J Bioenerg Biomembr (2008) 40:337–345
DOI 10.1007/s10863-008-9152-z

Computer simulation of synchronization of Na/K pump molecules

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Received: 2 August 2007 / Accepted: 28 June 2008 / Published online: 5 August 2008

Abstract The behavior of Na/K pump currents when exposed to an oscillating electric field is studied by computer simulation. The pump current from a single pump molecule was sketched based on previous experimental results. The oscillating electric field is designed as a symmetric, dichotomous waveform varying the membrane potential from −30 to −150 mV around the membrane resting potential of −90 mV. Based on experimental results from skeletal muscle fibers, the energy needed to overcome the electrochemical potentials for the Na and K-transports are calculated in response to the field's two half-cycles. We found that a specially designed oscillating electric field can eventually synchronize the pump molecules so that all the individual pumps run at the same pumping rate and phase as the field oscillation. They extrude Na ions during the positive half-cycle and pump in K ions during the negative half-cycle. The field can force the two ion-transports into the corresponding half-cycles, respectively, but cannot determine their detailed positions. In other words, the oscillating electric field can synchronize pumps in terms of their pumping loops but not at a specific step in the loop. These results are consistent with our experimental results in measurement of the pump currents.

Keywords Computer simulation · Na/K pump · Oscillating electric field · Synchronization

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Introduction

In a living system, there are many kinds of pump molecules, or ion-exchangers, moving one kind of ion out of the cell by exchanging if for another kind of ion. One example is the Na/K pump, which extrudes 3 Na ions and pumps 2 K ions in, consuming one ATP in each cycle. The pump molecules are critical to cells' physiological functions, including maintaining ionic concentration gradients across the cell membrane, generating electrical signals, and providing energy to other membrane transporters.

Microscopically, there exist two components of the pump currents, an outward current representing extrusion of ions and an inward current corresponding to ion influx. Because of structural independence, individual pumps may run at different pumping rates and random pumping phases. Therefore, the two opposite current components cannot be distinguished in a steady-state current measurement of a group of pumps. For example, Na/K pump currents measured under physiological conditions exhibit only unidirectional outward currents without distinguishable inward components.

The two components of the Na/K pump current have been studied separately (Apell and Bersch 1987; Bamberg et al. 1993; Sokolov et al. 1998; Hilgemann 1994; Holmgren et al. 2000). In these studies, the pumping loops of individual pumps were purposely interrupted by various methods in order to force all the pumps to stay in the same state. Then, an optical signal or an electrical stimulation was used to trigger the movements of the molecules to the next state. Consequently, the transient pump currents corresponding to this specific step are generated simultaneously by all the pumps.

Synchronization of the pump molecules in physiological running mode had not been studied. That is probably

because of the difficulty in treatment of two ion-transports separately in a running loop. In this paper we present the results of computer simulation in study of the Na/K pump synchronization in a physiological running mode (Lauger and Apell 1986; Smith and Crampin 2004) by a specially designed oscillating electric field. The results are compared with our experimental results (Chen and Zhang 2006; Chen et al. 2008).

Concept of pump synchronization is similar to that of electronic synchronization, but more complicated in practice. In a synchrotron, an acceleration electric field can be applied specifically to the pathway of the electronic beam. Practically, it is impossible to influence one transport without affecting the other in a running loop. Any electric field applied to the cell membrane either depolarizing or hyperpolarizing the membrane potential can only facilitate one ion-transport but inevitably hinder the other. Therefore, we have to consider the field effects on both ion-transports simultaneously.

Mechanisms involved in synchronization of pump molecules

Based on the Post-Albers model (Albers 1967; Post et al. 1972), the Na/K pump extrudes Na ions out of cell and then pumps in K ions in a sequential pattern, generating outward Na and inward K currents alternatively in a loop. When a pulsed oscillating electric field is applied to the cell membrane, the field at two half-pulses has different effects on each ion-transport which can be quantified by the field-induced energy changes in the ion-transports. For skeletal muscle fibers with physiological ionic concentrations, Nerstian equilibrium potential for the Na and K ions are about 60 and −90 mV, respectively, intracellular with respect to the extracellular fluid, and the membrane resting potential is about −90 mV (Hille 2001).

For a symmetric, pulsed oscillating electric field alternating the membrane potential from −30 to −150 mV, extrusion of 3 Na-ions when the step falls into a negative half-pulse requires 3×60=180 meV to overcome the ionic concentration gradient, and 3×150=450 meV for the electric potential. A total energy of at least 630 meV is needed to complete the Na-transport. If the step falls into a positive half-pulse, the total energy needed is significantly reduced to 3×(60+30)=270 meV. Considering that the metabolic energy from hydrolysis of a single ATP is about 550 meV (Lauger 1991; Stein 1990; Weiss 1996), 3 Na ions can be easily extruded during the positive half-cycle but difficult during the negative half-cycle. Thereafter, we call the positive and negative half-cycles the facilitating and hindering half-cycles or half-pulses, respectively, for the Na-transport.

Similarly, if the K-transport falls into a positive half-pulse the energy needed for pumping-in 2 K ions to overcome the electrochemical potential is 2×(90–30)=120 meV, which is significantly reduced to a negative value of 2×(90–150)=−120 meV when falling into a negative half-pulse. Therefore, the positive half-pulse becomes a hindering half-pulse and the negative half-pulse a facilitating half-pulse. Figure 1 shows the energies needed to overcome the electrochemical potentials at different half-pulses.

Let's consider two extreme situations. Assuming that the Na-extrusion and K-influx fall into the negative and positive half-cycles, respectively, the energy needed to overcome the electrochemical potential for both ion-transports is 630+120=750 meV. Considering the protein's conformational change, more energy may be needed. The total energy needed for the entire loop is much larger than the ATP hydrolysis energy. Therefore, the pump will be fully inhibited until the field changes its polarity.

On the other hand, once the Na-extrusion and K-influx fall into the positive and negative half-cycles, respectively, the energy barriers for the two transports are significantly reduced. The total energy needed to overcome the electro-

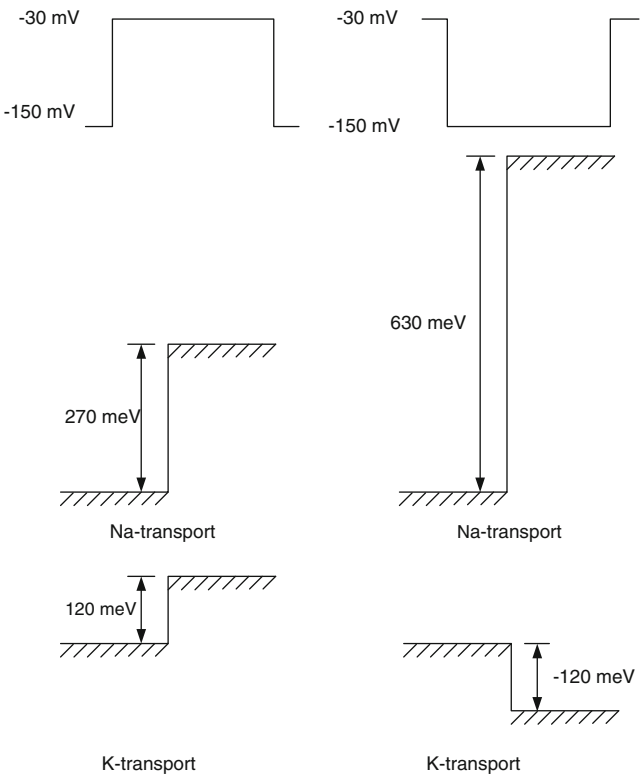

Fig. 1 Energy needed for the Na- and K-transports when falling into the positive and negative half-pulses, respectively. When 3 Na-extrusion falls into a positive half-cycle, the energy needed is 270 meV, which increases significantly to 630 meV when falling into a negative half-cycle. In contrast, the 2 K-influx needs energy of 120 meV when fall into a positive half-cycle and becomes a negative value of −120 meV when into a negative half-cycle

chemical potential for the whole loop becomes 270–120= 150 meV, much smaller than the ATP hydrolysis energy of 550 mV. The two ion-transports can easily overcome this barrier, and will repeatedly fall into the positive and negative half-pulses, respectively, or the pumps are synchronized to the oscillating electric field.

Parameters setup and computational results

Single pump current

The first attempt is to sketch the pump current from a single pump molecule. Pump currents at steady-state have been widely studied (Rakowski et al. 1997; Artigas and Gadsby 2002; De Weer et al. 1988; Rakowski et al. 1991; Glynn 1984; Gadsby and Nakao 1989). However, measurement of a single pump current in a running mode has not been reported. We can draw the single pump current based on the previous studies. First, it has been shown that each ion-transport in the pumping loop consists of a sequence of reaction steps of ion binding, occlusion, protein's conformational change, deocclusion, and ion unbinding or releasing (Holmgren et al. 2000; Apell 2003; Humphrey et al. 2002; Apell 2003). The electrogenic steps are mainly the ion binding and unbinding steps in the two side accessory channels (Holmgren et al. 2000; Apell 2003, 2004).

At a steady-state, the pumping flux throughout the entire pumping cycle is a constant. Three Na ions access to the binding sites from the narrow access channel which is controlled by a gate. Proteins conformational change closes the gate making the ion in an occluded state followed by deocclusion of the ion into the other side accessory channel where they will be released (Hilgemann 1994; Holmgren et al. 2000; Artigas and Gadsby 2002; Apell 2003, 2004). The electrogenic steps of binding and releasing in the two accessory channels are represented by two pulsed outward currents, correspondingly, while the steps in between, including occlusion, conformation transition, deocclusion, phosphorylation and so on are not electrogenic and thus are electrically silent. Similarly, the movement of 2 K ions in the binding and unbinding accessory channels on the two sides during the K-transport generates two pulsed inward currents separated by those voltage-independent steps again including occlusion, conformational transition, deocclusion and protein dephosporylation. Therefore, a single pump current can be sketched as two outward current-bars separated by a time-interval followed by two separated inward current-bars as shown in Fig. 2. Ratio of the areas underneath the outward and inward current-bars represents the stoichiometric ratio of 3:2.

Secondly, previous studies have shown that the apportionment factor of the applied membrane potential on the

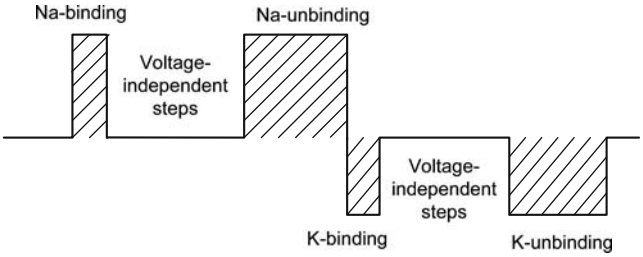

Fig. 2 Components of pump current form a single pump. Two outward current-bars stand for the binding and unbinding steps for the Na-extrusion. Area of each pulse represents movement of 3 Na ions. Because the membrane potential apportionment factor for the unbinding step is greater than that for the binding step, the second pulse has a wider duration and larger area. Similarly, two inward current-bars represent the binding and unbinding steps of 2 K ions influx

binding step (p=0.1–0.2) is smaller than that on the unbinding step (p=0.4–0.5) (Apell 2004). Therefore, duration of the binding current-bar is narrower than that of the unbinding bar. In our calculation, the duration ratio is set 1:3. Thirdly, because neither the binding nor unbinding steps are rate-limiting steps in the loop when the membrane is depolarized (Lauger 1991), the current-bar durations should be shorter than their time-intervals. Fourthly, experimental results showed that the release of Na ions is immediately followed by the binding of K ion without time delay (Apell 2003). There should be no time delay between the Na-unbinding and K-binding current-bars.

Durations of the Na- and K-current-bars in response to different half-pulses

Based on these studies, we select the durations of the Na-binding and unbinding current-bars, and their time-interval as 1, 3 and 4 ms, respectively, when the Na-transport falls into a positive half-pulse. Similarly, when the K-transport falls into a negative half-pulse, durations of K-binding and unbinding current-bars are selected as 0.9 and 2.7 ms, respectively, with a time-interval of 3.8 ms.

Time courses of the binding and unbinding steps when the transports fall into the corresponding hindering half-pulses can be estimated from the chemical reactions. The ion-binding or unbinding step can be expressed as a two-state model with a forward and backward rate-coefficients α and β between c_1 and c_2. The reaction flux is

$$\phi = -\frac{\mathrm{d}c_1}{\mathrm{d}t} = \alpha c_1 - \beta c_2 \qquad (1)$$

where the rate-coefficient can be expressed by Boltzmann distribution based on the energy difference. The energy difference between the two states includes two components, intrinsic energy such as ATP hydrolysis energy, and the field-induced electric energy. We are interested in the

electric field-induced changes so that we only have to focus on the electric energy. When the membrane potential is changed ΔV, charge particles $Q=ze$ will find their binding energy changed by about $\Delta E_b=Q\Delta Vp_b$ and their unbinding energy on the other side by about $\Delta E_b=Q\Delta Vp_u$, where p_b and p_u are the apportionment factors for the binding and unbinding steps, respectively. Each of these energy changes has to be distributed over the forward and backward reactions in each step. Considering a simple situation that the energy barrier is symmetric, then 50–50% of the electric energy will be over the forward and backward rate-coefficients. Assuming that α_f and β_f are the corresponding rate-coefficients in the facilitating half-pulse, and the membrane potential changes ΔV=120 mV from the facilitating half-pulse to the hindering half-pulse, based on the theory of absolute reaction rate, the forward and backward rate-coefficients α_h and β_h in the hindering half-pulses can be expressed as follows:

$$\begin{aligned}\alpha_h &= \alpha_f e^{-Q\Delta Vp/2KT} = \alpha_f e^{-ze\Delta Vp/2KT} = \alpha_f e^{-z\Delta Vp/52}\\ \beta_h &= \beta_f e^{-Q\Delta Vp/2KT} = \beta_f e^{-ze\Delta Vp/2KT} = \beta_f e^{z\Delta Vp/52}\end{aligned} \quad (2)$$

where the relation of $e/KT=F/RT$=(1/26) mV at room temperature are considered. The reaction flux can be obtained by substituting the rate-coefficients into Eq. 1. Because of an increment in the energy barrier, the reaction will be slowed down. The flux for the Na-binding step when falling into a negative half-pulse becomes:

$$\begin{aligned}\phi &= -\frac{dc_1}{dt} = \alpha_h c_1 - \beta_h c_2 = \alpha_f e^{-z\Delta Vp_b/52} c_1 - \beta_f e^{z\Delta Vp_b/52} c_2\\ &= \alpha_f e^{-3(120)0.2/52} c_1 - \beta_f e^{3(120)0.2/52} c_2\\ &\approx (1/4)\alpha_f c_1 - 4\beta_f c_2\end{aligned}$$

where an apportionment factor p_b=0.2 was used. Clearly, the flux is significantly reduced when the binding step falls into a negative half-pulse. It will take much longer for the movement of 3 Na ions in the binding access channel. We select 3 ms as the duration of the binding current-bar when it falls into the negative half-pulse. Considering a larger apportionment factor, p_u, the unbinding current-bar is estimated to have 9 ms duration. Indeed, an increment in the energy difference many not be able to fully block the ion movement, it takes much longer for the movements in the access channels. Similarly, the durations of the two current-bars for the K-transport increase to 8 and 24 ms, respectively, when falling into the positive half-pulse. Because the areas of current-bars remain unchanged, the bar-heights will be significantly reduced whenever falling into the hindering half-pulses. Upper panel of Fig. 3 shows

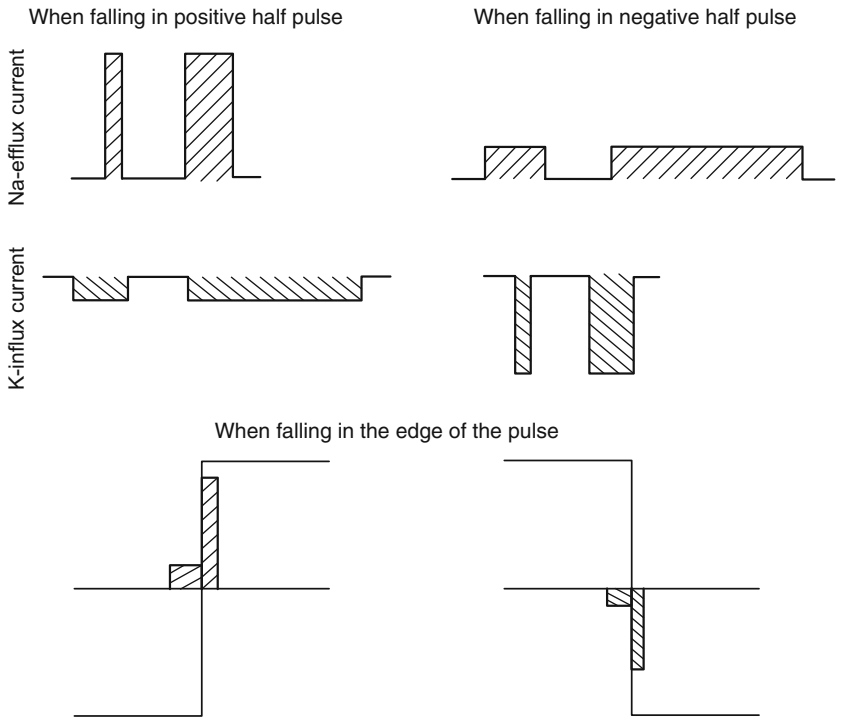

Fig. 3 *Upper panel* Durations of the binding and unbinding steps when falling into different half-cycles. Both current-bars are elongated significantly when falling into the hindering half-cycles. *Lower panel* Current-bars when the field changes its polarity during the binding and unbinding steps. The *left column* represents for the Na-binding step and the right column for the K-binding step. The area of the current-bars remains a constant

the durations of the Na- and K current-bars in responding to the two half-pulses.

Whenever a current-bar falling onto the edge of the oscillating pulse, or the pulse changes its polarity during the binding or unbinding step, the current-bar duration and magnitude will be changed correspondingly. However, each current-bar remains the same area. The pump currents when falling onto the pulse edge are presented in the lower panel of Fig. 3.

Synchronization of a single pump to the oscillating electric field.

A MATLAB program implements the simulation which is described below. (1) A pulsed oscillating electric field is generated. The membrane potential is held at −90 mV and starting from the third pulse, the membrane potential is alternated by ΔV. (2) The pumping loop is mimicked by alternating the two outward Na current-bars and two inward K current-bars. All the pumps initially have a random phase determined by the computer generated random signals. (3) As long as the membrane potential oscillates the duration and temporal position of each current-bars and their intervals are calculated based on the half-pulse the current-bar fallen in. When falling into the facilitating half-pulse, the current-bar duration becomes shorter so that the reaction may be completed within the pulse. When falling into the hindering half-pulse, the current-bar duration is significantly elongated, so that the reaction can not be finished in the pulse. Whenever a current-bar falls onto the edge of the oscillating pulse, the current-bar will consists of two parts, the duration and magnitude of the two parts determined by the corresponding half-pulses, respectively, but remain the same total area.

Figure 4 shows the computer simulation results from a single pump when exposed to the oscillating electric field

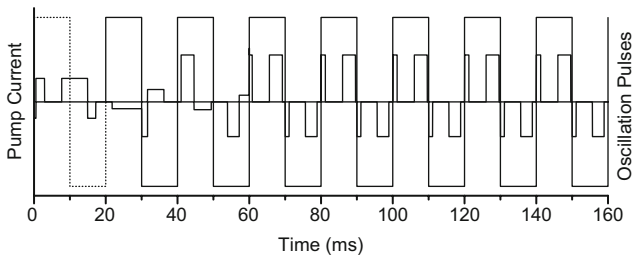

Fig. 4 Synchronization of a single pump by an oscillating electric field. The electric field is applied from the second pulse remarked by a solid line. The first oscillating cycle in *dotted line* means that no field is applied. Durations of the current-bars change significantly depending on the field polarity. After about four oscillating pulses, the Na and K current-bars are locked into the positive and negative half-pulses, respectively. It indicates that the pumping loop has reached synchronization to the oscillating electric field

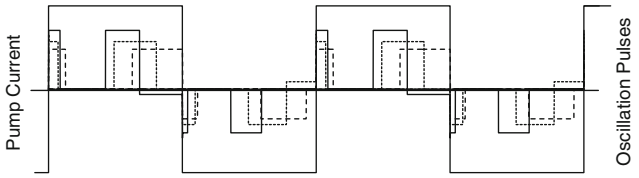

Fig. 5 Synchronization of three pumps with different initial pumping paces (different current-pulse durations and time-intervals). Final durations and positions of the current-bars from individual pumps are not the same spreading throughout the half-pulses

having a half-pulse duration of 10 ms. The initial pump duration is 24 ms. In this case, the current-bars increase (decrease) by a factor of 2 when it falls into the facilitating (hindering) half cycle of the electric field. After several oscillations, the Na-binding and unbinding current-bars are repeatedly trapped into the positive half-pulses and the K current-bars into the negative half-pulses, respectively. In other words, the pumping cycle is synchronized to the field frequency.

Figure 5 shows the superimposed computational results of three synchronized pumps with different initial pumping rates. They have different initial durations of binding and unbinding current-bars and their time-intervals, as well as random pumping phases. The results show that once synchronized the current-bars for the Na- and K-transports from all three pumps are trapped in the positive and negative half-pulses, respectively. However, the positions of current-bars within the half-pulses are not the same. They are spread throughout the entire the half-pulses.

When cells are exposed to an external electric field, the field-induced membrane potentials differ significantly depending on the shape, size of the cells, and the location of the membrane. Even in experiments using the voltage-clamp technique, the series resistance of the current injection circuit and the membrane capacitance make the membrane potential non-uniform, especially in response to an oscillating electric field. Therefore, individual pumps may undergo different membrane potentials. As a result, the field-induced changes in the rate-coefficients of the voltage-dependent steps and therefore the current-bar durations may not be the same. Consequently, the number of pulses needed to synchronize individual pumps and the resultant synchronized pump currents may differ significantly. Figure 6 shows superimposed pump currents from three pumps with different initial pumping rate synchronized by three different pulse-magnitudes.

Synchronization of a group of pumps

In the cell membrane, there are hundreds of thousands of pump molecules running at different pumping rates and random phases. We have previously studied distribution of

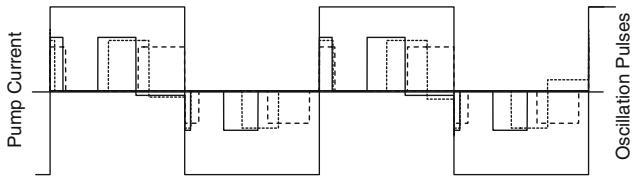

Fig. 6 Synchronization of three pumps in response to different membrane potentials and initial pumping paces combination. Durations and the positions of the current-bars are not the same, spreading throughout the half-pulse

the pumping rates as a function of the membrane potential, temperature, and ionic concentration gradients in the physiological ranges, which is a Gaussian-curve-like distribution shown as the left curve in Fig. 7 (Huang F, Rabson D, and Chen W, Distribution of the Na/K pumps' turnover rates as a function of membrane potential, temperature, and ion concentration gradients and effect of fluctuations. Manuscript for publication). For a given probability distribution of the Na/K pumping rates at the holding potential, the percentage of the pumps that can be synchronized is dependent upon the frequency and amplitude of the oscillating electric field. With a given amplitude and a fixed frequency, a pulsed oscillating electric filed can only synchronize within a certain range of the pumping rates of the pump molecules. The percentage of the synchronized pumps can be calculated by integrating the probability distribution function in that range of pumping rates. In our calculation, the criteria of the pump synchronization is more than 80% of both the Na and K current-bars repeatedly fallen into the corresponding facilitation half-cycles.

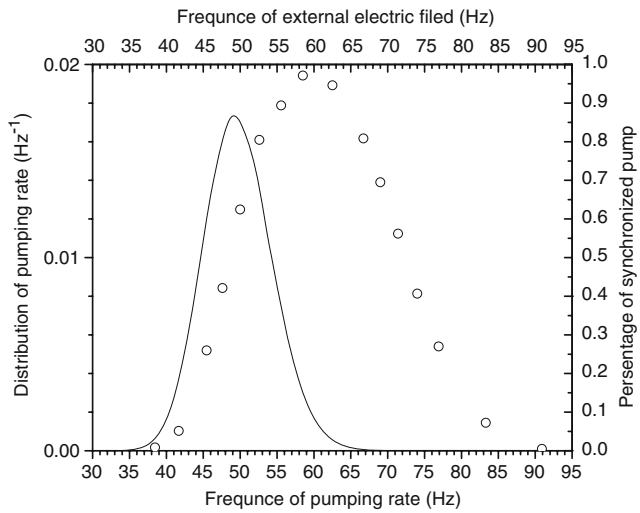

Fig. 7 The *solid line* is a given probability distribution of the Na/K pumping rate at holding potential 90 mV. The *circles* are the percentages of the synchronized pump as a function of the frequency of the applied external electric field as a square pulse series. The percentage of the synchronized pumps can be calculated by integrating the probability distribution function at that range of pumping rate

As an example, for a fixed field-strength, the percentage of the synchronized Na/K pumps as a function of the field oscillating frequency were obtained and shown as the right curve in Fig. 7. The trend of the curve is similar as that of the pumping rate distribution. The width of the percentage profile is about two times wider than that of the probability distribution profile of the pumping rate. The peak is shifted to higher frequency. At the peak of the curve most of the pumps can be synchronized. For this specific situation, the shift is about 10 Hz. In fact, this frequency shift depends on the field-strength. The higher the field-strength, the more the curve shifts to the peak. This gives us a theoretical method to identify the optimal synchronization frequency.

In the simulation of synchronization of a group of pumps, for simplicity, we chose the band of pumping cycle distribution from 40 to 60 ms at a holding potential of −90 mV. Another MATLAB program implements the synchronization simulation. The algorithm is the single pump algorithm repeated for N pumps. The pumps' initial pumping rates and the corresponding pump number is determined by the distribution. Figure 8 shows the results of the synchronized pump currents from this group of pumps.

The Na-extrusion outward currents are represented by a darker line and the inward K-pump currents by a brighter line. The dotted pulses means no field is applied to the pumps so that the Na-extrusion and K-influx currents are relatively uniformly distributed as outward and inward currents, respectively. The ratio of the outward over inward currents is about 3:2. After the field application, marked by the solid pulses, even for the first two half-pulses, the current distribution is still relatively uniform. As the field oscillates, more and more Na-extrusion currents fall into the positive half-pulse, and less and less are left in the negative half-pulses. Starting from the fourth half-pulse, very little Na-outward current is left in the negative half-pulses.

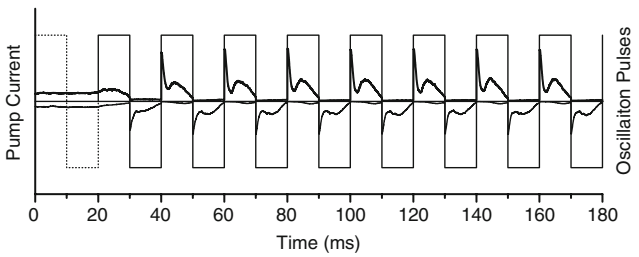

Fig. 8 Synchronization of a group of pump molecules with initial pumping duration in a range from 15 to 60 ms. The oscillating electric field is applied from the second pulse remarked by a *solid line*. Before the field application, the Na- and K-currents are equally distributed as outward and inward currents. Magnitude ratio is 3:2. Even for the first oscillating pulse, they remain the same. Then, the Na-currents are gradually trapped into the positive half-cycle, and the K-currents into the negative half-cycle. Magnitude of outward current within the positive half-pulse is gradually increased, and so do the inward current within the negative half-cycle

Table 1 Integration of the synchronized pump currents or ions transported during the positive and negative half-pulses, respectively

Integration	Positive half-cycle	Negative half-cycle	Inward/outward currents ratio
Outward current	630	40	
Inward current	−44	−414	
Net current	586	−374	3.1:2

The result in the positive half-cycle for Na ions is 651 arbitrary units (au) which is much larger than that in the negative half-cycle of 91 au. Similarly, the result of K ions in the negative half-cycle of 400 au is much larger than that in the positive half-cycle of 98 au. The results indicate that majority of the pumps were synchronized. Ratio of the net outward over inward pump currents is 3.1:2, which is close to, but larger than, the stoichiometric number of the Na/K pumps (3:2). The discrepancy means that not all the pumps were synchronized.

Simultaneously, more and more K-influx currents fall into the negative half-pulses. Integration of the pump currents within the positive and negative half-cycle, respectively, shows a ratio larger than, but close to, 3:2, the stoichiometric number of the Na/K pump, as shown in Table 1. This result shows that a large number of the pumps are gradually synchronized to the oscillating electric field.

Figure 9 displays the experimental results of the Na/K pump currents when exposed to the oscillating electric field alternating the membrane potential from −150 to −30 mV (Chen et al. 2008). The pump currents were measured from single skeletal muscle fibers of frogs by using double Vaseline-gap voltage clamp techniques. As the membrane potential was continuously oscillated, the pump currents gradually changed from the initially unidirectional outward current to the separated outward and inward pump currents. The ratio of the outward over inward pump currents is about 3.:2. The results are consistent with our computer simulation study.

Conclusion and discussions

Previous studies showed that an oscillating electric field at a frequency of kilo Hz can facilitate Rb accumulation, and at a frequency of mega hertz can facilitate Na-extrusion (Teissie and Tsong 1980) in erythrocytes. The phenomenon was explained later by an optimal frequency window model

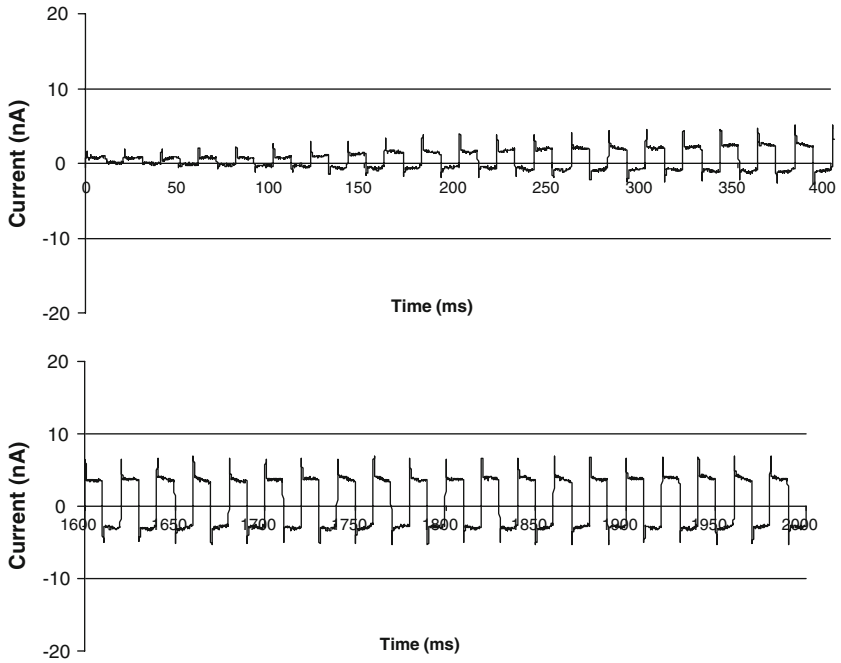

Fig. 9 Experimental results of the Na/K pump currents when exposed to a symmetric, pulsed oscillating electric field alternating the membrane potential from −30 to −150 mV at the membrane resting potential of −90 mV. The results were obtained from a single skeletal muscle fiber by using double Vaseline voltage-clamp techniques. As the membrane potential is oscillating, the initially unidirectional outward current is gradually changed to the separated outward and inward pump currents. When reach a steady-state, the ratio of the outward over inward pump currents shows larger than, but close to 3:2

where the pump molecules have been postulated to have Loranzian frequencies (Robertson and Astumian 1991; Markin et al. 1992). Accumulation of Rb and facilitation of Na-extrusion are due to the applied field frequency falling into the pumps' optimal frequency windows, resonance, or synchronization to the pumps' Loranzian frequency.

In this study, we are not dealing with the postulated Loranzian frequency of the pumps in a range of kilo and mega Hz. Instead, we focus on the experimentally identified natural pumping rates of about 50 Hz. The results show that individual pumps with initially different pumping rates and random pumping phases can be synchronized by a well designed oscillating electric field. As a result, the Na- and K-transports, are eventually trapped into the positive and negative half-pulses, respectively and repeatedly. The positive currents elicited by the positive half-pulse mainly represent the Na outward currents and the negative currents generated by the negative half-pulse correspond to the K-inward currents. The frequency of the electric field can not be the same as, but must be higher than, the initial natural pumping rates. The specific value depends on the distribution of the initial pumping rates, the waveform and the magnitude of the applied oscillating electric field.

It has been well accepted that membrane potential can change the pump currents or the pumping rate by altering the time course of the voltage-dependent steps. In this study, we used an isolated two-state model to estimate the changes in the time-course of the ion-binding and -unbinding steps. The estimated values may not be accurate. However, the results of the oscillating electric field-induced synchronization of the Na/K pumps do not lose generality.

From both computer simulation and experimental results, the synchronized Na- and K-pump currents are different from those obtained in the experiments of simultaneously-triggering a single step in an interrupted mode, which showed an initial maximum current followed by an exponential decay. In physiological running mode the synchronized pump currents spread throughout the corresponding half-pulses even though the current-density at the early stage in the half-pulse is higher than that at the later stage, as shown in Figs. 8 and 9. The discrepancy can be explained as follows. First, in the simultaneously-triggering experiments, the pumping loop was interrupted so that all the individual pumps do not reach the electrogenic step. Stimulation triggers the following electrogenic step generating a transient pump current followed by an exponential decay. In physiologically running mode, the binding and unbinding steps from both transports continuously generate pump currents. When synchronized, there should be two separately pulsed currents representing binding and unbinding steps in each half-pulse. Even though the first current pulse may continue with the rising phase of the half-pulse, the second one will not.

Secondly, in the simultaneously-triggering experiments, all the pumps were initially inhibited so that the generated pump currents have the same phase. In physiological running mode, only the pumping loops are synchronized, steps from individual pumps may remain in different phases. In other words, the Na- and K-currents are trapped into the positive and negative half-pulses, respectively, but the positions of the current-bars from individual pumps may not be the same, which can be spread throughout the entire half-pulse.

Thirdly, considering a band distribution of the initial pumping rates, each individual pump has different durations for the current-bars and the time-intervals. Even through synchronized, these values may not equalize so that the current-bars may spread within the half-pulses. Finally, thermal effects or any fluctuation in environmental parameters may affect the pumping rates so that the current-bars further spread within the half-pulses.

Experimental results showed in Fig. 9 exhibit more uniform distribution of the synchronized pump currents within the corresponding half-pulses compared to the results from the computer simulation showed in Fig. 7. The discrepancy can be understood in light of two factors, the non-uniformly distributed membrane potential, which spreads the field-induced effects on the pumping rates, and the non-synchronized pump currents which are always evenly distributed.

In summary, this study shows that individual pumps with initially different pumping rates and random pumping phases can be synchronized by a well designed oscillating electric field. As a result, the pumps all extrude Na ions during the positive half-cycle and then pump in K ions during the negative half-cycle. The measured positive currents generated by the positive half-pulse are mainly the outward Na currents and the negative currents elicited by the negative half-pulse represent the inward K-currents. In contrast to simultaneously-triggering a specific step in an interrupted mode, pump synchronization in running mode only confines the ion-transports to the corresponding half-cycles, but the detailed position within the half-pulse cannot be determined.

Acknowledgement The authors wish to thank Dr. D. Rabson for his instructive suggestions and reading the manuscript. This work is partially supported by our research grants from NIH, 2NIGM50785, and NSF PHY-0515787.

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