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Review

Oscillatory enzyme reactions and Michaelis–Menten kinetics

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ABSTRACT

Oscillations occur in a number of enzymatic systems as a result of feedback regulation. How Michaelis–Menten kinetics influences oscillatory behavior in enzyme systems is investigated in models for oscillations in the activity of phosphofructokinase (PFK) in glycolysis and of cyclin-dependent kinases in the cell cycle. The model for the PFK reaction is based on a product-activated allosteric enzyme reaction coupled to enzymatic degradation of the reaction product. The Michaelian nature of the product decay term markedly influences the period, amplitude and waveform of the oscillations. Likewise, a model for oscillations of Cdc2 kinase in embryonic cell cycles based on Michaelis–Menten phosphorylation–dephosphorylation kinetics shows that the occurrence and amplitude of the oscillations strongly depend on the ultrasensitivity of the enzymatic cascade that controls the activity of the cyclin-dependent kinase.

1. Michaelis–Menten kinetics and biochemical oscillations

The equation proposed by Michaelis and Menten in their classic paper of 1913 [1,2] represented a major advance in the quantitative study of enzyme action [3]. Building on previous work by Victor Henri [4], it provided an explanation for the observation that the enzyme reaction rate reaches a plateau at saturating substrate concentrations, as a result of the formation of an enzyme–substrate complex. Enzyme kinetics operates in a linear regime with respect to the substrate concentration when the latter is sufficiently low, before reaching a maximum value as the substrate concentration increases. The equation derived by Michaelis and Menten allowed them to determine the initial rate of the reaction, as well as the time course of the substrate and product in the course of the reaction [1–3].

The Michaelis–Menten equation is commonly used to determine the kinetic properties of isolated enzymes. It is also used in modeling the dynamics of enzyme systems, among which those that display oscillatory behavior. If oscillatory reactions are not so common among the many biochemical processes catalyzed by enzymes, known examples are responsible for some important cellular rhythms [5]. Two types of oscillatory enzyme reactions can be distinguished. Either the enzymes are at the core of the oscillatory mechanism or they are driven by some external oscillation. To the first class belong the phosphofructokinase (PFK) reaction that underlies glycolytic oscillations in yeast [6], the oscillatory synthesis of cyclic AMP by adenylate cyclase in *Dictyostelium* amoebae [7,8] with associated periodic changes in the kinase PKA [9,10], and (iii) the oscillatory activity of cyclin-dependent kinases (Cdks) that govern progression in the cell cycle [11,12]. To the class of periodically driven enzyme reactions belong a large variety of enzymes expressed under control of the circadian clock [13–16]. Another exogenous enzymatic rhythm is observed for the activity of calmodulin kinase II, which is modulated by Ca^{++} oscillations [17,18].

The systems biology of cellular rhythms was recently reviewed [19,20]. Although new cellular rhythms have been uncovered in recent years, most of which are based on the control of gene transcription, the number of known oscillatory enzyme reactions has not increased significantly since they were reviewed decades ago [21,22], with the notable exception of Cdk oscillations in the cell cycle. The purpose of this article is to examine the link between Michaelis–Menten kinetics and endogenous oscillations in enzymatic reactions. Are Michaelis–Menten enzymes capable of producing biochemical oscillations, and how do they affect oscillations in systems in which the mechanism of periodic behavior relies on allosteric enzyme regulation? While it was initially proposed for isolated enzymes, can the Michaelis–Menten equation be used in describing the dynamics of systems consisting of coupled enzyme reactions? We shall address these questions by focusing on periodic behavior in models centered on phosphofructokinase in glycolysis and on cyclin-dependent kinases in the cell

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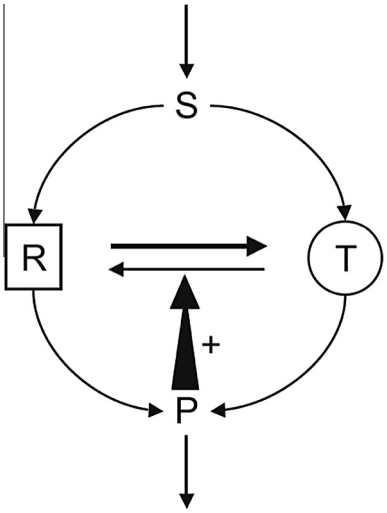

Fig. 1. Scheme of a model for a product-activated allosteric enzyme reaction proposed for glycolytic oscillations [5,30]. The substrate *S*, injected at a constant rate, binds to the two conformations *R* (active) and *T* (less active or inactive) of an allosteric enzyme that transforms it into product *P*. The latter is removed in a sink reaction catalyzed by an enzyme possessing linear or Michaelis–Menten kinetics. The allosteric enzyme consists of multiple subunits (not shown) which undergo a concerted transition between the two conformational states. The product, a positive effector, binds exclusively to the *R* state and thereby elicits the transition from the less active to the most active state of the allosteric enzyme.

cycle, which pertain to the best-known examples of biochemical oscillations that occur in cells as a result of enzymatic regulation.

2. Role of cooperative and Michaelian kinetics in a model for glycolytic oscillations

Glycolytic oscillations, discovered some 50 years ago, still represent the prototypic example of periodic behavior in a metabolic pathway [5,19–22]. They occur with a period of the order of 5–10 min in yeast under conditions where glucose is injected at a constant rate. The glycolytic substrate, supplied at a constant rate, is transformed in a periodic manner by the metabolic pathway. Glycolytic oscillations were first studied in yeast cell populations and in yeast cell extracts [21,23,24]. They were later demonstrated in pancreatic β cells where they are thought to underlie the pulsatile section of insulin [25]. More recently they were demonstrated in individual yeast cells [26,27]. Early on, theoretical models suggested that the mechanism of glycolytic oscillations largely relies on the reaction catalyzed by phosphofructokinase (PFK) [28–31]. The role of PFK is still considered to be at the core of the oscillatory phenomenon [24]. Models proposed for glycolytic oscillations are of two kinds: either they are centered on the PFK reaction [28–31], or they provide a comprehensive description of the glycolytic pathway [32,33]. The former type of model permits a more detailed analysis of the conditions in which oscillations arise, while comprehensive models allow one to establish the phase relationships between a large number of oscillating variables in the glycolytic pathway.

The reason why PFK produces oscillations can be related to its peculiar regulation: the enzyme is activated by one of its reaction products, ADP, via AMP. Moreover, PFK is an allosteric protein, composed of multiple subunits that interact in a cooperative manner. A model for an allosteric enzyme activated by its reaction product has been proposed for glycolytic oscillations [30,31]. It is based on the concerted transition model for allosteric enzymes [34], to which is added the positive feedback exerted by the product. To display oscillations such a system must be open and operate in non-equilibrium conditions. Therefore, in addition to PFK, the model incorporates the substrate input and the consumption of product in a second enzyme reaction, which may be of Michaelian nature (see scheme in Fig. 1). The model is governed by the following kinetic equations [30,31]:

$$\frac{dS}{dt} = v - \sigma\phi$$

$$\frac{dP}{dt} = q\sigma\phi - f(P) \tag{1}$$

In the simple case where the substrate *S* binds exclusively to the most active conformation of the enzyme, the enzyme rate function ϕ is given by:

$$\phi = \frac{S(1+S)^{n-1}(1+P)^n}{L + (1+S)^n(1+P)^n} \tag{2}$$

and the product sink function, or decay term, takes a linear form:

$$f(P) = k_S P \tag{3}$$

Parameters v and σ in Eq. (1) are the normalized substrate injection rate and maximum rate of the enzyme reaction; q is a normalization parameter, and $L>>1$ is the allosteric constant of the enzyme measuring the ratio of inactive (T) to active (R) conformation in the absence of ligand; S and P denote the normalized, dimensionless substrate and product concentrations (see [5,30,31] for further details). The terms $(1 + P)^2$ in function ϕ reflect activation of the enzyme by the reaction product P, which binds exclusively to the most active state of the enzyme. Parameter L markedly influences the degree of cooperativity of the allosteric enzyme.

The study of this model shows that sustained oscillations can occur in a range bounded by two critical values of the constant substrate input provided that the enzyme cooperativity exceeds a threshold value [5,30,31,35]. This result seems to rule out the possibility of sustained oscillations if the enzyme were a monomer obeying Michaelis–Menten kinetics. However, the requirement for cooperativity of the regulated enzyme lessens when the kinetics of the step involving the enzymatic degradation of the reaction product acquires a saturatable rather than linear nature [36]. Then the sink function takes the form of a Michaelis–Menten equation:

$$f(P) = \frac{V_d P}{K_m + P} \tag{4}$$

where K_m denotes the normalized Michaelis constant of the enzyme. At large values of K_m, $f(P)$ tends to a linear form like in Eq. (3), with an apparent first-order rate constant equal to (V_d/K_m). Sustained oscillations in these conditions can already occur when the positively regulated enzyme is monomeric [36]. This shows that the relatively mild non-linearity provided by the Michaelis–Menten equation contributes to the overall non-linearity of the system centered on the product-activated enzyme reaction, and allows the occurrence of sustained oscillations even when the degree of cooperativity of the regulated enzyme diminishes down to unity, though cooperativity favors the occurrence of oscillatory behavior [35,36].

If oscillations can in principle occur in the reaction catalyzed by a monomeric, product-activated enzyme when the sink of the product becomes Michaelian instead of linear, it should be noted that these oscillations then acquire an extremely long period while metabolite concentrations reach extremely large levels [36]. The period and the metabolite levels markedly decrease in the model as the number of protomers of the regulated allosteric enzyme increases from 1 to 4 or more. This observation illustrates the role of enzyme cooperativity in the mechanism of glycolytic oscillations: the positive feedback is amplified by cooperative interactions between the enzyme subunits, so that the explosive synthesis of