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Biothermokinetics of energy conversion

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Biothermokinetics is a collective notation indicating that the processes in a biological system are analyzed in terms of non-equilibrium thermodynamics and kinetics. Thermodynamics requires that an experimental system be first translated into a thermodynamic system [1], which forces us to define the elements present in the experimental system. Then we have to decide which of them are essential for the actual experiment and which, though present, are unimportant because they do not really contribute to the experiment. A rather complex experimental system may thus be translated into a relatively simple thermodynamic system consisting of two compartments separated by a membrane (see Fig. 1).

Thermodynamics serves to define what is the flow, J_p, of the pth process discernible or anticipated to occur in the system, and what is its conjugate force, X_p [1]. The flows are defined by the time derivatives of the mole numbers of the species involved which, in the case of chemical reactions, are divided by the stoichiometric coefficients [1]. The force of the jth reaction in compartment k is its affinity,

$$\mathscr{A}_{j,k} = -\sum_r \nu_{\mathrm{Rr}(j,k)} \tilde{\mu}_{\mathrm{Rr}(j,k)} = RT \ln\left\{ K_{cj,k} \Big/ \left[\prod_r c_{\mathrm{Rr}(j,k)}^{\nu_{\mathrm{Rr}(j,k)}} \right] \right\} \qquad (1)$$

where reactant $\mathrm{R}_{\mathrm{r}(j,k)}$ ($r = 1, 2, \ldots$) is the general notation for substrates and products whose stoichiometric coefficients $\nu_{\mathrm{Rr}(j,k)}$ are counted negative and positive, respectively. R is the gas constant and T the absolute temperature. $K_{cj,k}$ is the equilibrium constant of the reaction, while $\tilde{\mu}$ and $c_{\mathrm{Rr}(j,k)}$ denote the electrochemical potential and the concentration of reactant $\mathrm{Rr}(j,k)$, respectively. When dealing with transport of species it is necessary to arbitrarily choose a positive direction which has to be the same for all transport processes. With the direction from compartment 1 to compartment 2 chosen as positive (cf. Fig. 1), the thermodynamic force for the transport of the ith species is

$$\Delta\tilde{\mu}_i = \tilde{\mu}_{i,1} - \tilde{\mu}_{i,2} = RT \ln\{c_{i,1}/c_{i,2}\} + z_i F(\phi_1 - \phi_2) \qquad (2)$$

Here F and ϕ_k are the Faraday constant and the electrical potential of compartment k, respectively, while z_i is the valence of the species.

The flows and forces of the processes in a system are subject to the fundamental equation of non-equilibrium thermodynamics called the dissipation function Φ

$$\Phi = T\,\mathrm{d}S/\mathrm{d}t = -\mathrm{d}G/\mathrm{d}t = -\sum_k \sum_i \tilde{\mu}_{i,k}\,\mathrm{d}n_{i,k}/\mathrm{d}t = \sum_p J_p X_p \geq 0 \qquad (3)$$

Here $\tilde{\mu}_{i,k}$ and $n_{i,k}$ denote, respectively, the electrochemical potential and the mole number of the ith species in the kth phase or compartment. The sums have to be taken over all species, all phases and compartments, and all processes. Eqn. 3 indicates that free energy, G, of the system is 'dissipated' and entropy S is

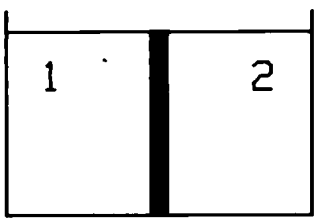

Fig. 1. A thermodynamic system. For the type of experiment performed with an isolated muscle fiber (see text) the elements of the experimental system are translated into a compartment 1 filled with an aqueous phase, which is separated by a membrane from a compartment 2 containing also an aqueous phase. These elements correspond to the myoplasm, the sarcoplasmic reticulum and its lumen, respectively.

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'produced' due to the running processes. $\Phi = 0$ is only attained at the equilibrium state of the system where all processes cease, i.e., when the system is non functional (or dead).

Only the sum of flow-force products $J_p X_p$ in Eqn. 3 has to be positive, individual products can be positive or negative or vanish. In the latter case the pertinent process (but not the whole system) is at equilibrium. $J_p X_p > 0$ means that the process runs spontaneously, i.e., energetically 'downhill' and driven by its own force. $J_p X_p < 0$ means that the process is driven 'uphill' against its own force, in which case it has to be coupled to another and driving process (or processes). *Energy conversion* from the driving to the driven process then takes place which is a *dynamic phenomenon*. Hence the 'input' power (i.e., energy per time) invested by the driving process has to be compared to the 'output' power gained by the driven process, as expressed by the pertinent flow-force products $J_{in} X_{in}$ and $J_{out} X_{out}$, respectively. It is important to note that in general $J_{in} X_{in} + J_{out} X_{out} > 0$. As a consequence not all of the free energy associated with the driving process can be recollected in the driven process but some has to be spent in entropy production. If $J_{in} X_{in} + J_{out} X_{out} = 0$ the coupled processes are at equilibrium where the forces balance each other but $J_{in} = J_{out} = 0$, i.e., nothing moves and no energy is actually converted.

Thermodynamics also takes care of mass and charge balance, which lead to relations describing the effect of the flows on the parameters of the system such as the concentrations of species and membrane potentials. The important quantities involved in these relations are the chemical capacitances for species and electrical capacities of membranes for charges [1]. All these statements are generally valid and independent of the mechanisms by which the processes proceed. This is the great advantage of thermodynamics but, at the same time, its severe limitation. Thermodynamics cannot tell us how the flows are related to the forces. Answers to this question can in general be obtained only on the basis of molecular or kinetic schemes. However, thermodynamics and kinetics are not disjointed approaches to the description of processes. There is a strict connection between the two which is expressed in the relations called detailed balancing and thermokinetic balancing [1,2]. These relations set boundaries with which the parameters of a kinetic scheme have to comply in order not to violate thermodynamic laws.

The analysis of a process in terms of a kinetic scheme yields a relation which indicates how the flow depends on the *concentration* of the species involved in the process. The force of this process, however, comprises the ratio of these concentrations (see Eqns. 1 and 2). Hence the flow-force relation is ambiguous because the same force can arise from a multitude of concentrations. By means of additional conditions which apply to many experimental systems the ambiguity can be removed [1,3,4]. Such a condition is fulfilled for a transport process if the total mole number of the transported species is constant. For a chemical reaction a possible condition requires that the sum of the concentrations of a substrate and a product is constant while the concentrations of the remaining substrates and/or products are (approximately) constant. Under these circumstances the dependence of the flow on the force follows a hyperbolic tangent function which is determined by the two extreme flows attained at very low and very high forces [1].

Another aspect emerging from the kinetic analysis of an enzyme catalyzed transport of a charged species across a membrane relates to the *kinetic inequivalence* of the thermodynamically equivalent chemical and electrical part of the force (see Eqn. 2). In order to transport such a species the enzyme has to bind and to release it on both sides of the membrane, and the transport proceeds at reasonable rates only if association and dissociation rate are commensurable. Otherwise, either binding is insufficient if the dissociation rate is unproportionally large, or the species remains bound for too long time intervals if the dissociation rate is too slow. Since the association rate is determined by the pertinent rate constant and the concentration of the species the condition of commensurable rates is fulfilled only in a limited range of concentrations where the latter are of the orders of the dissociation constant for binding. As a consequence an enzyme designed for the (coupled or uncoupled) transport of a species between two comparable concentrations but across a large potential difference (cf. Eqn. 2) is likely to fail if exposed to a substantial concentration difference with a small potential difference, and vice versa. The enzyme then experiences what is called kinetic control [1] which expresses the general phenomenon that rates may become vanishingly small despite of a large thermodynamic force if concentrations and/or potentials attain values outside the range within which an enzyme can work. Kinetic equivalence, however, exists as demonstrated by the H^+-ATPsynthase of thylakoids [5] and chromatophores [6].

Which level of description of a system is adequate depends on the type of experiments performed and the parameters of the system thereby measured. This becomes evident when considering the system investigated by Klein et al. [7]. These authors carefully measured the time course of the Ca^{2+} concentration in the myoplasm of isolated muscle fibers starting about 1 s after electrical stimulation. The decrease of this concentration was attributed to the activity of the Ca^{2+}-ATPase which pumps Ca^{2+} from the myoplasm into the lumen of the sarcoplasmic reticulum on expense of ATP-hydrolysis. When taking the binding of Ca^{2+}

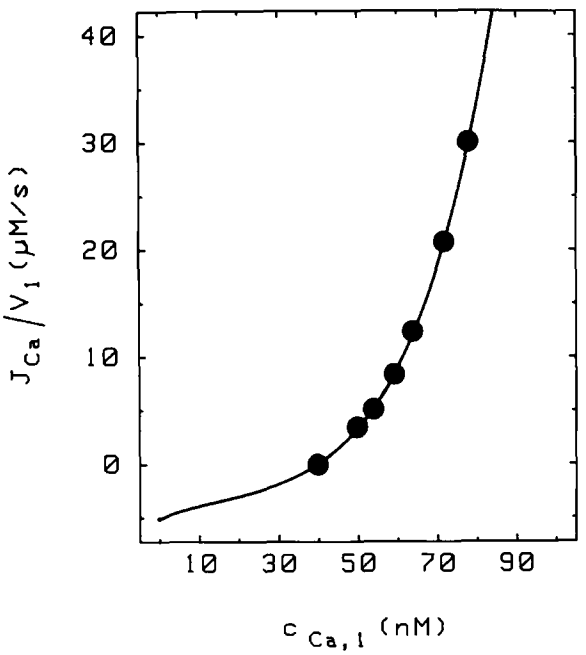

Fig. 2. Dependence of the total flow J_{Ca} on the Ca^{2+} concentration $c_{Ca,1}$ in the myoplasm. Experimental data (•) taken from Fig. 4 of Ref. 7 were fitted to Eqns. 4 and 5 (solid line). Values of parameters: $n_E/V_1 = 200$ μM (V_1 is the volume of the myoplasm), $X_{ph}/(RT) = 22.2$, $A = 450$ s, $B \approx 2 \cdot 10^6$, $K_1^* \approx 0.2$ μM^2, $K_2^* = 0.015$ mM^2, $P_{Ca}/V_1 = 0.0011$ s^{-1}, $V_1/V_2 = 5$ (V_2 is the volume of the lumen of the sarcoplasmic reticulum), $c_{Ca,tot} = n_{Ca,tot}/(V_1 + V_2) = 0.985$ mM. Values of parameters describing binding of Ca^{2+} were taken from Ref. 7.

(primarily to parvalbumin) into account, the dependence of the total flow, J_{Ca}, on the concentration $c_{Ca,1}$ in the myoplasm could be established (Fig. 2).

This experimental system is adequately represented by the thermodynamic system shown in Fig. 1. Moreover, the concentrations of ATP, ADP and phosphate, and hence the force X_{ph} of ATP-hydrolysis (cf. Eqn. 1) can safely be assumed as constant. The electrical potential difference between the compartments is negligible due to high enough permeabilities of other ions. The performance of the Ca^{2+}-ATPase can be assessed by means of a seven-state enzyme cycle [2,8] which yields for the flow of pumped Ca^{2+}

$$J_{Ca,E} = 2n_E \frac{(c_{Ca,1}/c_{Ca,2})^2 \exp\{X_{ph}/(RT)\} - 1}{A[1 + B(c_{Ca,1}/c_{Ca,2})^2 + c_{Ca,1}^2/K_1^* + K_2^*/c_{Ca,2}^2]} \quad (4)$$

where n_E denotes the mole number of enzyme, while A, B, K_1^*, and K_2^* are abbreviations for terms comprising the concentrations of ATP, ADP, and phosphate as well as the transition probabilities between different states of the enzyme cycle. The Ca^{2+} concentration, $c_{Ca,2}$, in the lumen of the sarcoplasmic reticulum can be calculated from the mass balance for these ions, while their leak from this compartment is assessed by

$$J_{Ca,\ell} = P_{Ca}[c_{Ca,1} - c_{Ca,2}] \quad (5)$$

where P_{Ca} is the overall permeability. The total flow is $J_{Ca} = J_{Ca,E} + J_{Ca,\ell}$, and a non-linear fitting procedure for the parameters yielded the result shown as solid line in Fig. 2. This result is at variance with the conclusion arrived at by Klein et al. [7]. Their analysis of the data is based on the simple Michaelis-Menten relation

$$v = v_{max} c_{Ca,1}^n / [K_m + c_{Ca,1}^n] \quad (6)$$

for the pump with the assumption that $K_m \gg c_{Ca,1}^n$. The power n obtained in a fitting procedure turned out to be close to 4 which led the authors to conclude that the functional unit of the Ca^{2+}-ATPase is a dimer. Inspection of Eqn. 4 shows that Eqn. 6 is valid only for $c_{Ca,2} = 0$, which is certainly not the case. In fact, the present analysis reveals that $c_{Ca,2}$ is of the order of millimolar. This example clearly demonstrates that a proper thermokinetic description of a system is essential.

Acknowledgement

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