FEBS Letters 587 (2013) 2744–2752

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Exploration of the spontaneous fluctuating activity of single enzyme molecules

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ARTICLE INFO

Article history:
Received 7 June 2013
Revised 1 July 2013
Accepted 2 July 2013
Available online 12 July 2013

Edited by Athel Cornish-Bowden

Keywords:
Enzyme kinetic
Michaelis–Menten
Stochastic process
Mathematical Modeling
Single-molecule phenomenon
Biophysics

ABSTRACT

Single enzyme molecules display inevitable, stochastic fluctuations in their catalytic activity. In metabolism, for instance, the stochastic activity of individual enzymes is averaged out due to their high copy numbers per single cell. However, many processes inside cells rely on single enzyme activity, such as transcription, replication, translation, and histone modifications. Here we introduce the main theoretical concepts of stochastic single-enzyme activity starting from the Michaelis–Menten enzyme mechanism. Next, we discuss stochasticity of multi-substrate enzymes, of enzymes and receptors with multiple conformational states and finally, how fluctuations in receptor activity arise from fluctuations in signal concentration. This paper aims to introduce the exciting field of single-molecule enzyme kinetics and stochasticity to a wider audience of biochemists and systems biologists.

1. Introduction

Essentially all cellular reactions are catalyzed by enzymes, regardless of whether metabolism, signal transduction, or gene expression is considered. In metabolism, enzymes interconvert metabolites to generate the energy and precursors for macromolecular synthesis. As passive or active transporters, enzymes transport molecules in and out of the cell. Other enzymes covalently modify signaling proteins, metabolic enzymes, and nucleosomes, or transport macromolecules across the cell by free energy-driven translocation along the cytoskeleton. Thus, enzymes can operate as catalysts, either coupled to free energy transduction or not, or as molecular motors. In the latter case free energy dissipation is coupled to, for instance, directed motion of enzymes along actin or to the bacterial flagella in chemotaxis. When environmental conditions change, organisms adjust enzyme concentrations to rewire their molecular networks to better meet current demands. Natural selection acts on beneficial, genetic mutations that alter the concentrations or kinetic properties of enzymes. In other words, enzymes lie at the basis of cell functioning and are central to any quantitative understanding in cell biology. Moreover, molecular systems biology studies require knowledge of enzyme properties to be able to assess how the concerted activities of enzymes, organized in networks, give rise to cell function.

The quantitative understanding of enzyme kinetics was pioneered by Michaelis and Menten in 1913 [1], followed by Briggs and Haldane in 1925 [2]. About 50 years later, Cleland unified most of this work in a comprehensive theory of monomeric enzyme kinetics in a series of seminal papers [3–5]. The work about feedback-regulation of metabolic enzymes [6,7] initiated studies on the kinetics of oligomeric enzymes, which were later followed by many studies on hemoglobin (reviewed in [8]). Oligomeric enzymes are composed out of several, interacting subunits that can display cooperativity and may be under allosteric control [9]. This lead to the development of allosteric and cooperative enzyme kinetics: the concerted symmetry model of Monod, Wyman, and Changeux [10], the sequential model by Koshland, Nemethy, and Filmer [11], and more recently, the reversible Hill equation by Hofmeyr and Cornish-Bowden [12]. All these theories of enzymology are macroscopic theories of enzyme kinetics, considering the average properties of ensembles of enzymes.

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0014-5793/$36.00 © 2013 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.febslet.2013.07.005

Macroscopic theories in enzymology about monomeric and multimeric enzyme kinetics do not refer to the inherently stochastic aspects of the activity of single enzyme molecules. Enzyme ensembles concern thousands to millions of proteins that each function independently. Much can be learned about enzymes from those studies. For example, in metabolism the simultaneous activity of a huge number of enzymes matters most often. Consequently, the ensemble perspective gives the relevant picture. However, from the study of single enzyme molecules valuable additional insight can be gained about enzyme mechanisms [13,14]. Moreover, in many cellular functions it is the activity of single enzymes that matters. Examples are protein transport along the cytoskeleton or mRNA synthesis via transcription, which are inherently single-enzyme activities. In those latter examples, spontaneous, stochastic fluctuations in the activity of a single enzyme can have profound consequences that can be propagated to the phenotypic properties of a cell, on which natural selection acts. Thus in those cases the study of single enzyme molecules is required to understand fundamental aspects of cell biology.

The experimental study of single enzyme copies was pioneered by B. Rotman in 1961 [15], but gained real momentum only three decades later with the advance of single-molecule fluorescence microscopy techniques (reviewed in [14]). Here we limit ourselves to the theoretical aspects of the activity of single enzyme molecules. We use several illustrative examples of the activity of single enzyme molecules to introduce the reader to the main stochastic models (Supplemental material) and concepts of this exciting field in enzymology.

2. Results

2.1. Fluctuations in the activity of a Michaelis–Menten enzyme

In this section we derive some of the microscopic properties of enzyme kinetics, which concern the stochastic activity of the single enzyme molecules, and relate those to classical enzyme kinetic parameters (i.e. K_M and V_{MAX}). In particular, we discuss the distribution of times between subsequent product formations catalyzed by a single enzyme, the turnover time distribution, and how this relates to the catalysis rate of the enzyme. In the Supplemental material we present two methods how this can be done for arbitrarily complex enzyme mechanisms. When the Michaelis–Menten (MM) mechanism is considered, the mean turnover time turns out to give rise to the well-known MM rate equation.

The simplest model that gives rise to the MM enzyme mechanism considers one enzyme (E), one substrate (S), one enzyme-substrate complex (ES), and one product (P). The enzyme-substrate complex can either dissociate into the enzyme and substrate or catalysis can occur giving rise to product and the original enzyme (Fig. 1A).

Deterministic models of enzyme activity fail to capture the discreteness and stochasticity effects that occur when enzyme molecules are present at low copy numbers. If we assume that the cell is a well-stirred compartment, we can ignore molecular positions and diffusion. Then, we can use the chemical master equation (CME) [16] to describe the stochastic activity of a single enzyme (reviewed in [17]). The CME determines the probability that the system is in a specific state at a given (future) time. A single state is a particular combination of the number of E, ES, S and P molecules per cell. Note that from the stochastic perspective the state is a vector of integers (the (copy) number of a specific molecule per cell) and not of real numbers as one would have in a deterministic, macroscopic description when concentrations are considered. The general description of the CME is given in Eq. (S-6) and describes the rate of change in the probability mass to observe the system in a particular state. It is a balance equation for the probability mass of the states. A more specific description of the CME is given by:

$$\frac{dP(\boldsymbol{x},t|\boldsymbol{x_0},t_0)}{dt}=\sum_{r\in R}a_r(\boldsymbol{x}-\boldsymbol{\nu}_r)\cdot P(\boldsymbol{x}-\boldsymbol{\nu}_r,t|\boldsymbol{x_0},t_0)-\sum_{r\in R}a_r(\boldsymbol{x})\cdot P(\boldsymbol{x},t|\boldsymbol{x_0},t_0) \tag{1}$$

Here, $\boldsymbol{x}$ is the state vector which contains the number of molecules (denoted later by N) of each species in time, $P(\boldsymbol{x},t|\boldsymbol{x_0},t_0)$ is the probability to observe the system in state $\boldsymbol{x}$ at time t given the initial state $\boldsymbol{x_0}$ at time t_0, $\boldsymbol{\nu_r}$ is the state-change vector of reaction r (vector with stoichiometric coefficients) and $a_r(\boldsymbol{x})$ is the propensity function of reaction r, i.e., the probability per unit time that reaction r fires given that the system is currently in state $\boldsymbol{x}$.

For the simple model shown in Fig. 1A, the propensity functions are given by (very similar to mass-action kinetics),

$$\begin{aligned} a_1(\boldsymbol{x}) &= k_1^+\cdot N_E\cdot N_S \\ a_2(\boldsymbol{x}) &= k_1^-\cdot N_{ES} \\ a_3(\boldsymbol{x}) &= k_c\cdot N_{ES} \end{aligned} \tag{2}$$

where N_E, N_S, and N_{ES} are the copy numbers – number of molecules – of E, S, and ES, respectively. Note that rate constants of unimolecular reactions are independent of the system volume (V), whereas rate constants of bimolecular reactions (e.g. k_1^+) are inversely proportional to V. This is because the search time for two reactant molecules depends on volume [18].

If we assume that the number of substrate molecules, N_S, is held constant the state-change vectors for the simple enzyme kinetics model are given by,

$$\begin{aligned} \boldsymbol{\nu_1} &= (-1,1,0) \\ \boldsymbol{\nu_2} &= (1,-1,0) \\ \boldsymbol{\nu_3} &= (1,-1,1) \end{aligned} \tag{3}$$

of which the entries correspond to the stoichiometric coefficients of the molecules in the same order as they occur in the state vector $\boldsymbol{x}$:

$$\boldsymbol{x}=(N_E,N_{ES},N_P) \tag{4}$$

Thus, reaction (2) produces one molecule E and consumes one molecule ES (S is considered fixed and therefore not specified).

We consider only a single enzyme copy, which implies that N_{ES} is 0 if N_E is 1 and vice versa (Fig. 1B and 1C). Production events of P will occur at irregular intervals when a single enzyme is modeled with the CME. An example is shown in Fig. 1D where three different trajectories of a stochastic simulation are shown. Each simulation started from the same initial conditions and with the same kinetic parameters. These trajectories fluctuate around the analytical solution for a large ensemble of the same enzyme molecules. This ensemble is described by the set of ordinary differential equations that characterize the deterministic, macroscopic dynamics of an ensemble of independent enzyme molecules.

We are interested in the time to make one product molecule, i.e., to increase N_P by 1. Directly after the previous product molecule has been synthesized the enzyme is in the unbound state. Therefore, we consider an initial condition with $N_E(0)=1$, $N_{ES}(0)=0$ and use the CME to determine how the probability $P(N_E, N_{ES}, N_P, t)$ changes over time from this initial condition. Note that the number of molecules of P does not matter in this case, as it does not influence any of the elementary enzyme rates.

Substituting Eqs. (2)–(4) into Eq. (1) gives the CME for our simple enzyme kinetics model:

$$\begin{aligned} \frac{dP(1,0,N_P,t|x_0,t_0)}{dt} &= k_1^-\cdot P(0,1,N_P,t|x_0,t_0)+k_c\cdot P(0,1,N_P \\ &\quad -1,t|x_0,t_0)-k_1^+\cdot N_S\cdot P(1,0,N_P,t|x_0,t_0) \\ &\quad \times\frac{dP(0,1,N_P,t|x_0,t_0)}{dt} \\ &= k_1^+\cdot N_S\cdot P(1,0,N_P,t|x_0,t_0)-\left(k_1^-+k_c\right) \\ &\quad \cdot P(0,1,N_P,t|x_0,t_0) \end{aligned} \tag{5}$$