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Entropy facilitated active transport

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We show how active transport of ions can be interpreted as an entropy facilitated process. In this interpretation, a particular change in the pore geometry through which substrates are transported gives rise to a driving force. This chemical energy provided by the chemical reaction is then used to create a protein geometry favorable for the uphill transport of ions. Attempts to estimate the energy available by this change in several proteins shows that an entropic contribution from the pore geometry is significant. We discuss how this effect can be used to understand how energy transduction in active transport can take place over a relatively long distance. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4982799]

I. INTRODUCTION

Active transport is of major importance in biology; meaning the transport of a compound against its chemical potential by means of a chemical reaction. A notable example is the large P-type ATPase protein family, which functions as ion or lipid pumps, crucial for a wide range of processes in almost all forms of life.[1] P-type ATPases share a common topology and their operation can be described using a post-Albers cycle with four key conformations: E1, E1P, E2P, and E2.[2] The transitions E1P ↔ E2P and E2 ↔ E1 in the catalytic cycle are associated with large conformational changes,[2] and it is clear that the energy released by the hydrolysis of adenosine triphosphate (ATP) drives the enzyme between these states. Using Ca^{2+}-ATPase as an example, Ca^{2+} ions are moved against a concentration ratio which can be as high as 10^4, corresponding to a Gibbs energy of 24 kJ/mol ion. A typical chemical energy available from the ATP reaction[3] is $\Delta G = -50$ kJ/mol which shows that the transport of one or two Ca^{2+} ions is spontaneous. The still open question after many years of research is how the energy is transferred to the ionic binding site, to help push the ion against its chemical potential. Part of the difficulty has been to explain how this can happen with a relatively large distance (5 nm)[4] between the ATP binding site and the ion-binding site.

In this context, we propose to examine more closely the meaning of the large conformational changes that take place in all P-type ATPases during active transport. A common feature which appears, the formation of a wide funnel-shaped outlet channel, is observed in several P-type ATPases[5–8] when the enzyme changes from the E1P state to the E2P state. The ion, first occluded from the cytosol, is eventually exposed to the lumen. But why does the ion leave its binding site when the overall chemical potential increases in the direction of transport? We ask here whether this increasing chemical potential profile can vary during the conformational cycle and reach a maximum that eventually allows the ion to proceed to the lumen. Can this be facilitated by the observed change in the pore geometry? We propose so and provide arguments in favor of it. Doing so, we also address the old question related to active transport in biology: How can a scalar chemical reaction couple to ion transport which is vectorial? Chemical energy is released locally, while transport has a direction. At the membrane interface, however, we need to consider only the scalar normal component of the ion flux. This flux now needs to be coupled to the reaction which takes place at another location. How is that possible? It is well known that the ATP hydrolysis leads to large conformational changes in ATPase. We actively use this observation to explain that the conformational change itself contributes significantly to the ion transport. We clarify therefore how a local reaction can couple to transport, implicitly reducing the need for a change in the binding energy of Ca^{2+}. Presumably, a large change in the binding energy is not likely for a translocation process that is known to be reversible. In summary, we help explain the mechanism for how a change in Gibbs energy by a chemical reaction, taking place relatively far away from the binding site (see, e.g., Ref. 4) can be used for ion transfer. Active transport, which has been discussed since the discovery of the pumps, has often been explained by mechanical pump models. Our explanation provides an alternative to these.

We shall argue that active transport can be better understood by shifting focus from an energy to an entropy gradient in a specific part of the translocation process. When the outlet channel is formed in the E2P state, the ion in the channel will experience a force directed to the lumen because the shape of this channel allows its entropy to increase or, equivalently, its chemical potential to decrease from the E2P state to the lumen, as we will demonstrate. By bringing about such a change in the chemical potential profile of the ion, the chemical energy

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of the reaction may transfer energy to the ion. Via the formation of a particular pore geometry, the reaction contributes to active transport. The idea is not new for passive transport. It stems from previous studies which have shown how entropic barriers can play a major role for separation purposes.[9–11] It has not been applied in biology, however, and in particular not to active transport.

The article is organized as follows. In Section II, we present the entropic transport model which considers ion diffusion in the channel as one-dimensional diffusion mediated by an entropic gradient resulting from the irregular shape of the channel. In Section III, we show how entropy drives active transport in P-type ATPases and compute the ion translocation rate. In Sec. IV, we emphasize our main results.

II. ENTROPIC TRANSPORT MODEL FOR ACTIVE TRANSPORT

Ion channels are quasi-1D structures in which ions move along a main transport direction, say the x-direction. The confinement in the cross section of the channel establishes a drastic separation of time scales with a rapid local equilibration of the ions moving in the (y,z)-plane, as compared to that for the x-direction. Under these conditions, one may adopt a coarse-graining description in which transport can approximately be studied in terms of only one coordinate, the x-axis of the channel. The shape of the channel considered will be accounted for by an entropic gradient. A global criterion for the consistency of this approach, formulated in terms of the shape of the structure and the strength of an applied force, was established by Burada *et al.*[12]

To explain why entropy varies along the main transport coordinate, thus establishing an entropic gradient, we analyze the equilibrium ion distribution, $c(x)$, neglecting for the moment enthalpic effects. This distribution is obtained by integrating over the (y,z)-coordinates[9]

$$c(x) = c_0 \frac{A(x)}{A_0}, \tag{1}$$

where $A(x)$ is the cross-sectional area of the 3D-channel and A_0 is the cross-sectional area at a reference point, say $x = 0$. The ion concentration at this point is c_0. The size of the particles has been shown to influence the transport properties.[13] Both $A(x)$ and A_0 measure the available area, e.g., for a circular geometry $A(x) = \pi\,(r(x) - a)^2$, where $r(x)$ is the radius of the pore at position x and a is the radius of the ion.

Under local equilibrium conditions for the transverse motion, the density given by Eq. (1) can be viewed as the canonical distribution function

$$c(x) = c_0 \exp\left(\frac{\Delta S_{\text{pore}}(x)}{R}\right), \tag{2}$$

where R is the gas constant and we have introduced the change in entropy, $\Delta S_{\text{pore}}(x) = S_{\text{pore}}(x) - S_{\text{pore}}(0)$, associated with the change in the cross-sectional area. From these equations, we conclude that the change in the entropy during ion motion along the pore is directly related to the cross-sectional area

$$\Delta S_{\text{pore}}(x) = R \ln \frac{A(x)}{A_0} \tag{3}$$

and that the position dependent entropy along the ion trajectory is giving rise to a (thermodynamic) force of entropic nature, F_{ent}, acting on the ions

$$F_{\text{ent}} = T \frac{\partial \Delta S_{\text{pore}}}{\partial x} = RT \frac{1}{A(x)} \frac{\partial A(x)}{\partial x}. \tag{4}$$

The direction of the force directly depends on the rate of change of the channel cross section. The total entropy change at x, $\Delta S(x)$, has also the normal contribution due to the ion concentration $\Delta S_{\text{Ca}}(x) = -R \ln[c(x)/c_0]$, giving

$$\Delta S(x) = R \ln \frac{c_0 A(x)}{c(x) A_0} \tag{5}$$

which reflects the distribution given in Eq. (1). The value is zero (i.e., equilibrium) for a concentration ratio that is equal to $A_0/A(x)$.

The full difference in the Gibbs energy (chemical potential) between the points x and 0 is

$$\Delta\mu(x) = \Delta H(x) - T\Delta S(x) = \Delta H(x) - RT \ln \frac{c_0 A(x)}{c(x) A_0}, \tag{6}$$

where $\Delta H(x) = H(x) - H_0$ is the (possible) enthalpic contribution to the ion's energy.

The entropy production along the coordinate, $\sigma(x)$, is the product of the flux $J(x)$ and the driving force $-(1/T)(\partial\mu(x)/\partial x)$,

$$\sigma(x) = -J(x) \frac{1}{T} \frac{\partial \mu(x)}{\partial x}. \tag{7}$$

The flux-force relation is linear on this meso-level

$$J(x) = -\frac{l(x)}{T} \frac{\partial \mu(x)}{\partial x}, \tag{8}$$

where $l(x)$ is the conductivity. The entropy production is everywhere positive along the x-coordinate. Consider a time frame where we can assume a quasi-stationary state, in which the flux is constant along the x-coordinate, $J(x) = J$. We integrate Eq. (7) for this case and obtain

$$\sigma_{\text{t}} = -J \frac{\Delta \mu}{T}, \tag{9}$$

where $\sigma_{\text{t}} = \int \sigma(x) \mathrm{d}x$ is the entropy production, measuring x in units of the channel length. The overall driving force defined by this expression is

$$-\frac{\Delta \mu}{T} = -\frac{\Delta H}{T} + R \ln \frac{c_0 A(1)}{c(1) A_0}. \tag{10}$$

Here Δ refers to the whole channel length. The remaining problem, to find the flux, is discussed below.

III. ENTROPY DRIVEN ACTIVE TRANSPORT IN P-TYPE ATPases

To exemplify the impact of the pore geometry, we will take the Ca^{2+} transporting Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) but we note that the ideas presented apply more generally. For Ca^{2+}-ATPase, we illustrate the variation of the chemical potential as ions are transported from the cytosol to the lumen of the SR in Fig. 1. It is known that the binding of Ca^{2+} is fast[14] and can be regarded as being in equilibrium. The chemical potential of Ca^{2+} in the E1 state, $\mu_{\text{Ca2.E1}}$, is then in good approximation equal to the chemical potential in the

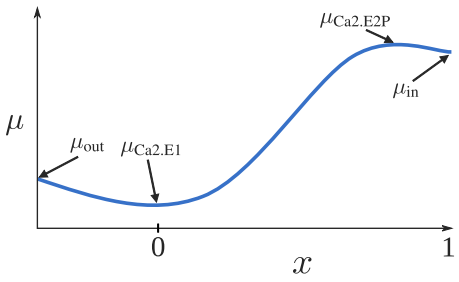

FIG. 1. Illustration of the variation in the chemical potential μ of a Ca^{2+}-ion as it is transported by Ca^{2+}-ATPase from the cytosol to the lumen of SR. The coordinate x denotes the extent of transport inside the funnel-shaped pore from the binding site at $x = 0$ to the lumen at $x = 1$. The chemical potential is indicated for four states: in the cytosol/outside (μ_{out}), lumen/inside (μ_{in}), the E1 ($\mu_{Ca2.E1}$), and the E2P ($\mu_{Ca2.E2P}$) states. For a concentration ratio of 10^4 at 37 °C, the energy requirement is 24 kJ/mol ion transported.

cytosol, μ_{out}, as depicted in Fig. 1. The concentration dependent contribution to the chemical potential in the final state, μ_{in}, is always larger than μ_{out} during active transport and the corresponding ion concentrations are $c_{in} > c_{out}$.

The difference between μ_{in} and the chemical potential of Ca^{2+} when the enzyme is in the state E2P, $\mu_{Ca2.E2P}$, is unknown. We argue that it must be negative to allow the ion to enter the lumen. In Fig. 1 the two chemical potentials are pictured as being close to each other, enabling the ion to pass to the lumen. The ion-binding energy is frequently discussed in this context. The variation in the binding energy between the E1 and E2P sites can be attributed to the enthalpic part of the chemical potential, as the enthalpy gives a measure of the bond strength. The E1 site is attractive (fast binding), and repulsive forces can raise the enthalpic part of the chemical potential of the E2P site, $\mu_{Ca2.E2P}$, providing a low affinity binding site from which the ions may leave spontaneously. Alternatively, the leap from $\mu_{Ca2.E1}$ to $\mu_{Ca2.E2P}$ can be caused also by entropic effects: the ATP hydrolysis can bring about a change in the actual shape of the channel where the ion is transported, which lowers the entropy contribution to $\mu_{Ca2.E2P}$.

The special common property of outlet channels observed in P-type ATPases is their conical shape. They are narrow but widen towards the exit of the channel, as illustrated in Fig. 2. For such a structure, the entropic force is positive and will facilitate translocation in the direction of the wider opening. The overall entropy change (ΔS) for the ion

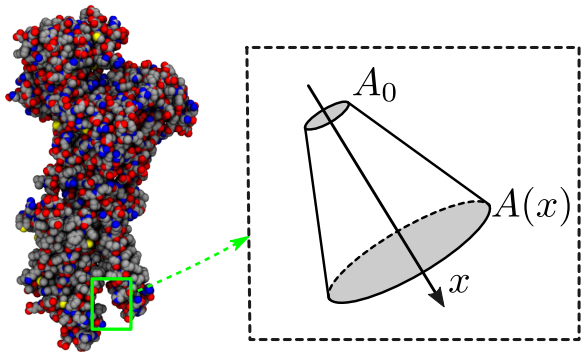

FIG. 2. The E2P state for Ca^{2+}-ATPase (Protein Data Bank ID: 3B9B[5]) showing the funnel-shaped channel. The inset shows a conical funnel where the area increases from the start of the channel (A_0) to the exit (A_{in}) where $0 \le x \le 1$ indicates the position along the channel.

translocation will thus have two contributions: the normal contribution from the ion concentration difference ($\Delta S_{Ca} = -R\ln(c_{in}/c_0) = -\Delta\mu_{Ca}/T$) and a special contribution from the pore shape. The entropy variation per mol of ion is given by $\Delta S = \Delta S_{Ca} + \Delta S_{pore}$ and by introducing Eq. (5) for $x = 1$, we obtain

$$\Delta S = R\ln\frac{c_0 A_{in}}{c_{in} A_0}. \tag{11}$$

The entropic force depends on the concentration ratio but also on the ratio of the cross-sectional areas at the two sides of the channel. A large c_0/c_{in} ratio may be counteracted by a smaller A_0/A_{in} ratio, meaning that translocation may take place against the gradient in concentration. In other words, it may be facilitated by entropic forces induced by the formation of a conical pore, and the larger the ratio A_{in}/A_0, the larger is the co-acting entropic force.

Consider now the state E2P. The large enthalpy change of the hydrolysis of ATP has at an earlier moment been transferred into the structural energy of the conical outlet channel in E2P and possibly also changed the ion-binding site into a low(er) affinity site. We consider the last step in the translocation process. The overall driving force, $\Delta\mu$, for transport in the channel was given above, cf. Eq. (10). The ion translocation can be regarded as a diffusion process along the x-coordinate. The conductivity $l(x)$ in Eq. (8) is usually proportional to $c(x)$. We introduce the constant diffusion coefficient, $D \equiv Rl(x)/c(x)$, into the flux equation and obtain for constant J

$$J = -\frac{Dc_0}{RT}\frac{\partial\mu}{\partial x} = -Dc_0\frac{A(x)}{A_0}\exp\left[-\frac{H(x)}{RT}\right]\frac{\partial}{\partial x}\exp\left[-\frac{\mu(x)-\mu_0}{RT}\right]. \tag{12}$$

After multiplying this equation left and right with $A_0/A(x)\exp(-H(x)/RT)$ and integrating, we obtain a common expression[15] for the overall process,

$$J = -L\left[1 - \exp\left(-\frac{\Delta\mu}{RT}\right)\right], \tag{13}$$

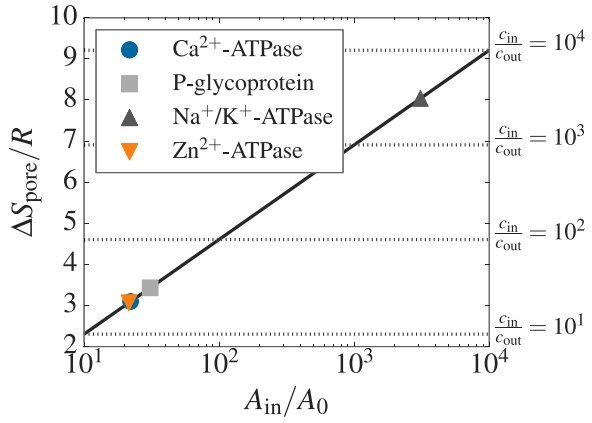

FIG. 3. Entropic contribution, ΔS_{pore} (solid black line), to the driving force as a function of the ratio of cross-sectional areas. Contributions for several enzymes are also shown: Zn^{2+}-ATPase,[2] P-glycoprotein,[16] Ca^{2+}-ATPase,[5] and Na^+/K^+-ATPase.[6] We have considered the size of the ions using the ionic radii reported by Marcus[17] (Ca^{2+}: 1.03 Å, Na^+: 0.97 Å, K^+: 1.41 Å, Zn^{2+}: 0.70 Å) and for Na^+/K^+-ATPase we only considered the smallest ion (Na^+). As P-glycoprotein may transport many different compounds, we have omitted the size of the transported compound and the estimate represents a lower bound.

TABLE I. Pore geometry for the enzymes considered here: Zn^{2+}-ATPase,[2] P-glycoprotein,[16] Ca^{2+}-ATPase,[5] and Na^+/K^+-ATPase.[6] For Zn^{2+}-ATPase, the widths of the channel were estimated using the crystal structure (Protein Data Bank ID: 4UMV).

Protein	r_0 (Å)	$r(1)$ (Å)
Ca^{2+}-ATPase	4	15
P-glycoprotein	9	50
Na^+/K^+-ATPase	1.1	8.2
Zn^{2+}-ATPase	4	16

where l can be interpreted as the backward reaction rate[15] and has an explicit form

$$L = Dc_0 \left[\int_0^1 \frac{A_0}{A(x)} \exp\left(\frac{H(x)}{RT}\right) \mathrm{d}x \right]. \tag{14}$$

This means that we can determine ΔH from the temperature dependence of the flux or the chemical reaction. The equation gives an Arrhenius behavior of J through the dependence on ΔH.

Assuming a circular cross-sectional area, the entropic contribution to the driving force can be further adjusted,

$$-T\Delta S = RT \ln \left[\left(\frac{c(1)}{c_0}\right) \left(\frac{r_0 - a}{r(1) - a}\right)^2 \right], \tag{15}$$

where r_0 is the radius at the start of the channel, $r(1)$ at the exit of the channel, and a the radius of the ion.

Figure 3 shows the entropic contribution to the driving force for several enzymes as computed from Eq. (15). The data used in the calculations are given in Table I.

As the results show, the entropic contribution can be sizable. It is not sufficient to explain active transport by itself for all the enzymes, as can be seen by the results for Ca^{2+}-ATPase. In this case, the entropic contribution is on the order of 10 kJ/mol ion transported at 37 °C. This accounts for 40% of the minimum chemical potential difference needed (25 kJ/mol), implying that other contributions, for instance, enthalpic effects, also are important for this protein. For Na^+/K^+-ATPase we find that, due to the narrowness of the pore, the entropic contribution (20 kJ/mol) is relatively large compared to the other enzymes. This is sufficient to overcome the concentration ratio of Na^+ at physiological conditions. In this case, the Na^+ ions are transported against the resting membrane potential, ~ -60 mV. Including this in the change in Gibbs energy when transporting the Na^+ ion and assuming a concentration ratio on the order of 10, the required Gibbs energy is 12 kJ/mol ion transported. This means that the entropic force estimated in Fig. 3 is sufficient for this enzyme. The entropic contribution for the two other enzymes is similar to that of Ca^{2+}-ATPase. We have neglected the possibility of complexing the ions with other species such as water which could increase the effective radius and the entropic force. Further, we have not considered contributions from the counter-transport of other ions and water.

IV. CONCLUSIONS

We have shown that actual pore geometry, of conical form at the outlet of the channel, observed in several transport enzymes may give rise to an entropic force that facilitates the transport of ions through the channel. The force originates from variations of the cross-sectional area of the channel which induces an entropy gradient along the main transport direction. This entropic effect can be used, together with enthalpic effects, to explain how conformational changes influence the binding site and allow the transport of compounds against their concentration gradients. In particular, the entropic force due to the conformational change gives the link between the energy released by the hydrolysis of ATP and the ion transport. This provides a mechanism for the coupling of the scalars involved, the chemical reaction rate, and the normal component of the ion flux. The purpose of the modified ATPase conformation is the modified pore geometry. This in addition to a lower affinity-site will then give the ion sufficient energy to enter the lumen.

The deformation of the channel to form a cone is the result of the hydrolysis of ATP and has been observed in crystallization experiments in the E2-form of the different proteins we have analyzed. Using an entropic transport model, proposed for studies of diffusion in quasi-1D structures, we have obtained an expression for the entropy change of the ions in terms of the concentrations and cross-sectional areas of the start and end points of the conical region of the channel.

Using experimental measurements of the concentrations at both sides of the conical region and of the cross-sectional areas obtained from crystallization experiments, we can obtain the entropic contribution to ion translocation. Our formula is general, as it can be used to describe active transport for different P-type ATPases in terms of simple measurable parameters. From it, we can obtain the ion translocation rate, which explains why ions move forward. Additionally, our model provides a thermodynamic justification of the validity of a mean field description usually proposed in ion pumps.[18] The reduction of the dimensionality of the system, from 3D to 1D, is only possible under local thermodynamic equilibrium conditions. The mechanism fits well with a reversible process.

Our model adds to the understanding of the active transport mechanism, as it explains much of the energy needed to move "uphill." It can be contained in a more general description.[15,19]

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