Synchronization of Ion Exchangers by an Oscillating Electric Field: Theory

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Received: July 12, 2007; Revised Manuscript Received: April 22, 2008

Can we physically manipulate functions of membrane proteins, such as ion exchangers, especially the active transporters? This is a fascinating question which has attracted many scientists. Recently, we developed a new technique that we call synchronization modulation with which we realized significant (many-folds) activation of Na/K pumps by a well-designed oscillating electric field. In this technique, we consider activation of the pump molecules as a dynamic entrainment procedure, where individual pumps are first entrained to run at the same pumping rate and phase as the oscillating electric field and then the two transports are electrically facilitated separately and alternately by gradually increasing the field oscillating frequency. The procedure consists of two steps: synchronization and modulation. In this paper, we discuss the underlying mechanism involved in the first step: synchronization of the pump molecules.

Introduction

Many ion exchangers move one kind of ion out of cells by exchanging for another kind of ion. Some ion exchangers, such as Na/K ATPase, consume ATP; others do not. Significant work has been done by many scientists aimed at physically manipulating functions of the ion exchangers, especially the active transporters.[1–10] The pioneer works of Teissie and Tsong demonstrated that a kilohertz oscillating electric field can facilitate Rb accumulation in erythrocytes in the absence of ATP, which indicated the transduction of electrical energy to the pump.[1] Later, Tsong and co-workers and Robertson and Astumian[4,5] developed the resonance frequency model that predicted the existence of the pump intrinsic or characteristic frequencies. When an applied oscillating electric field resonates with the intrinsic frequency(s), the field can reduce the energy barrier and therefore activate pump functions. These works have been reviewed in an excellent paper.[9]

Recently, we developed a new technique that we call synchronization modulation. On the basis of the experimental results that the pumping rate is adjustable, we have predicted that a well-designed oscillating electric field, if both the oscillating frequency and phase are continuously matched to the pump's turnover, can significantly accelerate the pumping rate, showing an exponential-like $I-V$ curve.[11] However there are hundreds of thousands of pumps in the cell membrane, each of which may run at a different pumping rate and random pumping phase. Therefore, the first step in this technique is to synchronize the individual pumps to run at the same pumping rate and pumping phase.

Microscopically, for each ion exchanger, there should be two components of the currents in each running loop, an outward current representing the outward ion transport and an inward current representing the inward transport. However, because of environmental differences, individual ion exchangers may not have the same turnover rate and may run at random phases due to structural independence. As a result, the two current components cannot be distinguished with the steady-state current measurements. For example, the Na/K pump extrudes three Na ions and pumps in two K ions by consuming metabolic energy from the hydrolysis of one ATP in each pumping cycle. The available pump currents measured during a normal running mode only show a unidirectional outward current without a distinguishable inward component.

The two components of the Na/K pump currents have been studied separately.[12–16] The pumping loop was purposely interrupted by various methods in order to restrict all the pumps to stay at the same state right before the Na- or K-transport step is initiated. Then, either an optical signal or an electrical stimulation was used to trigger all the pump molecules moving them to the next state generating a transient pump current.

In contrast, we are studying synchronization of the pump molecules in a physiological running mode by applying the concept of an electronic synchrotron to the biological system. Synchronization of the pumping rate is more complicated than the synchronization of an electronic beam. In a synchrotron, the acceleration electric field can be applied specifically to the pathway of the electronic beam. Practically, it is impossible to apply an electric field to specifically influence one transport without affecting the other. Any applied electric field will inevitably affect the two transports oppositely.

In this paper, we first briefly show our experimental results in the study of synchronization of the Na/K pumps by a specially designed oscillating electric field. Then, we discuss the underlying mechanisms involved in the synchronization of the ion exchangers using the Na/K pumps as an example. The results show that the pumping loops of individual pump molecules can be synchronized by a well-designed oscillating electric field. The synchronized pumps not only have the same turnover rate as the field oscillation but also have the same phase so that all the individual pumps extrude Na ions during the positive half-cycle and then pump in K ions in the negative half-cycle.

Experimental Results

We have conducted a series of experiments to investigate the effects of an oscillating electric field on the Na/K pump functions. The pulsed oscillating electric field was applied to the cell membrane of single fibers of frog skeletal muscles, and

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10.1021/jp0754637 CCC: $40.75
Published on Web 07/23/2008

the Na/K pump currents were monitored by our improved double Vaseline-gap voltage clamp technique. The detailed technique has been described previously.[17–19] Briefly, single skeletal muscle fibers were hand dissected under a microscope from twitch muscles, semitendonosis of *Rana pippiens* frogs, and mounted into a custom-made chamber. The fibers were held by two clips and stretched to a sarcomere length of 3 μm to avoid contraction during electric stimulations. Then, the fibers were electrically and ionically separated by two Vaseline partitions into three segments. The dimensions of the partitions and the central segment were 100 and 300 μm, respectively. The membranes of two end segments were chemically punctured, and then the three segments were connected to a voltage clamp (Dagan TEV 200) through six Ag/AgCl pellets. The electric field we applied was provided through the central pool and end pool to clamp the potential across the cell membrane in the central pool. Two electrodes were used, one for measuring the potential difference across the cell membrane and another for injecting currents. With consideration for the intracellular resistance, a custom-made negative feedback amplifier was used to compensate for the voltage drop on this series resistance in order to improve the voltage distribution throughout the central pool, which has been proven by the intracellular measurement using a microelectrode.[17]

The end pool solution or the intracellular solution contained a physiological concentration of ATP, and Na and K channel currents were blocked by tetrodotoxin and 3,4-diaminopyridine. All experiments were performed at room temperature, 24 °C.

In all our experiments, the membrane potential was held at −90 mV, the membrane resting potential of skeletal muscle fibers. The membrane potential was first hyperpolarized to −110 mV followed by four negative *p*/4 pulses whose waveforms are identical to, but one-fourth the magnitude of, the following corresponding stimulation pulses. The generated membrane leakage current and the leakage current through the Vaseline seals are added up and subtracted from the currents elicited by the following full-strength stimulation pulse.

The stimulation pulses alternated the membrane potential from −30 to −150 mV at a membrane holding potential of −90 mV. The oscillating frequency is 50 Hz. The elicited transmembrane currents were measured in the presence and absence of ouabain, a specific inhibiter of the Na/K pumps. After removal of the leakage currents, the Na/K pump currents were defined by subtracting the currents in the presence of ouabain from those in the absence of ouabain.

The measured Na/K pump currents in response to the pulsed oscillating membrane potential are shown in Figure 1. The upper panel shows the pump currents elicited by the first 20 pulses. The lower panel shows the pump currents evoked by the 60th to 80th oscillating pulses. As the number of the oscillating pulses increases, the characteristics of the pump currents change significantly.

Initially, the pumps run at random paces with different pumping rates at random phases. The currents elicited by the first pair of half-pulses shown in the upper panel represent the randomly paced pump currents. The positive half-pulse elicited net outward pump currents, and the negative half-pulse elicited very little current. However, as the membrane potential continued to oscillate; the elicited pump currents began to exhibit the following characteristics: (1) The negative half-pulses gradually generated distinguishable inward pump currents which were alternated with the outward components; (2) the magnitude of the outward pump current was about 3-fold greater than the analogous current from the randomly paced pumps; (3) the magnitude ratio of the outward to inward pump currents was about 3:2.

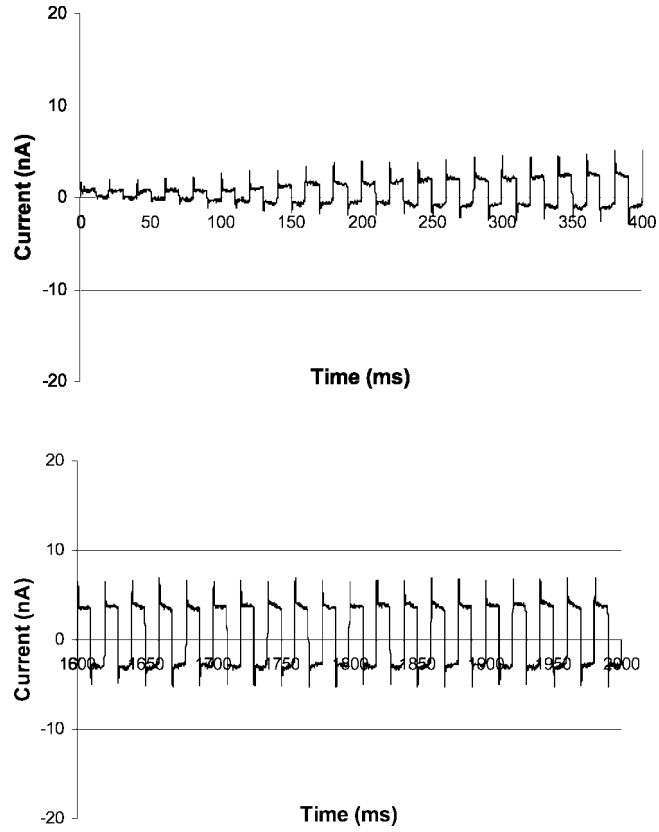

Figure 1. Na/K pump currents measured from skeletal muscle fibers using whole-cell voltage clamp techniques, in response to a rectangular pulsed oscillating electric field alternating the membrane potential from −30 to −150 mV at a membrane holding potential of −90 mV. The upper panel shows the pump currents elicited by the first 20 pulses, while the lower panel shows the pump currents evoked by the fourth 20 pulses.

This result along with the results of our systemic experimental studies[20–22] demonstrates synchronization of the Na/K pumps through the application of a well-designed oscillating electric field. Each pump molecule extrudes three Na ions out of the cell and pumps in two K ions in each cycle, resulting in one net charge outflux. The pump currents measured from random paces exhibits only this net unidirectional outward current. Once synchronized, all pumps extrude Na ions during the positive half-pulse and then pump in K ions. The ratio of the outward to inward current is about 3:2, reflecting the stoichiometric number of the Na/K pump.[23–25]

Theoretical Analysis

Six-State Model of the Na/K Pump and the Energy Barriers for Two Ion Transports. On the basis of the Post−Albers model,[26,27] we can simplify the Na/K pump to a six-state model where all the electrogenic steps in each ion-transport limb are attributed to one voltage-dependent step (Figure 2). Because the Na efflux and K influx occur sequentially in the pumping loop, there is a time window to specifically treat each ion transport. In addition, because the Na and K ions are moving in the opposite directions, the two ion transports have reverse voltage dependences. Therefore, we may be able to design an oscillating electric field to distinguishably influence the two transports.

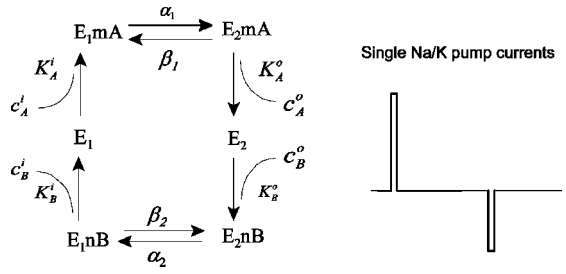

Figure 2. Left panel: Simplified Post−Albers model for the Na/K pump. Right panel: transient pump current of a single Na/K pump where the outward current represents Na extrusion and the inward current represents K influx. The transient pump currents have time courses from tens of microseconds to sub-microseconds much shorter than the time interval between two transports.

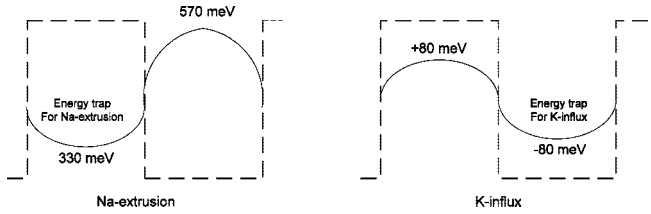

Figure 3. Schematics of energy differences and energy traps for the Na extrusion and K influx in positive and negative half-pulses, respectively.

We will discuss the pump synchronization in two steps, starting from the design of an oscillating electric field that can alternatively change the energy differences in the two ion transports and then discuss how the oscillating electric field forces the individual pumps to run at the same pumping rate and phase.

The energy differences in the two ion transports can be easily calculated. For skeletal muscle fibers, the intra- and extracellular Na concentrations are about 4.5 and 120 mM, respectively.[28,29] The chemical energy difference can be expressed as the equilibrium potential of 60 mV based on the Nernst equation.[28,29] If we choose a symmetric pulsed oscillating waveform alternating the membrane potential from −50 to −130 mV, extrusion of a single Na ion out of the cell requires (60 + 130) = 190 meV of energy during the negative half-pulse. To extrude three Na ions, we need 570 meV, which is higher than at the membrane resting potential of 3(60 + 90) = 450 meV, where 90 represents the membrane resting potential Therefore, the Na extrusion will be hindered during the negative half-pulse, which we called a hindering half-pulse.

During the positive half-pulse of −50 mV, the energy difference is significantly reduced to 3(60 + 50) = 330 mV lower than at the membrane resting potential (Figure 3). Consequently, Na extrusion will be facilitated during this period, which we called a facilitating half-pulse.

Similarly, based on the intra- and extra-cellular K ion concentrations of 115 and 5 mM, respectively,[28,29] the chemical potential can be expressed as the K equilibrium potential of 90 mV.[28,29] The energy needed to pump in two K ions during the positive half-pulse is 2(90−50) = 80 meV, whereas during the negative half-pulse it is significantly reduced to a negative value of 2(90−130) = −80 meV (Figure 3). In comparison with the energy difference at the membrane resting potential of 2(90−90) = 0 meV, the K-influx step will be facilitated during the negative half-pulse and hindered during the positive half-pulse.

Manipulation Effects of the Oscillating Electric Field on the Pumping Rate. We now apply this oscillating electric field to a group of pump molecules with different pumping rates and random pumping phases. To obtain a general result, we have to consider all the possibilities. There are three possibilities or cases.

Case 1: The initial pumping rate is far higher than the field oscillating frequency, or $d_o < T/2$, where d is the time interval between the two ion transports in the pumping loop, d_o is the initial time interval without the field application, and T is the half-pulse duration of the oscillating electric field. In this case, both transports can fall into either a single pulse or two consecutive pulses as the pulse changes its polarity.

Case 2: The initial pumping rate is comparable to the field frequency, $T/2 < d_o < 2T$. The two transports are most likely falling into two half-pulses, alternately. There are two situations: (i) both transports are hindered, and (ii) both are facilitated.

Case 3: Finally, case 3 is that the initial pumping rate is far lower than the field frequency, $d_o > 2T$.

Case 1. The initial pumping rate is far higher than the field oscillating frequency, $d_o < T/2$. When both transports fall into the same half-pulse, the electric field facilitates one transport and hinders the other (Figure 4). If it is a positive half-cycle which is equivalent to a dc depolarization pulse, the total energy needed for the two ion transports is about 330 + 80 = 410 meV, smaller than that at the membrane resting potential of 450 + 0 = 450 meV. The pumping rate is accelerated but not significantly because only the Na extrusion is facilitated while the K influx is hindered. If it is a negative half-cycle, which is similar to a dc hyperpolarization pulse, the energy needed for the entire pumping loop is 570 − 80 = 490 meV, a little higher than at the membrane resting potential. The pumping rate is reduced because of the hindering of the Na extrusion even though the K influx is facilitated; the former is generally slower than the latter.

Since the electric field always facilitates one transport and hinders the other, the field effects on the two transports cannot be accumulated to affect the whole pumping rate. Therefore, only the first pair of transports is affected, resulting in a change in the time interval, d, so that the pumping rate quickly reaches a steady state. This can be seen from the experimental results using a single pulse to study the Na/K pumps. The elicited pump currents show a quick response to the rising phase of the pulse.

When the pulse changes its polarity, the Na extrusion may fall into a negative half-pulse and the K influx into a positive half-pulse. Both transports are hindered. The total energy needed for a loop will be 570 + 80 = 650 meV so that the time interval d will increase more significantly than in the above situation. However, this situation can only happen when the pulse changes its polarity, and then quickly returns to the above situation (Figure 4). It has been shown that neither the Na nor K transient current represents a rate-limiting step of the loop.[29−33] One time hindering for the two transports cannot significantly alter the pumping rate.

On the other hand, the Na extrusion may fall into a positive half-pulse and K influx into a negative half-pulse when the pulse changes its polarity. Both transports are facilitated. The energy needed for a loop is 330 − 80 = 250 meV, much lower than at the membrane resting potential. The time-interval d will be decreased. Again, due to not being a rate-limiting step in the loop, one time facilitation in two ion transports will not significantly accelerate the pumping rate.

In summary, because the initial pumping rate is far higher than the field oscillation frequency so that the field always affects the two transports oppositely, the field effects cannot be accumulated. The pump rate quickly changes in response to

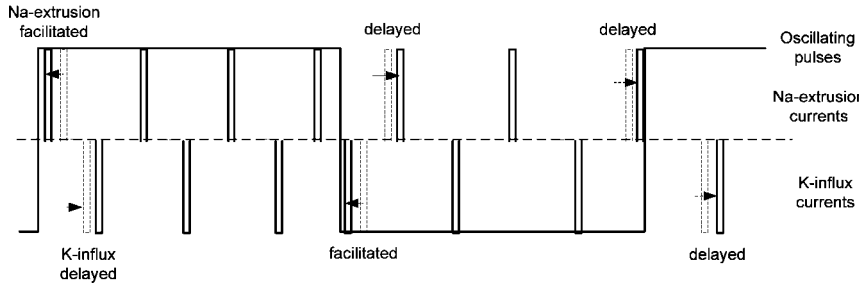

Figure 4. Pumping rate which is much faster than the field frequency, $d_o < T/2$. The dotted lines represent original pump currents without electric field, and the solid lines represent those under the electric field. When both falling in a positive half-pulse, Na extrusion is accelerated and K influx is hindered. This situation is similar to an application of a dc pulse depolarizing the membrane potential. When both falling into a negative half-pulse, Na extrusion is delayed and the K influx is facilitated, similar to a hyperpolarizing pulse. Because of opposite effects on the two transports, the effects cannot be accumulated, and the pumping rate quickly reaches a steady state. When the pulse changes its polarity, Na extrusion may fall into a positive half-pulse and K influx into a negative half-pulse. Both transports are facilitated. Or, Na extrusion may fall into a negative half-pulse and K influx into a positive half-pulse. Both transports are hindered.

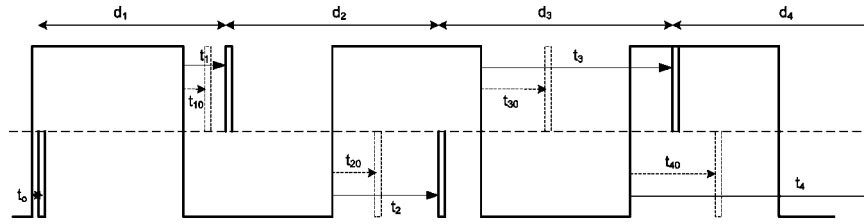

Figure 5. Pumping rate, being a little lower than the field frequency, $T < d_o < 2T$. Both transports fall into the hindering half-pulses. The phase difference, t_{10}, t_{20}, t_{30},..., without considering the field effects, is linearly accumulated as a function of n. When considering the field effects, they, t_1, t_2, t_3,..., will be significantly accelerated until the transports fall into the following facilitating half-pulse, $t_3 > T$. The time interval d between the two ion transports is also linearly increased.

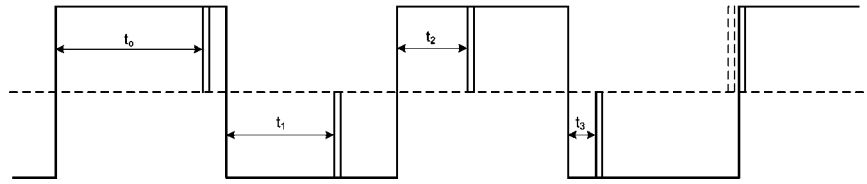

Figure 6. Pumping rate which a little higher than the field frequency, $T/2 < d_o < T$. Both transports fall into facilitating half-pulses. The phase difference t is accumulated to be smaller and smaller even without considering the field effects. Eventually, the transient pump current will catch the rising phase of the pulse. If considering the field facilitation, the phase accumulation will be accelerated (not shown in the figure).

the two half-cycles of the oscillating electric field and repeats again and again.

Case 2. The pumping rate is comparable to the field oscillating frequency, or $T/2 < d_o < 2T$. In contrast to the case 1 where the pumping rate quickly reaches two steady states alternately in response to two half-pulses, the field effects on the pumping loop will be accumulated if the two transports repeatedly fall into either hindering or facilitating half-pulses. Facilitation (hindering) on the first ion transport increases (decreases) its products which go through the intermediate steps and eventually increases (decreases) the reactants of the second transport. This reactant increase (-decrease) and the electrical facilitation (hindering) on the second transport will in turn further facilitate (hinder) the first transport. Consequently, the change in the time interval, d, will be accumulated in the succeeding half-pulses, and therefore the pumping rate will be changed.

To describe this situation, we define a phase difference, t, which is the time interval between the transport and the rising phase of the half-pulse where the transport falls in, and assume that the initial phase difference is t_o. Because of the difference in the pumping rate and the oscillating electric field, this phase difference will be accumulated in the succeeding half-pulses.

For the first situation, both transports alternately fall into the hindering half-pulses repeatedly. Each half-pulse will hinder the corresponding transport and therefore increase d with respect to the previous transport. As the field oscillates, d becomes larger and larger. For simplicity, it is assumed that the hindering effects on both transports are very similar, each half-pulse increasing d by a factor of a. d will be gradually increased in the succeeding half-pulses, which will significantly affect the phase difference.

When the initial time-interval d_o between the two transports is a little shorter than the half-pulse duration, $T/2 < d_o < T$, the phase difference t_n in the succeeding nth half-pulses can be expressed.

$$t_1 = t_o - (T - d_1) = t_o - [T - (d_o + a)] = t_o - (T - d_o) + a$$

$$t_2 = t_1 - (T - d_2) = t_1 - [T - (d_o + 2a)] = t_o - [T - (d_o + a)] - [T - (d_o + 2a)] = t_o - 2(T - d_o) + 3a$$

$$t_3 = t_2 - (T - d_3) = t_2 - [T - (d_o + 3a)] = t_o - 2(T - d_o) + 3a - [T - (d_o + 3a)] = t_o - 3(T - d_o) + 6a$$

$$\vdots$$

$$t_n = t_o - n(T - d_o) + a\sum_{i=1}^{n} i \quad n = 0, 1, 2, \ldots \quad (1)$$

Here the second term represents a down accumulation of the phase difference due to initially $d_o < T$, and the third terms

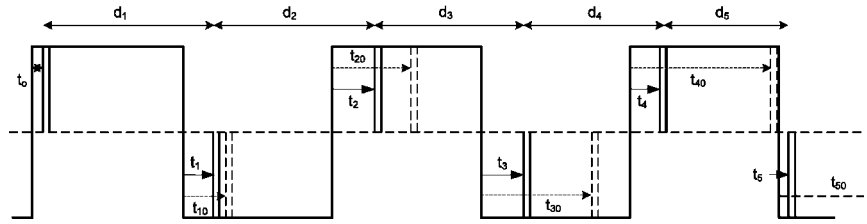

Figure 7. Pumping rate, being a little lower than the field frequency, $T < d_o < 2T$. Both transports fall in the facilitating half-pulses. t_{10}, t_{20}, t_{30},... represent the phase differences without the field application which is linearly accumulated up, while t_1, t_2, t_3, . . . represent the real phase differences under the field facilitation which is initially accumulated up and then quickly accumulated down until hitting the pulse's rising phase. The time interval d between the two ion transports is linearly reduced.

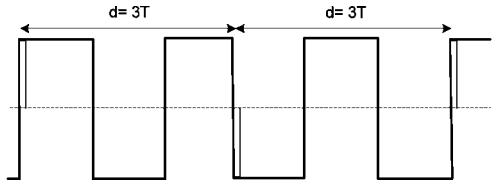

Figure 8. Pumping rate being much lower than the field frequency, $d_o > 2T$. The two ion transports will be eventually trapped into the corresponding facilitating half-pulses, which are not consecutive but close to the initial pumping rate.

represents the field-hindering effects. The second and third terms have opposite signs. The second term increases linearly with n, while the third term increases by the much faster rate of $n(n + 1)/2$. Consequently, when the number n increases, the phase difference, t_n, is eventually accumulated up to be larger than the half-pulse duration, T, or the transport falls into the following facilitating half-pulses.

When d_o is a little longer than the half-pulse duration, $T < d_o < 2T$, the phase difference up-accumulation is even faster:

$$t_1 = t_o + (d_1 - T) = t_o + [(d_o + a) - T] = t_o + (d_o - T) + a$$
$$t_2 = t_1 + (d_2 - T) = t_1 + [(d_o + 2a) - T] = t_o + [(d_o + a) - T] + [(d_o + 2a) - T] = t_o + 2(d_o - T) + 3a$$
$$t_3 = t_2 + (d_3 - T) = t_2 + [(d_o + 3a) - T] = t_o + 2(d_o - T) + 3a + [(d_o + 3a) - T] = t_o + 3(d_o - T) + 6a$$
$$\vdots$$
$$t_n = t_o + n(d_o - T) + a\sum_{i=1}^{n} i \quad n = 0, 1, ... \quad (2)$$

When the field strength is very small so that a can be ignored and the third term eliminated, the phase difference (second term) is accumulated up linearly due to initially $d_o > T$. The electric field accelerates the phase accumulation so that the transport quickly falls into the following facilitating half-pulse (Figure 5).

In summary, if the two transports initially fall into two hindering half-pulses, respectively, the electric field forces them further out of phase with respect to the hindering pulses so that both transports fall into the following facilitating half-pulses.

When the two transports fall into the facilitating half-pulses alternately (the second situation), the field effects will be significantly different. If the pumping rate is a little higher than the field frequency, or $T/2 < d_o < T$, the phase difference will be accumulated down in the succeeding half-pulses, $t_0 > t_1 > t_2 > t_3 >$..., even the field strength is very small (Figure 6). The field facilitation accelerates this down-accumulation. Again, for simplicity, it is assumed that the facilitating effects on the two transports are similar and each facilitating half-pulse reduces d by a factor of b. d_o will be continuously reduced in the succeeding half-pulses. The phase difference at the nth half-pulse will be

$$t_1 = t_o - (T - d_1) = t_o - [T - (d_o - b)] = t_o - (T - d_o) - b$$
$$t_2 = t_1 - (T - d_2) = t_1 - [T - (d_o + 2b)] = t_o - [T - (d_o - b)] - [T - (d_o - 2b)] = t_o - 2(T - d_o) - 3b$$
$$t_3 = t_2 - (T - d_3) = t_2 - (T - (d_o + 3b)] = t_o - 2(T - d_o) - 3b - [T - (d_o - 3b)] = t_o - 3(T - d_o) + 6b$$
$$\vdots$$
$$t_n = t_o - n(T - d_o) - b\sum_{i=1}^{n} i \quad n = 0, 1, 2, ... \quad (3)$$

Again, the second term represents the down-accumulation of the phase difference due to initially $d_o < T$, and the third terms represent the field-facilitating effects. When the number n increases, the phase difference quickly reduces to zero, or the transient pump current catches the rising phase of the pulse. The number of half-pulses needed is as follows:

$$0 = t_o - n(T - d_o) - b\sum_{i=1}^{n} i \quad (4)$$

When $T < d_o < 2T$, the phase difference can be expressed as

$$t_1 = t_o + (d_1 - T) = t_o + [(d_o - b) - T] = t_o + (d_o - T) - b$$
$$t_2 = t_1 + (d_2 - T) = t_1 + [(d_o - 2b) - T] = t_o + [(d_o - b) - T] + [(d_o - 2b) - T] = t_o + 2(d_o - T) - 3b$$
$$t_3 = t_2 + (d_3 - T) = t_2 + [(d_o - 3b) - T] = t_o + 2(d_o - T) - 3b + [(d_o - 3b) - T] = t_o + 3(d_o - T) - 6b$$
$$\vdots$$
$$t_n = t_o + n(d_o - T) - b\sum_{i=1}^{n} i \quad n = 0, 1, 2, ... \quad (5)$$

The second and third terms have opposite signs. The second term represents the phase-difference up-accumulation due to initially $T < d_o$, while the third term represents the down-accumulation due to the field facilitation. The third term increases much faster by $n(n + 1)/2$ than the linear increase of the second term as n increases (Figure 7). The up-accumulation quickly becomes down-accumulation as long as the two terms equalize:

$$n(d_o - T) = b\sum_{i=1}^{n} i \qquad \frac{d_o - T}{b} = \frac{1}{n}\sum_{i=1}^{n} i \qquad (6)$$

Again, the transport will catch the rising phase of the facilitating half-pulses. In other words, the two ion transports are kept within the facilitating pulses.

When the ion transport falls into the preceding half-pulse, a hindering half-pulse; the transports will be delayed until the pulse changes its polarity to a facilitating half-pulse. As a result, both transports will be trapped in the consecutive facilitating half-pulses, alternately. Consequently, all individual pumps extrude Na ions during the positive half-pulses and pump in K ions during the negative half-pulses. In other words, the pump molecules are synchronized to the oscillating electric field.

It is clear that the field-induced hindering factor a and facilitating factor b are independent of the phase difference t_i. That is because the electric field has a dichotomous waveform. The field effects remain the same regardless of the position within the half-pulse in which the transport occurs. For simplicity reasons, we use the factor a to represent the hindering effects induced by each half-pulse for both the Na and K transports. Similarly, we assume the same facilitating effect d for both the Na and K transports. Results based on these assumptions should not lose their generality in terms of understanding the underlying mechanisms involved in the pump synchronization. Further distinguishing the different field effects on the two transports will be useful when focusing on the quantitative study of how many pulses are needed to realize the synchronization.

Case 3. Finally, when the initial pumping rate is much lower than the field oscillating frequency, or $d_o > 2T$, the patterns of the field-induced effects on the pump molecules remain the same as above. The only difference is that the two transports cannot fall into two consecutive half-pulses due to the initial $d_o > 2T$. Similarly, the electric field keeps the two ion transports out of the corresponding hindering half-pulses. As a result, both the Na and K transports will be trapped into the corresponding facilitating half-pulses. For example, for a pump whose d_o is in a range of $4T > d_o > 2T$, if the Na extrusion is trapped into a positive half-pulse, the following K influx will be into the second negative half-pulse, or the pumping rate is synchronized to one-third of the field frequency (Figure 8). In summary, the pump molecules whose d_o is much longer than the half-pulse duration, or whose pumping rate is much lower than the field oscillating frequency, will be synchronized to a fraction of the field frequency.

In summary, due to different effects of the two half-pulses of the oscillating electric field on the two ion transports, energy barrier and energy trap, the two ion transports of the individual pumps will be eventually kept in the consecutive facilitating half-pulses. Consequently, the pump currents elicited by the positive half-pulses mainly represent the outward Na currents, and those evoked during the negative half-pulses represent the inward K currents.

Figure 9 explains the features of the measured pump currents from a group of synchronized and unsynchronized pumps. The two separated transient pump currents in the upper panel represent the two ion transports of a pumping loop. Without synchronization, the inward K currents cannot be distinguished from the outward Na currents in a steady-state current measurement. The measured pump currents, which is a current summation from all the individual pumps, only exhibits a net outward current, as shown in the left column of the lower panel. Once synchronized, all the pumps extrude Na ions during the positive half-cycle and then pump in K ion during the negative half-cycle. The two components of the pump currents are separated, which is shown in the right column of the lower panel.

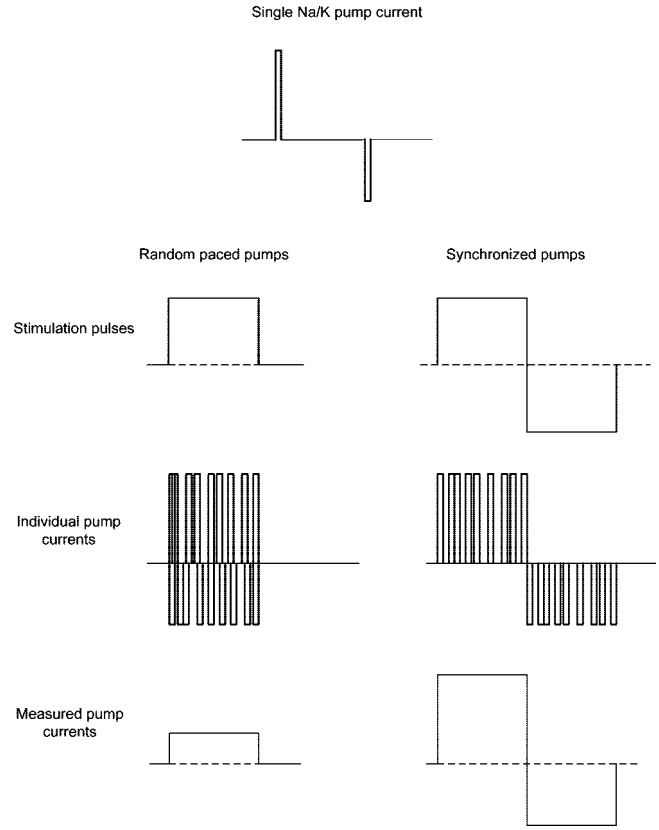

Figure 9. Schematic drawing of the Na/K pump currents. The upper panel shows the pump current elicited by a single Na/K pump molecule based on the previous studies from other laboratories. The left column of the lower panel shows the pump currents from the randomly paced pumps, and the right column shows the pump currents from the synchronized pumps.

Synchronization, a Dynamic Process. Even after the pumps are synchronized, the ion transports are not static within the facilitating half-pulses. On one hand, the phase accumulation may lead the pumping loop out of the phase from the oscillating electric field. The phase difference can be accumulated in the succeeding half-pulses. On the other hand, the field facilitation effects move the transports toward the rising phase of the facilitating half-pulses. Because the facilitation effects are more significant than the phase accumulation in the following pulses (eq 5), the ion transports will be trapped in the facilitating half-pulse and eventually move toward the rising phase. Once reaching the preceding hindering half-pulse, the ion transport will be hindered until the pulse changes its polarity, and return to the facilitating half-pulses.

Furthermore, due to environmental changes, such as the ionic concentration changes induced by ion channel opening and the thermal effects, the pumping rate may be fluctuated resulting in a new d and a new t. This fluctuation can happen at any time in the synchronization process. Some ion transports may even fall into the following half-pulse, the hindering half-pulse. Then, the transport will experience the competition of facilitation and phase accumulation again and eventually be trapped in the facilitating half-pulses.

In summary, synchronization of pump molecules is a dynamic process. Each individual pump tends to be out of phase with respect to the oscillating electric field due to the phase accumulation. However, because of the stronger field-facilitating

effects, most of the pump molecules or most of the time of each individual pump will be trapped in the facilitating half-pulses. Certainly, whenever the field oscillation ceased, the pumps quickly lose their synchronization, becoming randomly paced, as proven by our experimental results.[21]

The experimentally measured steady-state pump currents shown in Figure 1 show that the pump currents span the entire half-pulses and the current density at the rising phase is higher than for the rest of the pulse. In addition to the above discussion, the following explanation will be helpful to understand the experimental results. To explore the mechanisms involved in the synchronization, we employ a simple six-state model, where all of the currents in the Na extrusion limb are attributed in a single narrow pulsed current and likewise for the K pumping in limb. In fact, more than one step is electrogenic in each ion transport limb. Experimental results from the study of Na/K pump have shown that there are at least two electrogenic steps in each transport limb: ion movements in the binding access channel and in the unbinding access channel separated by the intermediate steps which are voltage-independent. Therefore, even though the first current is close to the rising phase, the second one will certainly not be. It may be spread in the half-pulse due to different time courses of the intermediate voltage-independent steps.

Our computer simulation[34] from a large group of pumps with separated binding and unbinding currents in each limb of the individual pumps have shown that the synchronized pump current spreads throughout the entire facilitating half-pulse, with more current density at the rising phase of each pulse, which is similar to those shown in Figure 1.

Conclusion

In summary, we used the Na/K pumps as an example to study the ion-exchanger synchronization. We found that the pumping loops of individual pump molecules can be synchronized by a well-designed oscillating electric field so that all the individual pumps extrude Na ions during the positive half-cycle and then pump in K ions during the negative cycle. As a result, the inward pump currents mainly represent the K-influx and the outward current represent the Na-extrusion. The magnitude ratio of the outward to inward currents reflects the pump's stoichiometric ratio of 3:2. A sister paper will further discuss electrical modulation of the pumping rate.

References and Notes

(1) Teissie, J.; Tsong, T. Y. Evidence of voltage-induced channel opening in Na/K ATPase of human erythrocyte membrane. *J. Membr. Biol.* **1980**, *55*, 133–140.

(2) Blank, M.; Soo, L. The effects of alternating currents on Na, K-ATPase. *Bioelectrochem. Bioenerg.* **1989**, *22*, 313–322.

(3) Weaver, J. C.; Astumian, R. D. The response of living cells to very weak electric fields: the thermal noise limit. *Science* **1990**, *247*, 459–462.

(4) Robertson, B.; Astumian, D. Frequency dependence of catalyzed reactions in a weak oscillating field. *J. Chem. Phys.* **1991**, *94* (11), 7414–7418.

(5) Markin, V. S.; Liu, D. S.; Rosenberg, M. D.; Tsong, T. Y. Resonance transduction of low level periodic signals by an enzyme: an oscillatory activation barrier model. *Biophys., J.* **1992**, *61* (4), 1045–1049.

(6) Tsong, T. Y.; Chang, C. H. Catalytic wheel, Brownian motor, and biological energy transduction. *AAPPS Bull.* **2003**, *13* (2), 12–18.

(7) Blank, M.; Soo, L. A proposed explanation for effects of electric and magnetic fields on the Na, K-ATPase in terms of interaction with electron. *Bioelectromagnetics* **2005**, *>26*, 591–597.

(8) Astumian, R. D. Adiabatic pumping mechanism for ion motive ATPases. *Phys. Rev. Lett.* **2003**, *91* (11), 118102(4)

(9) Tsong, T. Y. Electrical modulation of membrane proteins: enforced conformational oscillations and biological energy and signal transductions. *Annu. Rev. Biophys. Biophys. Chem.* **1990**, *19*, 83–106.

(10) Clausen, T.; Nielsen, O. B. Rapid activation of the Na/K pump: mechanisms and functional significance. *Biol. Skr.−K. Dan. Vidensk. Selsk.* **1998**, *49*, 153–158.

(11) Chen, W. Voltage-dependence of the synchronized Na/K pump currents, submitted for publication.

(12) Apell, H. J.; Bersch, B. Oxonol VI as an optical indicator for membrane potentials in lipid vesicles. *Biochim. Biophys. Acta* **1987**, *903*, 480–494.

(13) Bamberg, E.; Tittor, J.; Oesterhelt, D. Light-driven proton or chloride pumping by halorhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90* (2), 639–643.

(14) Sokolov, V. S.; Stukolov, S. M.; Darmostuk, A. S.; Apell, H. J. Influence of sodium concentration on changes of membrane capacitance associated with the electrogenic ion transport by the Na,K-ATPase. *Eur. Biophys. J.* **1998**, *27*, 605–617.

(15) Hilgemann, D. W. Channel-like function of the Na, K pump probed at microsecond resolution in giant membrane patches. *Science* **1994**, *263*, 1429–1432.

(16) Holmgren, M.; Wagg, J.; Bezanilla, F.; Rakowski, R. F.; De Weer, P.; Gadsby, D. C. Three distinct and sequential steps in the release of sodium ions by the Na^+/K^+-ATPase. *Nature* **2000**, *402* (24), 898–901.

(17) Chen, W.; Lee, R. An improved double Vaseline gap voltage to study electroporated skeletal muscle fibers. *Biophys. J.* **1994**, *66*, 700–709.

(18) Hui, C. S.; Chen, W. Separation of Q_β and Q_γ charge components in frog twitch fibers with tetracaine. *J. Gen. Physiol.* **1992**, *99*, 985–1016.

(19) Chen, W.; Wu, W. H. The asymmetric, rectifier-like I-V curve of the Na/K pump transient currents in frog skeletal muscle fibers. *Bioelectrochemistry* **2002**, *56*, 199–203.

(20) Chen, W.; Zhang, Z. S. Synchronization of Na/K pump molecules by a train of squared pulses. *J. Bioenerg. Biomembr.* **2006**, *38* (5-6), 319–325.

(21) Chen, W.; Zhang, Z. S.; Huang, F. Synchronization of the Na/K pumps by an oscillating electric field. *J. Bioenerg. Biomembr.*, in press.

(22) Chen, W.; Zhang, Z. S.; Huang, F. Entrainment of Na/K pumps by synchronization modulation electric field. *J. Bioenerg. Biomembr.*, **2007**, *39*, 331–339.

(23) De Weer, P.; Gadsby, D. C.; Rakowski, R. F. Stoichiometry and voltage dependence of the Na/K pump. In *The Na, K-ATPase, Part A: Molecular aspects*: Progress in Clinical and Biological Research; Skou, J. C.; Norby, J. G.; Maunsbachy, A.B., Esmann, M., Eds.; A. R. Liss: New York, 1988; Vol. 268A, pp 421−434.

(24) Rakowski, R. F.; Gadsby, D. C.; De Weer, P. Stoichiometry and voltage dependence of the sodium pump in voltage-champed, internally dialyzed squid giant axon. *J. Gen. Physiol.* **1989**, *93*, 903–941.

(25) Glynn, I. M. The electrogenic sodium pump. In *Electrogenic transport. Fundamental principles and physiological implications*. Blaustein, M. P., Lieberman, M., Eds.; Raven Press: New York, 1984; pp 33−48.

(26) Albers, R. W. Biochemical aspects of active transport. *Annu. Rev. Biochem.* **1967**, *36*, 727–756.

(27) Post, R. L.; Hegyvary, C.; Kume, S. Activation by adenosine triphosphate in the phosphorylation kinetics of sodum and potassium ion transport adenosine triphosphatase. *J. Biol. Chem.* **1972**, *247*, 6530–6540.

(28) Hille, B. *Ionic channels of excitable membranes*, *3*rd ed.; Sinauer Associates: Sunderland, MA, 2003.

(29) Lauger, P. *Na/K ATPase, in Electrogenic Ion Pumps*; Sinauer Associates: Sunderland, MA, 1996; pp 201−204.

(30) Chen, W. Voltage dependence of the carrier-mediated ion transport. *Phys. Rev. E* **200**, *73* (2), 021892.

(31) Lauger, P.; Apell, H. J. A microscopic model for the current-voltage behavior of the Na-K pump. *Eur. Biophys. J.* **1986**, *13*, 309–321.

(32) Apell, H. J. Toward an understanding of ion transport through the Na, K-ATPases. *Ann. N. Y. Acd. Sci.* **2003**, *986*, 133–140.

(33) Smith, N. P.; Crampin, E. J. Development of models of active ion transport for whole-cell modelling: Cardiac sodium-potassium pump as a case study. *Prog. Biophys. Mol. Biol.* **2004**, *85*, 387–405.

(34) Chen, W.; Huang, F. Computer simulation of synchronization of Na/K pump molecules. *J. Bioenerg. Biomembr.*, in press.

JP0754637