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Article

Metabolic pathways: does the actual Gibbs free-energy change affect the flux rate?

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Abstract

Energy dissipation fulfils an important function in metabolism driving the flux of matter through metabolic pathways. The flux rate is a multivariable function. One of such variables affecting the flux is the actual free energy change. A particular case of metabolic pathway is analysed to illustrate this fact, using only general enzymic and thermodynamic concepts. © 2001 IUBMB. Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

In biochemistry, it is important to know the factors affecting the flux rate through a metabolic pathway, especially, in the context of intermediary metabolism. The flux rate is a multivariable function, so that no single factor necessarily controls the flow [1]. In a recent article published in *Biochemical Education*, Wadell et al. [2] proposed that the rate of glucose consumption through the glycolysis pathway is proportional to the actual Gibbs energy change for the overall process. Although this proposition is well-founded within the framework of irreversible thermodynamics, it has caused some controversy [3,4].

The arguments used against Wadell and co-workers, were based on the assumption "that thermodynamics cannot be used to predict the rate of any reaction, except when the overall rate is zero, due to equilibrium with respect to the reaction". Although this claim is not necessarily incorrect, the conditions under which it is true should be clarified in order to avoid misleading conclusions. Under equilibrium conditions, that is, when only classic thermodynamics is applicable, the claim is correct and the Gibbs free energy indicates whether a reaction can spontaneously proceed in a certain direction, but it provides no information concerning the rate of the reaction. However, metabolism within living cells occurs far from equilibrium. In fact, empirical analyses of different cellular processes have reported a linear relationship between the flow and the driving force of the process ([5] and references therein).

I am aware that non-equilibrium thermodynamics is beyond the scope of most general biochemistry courses. For that reason, I have used only enzymic and general thermodynamic concepts to analyse a particular case of metabolic pathway, where the flow rate can be explicitly expressed as a function of the actual free energy change.

2. Linking thermodynamics and kinetics

Let us consider a chain of unsaturated enzymes, E_1, E_2, …, E_n, carrying out the overall conversions of an external substance, X_1, to another external substance, X_2, via successive intermediary metabolites S_1, S_2, …, S_{n-1}. This is indicated in the following equation

$$X_1 \overset{E_1}{\rightarrow} S_1 \overset{E_2}{\rightarrow} S_2 \overset{E_3}{\rightarrow} \cdots \rightarrow S_{n-1} \overset{E_n}{\rightarrow} X_2. \quad (1)$$

Suitable rate expressions to represent the reversible enzyme-catalysed reactions at each step, are given by the net rate equation

$$v_i = \frac{(V_{\max_i}/K_{M_i})[S_{i-1}] - (V'_{\max_i}/K'_{M_i})[S_i]}{1 + [S_{i-1}]/K_{M_i} + [S_i]/K'_{M_i}}, \quad (2)$$

where S_{i-1} and S_i refer to the substrate and product of enzyme E_i, respectively. $V_{\max_i}$ and K_{M_i} are the maximal velocity and Michaelis constant measured in the forward direction. $V'_{\max_i}$ and K'_{M_i} are the maximal velocity and Michaelis constant for the backward direction.

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We then go on to consider the limiting case, amenable to immediate analysis, in which we assume that all the enzymes concerned are unsaturated, that is $[S] \ll K_M$. Then, the denominator of Eq. (2) can be ignored.

$$v_i = (V_{max_i}/K_{M_i})[S_{i-1}] - (V'_{max_i}/K'_{M_i})[S_i]. \quad (3)$$

The relationship between the equilibrium constant of the ith reaction, K_{eq_i}, and the enzymic parameters of enzyme E_i, is given by Eq. (4), which is known as Haldane's relationship

$$K_{eq_i} = [S_i]_{eq}/[S_{i-1}]_{eq} = (V_{max_i}/K_{M_i})/(V'_{max_i}/K'_{M_i}). \quad (4)$$

Note that Eq. (4) can be easily derived from Eq. (2), simply by assuming equilibrium conditions: the net rate, v_i, must be zero at equilibrium, and $[S_{i-1}]$ and $[S_i]$ become $[S_{i-1}]_{eq}$ and $[S_i]_{eq}$, respectively. Haldane's relationship can also be expressed as

$$(V'_{max_i}/K'_{M_i}) = (V_{max_i}/K_{M_i})1/K_{eq_i}. \quad (5)$$

Using Eq. (5) to substitute the term (V'_{max_i}/K'_{M_i}) in Eq. (3), the net rate equation can be expressed as

$$v_i = V_{max_i}/K_{M_i}\,([S_{i-1}] - [S_i]/K_{eq_i}). \quad (6)$$

At the steady state, all the v_i rates must equal the pathway flux, J. Thus, we can write the set of equations:

$$v_1 = J = V_{max_1}/K_{M_1}\,([X_1] - [S_1]/K_{eq_1}),$$

$$v_2 = J = V_{max_2}/K_{M_2}\,([S_1] - [S_2]/K_{eq_2}),$$

$$v_n = J = V_{max_n}/K_{M_n}\,([S_{n-1}] - [X_2]/K_{eq_n}). \quad (7)$$

The unknown metabolite levels, S_i, can be eliminated from Eq. (7) by dividing each equation by the appropriate V_{max_i}/K_{M_i} terms and also by K_{eq_1} for the second equation, $K_{eq_1}K_{eq_2}$ for the third, and so on. Addition of all the equations in this form eliminates the S_i concentrations and leads to

$$J\left(\frac{K_{M_1}}{V_{max_1}} + \frac{K_{M_2}}{V_{max_2}K_{eq_1}} + \cdots + \frac{K_{M_n}}{V_{max_n}K_{eq_1}K_{eq_2}\cdots K_{eq_{n-1}}}\right) = [X_1] - ([X_2]/K_{eq_1}K_{eq_2}\cdots K_{eq_n}). \quad (8)$$

Hence, the required solution for J is, as proposed by Kacser and Burns [1]:

$$J = \frac{[X_1] - ([X_2]/K_{eq_1}K_{eq_2}\cdots K_{eq_n})}{[K_{M_1}/V_{max_1} + K_{M_2}/(V_{max_2}K_{eq_1}) + \cdots + K_{M_n}/(V_{max_n}K_{eq_1}K_{eq_2}\cdots K_{eq_{n-1}})]}. \quad (9)$$

An inspection of Eq. (9) indicates that the flux is a function of all the pathway's enzymic parameters (V_{max_i} and K_{M_i}) as well as of its thermodynamic parameters K_{eq_i}. To simplify the formulation, let the symbol α^{-1} represent the denominator of Eq. (9). Then

$$J = \alpha\,([X_1] - [X_2]/K_{eq}), \quad (10)$$

where $K_{eq} = [X_2]_{eq}/[X_1]_{eq} = K_{eq_1}K_{eq_2}\cdots K_{eq_n}$.

Eq. (10) clearly states that the pathway flux is proportional to a difference term that accounts for the degree to which the pathway is out of equilibrium, the main point of this paper. Since $\Delta G = \Delta G^{0'} + RT\ln([X_2]/[X_1])$ and $\Delta G^{o'} = RT\ln K_{eq}$, the flux rate is also a function of the thermodynamic disequilibrium:

$$\Delta G = RT\ln([X_2]/[X_1]/K_{eq}). \quad (11)$$

Using Eq. (11) to replace the ratio $[X_2]/K_{eq}$ in Eq. (10), we obtain the required relationship between flux rate and ΔG as

$$J = \alpha[X_1](1 - e^{\Delta G/RT}). \quad (12)$$

3. Conclusions

There is indeed a relationship between the flux rate and the free-energy change under certain non-equilibrium conditions. Although this fact is widely accepted throughout the specialised literature, it confuses most students, even after they have completed biochemistry courses. To correct this situation, students and teachers alike must be aware that a sound knowledge of the laws of thermodynamics will facilitate the understanding of cellular metabolism. This includes energy transformation processes which have two sides: energy conservation and energy dissipation, both are equally necessary to maintain metabolism.

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