Na,K-ATPase as a Brownian Motor: Electric Field-Induced Conformational Fluctuation Leads to Uphill Pumping of Cation in the Absence of ATP

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Abstract. Na,K-ATPase uses chemical bond energy of ATP to pump K^+ into, and Na^+ out of a cell. Both are uphill transports. During the catalytic cycle the enzyme alternates between two conformational states, E_1 and E_2 . This communication describes an experiment, which employs electric field to drive oscillation or fluctuation of enzyme conformation between the E_1 and the E_2 states. It is shown that the field-induced conformational oscillation or fluctuation leads to uphill pumping of the cation by the enzyme without consumption of ATP. Biochemical specificity of the catalysis is preserved. Data indicate that Na,K-ATPase can harvest energy from the applied electric field to perform chemical work, and a ratchet mechanism is inherent in this energy transduction process. A Theory of Electroconformational Coupling (TEC) that embodies essential features of the Brownian Ratchet successfully simulates the field-frequency and field-amplitude optima and other features of the ion pumping activity. A four-state TEC motor can achieve high efficiency of the energy transduction, asymptotically reaching 100% under the optimal condition. Pumping by ion rectification fails to reach high efficiency. The TEC concept is also mused to understand other biological motors and engines.

Key words: Brownian ratchet, conformational fluctuation, ion pump, molecular engine

1. Introduction

A cell uses molecular scale devices and engines to perform all its functions. Among these devices and engines an universal instrument for pumping K^+ into, and Na^+ out of the cell is the Na,K-ATPase [19, 23]. Because of its highly specific purposes it is also referred to as Na,K-Pump. Na,K-ATPase is a membrane integral protein. While there may be minor variations in structural details to gear toward different environments in which different types of cells are to survive the basic construct of the pump is known. The molecule is composed of two α -subunits, each of 115 kDa and two β -subunits each 35 kDa. The α -subunit is the catalytic subunit and there is an ATP binding and hydrolysis site. The α -subunit also has binding sites for K^+ facing the external medium and for Na^+ facing the internal medium, the cytoplasm. The β -subunit is the regulatory subunit. Skou discovered the enzyme

in 1957 and today its biochemical mechanism of action is to a great extent understood. Basically there are two biochemical steps in a pump cycle. One is the Na^+ -dependent phosphorylation and the other is the K^+ -dependent dephosphorylation. The phosphorylation/dephosphorylation cycle triggers conformational changes with concomitant transport of Na^+ in the first step and of K^+ in the second step [8, 19, 23]. An ATP is hydrolyzed in one transport cycle. An interesting property of the transport process, which portended our selection of electric field to generate conformational oscillation, is the asymmetric transport of ions by the enzyme [20, 21, 25, 26]. In the cell the enzyme hydrolyzes one ATP to pump three Na^+ out of the cytoplasm with a concurrent pumping-in of two K^+ from the external medium. If no other biochemical process came into play to offset this charge imbalance the cytoplasm would be stocked with negative charges. The asymmetric cation pumping is perhaps one of many mechanisms with which a cell can maintain a negative electric potential inside its plasma membrane. The enzyme is thus electrogenic. Many studies have confirmed that the conformational states of enzyme are voltage-sensitive, i.e. a change in the transmembrane electric potential of a cell will shift the chemical equilibrium of the enzyme between the cytoplasm-facing E_1 and the external-cellular-facing E_2 states [18, 22]. This characteristic of the enzyme has been investigated in greater detail [1, 11, 20, 21, 25, 26, 35, 36]. We ask the question, can conformational fluctuation induced by forces other than ATP hydrolysis, e.g. electric potential, also lead to uphill pumping of the cations? And, if so are the mechanisms of action similar in both cases?

2. Human Erythrocyte as Model System

A specific inhibitor of Na,K-ATPase is available which greatly facilitates our experimental design. Digitalis, such as ouabain, is an effective blocker of the K^+ binding to the E_2 state. Blocking the K^+ binding effectively inhibits the pump activity. The inhibitor action is selective and at the sub-millimolar concentration other ion transporters are not affected [19, 23]. Thus, ion fluxes which can be suppressed by ouabain are attributable to the Na,K-Pump activity. Human erythrocyte is the cell of our choice and ion movements are measured with the radioactive tracers of Na^+ , K^+ and Rb^+ . *Our experimental system preserves all the native environment of the enzyme and any effect observed would have some relevancy to its biological function.* Other evidence, which will be presented later, will also show that the phenomenon we report here is related to its physiological pump activity. In many of our experiments Rb^+ was substituted for K^+ because of the longer decay half-life of Rb^{86} than of K^{45} . Na,K-ATPase pumps Rb^+ inward just as effectively as it does K^+ [8].

A typical experiment is done as follows [11, 20, 21]. Freshly drawn human blood is washed several times with isotonic saline, which contains heparin to prevent coagulation. The washed cells are then re-suspended in an isotonic medium, which contains the desired ionic compositions. Appropriate radioactive tracer of

Rb⁺, K⁺, or Na⁺ is added. To control cytoplasmic ion concentration we use electroporation/resealing technique [17, 27]. In some experiments cytoplasmic ATP is depleted to less than 10 μ M. A complete depletion of ATP was found detrimental to cell integrity and was avoided. As 10 μ M is about 100 times below the K_m , of the ATP hydrolysis, its presence should pose little problem in our interpretation of data. Furthermore, most experiments have been done at a low temperature (3–4 °C) at which ATP hydrolysis is negligibly small.

Voltage stimulation is done to cells in suspension and ion influx and efflux measured with radioactive counting. As stated, ouabain-sensitive fluxes are attributed to the activity of Na,K-ATPase. A voltage-stimulated sample is compared with a control sample, which is kept at the same temperature for the same length of time. For experimental setup and details please refer to publications elsewhere [20, 21].

3. Voltage Stimulation with Different Waveforms

If the chemical equilibrium between the E_1 and the E_2 states of the enzyme is amendable to electric perturbation as the literature indicates, the system will experience a driving force of $\Delta G = -\Delta \mathbf{M} \cdot \mathbf{E}_{\text{eff}}$ when an electric field is applied to the system [27–31]. A device of proper design should be able to harvest this free energy to do chemical work. As is clear from the equation while the ΔG is a scalar quantity it varies with time depending on the dot product of the two vector quantities. This point will be further elaborated later. Here $\Delta \mathbf{M}$ is the difference in the molar electric moments of the two states and $\mathbf{E}_{\text{eff}}$ is the effective field strength experienced by the enzyme. As the enzyme is a transmembrane protein the electric potential it experiences is simply the transmembrane potential, which for our present discussion is, $\mathbf{E}_{\text{eff}}/d = 1.5R_{\text{cell}}\mathbf{E}_{\text{ap}}$, where d , R_{cell} and $\mathbf{E}_{\text{ap}}$ are respectively the thickness of the cell membrane (~ 5 nm), the radius of the cell and the applied electric field. In the absence of three-dimensional structures of the enzyme for the two states, $\Delta \mathbf{M}$ is expressed as,

$$\Delta \mathbf{M} = (\sum_i z_i \Delta \mathbf{r}_i + \Delta \mu + \Delta \alpha \mathbf{E}_{\text{eff}}) N_{av}.$$

In the equation z_i , $\Delta \mathbf{r}_i$, $\Delta \mu$, $\Delta \alpha$ and N_{av} are respectively the net charges of i -th group, vector displacement of the group, difference in the dipole moments, and the polarizabilities, of the two states, and the Avogadro's number. For human erythrocyte used in our experiment $\mathbf{E}_{\text{eff}}$ is roughly $1000 \times \mathbf{E}_{\text{ap}}$.

Electric pulses of several different waveforms have been tested of their effectiveness in driving uphill transport of Rb⁺, K⁺ and Na⁺, and all these waveforms fuel active transport within specific sets of electric parameters. These waveforms are sinusoidal waveform, square-topped waveform, random telegraph field (RTF) of constant amplitude, and RTF with amplitude varying according to Gaussian distribution function. An RTF has a pulse-width t expressed as,

$$t = -\tau^* \ln(R).$$

Figure 1. Pulsed electric field based on the Random Telegraph Function (RTF) and the Gaussian Distribution. In A, a square-top RTF of alternating polarity, with constant amplitude of 20 V cm^{-1} and a mean frequency of 1.0 kHz is shown. The lifetime of the RTF has a distribution shown in Panel D. In B, the pulsed bipolar electric field has a constant lifetime but its amplitude varies according to the Gaussian Distribution shown in Panel E. In C, both lifetime and amplitude are fluctuating. The frequency follows the RTF of D and the amplitude follows the Gaussian Distribution of E. These waveforms were generated by a PC to drive a pulse generator for the electric stimulation of Na,K-ATPase by Xie *et al.* [35, 36].

Here τ^* is the mean pulse-width and R is a random number between 0.01 and 0.99. RTF has a Poisson distribution of pulse width although their occurrence is random. For a Gaussian, the amplitude x follows the distribution function,

$$G(x) = (1/\sigma\sqrt{\pi}) \exp[(x - \mu)^2/2\sigma^2].$$

In the equation μ and σ are, respectively, the mean and the standard deviation of the distribution. Figure 1 gives examples of RTF and RTF-Gaussian waveforms.

The selection of electric field strength is critical and care must be given to avoid electroporation of cell membrane by the applied field. Electroporation of cell

Figure 2. Electric activation of the Rb^+ pumping mode of Na,K-ATPase in human erythrocyte. Panel (a) gives Rb^+ influx of human red cell stimulated with the RTF of mean frequency 1.0 kHz, as shown in Panel A of Figure 1, with varying field strength. Data in the filled circles are for the electric field stimulated samples; in the stars are for the stimulated samples in the presence of 0.20 mM of ouabain which inhibits Na,K-ATPase. Data in the crosses are for the control samples, without electric stimulation, with or without ouabain. The experiment is done at 3 ± 1 °C at which temperature ATP hydrolysis is insignificant. Panel (b) shows the result of an experiment with the constant amplitude of 20 V cm^{-1} with varied mean-frequency of the RTF. The curves, drawn across the data points of the stimulated samples are simulated results based on the four-state model (Scheme II) according to the Theory of Electroconformational Coupling (TEC) [35].

membranes will lead to leakage of ions and produce ion fluxes that are unrelated to the pump activity. Fortunately any ion flux due to electric damage of membrane will be a passive diffusion and it can be easily discerned from fluxes that belong to active pumping. The results presented below recount only the active pumping by the Na,K-ATPase with sinusoidal and the RTF waveforms [11, 20, 21, 32, 35, 36].

1. Sinusoidal waveform: The surprise finding is that an alternating current (a.c.) of amplitude 20 V cm^{-1} induces ion fluxes which are attributable to Na,K-ATPase activity. No passive leakage of ions is detected. The maximal fluxes at 3 °C are approximately 30 K^+ , or Rb^+ inward pumping per enzyme per

Figure 3. Effects of a broadband electric noise on the electric field-stimulated Rb^+ pumping activity of Na,K-ATPase. All experiments are done at $3 \pm 1^\circ\text{C}$ as described in Figure 2. Here a sinusoidal electric field with a frequency of 1.0 kHz is used for stimulation. The amplitude of the sinusoidal electric signal is varied: 20, 17.5, 15, 12.5, 10, 7.5, 0, and 20 V cm^{-1} , respectively, for Curves 1, 2, 3, 4, 5, 6, 7 and 8. The electric noise used has a relatively flat power spectrum above 100 kHz, and a cutoff frequency around 1.0 MHz. Samples for the experiments shown in Curve 8 also contain 0.20 mM of ouabain. 20 V cm^{-1} /1.0 kHz sinusoidal electric field is an optimal signal for the pump activity [11, 20, 21]. As shown in Curve 1, added noise at any level, reduces the efficiency of the pump. The threshold of the sinusoidal electric signal to stimulated pumping is 10 V cm^{-1} [11, 20, 21]. Data shown in Curve 6 indicate that appropriate levels of electric noise may elevate a sub-threshold signal to surface above the threshold. Effects of electric noise on intermediate levels of signal are positive at low levels but negative at high levels. The effect is compared with stochastic resonance reported in the literature. See main text for details. (Results of T.D. Xie).

second and 45 Na^+ outward pumping per enzyme per second. There is an optimal frequency associated with each ion transport. Here we are again astonished to find that the optimal frequency for the K^+ or Rb^+ influx at 1.0 kHz is very different from that for Na^+ efflux, at 1.0 MHz. The K_m (Michaelis-Menten constant) for the external K^+ or Rb^+ is 1.2 mM and for the cytoplasmic Na^+ is 8 mM, consistent with the regular activity of the enzyme with ATP as the energy source. Another important control experiment is to vary internal ATP concentration in the voltage stimulation experiment. Experiments shown that varying [ATP] from 10 μM – 5 mM has little effect on the voltage stimulated activity. Notice that these experiments are done at 3 °C where the ATP hydrolysis does not take place. Experiments done at higher temperatures do detect ATP hydrolysis activity but voltage stimulated samples still record higher pump activity. At 37 °C where the ATP hydrolysis is at its maximum and the pump is at its full capacity, no further stimulation by the electric field is observed.

2. Random Telegraph Field: As discussed below an a.c. field can turn a catalytic wheel in certain direction dependent on the thermodynamic and kinetic parameters of an enzyme catalytic mechanism. In a cell electric field on, around, or across a cell membrane is unlikely regularly oscillatory. If the enzyme adopts a ratchet mechanism for its action, at the molecular scale, it is likely Brownian [1, 6, 27–32]. The same argument can be made for the chemical fuel ATP [2–4, 27–32, 37]. ATP binding, hydrolysis and release of ADP and P_i while follow a certain sequence, each step is stochastic. Analysis of a simple four-state TEC enzyme shows that a ‘flashing ratchet’ can be very effective for harvesting energy from a fluctuating electric field to perform chemical work. Figure 2 gives the result of a typical experiment using RTF waveform of constant amplitude. The line drawn across the data points is the simulation of the TEC model (see Scheme II below). The good match indicates that the model has some merit. Not surprisingly for a larger standard deviation in a Gaussian/RTF, the transport activity, or efficiency of the pump, is greatly reduced. However, a careful study reveals minor mismatch in fine features [36]. This slight discrepancy could mean either the four-state model is too simple to account for Na,K-ATPase action or the TEC model needs further refinement.
3. Signal and Noise: These electric activation experiments have led us to define, at least for the purpose of the present discussion, the terms ‘signal’ and ‘noise’ [27, 28, 32]. An electric signal is taken as a pulsed electric field, which can productively interact with the ion pump under consideration and produce up-hill fluxes of the three cations. Electric fields, ambient or applied that are not recognized by the pump are considered noises. A noise may be periodic with an irrelevant frequency or fluctuating field with a time average of zero or of a finite quantity. This statement serves our purpose when effect of ‘white noise’ is intentionally added to the electric stimulation experiment.

A 'white noise' used in our experiment has a broad power spectrum with a cutoff frequency of approximately 1 MHz [32]. The power level of added electric noise is expressed as the root-mean-square voltage of the noise. The experiment is limited to Rb^+ pump measurement because for the Na^+ pump a white noise of broader power spectrum extending beyond 1 MHz would be required. All waveforms mentioned above have been used as electric signal against different power levels of electric noise. Figure 3 presents only data obtained with a sinusoidal signal. Data show that a low level of electric noise aids in signal transduction, i.e. driving cation uphill, when the signal is either below a threshold or below an optimal intensity. At the optimal level of ion pumping, electric noise exerts only negative effect, i.e. it suppresses the pump activity. Are the effects observed here, the stochastic resonance reported for other biological systems [5, 34]? A more systematic study will be published elsewhere.

4. Theory of Electroconformational Coupling (TEC)

As mentioned above the most widely adopted enzyme model, the Michaelis-Menten enzyme, when viewed from the present perspective, as illustrated below (Scheme I), is a catalytic wheel which is similar to our four-state TEC model (Scheme II).

Here S is the substrate and P, the product. When S to P conversion is a spontaneous process the mechanism becomes a catalytic wheel, turning clockwise in this case. The enzyme is recycled, or turned-over, in each turn of the wheel.

In Scheme II, K^+ in the extracellular medium is equivalent to substrate S in Scheme I and K^+ in cytoplasm is equivalent to product P. The main distinction between Scheme I in its most commonly used format and Scheme II is that $S \rightarrow P$ reaction is a down hill reaction (with a negative ΔG) while the $Rb_{out}^+ \rightarrow Rb_{in}^+$ reaction is an uphill transport (with a positive ΔG). However the distinction needs not be the case. When the applied field is turned off, Scheme II will become channel-like to allow passive transport of the cations. In a cell ATP synthesis from ADP and P_i has a ΔG of between 40 to 50 kJ. Oxidation of foodstuff provides energy to upset this large energy cost. Without this energy source F_0F_1 ATPase hydrolyzes ATP to pump proton uphill.

Na,K-ATPase is embedded in a cell membrane and there is restricted degree of freedom (see also the discussion of TEC model below). If the enzyme in Scheme I has similar restriction on its degree of freedom, for example, ATPase in a myosin head or RNA polymerase in DNA template, which is constrained and oriented in the thick filament or DNA polymer, many properties discussed below will also surface. In other words, Scheme I and Scheme II are equivalent schemes and the TEC concept is applicable to membrane integral enzymes as well as other enzymes with constrained motion. How will Scheme II behave if it is exposed to an oscillating or a RTF potential?

Figure 4 illustrates that Scheme II is a flashing ratchet. When the membrane is polarized (negative potential inside) the potential energy profile favors binding of the ion to E_1 and conversely when the membrane is depolarized (positive potential inside) the potential profile favors dissociation of the bound ion from E_2 . The applied electric field can force the conformational change between E_1 and E_2 , i.e. to drive change of potential energy profile from State 1 to State 2. In an a.c. field the system will be forced to oscillate between the two potential energy profiles [13, 27–32]. In a Gaussian/RTF electric field the system will fluctuates reminiscent of the Brownian Ratchet [32, 36]. Astumian and coworkers have discussed a flashing ratchet, in which the energy potential ratchets between a saw-teeth function and a flat function [2, 3]. In this case, an out-of-equilibrium thermal noise is shown to transport work load uphill, including myosin locomotion on actin cable, kinesin locomotion on microtubule, particle separation and uphill ion pumping, etc. The profiles shown in Figure 4 have been applied to simulate electric activation of mitochondrial ATPase and Na,K-ATPase by Tsong, Astumian, Markin, Chen and coworkers [1, 6, 11–16, 27–32, 35, 36]. Our analysis of the four-state TEC ratchet, Scheme II, clarifies many interesting aspects of the ion pump. These are outlined below.

1. Anisotropic chemical reaction: The application of vectorial driving force, periodic or fluctuating, to a system with constrained mobility and degree of freedom is characteristic of most biological motors. Both $\Delta \mathbf{M}$ and $\Delta \mathbf{E}_{eff}$ are vector quantities. Here the chemical reactions occur in a media that are anisotropic. Anisotropy simply means slow rotational and translational relaxation compare to an oscillatory or a fluctuating driving force [27, 28]. The motor

Figure 4. Conformational fluctuation of an *Ion Channel/Pump* is schematically illustrated. In E_1 , the channel opens its ligand binding side towards the left. The potential profile for the ligand to approach the channel is shown in 1b. When the channel opens the ligand binding side now faces the right (E_2) and the potential profile for ligand is altered, as shown in 2b. 3a,b and 4a,b are the ligand-bound species for the E_1 and the E_2 , respectively. When the conformation of the channel fluctuates between E_1 and E_2 , the potential profile for the ligand binding also fluctuates. The system mimics a Flashing Ratchet. As discussed in the main text, the E_1/E_2 fluctuation may be externally enforced [27]. And when the affinity of the ligand for the E_1 is greater than that for the E_2 , the channel becomes a left-to-right pump for the ligand, and vice versa [29]. The TEC model adopts the concept of the conformational fluctuation to construct a biological motor or engine. The model shown here is given a term *Channel Enzyme* [16]. In a special case, the affinity for the ligand is the same in E_1 as in E_2 the enzyme will function like an inefficient ion channel to conduct ion downhill [16].

molecule needs not be immobile. Absorption of polarized light even by a small molecule is anisotropic because rotational relaxation time of the molecule is much longer than the electronic transition process.

2. Asymmetry: For the efficient transduction of energy, asymmetry must also build into its chemical property [6, 29–31]. In Scheme II, the dissociation constant of Rb_{out}^+ must be much smaller than that for Rb_{in}^+ to enable the influx of Rb^+ . Reversing this asymmetry also reverses the direction of the pump. The out-pumping of Na^+ must be modeled with stronger binding inside than outside. This point may have relevance to the locomotion of a kinesin on a microtubule, or other systems which have similar constructs but different directional motions.

3. Optimal field frequency and amplitude: There is a high land or a window of field frequency for each pump to achieve maximal activity [29–31]. Likewise there is a window for the amplitude of the applied field. Frequency window is determined by the chemical rate constants of a TEC scheme. Amplitude window has more complex origin. In the later case many factors such as ΔM and protein stability come into play. Analysis done so far considers protein polarizability and protein stability.
4. Analysis predicts that a window of ligand concentration also exists. Search for such windows have been taken but only limited data are available for the Ca-ATPase of human erythrocyte [28]. For Rb^+ and Na^+ such windows may fall outside of our experimental condition, which is designed to demonstrate the uphill pumping activity.
5. Mitochondrial ATPase: F_0F_1 -ATPase can also be driven by electric field to synthesize ATP. In fact in an actively respiring mitochondrion the transmembrane electric potential across the inner mitochondrial membrane is unusually high, on the order of 200 mV, an E_{eff} approximately 400 kV cm^{-1} . If a mechanism exists to modulate its oscillation the TEC model may apply and aid us understand the inner working of the enzyme. We proposed that proton translocation through F_0 is an effective mechanism for modulating this steady-state potential to make it oscillatory [29–31]. If local oscillation can amount to $\pm 200 \text{ kV cm}^{-1}$ by proton translocation through the F_0 subunit, a 19 kJ of energy may be extracted in each translocation. It is now accepted that in the mitochondrion the stoichiometry of proton translocation is 3–4 per ATP synthesized. Translocation of 3 protons would have provided 54 kJ of energy by the TEC mechanism. A TEC model given in Scheme III summarizes such a construct.

The steady-state electric potential across the inner mitochondrial membrane is sustained by the electron transport chain, which degrades and oxidizes foodstuff to produce usable energy. In Scheme III the second subscript of F_0 indicates whether the proton channel is 'closed' or 'open', and star indicates that the ATPase complex is in an 'excited' state due to electroconformational change when the channel is closed. As the result of the electric excitation ATP is released and the channel is opened. The translocation of proton depolarized membrane and that allows binding of ADP and P_i with concomitant

resealing of the channel. This mechanism suggests to us that the enzyme spins with certain mean periodicity. Experiments were launched to search for such periodicity. Unfortunately the field required for ATP synthesis has to be high, in the kV cm^{-1} range when sub-mitochondrial particles were used and Joule heating easily denatured the membrane sample. With low amplitude a.c. only small amount of ATP is induced and the optimal frequency is roughly 10–50 Hz. How does the optimal frequency seen in our electric stimulation relate to the recent direct observation of Kinosita, Yasuda and coworkers (see related papers in this volume) remains unclear. Also, while we have used TEC concept to interpret ATP synthesis whether or not the mitochondrial ATPase is a molecular ratchet is debatable because of the relative bulk of the enzyme. Work by these authors show that the efficiency of the ATPase approaches 100%. As discussed below, our analysis suggests that the efficiency of a TEC enzyme may also asymptotically reach 100% [7, 12]. Other mechanisms considered by Markin *et al.* fail to achieve such high efficiency [13]. Fulinsky and coworkers have carried the concept of the local potential modulation further and done careful analyses of the behaviors of such ion pumps [9, 10].

6. Stochastic resonance: As reported in the literature noise may have beneficial effects, for example to enhance sensibility of signal transducers or to increase signal-to-noise ratio of ion channel [5, 34]. The phenomenon is known generally as stochastic resonance. Data shown in Figure 3 suggest that Na,K-ATPase also exhibits similar behavior. Our tentative interpretation is as follow. If a TEC enzyme is susceptible to the electric perturbation an imposed electric white noise is like thermal noise is to a Michaelis-Menten enzyme. Adding a high level of non-equilibrium electric noise to the system is equivalent to raising the temperature of the system. Increased temperature can facilitate the enzyme to overcome the activation barrier. However, a high level of noise can also impede normal enzyme action and in severe cases denature the protein catalyst as well. Two opposing effects balance to give rise to the observed enhancement and suppression of the electric activation results.
7. Generalization of the TEC concept: Since Michaelis-Menten enzyme is used to interpret most if not all of the biochemical processes in a cell and it has strong resemblance to the TEC enzyme it comes at no surprise that oscillation or fluctuation of other potential energies may also regulate or control these biochemical reactions [27, 28]. Transduction of sound and acoustic signal is a familiar case. Pressure is the relevant thermodynamic quantity for the process. In a well-constructed mechanism oscillation of pressure may allow transduction of energy amounting to $\Delta P \Delta V$, where ΔV is change in the molar volume of the E_1 and E_2 states. A piezoelectric model for transduction of sound waves has been proposed [14]. Oscillation of membrane curvature or tension may be coupled to changes in surface contact area of the enzyme with the membrane. Here the quantity of interest is $\Delta \gamma \Delta A$. Oscillation of concentration of a species may also serve as a signal to a biochemical process. Many

authors have discussed chemical ratchet in various chemical and biological systems. Notice that energy is a scalar quantity. The product of two conjugate-parameters must produce a scalar quantity to be consistent with the TEC model [28]. However, new biochemical phenomenon may require treatment of energy as a vectorial quantity. Is there any common thread among different kinds of nano-scale devices? If so, study of mechanisms of ion pumps and biological motors and engines will help us understand design and behavior of manmade nano-scale devices.

8. The efficiency of a TEC enzyme: Microscopic reversibility, detailed balancing and maximal energy transfer ensure a TEC enzyme to achieve its maximal efficiency [7, 12, 13, 29]. For the former, the equality, $k_{\text{conf}} \gg f_{\text{mean}}$ must hold true. Here k_{conf} is the rate of the E_1 to E_2 conversion and f_{mean} is the mean frequency of the pulsed electric field. To enable maximal transfer of energy, the equality, $f_{\text{mean}} \gg k_{\text{chem}}$ must be maintained. Here k_{chem} is the chemical rate process, or the ion binding/dissociation reaction. When these conditions are met and with a large amplitude field oscillation or fluctuation, the efficiency asymptotically approach unity. Markin *et al.* show that the efficiency is generally low when the ligand concentrations are similar on both sides of the membrane. However, Astumian's work indicates that Na,K-ATPase may function like an adiabatic electron pump (personal communication of R. D. Astumian). Two chemical relaxation processes have been introduced and these two rates are out-of-phase by nearly 90° , indicative of the adiabaticity of the two processes. The efficiency of such a pump will be high under all chemical conditions used in our experiment. It is not surprising that nature should design an ion pump that is close to an idealized adiabatic pump [24]. Detailed study remains to be done, however.
9. Internal oscillators: It follows from the above considerations that biochemical reactions in a cell are catalytic wheels of one form or another and a catalytic wheel has one or more inherent periodicities that are present by the chemical rates of the system. The range of frequency optimum available for a cell or organism should reflect on the range of molecular dynamics of the protein structure and the chemical kinetics of ion transport and other biochemical processes and is expected to be broad. These biological catalytic wheels are the engines, motors and signal transducers of life.

5. Effect of Electromagnetic Field

One important issue which continues to confront the public and the policy makers and to muzzle the environmental scientists is whether extremely weak and low frequency electromagnetic field can have any effect on our health. At the core of the issue is what is the limit of sensitivity a cell or an organism is susceptible to and for a susceptible field whether it can or cannot produce a beneficial or a detrimental effect. A clearly stated idea is that a signal that is above the ambient

thermal electric noise across the cell membrane will be recognizable by a cell [33]. Here two questions arise. First, what is a signal and what is a noise? Second, can a noise mask a signal or enhance a signal or having no effect on the transduction of a signal?

Earlier a signal is defined based on the TEC concept. Little ambiguity may arise by the simple extension of the TEC concept to include other types of potentials, e.g. chemical potential or acoustic wave. Again as stated an oscillation or fluctuation of a potential that is not perceptible will fall into the category of noise. A noise defined here differs from an equilibrium noise, thermal or thermal electrical. The data shown in Figure 3 indicate that as far as Na,K-ATPase is concerned the white noise at any level is a true noise. In the absence of a signal, increasing power level of the white noise does not trigger any uphill pumping of the cation. The sinusoidal field at 1.0 kHz and 20 V cm^{-1} is a signal. Data show that when a signal is below threshold, for example 10 V cm^{-1} no active influx of Rb^+ are induced. However adding a level of the noise raises the signal above its threshold value and the pump begins to transduce electric energy into a gradient energy. In all cases where the signal is not at its optimal value, addition of a low level of noise aids in the signal transduction of the enzyme.

Oscillatory Activation Barrier (OAB) Model: A signal may be weak, for example with E_{eff} in the V cm^{-1} range or the mV cm^{-1} range. In such a case the coupled energy $\Delta M \cdot \Delta E_{\text{eff}}$ will not suffice to pump an ion uphill or drive an uphill chemical process. However, because of the nonlinearity of the system a weak field is sufficient to alter the rate of a down hill reaction, e.g. the ATP hydrolysis. Markin *et al.* have put this idea into test [15, 28]. They have proposed a model based on electrical resonance between the applied weak signal and the activation barrier of a rate limiting process in the enzyme. They analyze an activation barrier, which is represented by a simple harmonic oscillator. When the oscillator resonates with a weak electromagnetic field, the rate of ATP hydrolysis is enhanced by 50%. An electromagnetic field does not need to provide a driving force to overcome the activation barrier of the reaction, as is the case in the TEC model. ATP hydrolysis is spontaneous under our experimental conditions and the free energy of the reaction serves as the driving force. Figure 5 illustrates how a non-linearity of a system enables a weak electromagnetic field to change the rate of a chemically downhill reaction. This study does not address the crucial question of what is the weakest electromagnetic signal a cell or an organism can perceive. However, it gives us some guidance to search in different regimes for the weak field effects and the strong field effects.

Finally, we ask the question are the biological molecules best described as macroscopic devices or as microscopic devices? Or do they fall in the boarder line between the two worlds? The experiment presented here argues for the second type, i.e. it is a microscopic machine, a Brownian Ratchet made with flexibility and capable of enormous level of conformational fluctuation. The simple construct for a Brownian Ratchet is the Flashing Ratchet. The system oscillates or fluctuates

Figure 5. Explanation of the Oscillating Activation Barrier (OAB) model. To explain effects of weak electric field on a spontaneous chemical reaction (ATP hydrolysis) we have tested an OAB model [15]. The activation barrier of a catalytic reaction is assumed to be oscillatory. If an applied electric field (sinusoidal, or otherwise) interacts with the transition-state structure of the chemical catalysis via electric coupling it will cause the activation barrier (X) to increase the amplitude of oscillation, e.g. by the amount $\pm \Delta X^\circ$, as shown in Panel A. Generally, a chemical rate depends on the barrier height in a non-linear fashion (Arrhenius equation), as illustrated in Panel B. In Panel C, the rate of the reaction in the absence of an external field is shown in the solid sinusoidal curve and its mean is A_0 . In the presence of an applied field the rate will oscillate as given in the dashed sinusoidal curve. The mean velocity $\langle A_{AC} \rangle$ in the presence of the field is elevated from A_0 . Whether the effect is to enhance the rate or to suppress the rate will depend on the mode of interaction and the behavior of the barrier oscillator. Marking *et al.* have tested a harmonic oscillator model [15].

between the two states with distinct potential profiles. A driving force is to sustain the oscillation or fluctuation of the system between these two states. This study also suggests that electric potential is the intermediate state for the transduction of proton gradient energy into the chemical bond energy of ATP. Thus, electric potential and its modulation into fluctuating potential and the energy conversion by the TEC mechanism may all the relevant events in the complex process of biological ATP synthesis. Future task is to search for the logical link to the observation done with single molecule techniques.

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