Active transport: A kinetic description based on thermodynamic grounds

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Abstract

We show that active transport processes in biological systems can be understood through a local equilibrium description formulated at the mesoscale, the scale to describe stochastic processes. This new approach uses the method established by nonequilibrium thermodynamics to account for the irreversible processes occurring at this scale and provides nonlinear kinetic equations for the rates in terms of the driving forces. The results show that the application domain of nonequilibrium thermodynamics method to biological systems goes beyond the linear domain. A model for transport of Ca\(^{2+}\) by the Ca\(^{2+}\)-ATPase, coupled to the hydrolysis of adenosine-triphosphate is analyzed in detail showing that it depends on the reaction Gibbs energy in a non-linear way. Our results unify thermodynamic and kinetic descriptions, thereby opening new perspectives in the study of different transport phenomena in biological systems.

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1 Introduction

Mitchell’s success in using the proton-motive force to explain energy conversion in mitochondria (Mitchell, 1961) led to a considerable effort over the next three decades to explore the usefulness of nonequilibrium thermodynamics in biology (Caplan and Essig, 1983; Westerhoff and van Dam, 1987; Walz, 1990). Even if substantial progress was made, it was eventually concluded that the theory had major drawbacks. The flux-force relations that were established, were linear, and could only describe the dynamics in a rather narrow range near total equilibrium. Since many biological reactions have an activation energy barrier and therefore have an intrinsically nonlinear dynamic behavior, such an overall thermodynamic analysis could not provide a full description of those processes. Existing kinetic descriptions, on the other hand, are not able to account for the coupling between transport and chemical reaction. That coupling has so far only rigorously been established within the framework of Onsager’s theory. These difficulties, which already were pointed out by Prigogine (Prigogine, 1955) and Eyring (Eyring and Eyring, 1965) in the context of activated processes, have hampered a wider application of nonequilibrium thermodynamics to biological systems, leaving the complete description of active transport processes to kinetic approaches. Kinetic descriptions take the tight coupling of transport and chemical reaction as a starting point and can therefore not address a varying degree of coupling or slippage.

Over the last few years, amazing new details have been revealed about the molecular mechanism of active transport by some ATPases (Berman, 2001).
Experimental evidence has been found that ATP hydrolysis leads to a rotation of the central parts of the enzyme, followed by an electric signal in the channel that spans the membrane, attributed to the ion that is being transported, see e.g. (Peinelt and Apell, 2004; Burzik et al., 2003; Sun et al., 2004). Active transport is accomplished by various types of molecular motors. A theory that relates these fluxes with driving forces in the system, however, is still lacking (Berman, 2001).

The purpose of this paper is to show that when irreversible processes are analyzed at a mesoscopic level, application of the method of nonequilibrium thermodynamics leads to a complete description of the process even in the nonlinear domain. The mesoscopic level has shorter time and length scales than we encounter on the macroscopic level. It is the level of description where fluctuations matter. Rate laws on this level are stochastic of nature. We shall use mesoscopic nonequilibrium thermodynamics (Reguera and Rubi, 2001; Vilar and Rubi, 2001) and go beyond the linear overall or 'black box' description offered by classical nonequilibrium thermodynamics. This gives a new perspective to the problem of biological transport phenomena.

The paper is organized as follows. In section 2, we introduce the main features of active transport of ions and discuss the limitations of a nonequilibrium thermodynamic description of the process. In Section 3, we present a kinetic description based on thermodynamic grounds which enables us to analyze active transport processes beyond the linear domain. Finally, in Section 4 we summarize our main results.
2 Active transport and nonequilibrium thermodynamics

We shall focus on the problem of active transport of an ion, the meaning of nonlinear flux-force relations and the coupling of vectorial and scalar forces in this context. As example we take Ca$^{2+}$ transport in sarcoplasmic reticulum by means of the Ca$^{2+}$-ATPase. We limit ourselves to the conversion between chemical energy and transport of the ion, neglecting a possible intervention of rotational energy. This does not exclude a torsional mechanism, however (Jain et al., 2004). The membrane is highly asymmetric, about 5 nm thick, and consists of a phospholipid bilayer with proteins embedded. Figure 1 gives a schematic picture of the Ca$^{2+}$-ATPase embedded in the phospholipid bilayer. The reaction takes place at the headgroup of the Ca$^{2+}$-ATPase. The ion, to be actively transported along the protein channel, binds to a separate site and triggers the chemical reaction, the ATP hydrolysis. Each channel transports only a few molecules or ions per second, and there are not many channels of the same type in one membrane. Experiments are done in a solution with many vesicles or organelles, so the observation refers to the average over the channels (Burzik et al., 2003). Thus, we have a chemical reaction on a time scale similar to that of diffusion (Peinelt and Apell, 2004; Burzik et al., 2003). Can we legitimately consider this dynamic situation with thermodynamic tools? We shall see that the answer is yes.

The spontaneous chemical reaction taking place at the membrane surface in Fig.1 is, schematically:

$$\text{ATP (s)} \rightleftharpoons \text{ADP (s) + P (s)}$$
Figure 1: The Ca\(^{2+}\)-ATPase that transports Ca\(^{2+}\) by hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and phosphate (P). The reaction takes place at the enzyme headgroup, and triggers the active transport through the protein stalk and channel. A surface area element is indicated by the dotted line.

where \(s\) stands for surface. The reaction Gibbs energy which arises is

\[
\Delta G = \mu^s_P + \mu^s_{ADP} - \mu^s_{ATP}
\]  

where \(\mu^s_j\) is the chemical potential at the surface of component \(j\). Calcium ions are transported across the membrane from side i to side o. The transport takes place against the chemical potential gradient as a consequence of its coupling to the above reaction:

\[
\text{Ca}^{2+} (i) \rightleftharpoons \text{Ca}^{2+} (s) \rightleftharpoons \text{Ca}^{2+} (o)
\]

There is probably also a countertransport of protons to keep the system’s local electroneutrality (Peinelt and Apell, 2004).

Non-equilibrium thermodynamics provides a description of the irreversible processes occurring at the surface (Albano and Bedeaux, 1987). It has been shown that the surface behaves as an autonomous thermodynamic system in
local thermodynamic equilibrium, even in the presence of strong driving forces (Røsjorde et al., 2000). This fact enables one to formulate the Gibbs equation for the surface element indicated in Fig.1 (Albano and Bedeaux, 1987):

\[ T ds^s = du^s - \mu^s_{\text{ATP}} dc^s_{\text{ATP}} - \mu^s_{\text{ADP}} dc^s_{\text{ADP}} - \mu^s_P dc^s_P - \mu^s_{\text{Ca}} dc^s_{\text{Ca}} \]  

(2)

Here \( c^j_s \) is the surface concentration of component \( j \) in mol/mg, referring to the total unknown area of transfer per mg of material, and \( u^s \) is the internal energy density of the surface. If the membrane potential contributes to transport of the calcium ions, one has to use electrochemical potentials. The conservation laws for the reacting species in Eq. (1) are

\[ \frac{dc^j_s}{dt} = \pm r + J^j_i \]  

(3)

where \( J^j_i \) are fluxes of species, \( r \) is the reaction rate (all in mol/s mg), and \( t \) is the time. The minus sign refers to ATP and the plus to the other species. The concentration of Ca\(^{2+} \) in mol/mg on the surface satisfies

\[ \frac{dc^s_{\text{Ca}}}{dt} = J^i_{\text{Ca}} - J^o_{\text{Ca}} \]  

(4)

where \( J^i_{\text{Ca}}, J^o_{\text{Ca}} \) are the inwards and outwards ion fluxes in mol/s mg. We omit the charge number in subscripts.

For the time being, we consider an isothermal surface. The entropy production (in W/mg K) then follows by using Eqs. (3) and (4) in combination with Gibbs equation, taking the temperature constant and \( \mu^s_j = \mu^i_j \):

\[ \sigma^s = -r \frac{1}{T} \Delta G - J^o_{\text{Ca}} \frac{1}{T} (\mu^i_{\text{Ca}} - \mu^o_{\text{Ca}}) \]  

(5)

The countertransport of protons to maintain the system’s electroneutrality (Peinelt and Apell, 2004) can be accounted for by modifying the driven force for \( Ca^{2+} \), see Eq. (20) below. From this one normally has inferred linear laws between
fluxes and driving forces (Caplan and Essig, 1983), with an expression for the flux of Ca\(^{2+}\)

\[
J_{Ca}^0 = -\frac{L_{dr}}{T} \Delta G - \frac{L_{dd}}{T} (\mu_{Ca}^o - \mu_{Ca}^i)
\]  

(6)

describing linear active transport for small values of \(\Delta G\) and constant Onsager coefficients \(L_{dr}, L_{rr}\). Equation (6) is normally taken for a ‘black box’ description of active transport from side i to o (Caplan and Essig, 1983). When the second term is negative, the coupling of \(J_{Ca}^0\) to the reaction (via \(L_{dr}\)) can compensate that negative contribution thus making transport against the concentration gradient possible. This is the coupling phenomenon that is referred to as active transport in the literature. No explanation has yet been given for the nature of the coupling (Berman, 2001), leaving the problem unsolved as how to characterize the total process far from equilibrium.

3 Kinetic description from thermodynamic grounds

The lack of equilibrium for the chemical reaction on a time scale similar to that for ion transport is the reason for energy transduction between reaction and transport. This energy transduction process shall now be described for shorter time scales at which the system evolves from the initial to the final states. The local states of the system correspond to successive molecular configurations and are parametrized by an internal reaction coordinate \(\gamma\) running from 0, at which there are reactants, to 1, at which there are products. The coordinate \(\gamma\) is dimensionless. One may then extend the assumption of local thermodynamic equilibrium to the mesoscopic level and formulate the corresponding Gibbs equation in \(\gamma\)-space:

\[
T ds^*(\gamma) = -G(\gamma)dc_r(\gamma) - \mu_{Ca}^o(\gamma)dc_{Ca}^o(\gamma)
\]  

(7)
where $c_r(\gamma)$ is the concentration of enzymes with a reaction mixture characterized by coordinate $\gamma$, and $c_{Ca}^{\gamma}(\gamma)$ is the average concentration of Ca$^{2+}$ over the state given by $\gamma$, both concentrations in mol/mg. Before the reaction, the Gibbs energy becomes $G(0)\mu_{ATP}$; after the reaction it is $G(1) = \mu_P + \mu_{ADP}$.

The relation between $\Delta G$ and $G(\gamma)$ is shown to the left in Fig. 2.

$$\sigma_s(\gamma)$$

Figure 2: Left, the expansion of $G(\gamma)$ in reaction coordinate space. Right, the activation energy $\Phi(\gamma)$ with a peak at $\gamma_{tr}$.

Mass conservation can be expressed at any position in $\gamma$-space through the conservation laws

$$\frac{dc_r(\gamma)}{dt} = -\frac{\partial}{\partial \gamma}r(\gamma) \quad \text{and} \quad \frac{dc_{Ca}^{\gamma}(\gamma)}{dt} = J_{Ca}^i(\gamma) - J_{Ca}^o(\gamma), \quad (8)$$

Here $r(\gamma)$ is the local reaction flux along the reaction coordinate. The fluxes $J_{Ca}^i(\gamma)$ and $J_{Ca}^o(\gamma)$ contributions to the diffusion fluxes on sides i and o respectively, averaged over enzymes in state $\gamma$. The integrals of the fluxes over $\gamma$ give the corresponding global fluxes. This choice for the conservation equation for Ca$^{2+}$ may capture the essence of the delivery of Ca$^{2+}$ to the other side in a pump that allows slippage.

The local entropy production $\sigma_s(\gamma)$, defined through the entropy change per
unit of time in the isothermal surface is then:

\[
\frac{ds^s(γ)}{dt} = σ^s(γ) + \frac{1}{T} \frac{∂G(γ)r(γ)}{∂γ} + \frac{μ_{Ca}^o}{T} J_{Ca}^o(γ) - \frac{μ_{Ca}^i}{T} J_{Ca}^i(γ),
\]

then follows from Eqs. (7) and (8)

\[
σ^s(γ) = -r(γ) \frac{1}{T} \frac{∂G(γ)}{∂γ} - \frac{1}{T} J_{Ca}^o(γ)(μ_{Ca}^o - μ_{Ca}^s(γ)) - \frac{1}{T} J_{Ca}^i(γ)(μ_{Ca}^i(γ) - μ_{Ca}^i)
\]

As has been assumed already, there is equilibrium for the Ca\(^{2+}\)-ion between the surface and the bulk solution \(μ_{Ca}^s(γ) = μ_{Ca}^i\). The local entropy production then simplifies to

\[
σ^s(γ) = -r(γ) \frac{1}{T} \frac{∂G(γ)}{∂γ} - \frac{1}{T} J_{Ca}^o(γ)(μ_{Ca}^o - μ_{Ca}^i)
\]

Due to the activation barrier, the reaction rate almost immediately reaches a quasi-stationary state in which it only depends on time. Using this property, the entropy production (4) is recovered upon integration of Eq. (11) with a varying calcium flux.

We shall assume that Ca\(^{2+}\) is not transported across an activation energy barrier. This means that the ion can escape from any surface state given by \(γ\) with \(μ_{Ca}^s(γ) = μ_{Ca}^i\) to side o with \(μ_{Ca}^o\), an explanation for why the flux depends on \(γ\). The local flux-force relations that follow from Eq. (11), are:

\[
\begin{align*}
    r(γ) &= \frac{l_{rr}(γ)}{T} \frac{∂G(γ)}{∂γ} - \frac{l_{rd}(γ)}{T}(μ_{Ca}^o - μ_{Ca}^i) \\
    J_{Ca}^o(γ) &= \frac{l_{dr}(γ)}{T} \frac{∂G(γ)}{∂γ} - \frac{l_{dd}(γ)}{T}(μ_{Ca}^o - μ_{Ca}^i)
\end{align*}
\]

which are valid along the path going from the initial to the final state and have been formulated under the equilibration assumption discussed previously. At each point of the path, the Onsager relation \(l_{rd}(γ) = l_{dr}(γ)\) is fulfilled and the coefficient do not depend on the driving forces. The last relation will give a non-linear equation for the flux of Ca\(^{2+}\) across the membrane.
The chemical reaction is an activated process. Along the internal coordinate, we can write the Gibbs energy as a combination of the ideal contribution and an activation energy per mole, $\Phi(\gamma)$:

$$G(\gamma) = G^0 + RT \ln c(\gamma) + \Phi(\gamma) \quad (13)$$

The boundary values of $G(\gamma)$ were defined above. The standard Gibbs energy is chosen to be $G^0 = \mu_0^{\text{ATP}}$. For the potential profile, correct boundary conditions are obtained with $\Phi(0) = 0$ and $\Phi(1) = \mu_0^{\text{ADP}} + \mu_0^{\text{P}}$. The activation energy barrier with the transition state, $\gamma_{tr}$, is illustrated in Fig. 2.

We do not know the coefficients in Eqs. (12). But, under the condition of constant stoichiometry, or tight coupling, we can make certain assumptions that allow us to integrate the expression for the calcium flux. A constant stoichiometry means that the number of Ca$^{2+}$ transported for every reacted ATP is fixed. When the stoichiometry is fixed, $J_{\text{Ca}}^0$ and $r$ are dependent, and the matrix of coefficients has a zero determinant. The number of Ca$^{2+}$ transported for every reacted ATP can also be defined in $\gamma$-space. We obtain:

$$n(\gamma) = \frac{J_{\text{Ca}}^0(\gamma)}{r} \frac{l_{dr}(\gamma)}{l_{rr}(\gamma)} = \frac{l_{dd}(\gamma)}{l_{rd}(\gamma)} \quad (14)$$

In order to show how the linear, local Eqs. (12) transform into nonlinear global laws, we choose as thermodynamic force the gradient of the local fugacity $z(\gamma)$,

$$z(\gamma) = \exp\left[\frac{(G(\gamma) - G^0)}{RT}\right] = c(\gamma) \exp\left[\frac{\Phi(\gamma)}{RT}\right] \quad (15)$$

The fugacity divided by the fugacity at equilibrium, can be understood as the probability distribution function in $\gamma$-space divided by the equilibrium distribution, $c_r(\gamma)/c_{r,eq}$. The use of probabilities is characteristic to a mesoscopic description. The local diffusion flux (12) can then be rewritten in terms of the
new thermodynamic force

\[
J_{Ca}^0(\gamma) = -\frac{nD'}{\exp[\Phi(\gamma)/RT]} \frac{\partial z(\gamma)}{\partial \gamma} - \frac{l_{dd}(\gamma)}{T} [\mu_{Ca}^0 - \mu_{Ca}^1] \tag{16}
\]

where we have used Eqs. (14) and (15) and defined \( D' = l_{rr}(\gamma)R/c(\gamma) \) in accordance with Kramers [?]. We find the macroscopic flux in terms of the driving forces by integrating over \( \gamma \). Using the quasi-stationary condition for the reaction flux, and constant \( n \), it follows that \( J_{Ca}^0 \) is constant. Using furthermore that \( D' \) is in good approximation constant, we obtain:

\[
J_{Ca}^0 = -nD_{ATP} \left( \exp \frac{\Delta G}{RT} - 1 \right) - L_{Ca} [\mu_{Ca}^0 - \mu_{Ca}^1] \tag{17}
\]

where

\[
D = D' \left( \int_0^1 \exp \Phi/RT \right)^{-1} \quad \text{and} \quad L_{Ca} = \int_0^1 l_{dd}/T d\gamma \tag{18}
\]

We have also used \( \exp(G(0) - G^0)/RT = c_{ATP} \).

This overall diffusion flux describes active transport of \( Ca^{2+} \) for arbitrary values of \( \Delta G \), and accounts thus for the transduction process also in the nonlinear regime, which could not previously be described through nonequilibrium thermodynamics. Nonlinearities inherent to the kinetics of the chemical reaction emerge in the broad description if one proceeds with a thermodynamic analysis at the mesoscopic level and subsequently coarsens the description by retaining only the initial and final states. At small values of the driving force, Eq. (21) reduces to expression (5) valid only in the linear regime. It is interesting to note that the prefactor of the nonlinear term contains an Arrhenius factor characteristic for activated processes. The first term in Eq. (21) is also present in the equation for active transport, derived by the King-Altmann-Hill method (Westerhoff and van Dam, 1987). This equation does not have a second term. The derivation and the understanding of Eq. (21) compared to equations that have been used earlier to describe \( Ca^{2+} \) transport, however, are very different.
Equation (21) can be used to plot experimental data and find the product \( nD \) and \( L_{Ca} \). The value of \( n \) obtained by imposing the static head condition \( J_{Ca}^0 = 0 \) to Eq. (17), may be different from the one given through the relation \( n = J_{Ca}^0 / r \) depending on experimental conditions. The chemical potentials for \( \text{Ca}^{2+} \) for state i or o:

\[
\mu_{Ca}^{i,o} = \mu_{Ca}^0 + RT \ln c_{Ca}^{i,o}
\]  

where the ideal mixture approximation and the same standard state has been used for both sides. The approximate difference in chemical potentials for \( \text{Ca}^{2+} \) is:

\[
\mu_{Ca}^o - \mu_{Ca}^i = RT \ln \frac{c_{Ca}^o}{c_{Ca}^i} \approx \frac{RT}{c_{Ca}^i} \left( c_{Ca}^o - c_{Ca}^i \right)
\]  

This driving force can be modified to also account for the countertransport of protons. The effective driving force for exchange of \( \text{Ca}^{2+} \) and \( \text{H}^+ \) becomes

\[
\Delta \mu = RT \ln \frac{c_{Ca}^i c_{H}^0}{c_{Ca}^o c_{H}^i}
\]  

Only a zero difference in pH between the sides has no influence on this force.

In the derivation of the flux equations (12), we have made the assumption that the surface is isothermal. Otherwise the model is general in the way that it allows slippage. We have further assumed that \( \text{Ca}^{2+} \) transport across the membrane is not an activated process, but rather that its contribution to the total flux is simply linear in its corresponding driving force, with a transport coefficient independent from the activation energy. Therefore, the only nonlinearity comes from the kinetics of the chemical reaction. This assumption may be valid for the concentrations of calcium that are physiologically relevant.

In order to integrate the flux on the mesoscopic level, we introduced one more assumption, namely that of a tight pump. This assumption is thus a
requirement for Eq. (21). The assumption may hold for an isothermal surface, but there are many conditions for which this is not true (Berman, 2001).

The theory we have presented could be useful in describing other types of active transport in biological systems, as the ones taking place in molecular motors for which translational (Bedeaux et al., 2004) or rotational motion may be induced at the expense of the energy provided by the chemical reaction (Sun et al., 2004). It can also be used for a more detailed look at pump slippage, believed to occur for high driving forces, and indicated by a variable stoichiometry (Zoratti et al., 1986; Walz, 1990). One advantage of the present formulation, not contained in kinetic approaches, is that the energy dissipated as heat (Berman; 2001) can be directly calculated from $T \sigma$. A large value of $T \sigma$ suggests that the surface may be nonisothermal, and $T \sigma$ has a meaning also for nonlinear flux-force relations.

4 Conclusions

The main aim of this paper has been to show that active transport processes can be described by means of mesoscopic nonequilibrium thermodynamics (Reguera and Rubi, 2001; Vilar and Rubi, 2001). We have been able to describe the coupling between the chemical reaction and transport, which is mandatory in a complete thermodynamic description of this type of system, and which was previously not obtained outside the range of linear flux-force-relations (Berman, 2001).

We have shown that when local equilibrium is formulated at shorter time scales, the overall diffusion flux is nonlinear in the macroscopic driving force. A new way to plot experimental data has thus been given, applicable for large values of $\Delta G$. The formulation we propose is of a mesoscopic nature and
may incorporate the presence of fluctuations in the dynamics consistent with a Fokker-Planck description (Vilar and Rubi, 2001). We have somewhat arbitrarily taken active transport of Ca\(^{2+}\) as an example, but the scheme proposed could be transferable to many kinds of transport processes that obtain their energy from chemical reactions such as molecular motors. This shows that nonequilibrium thermodynamic methods are not restricted to the linear response domain (Jülicher et al., 1997) but can be used in a broader context to perform a description of the dynamics also in the nonlinear domain. We hope in this way to provide a general scenario in which nonlinear transport processes occurring in biological system can be dealt with.

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