Nonlinearly Coupled Chemical Reactions

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Abstract. One can argue that the essence of life is in the ability to drive reactions that require energy by coupling them, via enzymes, to other reactions that release energy. Traditionally such reactions and the consequent energy flows are presented as being coupled linearly through a matrix. Below we present an example where the coupling is quadratic. We show how such coupling can arise chemically. When operated near equilibrium the resulting system is able to "ratchet" chemical conversions, i.e. allow an $A \rightarrow B$, but block the $B \rightarrow A$ reaction, even if A and B have the same energy. With quadratic coupling it is furthermore possible to convert the energy of oscillations into a steady metabolic flux.

Nowadays every textbook in molecular biology contains numerous examples of enzymes that catalyse a reaction and use the released energy of that reaction to drive another reaction energetically uphill [1]. The most notable example is the Na,K-ATPase. This protein takes an ATP (adenosine triphosphate, the common currency of energy in a living cell) molecule and hydrolyses it into ADP (adenosine diphosphate). Catalysing this hydrolysis involves a number of conformational changes of the protein and in case of the Na,K-ATPase these conformational changes include ion transport across the membrane against the electrochemical gradient. For every hydrolysed ATP three sodium ions are pumped from the inside to the outside of the cell and two potassium ions are pumped from the outside to the inside. It is in this way that the transmembrane potential, which is essential for the cell, is maintained. Fig. 1 shows a scheme to illustrate in terms of chemical kinetics how ATP hydrolysis can power the conversion of a substrate S into a product P.

In Fig. 1 ATP hydrolysis is depicted along the horizontal coordinate. Going from right to left energy decreases as ATP is hydrolysed. But part of the energy released in ATP hydrolysis is used to bring the enzyme from a state E into a new conformational state E^* with a higher energy. The enzyme can go back to state E by converting one molecule S into P. This will only work if the energy ΔU_{ATP} that is released in ATP hydrolysis is larger than the energy ΔU_{SP} that is required for an $S \rightarrow P$ conversion. This condition translates into $k_{+1}[\mathrm{ATP}]k_{+2}[\mathrm{S}] > k_{-1}[\mathrm{ADP}]k_{-2}[\mathrm{P}]$. In that case a net $S \rightarrow P$ conversion will

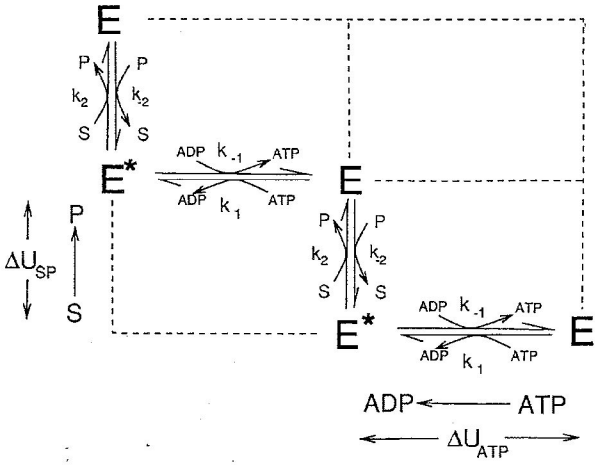

Fig. 1. A kinetic scheme to show how, in a catalytic cycle of an enzyme, the hydrolysis of ATP can be coupled to the conversion of a substrate S into a product P. The enzyme can exist in two forms, E and E*. ATP hydrolysis brings the enzyme from state E into state E*, which has a higher energy. Next this energy is transferred to an S $\rightarrow$ P conversion as the enzyme goes back from E* to E. For the process to occur in the upward right to left direction it is necessary that $\Delta U_{ATP} - \Delta U_{SP} > 0$.

occur. It is obvious that in case of $k_{+1}[\mathrm{ATP}]k_{+2}[\mathrm{S}] < k_{-1}[\mathrm{ADP}]k_{-2}[\mathrm{P}]$ the P $\rightarrow$ S conversion will power the production of ATP out of ADP and the net flow in Fig. 1 will be downward and from left to right. Thermodynamically such processes are described by a linear coupling [2]:

$$\begin{pmatrix} J_1 \\ J_2 \end{pmatrix} = \begin{pmatrix} L_{11} & L_{12} \\ L_{21} & L_{22} \end{pmatrix} \begin{pmatrix} X_1 \\ X_2 \end{pmatrix}. \tag{1}$$

Here J_1 is the ATP $\rightarrow$ ADP flow and J_2 is the S $\rightarrow$ P flow. X_1 represents the ATP/ADP chemical potential and X_1 represents the S/P chemical potential. Alternatively, one can denote with J_1 the energy lost through ATP-hydrolysis and with J_2 the energy gained in the S $\rightarrow$ P conversion.

In this paper we will present a kinetic scheme that is only slightly more complicated than the one in Fig. 1 to show that it is theoretically possible to drive an S $\rightarrow$ P conversion with either an ATP $\rightarrow$ ADP or ADP $\rightarrow$ ATP potential. In these situations the coupling is not linear, but quadratic. Taking $X_2 = 0$ we have at leading order:

$$\begin{aligned} J_1 &= L_1 X_1 \\ J_2 &= L_2 X_1^2. \end{aligned} \tag{2}$$

So the induced flow J_2 is always in the same direction. The Curie principle [2], which states that you cannot induce a directionality in the system that is

not already there from the outset, seems to no longer hold. It doesn't matter in which direction the force X_1 and the flow J_1 are, the induced coupled flow J_2 in Eq. (2) is always in the positive direction if L_2 is positive.

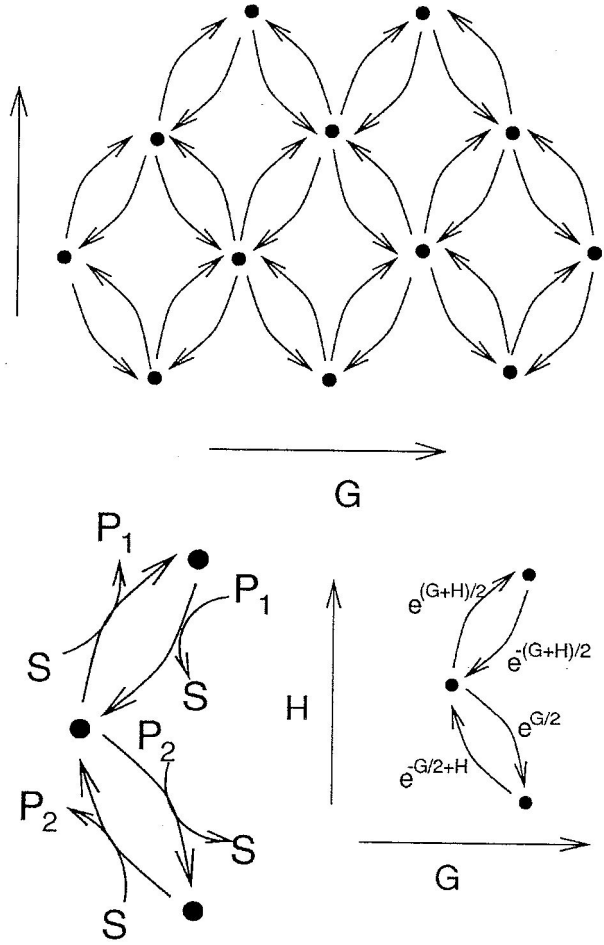

Fig. 2. (2a) A 2D one state Markov model. (2b) Transitions along the 45° line correspond to $S \rightleftharpoons P_1$ conversions. Transitions along the 135° line correspond to $S \rightleftharpoons P_2$ conversions. (2c) Transition rates have been picked such that the force dependence along the 45° line is equally apportioned, while along the 135° line the G dependence is equally apportioned, but the $S \to P_2$ transition depends on H and the $P_2 \to S$ transition does not. In the text it is shown how at $G = 0$ the force H leads to flux in the horizontal direction.

Next consider the 2D setup presented in Fig. 2. It is a one state model, but there are four different ways to get to a neighboring state (Fig. 2a). Fig. 2b

shows that going up on the 45° line the substrate S is converted into product P_1. Going up on the 135° line the substrate S is converted into product P_2. Going down on the 45° and the 135° line represents the conversion into S of P_1 and P_2 respectively. So moving from left to right P_2 is converted into P_1. Moving up represents the conversion of S into equal amounts of P_1 and P_2. Chemical gradients of the $S \rightleftharpoons P_1$, $S \rightleftharpoons P_2$ and $P_1 \rightleftharpoons P_2$ reactions translate into a vertical force H and a horizontal force G . The $[S] = [P_1] = [P_2]$ equilibrium corresponds to $G = H = 0$. The distance between the rows as well as the columns is taken to be unity and each state has a population of one. This implies that the energy difference between two states on the 45° line is $G + H$. The energy difference between two states on the 135° line is $-G + H$. The states of the enzyme are identical so the chemical forces are due solely to concentration differences among S, P_1 and P_2. When we take the energy in units of k_BT we have on the 45° line:

$$\frac{[S]}{[P_1]} = e^{(G+H)}. \tag{3}$$

On the 135° line the conversion of S into P_2 is a unit step against the force G. So we get

$$\frac{[S]}{[P_2]} = e^{(-G+H)}. \tag{4}$$

From these last two equations it is easily seen that $\frac{[P_2]}{[P_1]} = e^{2G}$.

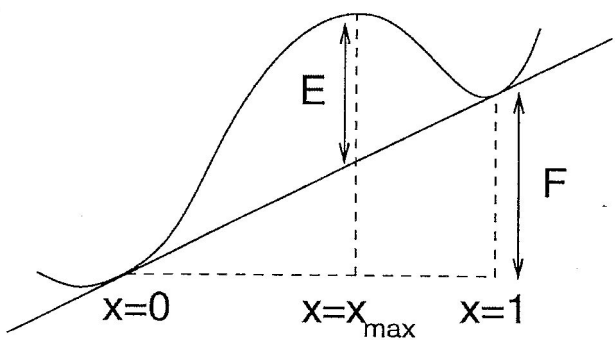

Fig. 3. A reaction coordinate between the states at $x = 0$ and $x = 1$. The transition rates over the barrier depend exponentially on the barrier height. When an external force F is applied the position on the x-coordinate of the maximum determines the apportionment (cf. Fig. 2) of the e^F factor over the two transition rates (see text).

If one of the states in Fig. 1 has a much smaller dwelling time than the other state we effectively look at a one state model. In this one state model S and ATP are like one substrate S′. ADP and P are like one product P′. The

picture is then one of an enzyme that is "hopping" along a line of identical states and dissipates energy as it converts S′ into P′. If the enzyme can turn S′ into either P'_1 or P'_2, we can get the situation described in the previous paragraph.

Fig. 3 shows a reaction coordinate that underlies chemical transitions like in Fig. 2. Going from the state at $x = 0$ to the state at $x = 1$ involves moving over an activation barrier between these states. The line with slope F represents the effect of a net force F along the x-direction. Were it not for the macroscopic force F, the states at $x = 0$ and $x = 1$ would have the same energy level. Transition rates depend exponentially on barrier height, so we get $k_{0\to1} = \alpha \exp[-E - x_{max}F] = \tilde{\alpha} \exp(-x_{max}F)$ and $k_{1\to0} = \alpha \exp[-E + (1 - x_{max})F] = \tilde{\alpha} \exp[(1 - x_{max})F]$. Obviously this approach is only valid when F is of the order of E or smaller. For $x_{max} = 1/2$ the force F is equally apportioned over the two states. It is only when $x_{max} \neq 1/2$ that an anisotropy is introduced in the system that results in unequal apportionment.

In the example in Fig. 2c there is equal apportionment in the 45° direction and unequal apportionment in the 135° direction. For convenience the two activation energies have been picked such that the prefactors before the exponentials are all equal to one. Next we will show that for this choice the vertical force H can bring about flow in the horizontal direction and that the flow force relations, at leading order, follow the pattern of the Eq. (2). We have in a steady state for the net flows in the horizontal and vertical direction respectively:

$$\begin{aligned} J_x &= 2 \sinh\left[\frac{1}{2}(G + H)\right] + 2e^{\frac{1}{2}H} \sinh\left[\frac{1}{2}(G - H)\right] \\ J_y &= 2 \sinh\left[\frac{1}{2}(G + H)\right] - 2e^{\frac{1}{2}H} \sinh\left[\frac{1}{2}(G - H)\right] \end{aligned} \tag{5}$$

If we take $G = 0$ and $H \neq 0$, then we get for the horizontal flow induced by the vertical force: $J_x = 2(\sinh \frac{H}{2})(1 - \exp \frac{H}{2})$, which at leading order behaves quadratically like $-\frac{1}{2}H^2$. For the vertical flow we get $J_y = 2 \sinh \frac{H}{2} + (e^H - 1)$, which at leading order behaves linearly like $2H$. So we have the structure of Eq. (2) with X_1 denoting H and J_1 and J_2 denoting the vertical and horizontal flows respectively.

So we take the system of Fig. 2 at $G = 0$. This implies $[P_1] = [P_2]$ and $[S]/[P_1] = [S]/[P_2] = e^H$. Chemically this system has some very curious properties and possibilities. A positive substrate - product potential ($H > 0$) leads to horizontal right to left flux, i.e. production of P_2 from S. But in case of a negative substrate - product potential ($H < 0$) we still get a net right to left horizontal flux and then it is P_1 that is consumed in order to produce S. So with this system we can produce a desired product P_2 when [S] is large and not consuming any P_2 when [S] becomes small. It is thus possible to produce an essential metabolite P_2 and not deplete the P_2 reserves if the supply of S ceases. Effectively we have created a chemical ratchet here: $S \to P_2$ occurs

when the chemical force is in that direction, but when the chemical force reverses the $P_2 \rightarrow S$ pathway is blocked. Another interesting feature is that with this system we can get a steady production of P_2 from P_1 by oscillating or fluctuating [S] around the $H = 0$ equilibrium. So we have a chemical AC to DC conversion. An oscillation or fluctuation of [S] can be brought about by an externally applied oscillating electromagnetic field. But there are also many autonomously generated oscillations in living cells (calcium oscillations [1] or glycolytic oscillations in yeast [3, 4]) and it is conceivable that these oscillations aid in the production of certain metabolites via the above mechanism.

The processes pointed out in the last paragraph only work when the involved reactions are close to equilibrium. Most *in vivo* biochemical reactions, however, are maintained far from equilibrium. The [ATP]/[ADP] ratio, for instance, is maintained very far above the Boltzmann equilibrium in a living cell. But there are examples of balances that stay close to a zero potential. For chloride and potassium, for instance, the electrochemical potential difference between intracellular and extracellular free ions is close to zero. However, locally there are are strong fluctuations of the electrical potential (about 10 mV [5, 6]), which are mostly due to the opening and closing of sodium channels. These fluctuations can cause the membrane passage energy of chloride and potassium to fluctuate strongly and the right environment may be created for the above described effects to occur.

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