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Transition from molecular chaos to coherent spiking of enzymic reactions in small spatial volumes

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Abstract

Theoretical study of an irreversible enzymic reaction with allosteric product activation reveals that, in small spatial volumes, it can undergo a transition to coherent spiking regime characterized by the presence of strong correlations between reaction events and the states of individual enzyme molecules.

Keywords: Enzymes; Molecular chaos; Coherent spiking; Allosteric activation

1. Introduction

Recently we have suggested that biochemical reactions in small spatial volumes, such as cellular compartments, have special properties and can proceed in a regime characterized by strong correlations between individual reaction events [1,2]. Here we present the results of a theoretical study of an irreversible product-activated reaction in a small system including only 100 allosteric enzyme molecules. When the strength of allosteric regulation is increased, this system goes from the state of molecular chaos to a highly ordered periodic spiking regime. The spikes are produced by synchronous firing of a few groups of enzyme molecules.

Under spiking conditions, each enzyme group collectively behaves as an excitable element (i.e. similar to a neuron) and the groups form a closed functional loop along which the excitation can indefinitely circulate. The transition from molecular chaos to coherent spiking could be understood as emergence of a functional structure in the originally uniform population. To demonstrate such a transition, we consider a simple example of an enzymic reaction. The microscopic process of this reaction consists of individual catalytic cycles.

A cycle begins when an enzyme molecule binds to a substrate molecule. The substrate–enzyme complex goes through a number of intermediate steps leading to the release of a product molecule and the return of the enzyme to its ground state. The first stage of the cycle is externally regulated: the enzyme affinity towards the substrate is increased when a regulatory molecule is bound. We suppose that the regulatory role is played allosterically by product

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molecules of the same enzymic reaction and hence the reaction is effectively autocatalytic.

A product molecule moves randomly through the volume and meets other enzymes. When it hits an atomic target group in an enzyme molecule in the ground state, binding between the enzyme and the product molecule occurs. The strength of allosteric regulation is characterized by the factor by which the binding of a regulatory product molecule increases the probability of binding a substrate. We assume that the substrate concentration is maintained constant by an external source. The product decays at a constant probability thus preventing its accumulation in the system; the mean life-time of product molecules is chosen shorter than the duration of a single enzymic cycle.

We suppose that the substrate–enzyme complex exists in the time interval $0 < t < T_0$ of the cycle, and a product molecule is fired at $t = T_0$ from its beginning. During the last recovery interval $T_0 < t < T$ the enzyme returns to its ground state where it can be activated again. The duration $T = 20$ ms of a single cycle of an enzyme molecule was chosen in our study in accordance with the typical maximal turnover numbers of enzymes [3], while T_0 was a varying parameter.

If a volume of linear size L contains only one enzyme target, a small product molecule performing Brownian motion finds it in about a traffic time $t_{\text{traffic}} = L^3/DR$ where D is the diffusion constant of the product and R is the radius of the atomic target group in enzyme molecules [2]. For $L = 0.1$ μm (a typical size of a small cellular compartment), $R = 10$ Å and $D = 10^{-6}$ cm^2 s^{-1}, this estimate yields $t_{\text{traffic}} = 10$ ms. In a volume containing N enzyme targets, a product molecule finds one of them in time $t_{\text{traffic}}/N = L^3/DRN$. If this time is larger than $t_{\text{mix}} = L^2/D$, mixing is so strong that a product molecule meets with equal probability any of the enzymes present inside the volume. This condition, which is assumed to hold below, is satisfied if the total number of enzymes is less than $N = L/R$. When $L = 0.1$ μm and $R = 10$ Å the critical number of enzymes is therefore $N = 100$.

Note that the length $L_c = (Dt_{\text{traffic}}/N)^{1/2}$ characterizes the mean distance passed by a released product molecule until it meets a target and thus initiates the catalytic cycle in the next enzyme molecule. Thus, it represents the correlation length for the considered diffusion-controlled reaction. When L_c is less than the linear size of a volume, the reaction in such a volume can break down into non-correlated spatial domains. As follows from our above estimates, this occurs when the number of enzymes exceeds its critical value $N_c = 100$.

If only one regulatory product molecule is present inside the reaction volume, it can activate a given enzyme molecule with probability $\Delta t/t_{\text{traffic}}$ within a short time interval Δt. The substrate binding rate of an activated enzyme is increased by a factor α which determines the strength of allosteric regulation. The probability of activated substrate binding within time Δt in the presence of one product molecule can therefore be estimated as $w_1 = \alpha(\Delta t/t_{\text{traffic}})w_0$ where w_0 is the probability of non-activated binding. If m product molecules are present in the volume, the total probability of free or activated substrate binding by a given enzyme molecule within time Δt is $p = 1 - (1 - w_0)(1 - w_1)^m$. The product molecules appear as a result of the reaction. They also decay at a constant rate γ and, hence, the probability that k out of m such molecules would die within time Δt is given by the binomial distribution $\pi(k|m) = [m!/k!(m - k)!]g^k(1 - g)^{m-k}$ where $g = \gamma\Delta t$.

The process of an enzymic reaction involving small numbers of molecules cannot be described by classical kinetics and a microscopic stochastic theory is needed [4,5]. Below we construct an approximate description of the considered reaction system. The enzyme dynamics is pictured here as a sequence of transitions at discrete moments. As a time step, $\Delta t = 1$ ms is chosen so that the total number of states in a single cycle is $K = T/\Delta t = 20$. A state of an enzyme is characterized by a phase variable taking values $\phi = 0, 1, \ldots K$. The enzyme ground state is $\phi = 0$, a product molecule is fired from the state $\phi = K_0$ where $K_0 = T_0/\Delta t$. The first transition, i.e. binding of a substrate, is probabilistic: an enzyme which is in the state $\phi = 0$ is found at the next moment in the state $\phi = 1$ with probability p. This probability depends on the number of regulatory product molecules m in the system. Subsequent transitions are assumed to be deterministic: at the next time moment the phase ϕ increases by 1 until the set value of K is reached from which it jumps back to

$\phi = 0$. Counting the number of enzymes passed through the state $\phi = K_0$ at a particular moment, we find the number of new product molecules added to the system. Due to the decay, the number of product molecules at the next time moment is further reduced by k with the probability $\pi(k|m)$.

By repeatedly applying this stochastic algorithm, the evolution of the reaction system is reproduced and can be investigated. The numerical analysis has revealed the following properties. When the allosteric regulation is weak, random enzymic activity is found (Fig. 1a) where the phases of catalytic cycles of individual enzymes are uniformly distributed (Fig. 2a). This regime can thus be described as molecular chaos. A maximum at $\phi = 0$ in Fig. 2a corresponds to enzymes in the ground state waiting to bind a substrate.

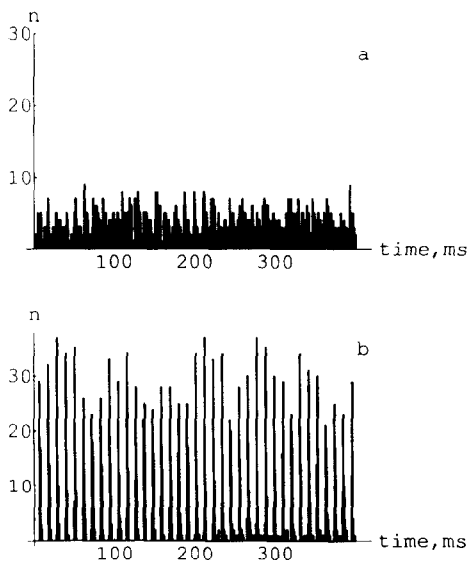

Fig. 1. Transition from molecular chaos to a coherent spiking regime of the enzymic reaction. The generation rate of product molecules, i.e. the number n of enzymes releasing a product molecule per time step, is shown as a function of time for two different allosteric regulation strengths (a) $\alpha = 10$ (molecular chaos) and (b) $\alpha = 70$ (spiking). The volume of linear size $L = 0.1$ μm contains $N = 100$ enzymes. The non-activated substrate binding rate is $w_0 = 0.01$ ms^{-1}, the decay rate and the diffusion constant of the product are $g = 0.3$ ms^{-1} and $D = 10^{-6}$ cm^2 s^{-1}. The duration of a catalytic cycle of a single enzyme molecule is $T = 20$ ms, a product molecule is fired after $T_0 = 11$ ms from its beginning, the discrete time step is $\Delta t = 1$ ms. Random initial conditions are taken.

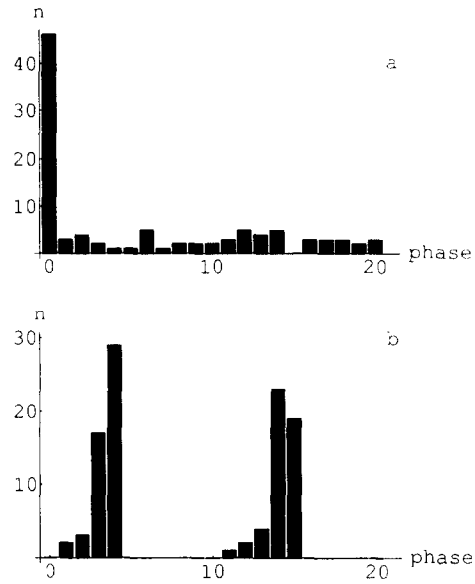

Fig. 2. The histogram of the distribution of $N = 100$ enzymes over their cycle phases ϕ in the regimes of (a) molecular chaos ($\alpha = 10$) and (b) spiking ($\alpha = 70$) at an arbitrarily chosen time moment after a long transient. The same parameters as in Fig. 1.

If the strength α of allosteric regulation is increased, coherent periodic spiking sets on (Fig. 1b). Now the population of enzyme molecules breaks into two synchronous groups with the phase shift about a half of the cycle duration (Fig. 2b). Each group generates pulses of the product which, because of the product's regulatory role, activate binding of the substrate by another group of enzyme molecules and thus trigger their catalytic cycles. Note that the product generation rate displays therefore spiking with a period about twice shorter than the duration of a single cycle. The numbers of enzymes in these two groups and heights of the spikes are fluctuating to a certain extent and enzymes occasionally change from one group to another.

When the phase inside the cycle where firing of a product molecule occurs was varied, the above regime with two synchronous enzyme groups was found for $T_0 = 10$, 11 and 12 ms. Spiking with three groups of enzyme molecules was observed for $T_0 = 7$, 13, 14 and 15 ms at higher strengths of the allosteric regulation. Synchronous spiking of the whole population developed when T_0 was less than 5 ms and at $T_0 = 19$ ms.

We note that these rapid spikes are principally

different from slow macroscopic rate oscillations and do not transform into them as the reaction volume is increased. The rate oscillations are macroscopic. Their period is determined not by the duration of catalytic cycles in individual enzymes, but by reaction rates of the system. Moreover, no kinetic oscillations are possible for the considered reaction scheme $S + E \rightarrow ES \rightarrow E + P$, $P \rightarrow 0$ if the substrate concentration is maintained constant, as assumed in our study.

As seen from our simulations, spiking disappears and the stationary state with random fluctuations is established when the traffic time, controlling the cooperativity parameter w_1, is increased. The traffic time is proportional to the reaction volume. Hence, even while the conditions of our model are still fulfilled, spiking is replaced by molecular chaos for the larger volumes. When the delays due to different spatial locations of the enzymes are taken into account, this would further wash off the coherence. Thus, spikes do not continuously transform into classical rate oscillations. They belong to a different branch, characterized by the presence of microscopic coherence in the reacting system.

The robustness of spiking is remarkable and points out to the general significance of this phenomenon. Though our analysis has been performed only for a simple example, it can easily be extended to more complex reaction networks. Such coherent regimes of chemical reactions in very small spatial volumes may be essential for molecular physiological processes in the living cells, as will be discussed in our subsequent publications. The mathematical analogs of such phenomena are seen in the ensembles of strongly interacting classical non-linear oscillators where synchronously oscillating clusters are known to spontaneously appear [6–9]. Development of new experimental techniques for the investigation of chemical reaction dynamics at a level of single molecules in intact cells, as theoretically analyzed here, is currently in progress [10].

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