FEBS Letters 587 (2013) 2891–2894

FEBS Letters

journal homepage: www.FEBSLetters.org

The average enzyme principle

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ARTICLE INFO

Article history:
Received 14 June 2013
Accepted 16 July 2013
Available online 23 July 2013

Edited by Athel Cornish-Bowden

Keywords:
Enzyme regulation
Michaelis–Menten
Metabolic network
Enzyme kinetics
Systems biology

ABSTRACT

The Michaelis–Menten equation for an irreversible enzymatic reaction depends linearly on the enzyme concentration. Even if the enzyme concentration changes in time, this linearity implies that the amount of substrate depleted during a given time interval depends only on the average enzyme concentration. Here, we use a time re-scaling approach to generalize this result to a broad category of multi-reaction systems, whose constituent enzymes have the same dependence on time, *e.g.* they belong to the same regulon. This "average enzyme principle" provides a natural methodology for jointly studying metabolism and its regulation.

1. Introduction

Biochemical reactions are embedded in complex metabolic networks. The dynamic features of these networks, such as the time-dependent regulation of proteins, underlie the capacity of the cell to cope with variable environmental conditions [1–3], to allocate resources efficiently[4], and to achieve complex adaptive strategies for survival [5]. Several classical studies have addressed the effect of changing enzyme levels on substrate kinetics [6–10]. Most recent efforts in systems biology, however, tend to focus either on understanding genome-scale metabolic networks under steady-state conditions [11,12], or on understanding transcriptional regulation irrespective of the underlying metabolism [13]. While this separation of biological complexity into metabolic and regulatory layers has given rise to extremely insightful techniques and analyses, the search for new approaches to merge these two layers in a unified manner is recognized as a fundamental, albeit difficult challenge.

Here, we revisit the classical Michaelis–Menten equation under the assumption of a time-dependent enzyme concentration. We first summarize our results from an earlier study [14], showing that, for an isolated reaction obeying Michaelis–Menten kinetics, the final substrate concentration depends not on the time-dependent details of enzyme concentration, but simply on its average. Indeed, any two enzyme profiles with the same average concentration and identical kinetic parameters yield the same final substrate concentration.

Next, we show how this "average enzyme principle" can be extended to more complex metabolic networks. This is best illustrated through the example of a linear metabolic pathway, which can be simulated numerically, and which helps formulate the problem in a way that is amenable to analytical proof. We prove that if all enzymes in the pathway follow the same dynamics, the final concentration of metabolites in the pathway depends only on the average enzyme level during the elapsed time. Importantly, the invariance to enzyme trajectories remains valid for a much broader category of metabolic networks whose constituent enzymes follow synchronous time-courses.

2. Results

Consider the following problem: a substrate S is degraded by an enzyme E. How much substrate is left after a given time ΔT? Under appropriate conditions and assumptions [15,16] the answer can be computed using the hundred-year-old Michaelis–Menten equation

$$\frac{dS}{dt} = \frac{-k_{cat} ES}{K_M + S} \quad (1)$$

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0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.07.032

As in most descriptions of enzymatic catalysis, the total amount of enzyme is assumed to be constant. Hence, an expression for the final concentration of substrate can classically be obtained by integrating:

$$\int_{S_0}^{S_f} \frac{(K_M + S)dS}{S} = \int_0^{\Delta T} -k_{cat} E dt \tag{2}$$

These integrals can be evaluated explicitly, yielding the result

$$K_m \ln\frac{S_f}{S_0} + S_f - S_0 = -k_{cat} E \Delta T \tag{3}$$

Thus, we can express the final concentration of the substrate as a function of its initial concentration, the time interval of interest, and the relevant enzymatic parameters. Note that while Eq. (3) constitutes an implicit function of S_f, an explicit expression can be obtained using the Lambert W function [17].

What if we pose the same problem, but assume that the enzyme concentration is instead a time-dependent quantity $E(t)$? Can we still easily compute the amount of substrate left after a given time ΔT? Behind the apparently open-ended challenge of addressing this question lies a simple answer, potentially rich of biological implications.

Under appropriate conditions [14], and in line with studies of hybrid metabolic-genetic systems [18,19], we can write the Michaelis–Menten equation in which the enzyme concentration is a time-dependent variable:

$$\frac{dS}{dt} = \frac{-k_{cat} E(t) S}{K_M + S} \tag{4}$$

Eq. (4) is still separable; in other words, enzyme concentration can be still isolated from the terms corresponding to substrate, yielding upon integration:

$$K_m \ln\frac{S_f}{S_0} + S_f - S_0 = -k_{cat} \int_0^{\Delta T} E(t) dt = -k_{cat} \Delta T E_{avg} \tag{5}$$

where E_{avg} is the average enzyme level during the time interval [0, ΔT]. Eq. (5) states that the final concentration of substrate depends only on the average concentration of enzyme in a time interval, rather than its kinetic details.

The potential implications of this simple result may best be seen by illustrating it in the following alternative way: if two enzyme time-course profiles E_1 and E_2 exhibit the same average enzyme concentration at time ΔT (*i.e.* $\int_0^{\Delta T} E_1(t)dt = \int_0^{\Delta T} E_2(t)dt$), the metabolic effects of these two enzymes are indistinguishable from each other at time ΔT. In other words, in order to degrade a certain amount of substrate S, any one of an infinite number of equivalent enzyme trajectories with identical averages may be used. While the validity of this statement should be apparent from the analytical nature of this result, a simple experimental assay reported earlier should relieve any further doubts [14]. As we explore in detail in [14], one consequence of this multiplicity of solutions is the possibility of identifying a trajectory that minimizes the cost a cell incurs when sequestering cellular resources for the production/degradation of enzyme.

Here we wish to show that this average enzyme principle holds not only for a single enzyme-catalyzed reaction, but also for a broad category of multi-step metabolic pathways, under certain assumptions on the time-dependence of the enzymes in the pathway. It is not obvious that the principle should extend: because the dynamics of each metabolite will depend on other metabolites in the network, it is not possible to simply integrate over time, as was done in the single-reaction case above.

The simplest case, which we examine in detail, is the one of a linear metabolic pathway of n metabolites in which the product of one reaction is the substrate for the next one. The dynamics of such a system is described by the following differential equations:

$$\frac{dS_1}{dt} = -E_1(t)\left(\frac{k_1 S_1}{K_{M,1} + S_1}\right) \tag{6}$$

$$\frac{dS_i}{dt} = E_{i-1}(t)\left(\frac{k_{i-1} S_{i-1}}{K_{M,i-1} + S_{i-1}}\right) - E_i(t)\left(\frac{k_i S_i}{K_{M,i} + S_i}\right),\ i = 2 \ldots n \tag{7}$$

where S_i corresponds to the ith substrate in the pathway, k_i is the rate constant of the ith reaction, and $K_{M,i}$ is the half-saturation constant of the ith reaction. We assume that all enzymes vary synchronously in time ($c_1E_1(t) = c_2E_2(t) = \ldots = c_nE_n(t)$, where each c_i is a positive constant). This assumption is reasonable for metabolic pathways (such as many linear ones) whose enzymes are co-regulated and exhibit similar time-dependent expression profiles [20,21]. A numerical solution of these equations shows that, for different enzyme time-courses with identical averages at time ΔT, metabolite concentrations at time ΔT are precisely identical (Fig. 1), exactly as in the case of a single enzyme.

The numerical result above is just a special case of a much more general analytical result, which we derive next. We start by writing the differential equations for a metabolic network comprised of n metabolites, denoted by the vector $(S_1, S_2, S_3, \ldots S_n) = \mathbf{S}$. We assume that each reaction in the network can be effectively described by irreversible Michaelis–Menten equations (as in Eqs. (6) and (7)). We further assume that all enzymes in the network have the same dependence on time (again, that $c_1E_1(t) = c_2E_2(t) = \ldots c_nE_n(t)$).

Then, using vector notation, we can write the differential equations for such as a system as

$$\frac{d\boldsymbol{S}}{dt} = E(t)\boldsymbol{f}(\boldsymbol{S}) \tag{8}$$

where the bold lettering denotes a vector. Note that $\boldsymbol{f}$ is a vector-valued function, which takes as input the vector of substrate concentrations, and outputs a vector of the same dimension. Because each individual c_i (the parameter corresponding to the scaling of each enzyme) is a fixed constant, it can be directly absorbed into the expression for $\boldsymbol{f}$. Now, let us consider the dynamics of the system above for two different enzyme time-courses, $E_A(t)$ and $E_B(t)$, with the same average concentration at time ΔT (*i.e.* $\int_0^{\Delta T} E_B(t)dt = \int_0^{\Delta T} E_A(t)dt$). We will show that the final concentration of each metabolite S_i at time ΔT is identical for the two enzyme time courses. To see this, let us make a change of variables by letting

$$\tau_A = \int_0^t E_A(x)dx \tag{9}$$

$$\tau_B = \int_0^t E_B(x)dx \tag{10}$$

Note that at $t = \Delta T$, τ_A and τ_B are equal (by our assumption of equivalent average enzyme levels). Then, we can use the chain rule and the Fundamental Theorem of Calculus to rewrite the system in Eqs. (9) and (10) as

$$\frac{d\boldsymbol{S}}{d\tau_A} = \frac{dt}{d\tau_A}\frac{d\boldsymbol{S}}{dt} = \frac{1}{E_A(t)} E_A(t)\boldsymbol{f}(\boldsymbol{s}) = \boldsymbol{f}(\boldsymbol{S}) \tag{11}$$

$$\frac{d\boldsymbol{S}}{d\tau_B} = \frac{dt}{d\tau_B}\frac{d\boldsymbol{S}}{dt} = \frac{1}{E_B(t)} E_B(t)\boldsymbol{f}(\boldsymbol{s}) = \boldsymbol{f}(\boldsymbol{S}) \tag{12}$$

Eqs. (11) and (12) can be interpreted as a re-scaling of the time variable. Then, since the form of Eqs. (11) and (12) is identical, they have identical solutions, except in differently scaled time variables. However, at time ΔT, $\tau_A = \int_0^{\Delta T} E_A(x)dx = \int_0^{\Delta T} E_B(x)dx = \tau_B$ and the solutions (*i.e.* the concentrations of substrate) are identical. Notably, an identical rescaling argument holds for any metabolic network that can be written in the form of Eq. (8). For any such metabolic network, including those with higher-order stoichiometry, branching, cooperativity, reversibility, and allosteric regulation, an