to obtain the net current. We discuss a limiting case of how this mechanism supports pumping against a concentration gradient and derive a maximum thermodynamic efficiency. First, we examine the special case of pumping near equilibrium.

With $\Delta\mu = 0$ the ratios $\vec{k}_1/\overset{\leftarrow}{k}_1 = \overset{\leftarrow}{k}_2/\vec{k}_2 = e^\epsilon$, and $\overset{\leftarrow}{k}_2/\vec{k}_1 = \overset{\leftarrow}{k}_2/\overset{\leftarrow}{k}_1 = e^u$. Using these relations in Eq. (2) we see that $F = (1 + e^u)^{-1}$. Further, we obtain the equilibrium binding probability $Q_{\text{eq}} = (1 + e^\epsilon)^{-1}$ by setting the currents $I_1 = I_2 = 0$ and solving the resulting equation. The adiabatic pump current can then be written

$$I_{\text{ad}} = \overline{FdQ_{\text{eq}}/dt} = \omega \oint FdQ_{\text{eq}}, \quad (4)$$

where ω is the frequency of the modulation.

The dependence of this expression on time arises only via the internal parameters u (through F) and ϵ (through Q_{eq}). The value of the integral is nonzero if ϵ and u vary out of phase with one another.

How can an externally applied ac electric field, by alternately changing the relative energies of states with different dipole moments [12,13], cause two such internal parameters of a protein to oscillate out of phase with one another? Consider the system shown on the left of Fig. 2, with two "major" conformational states.

One state, labeled *A*, has high affinity ($\epsilon_A > 0$ and $Q_{\text{eq},A} > 1/2$) and easy access between the well and the reservoir on the left ($u_A < 0$ and $F_A > 1/2$). The other state, labeled *B*, has low affinity ($\epsilon_B < 0$ and $Q_{\text{eq},B} < 1/2$) and easy access between the well and the reservoir on the right ($u_B > 0$ and $F_B < 1/2$). If *A* and *B* have different dipole moments, an external ac field will alternately favor first one, and then the other state, causing the average values of F and of Q_{eq} to oscillate. The physical motion by which the protein responds to the field is very complicated, involving many weak interactions and generally with at least several characteristic time scales. For simplicity let the conformational transition process be described by just two relaxation times, one "fast" and the other "slow" compared to the time scale for the external perturbation. If F is controlled by the fast relaxation, and Q_{eq} by the slow relaxation, F will rapidly increase to its equilibrium value $F > 1/2$ when the field favors "A," followed by an increase of Q_{eq} and binding of the ion from reservoir 1. When the field reverses, favoring *B*, F quickly adjusts to $F < 1/2$ followed by a decrease in Q_{eq} and release of the ion to reservoir 2.

If the amplitude of the external field is small enough that the amplitude of the oscillation of each state population is small compared to its steady state value we can

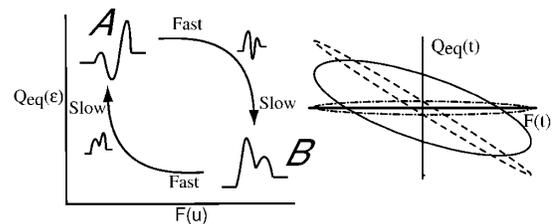


FIG. 2. A two state mechanism for adiabatic pumping. For clarity we have drawn energy diagrams for configurations along the pathways leading between the two states. An oscillating field that alternately favors one state and then the other will cause the ensemble average affinity and relative access to oscillate out of phase with one another, as shown by the parametric plot on the right using Eqs. (5) and (6). The number of ions transported per cycle of the field is proportional to the area enclosed [Eq. (4)] which is maximized when the product of the external frequency and relaxation time of the slow process is unity ($\tilde{\omega} = 1$) (solid line). The dashed diagonal curve shows the plot for low frequency ($\tilde{\omega} = 0.1$) and the horizontal dash-dotted curve shows the case for high frequency ($\tilde{\omega} = 10$).

use a linear response theory. If the relaxation along the “ F ” coordinate is fast compared to the external oscillation frequency ω we have

$$F(t) \approx F_0 + F_1 \cos(\omega t). \quad (5)$$

If the relaxation along the “ Q_{eq} ” coordinate is slower, with relaxation time τ , $Q_{\text{eq}}(t)$ can be written [13]

$$Q_{\text{eq}}(t) \approx Q_{\text{eq},0} - \frac{Q_{\text{eq},1}[\cos(\omega t) + \tilde{\omega} \sin(\omega t)]}{1 + \tilde{\omega}^2}, \quad (6)$$

where $\tilde{\omega} = \tau\omega$ is the reduced frequency. A plot of $F(t)$ vs $Q_{\text{eq}}(t)$ parametrized by time is shown on the right of Fig. 2. Inserting Eqs. (5) and (6) into Eq. (4) we find

$$I_{\text{ad}} \approx F_1 Q_{\text{eq},1} \frac{\tilde{\omega}}{1 + \tilde{\omega}^2}. \quad (7)$$

In previous work nonadiabatic flux arising from a two-state model was discussed [13]. This mechanism relies on the relaxation of the ion binding process, with relaxation time τ_{ion} . Defining the ratio $r = \tau/\tau_{\text{ion}}$ this nonadiabatic flux is given by the equation

$$I_{\text{nonad}} \approx A \frac{r^2 \tilde{\omega}^2}{1 + r^2 \tilde{\omega}^2}. \quad (8)$$

In Fig. 3 we fit the net current $I_{\text{net}} = I_{\text{ad}} + I_{\text{nonad}}$ to the data of Liu, Astumian, and Tsong [3] for ac field induced pumping of both Rb^+ (an analog of K^+) and Na^+ as functions of the reduced frequency, with $\tau = 10^{-6}$ s for sodium and $\tau = 10^{-3}$ s for rubidium.

The fit parameters $A/(F_1 Q_{\text{eq},1})$ and r are the ratios between the amplitude of the nonadiabatic and adiabatic pumping, and the ratio between the relaxation time for the internal conformational change controlling Q_{eq} to that for the external ion binding process, respectively. The parameter $A/(F_1 Q_{\text{eq},1})$ is quite small (0.14 in the fit shown in Fig. 3) indicating that the nonadiabatic contribution is essentially negligible except at very high frequencies $\tilde{\omega} \gg 1$, and the fit is insensitive to the value of r between 0.1 and 10 indicating that the rate for the ion binding process is roughly the same as that for the conformational change. Thus we conclude that the Na,K ATPase may work in many respects like an adiabatic pump, where two internal parameters are caused by the applied field to oscillate out of phase with one another.

A chemical kinetic model with three or more states (and therefore two or more relaxation times), however, can also give rise to a pumped current [7] $I_{\text{kin}} \approx (1 + a^2)\tilde{\omega}^2/[(a^2 + \tilde{\omega}^2)(1 + a^2\tilde{\omega}^2)]$ that can, with $a \approx 3$, fit the data of Liu *et al.* [3] quite well. This model is very different from the adiabatic model described above and relies on the external field driving the ion binding process out of equilibrium. Expression (4) for the adiabatic current is strictly odd with respect to frequency, depending on ω , ω^3 etc., while the expression (8) for I_{nonad} and for I_{kin} is strictly even with respect to frequency, depending on ω^2 , ω^4 etc. Thus, the two mechanisms should be

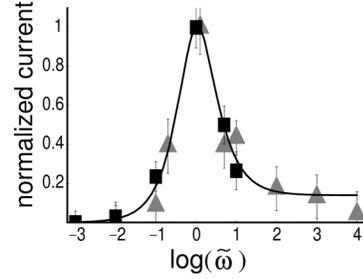


FIG. 3. Fit of data [3] for pumping Na^+ (black squares) and Rb^+ (grey triangles), which is an analog of K^+ , to the sum of Eqs. (7) and (8). We estimated the relaxation times $\tau_{\text{Na}^+} = 10^{-6}$ s and $\tau_{\text{Rb}^+} = 10^{-3}$ s from the frequency at which the flux was maximum [3]. Then we subtracted the baseline from each data point and normalized by dividing all fluxes by the maximum flux at 10^3 Hz and 10^6 Hz for Rb^+ and Na^+ , respectively. The fit parameters were $A/(F_1 Q_{\text{eq},1}) = 0.14$, which sets the high frequency saturation level, and the ratio $r = 1$, to which the fit is quite insensitive between $0.1 < r < 10$. The experimental data represent net flux obtained as the difference of field stimulated influx and field stimulated efflux measured using radiotracer exchange as described by Liu *et al.* [3]. In the experiments the applied fields induce an oscillation of the $\Delta\mu$. However, the conformational change of the protein from one form to another involves effectively two to three charges moving across the membrane, so the amplitude of the effect due to the oscillation of $\Delta\mu$ is smaller by about a factor of e^3 than that due to the effect on the protein conformation and we neglect it here.

distinguishable by very careful experiments in the low frequency regime, although the variability of biological samples may make this difficult in practice.

In the adiabatic mechanism for ion pumping by a protein the phase lag between F and Q_{eq} is caused by an internal conformational degree of freedom being out of equilibrium with the applied modulation. Even at low frequency the system is not in global equilibrium, but only in equilibrium with respect to the degree of freedom corresponding to ion transport. Recently an adiabatic electron pump based on the theory of Thouless [14] was experimentally realized using a quantum dot [6]. There the phase lag was introduced externally by separately modulating two parameters, so the pumping was truly globally adiabatic.

In the experiments of Liu *et al.* [3], the conformational oscillation was driven by an applied oscillating electric field. In chemically driven pumping, where, e.g., ATP hydrolysis drives transport, the stochastic binding of reactants and release of products cause transitions between states of the protein. In this case, after phosphorylation or dephosphorylation, the differential barrier height that controls the parameter F rapidly approaches its final value, followed by a slower relaxation of the well energy (i.e., Q_{eq}) to its new value. In this way, a stochastic input (ATP hydrolysis) is converted into two on average phase shifted outputs. Such hysteretic behavior is very general in proteins or, for that matter, for any relatively complex

molecule [13,15]. Chemical bonds can be characterized by their thermodynamic stability, i.e., bond strength, and by their kinetic lability, i.e., the rate at which the bonds are made and broken. When an external constraint is changed very slowly from one value to another and then back again chemical bonds are broken in the order of least stable to most stable and formed in the order most stable to least stable—i.e., the forward path from one state to another is the microscopic reverse of the backward path. On the other hand if the constraints are changed rapidly, bonds break in the order of most labile to least labile and also form in the order most labile to least labile. In this case the forward and backward pathways are not the microscopic reverses of one another.

It is important to note that for an “adiabatic” pump the direction of ion pumping is controlled by the internal relaxation dynamics of the protein. The two equilibrium states in Fig. 2 can be described in terms of the binding affinity and access to the two reservoirs. The state within the upper left-hand corner has strong affinity and easy access to the reservoir on the left, while the state in the lower right-hand corner has weak affinity and easy access to the reservoir on the right. The direction of pumping from left to right is consistent with the pictures of Lauger [1] and Jencks [2] given in terms of an ATP driven cycle through a sequence of equilibrium states. However, unlike those pictures, if here we reverse the relative relaxation times governing the access (F) and affinity (Q_{eq}) the direction of pumping is reversed.

We have focused on the case of pumping between reservoirs with identical ion chemical potentials because the rigorous absence of a leak current significantly simplifies the mathematical analysis. If $|u| \gg 0$ in each state, the leak current is approximately zero even for a nonzero gradient since the leak conductance is limited by the highest barrier. The maximum probability (achieved in the limit $|u| \rightarrow \infty$) for an ion to be pumped in one cycle of modulation is the difference in occupancy between the two states, which can be written $\tanh[(\Delta\epsilon - \Delta\mu)/2]$ since the well equilibrates with the reservoir to which it has finite access. Thus the maximum average output energy per cycle is $E_{out} = \Delta\mu \tanh[(\Delta\epsilon - \Delta\mu)/2]$. If we identify $\Delta\epsilon$ with the free energy released by ATP hydrolysis (i.e., the energy in kT provided by ATP hydrolysis under physiological conditions) the maximum efficiency is about 75%.

For an ion pump driven by a far-from-equilibrium chemical reaction such as ATP hydrolysis, there is little advantage to an adiabatic mechanism [16]. However, if the process would be driven by a reaction only slightly away from equilibrium, pumping ions up only a very small gradient of chemical potential, an adiabatic mechanism is much more efficient. The fact that pumping driven by an applied oscillating electric field is best fit by an almost adiabatic mechanism may reflect the evolutionary origins of biological pumps as channels that were incrementally modified to pump ions. It has been shown that

ion current down an ion electrochemical gradient can drive an ion channel to undergo directional cycling through a sequence of conformational states [17]. A chemical reaction that drives the cycling in reverse would drive uphill pumping.

A direct experimental test of the ideas discussed in this Letter has become possible with the recent development of methods to construct single ion pores from thin films [18]. With this technique a synthetic ion pump with several internal degrees of freedom engineered to control ion well energies and gate heights can be made [19]. By coupling into these internal degrees of freedom, ions can be pumped with an external stochastic field. Hopefully, the ideas discussed here, in conjunction with these experimental achievements, will pave the way for construction of engineered adiabatic ion pumps in the near future.

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