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Kinetic behavior of the general modifier mechanism of Botts and Morales with non-equilibrium binding

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ABSTRACT

In this paper, we perform a complete analysis of the kinetic behavior of the general modifier mechanism of Botts and Morales in both equilibrium steady states and non-equilibrium steady states (NESS). Enlightened by the non-equilibrium theory of Markov chains, we introduce the net flux into discussion and acquire an expression of the rate of product formation in NESS, which has clear biophysical significance. Up till now, it is a general belief that being an activator or an inhibitor is an intrinsic property of the modifier. However, we reveal that this traditional point of view is based on the equilibrium assumption. A modifier may no longer be an overall activator or inhibitor when the reaction system is not in equilibrium. Based on the regulation of enzyme activity by the modifier concentration, we classify the kinetic behavior of the modifier into three categories, which are named hyperbolic behavior, bell-shaped behavior, and switching behavior, respectively. We show that the switching phenomenon, in which a modifier may convert between an activator and an inhibitor when the modifier concentration varies, occurs only in NESS. Effects of drugs on the Pgp ATPase activity, where drugs may convert from activators to inhibitors with the increase of the drug concentration, are taken as a typical example to demonstrate the occurrence of the switching phenomenon.

1. Introduction

Modifiers or effectors, ligands that bind to enzymes and thereby alter their catalytic activity, play a crucial role in the study of biochemical problems, e.g., enzymatic catalysis and metabolic pathways (Cornish-Bowden, 2004; Todhunter, 1979; Bertucci, 2001; Malykh et al., 2001; Conway et al., 2003). Moreover, they have wide applications in pharmacology, toxicology, industry and agriculture. Activators and inhibitors are defined as modifiers that strengthen or weaken, respectively, the enzyme activity of the reaction system (Segel, 1993; Fontes et al., 2000). The enzyme activity is generally characterized in terms of the rate of product formation of the enzyme-catalyzed reaction in the steady state.

Most enzyme mechanisms that involve a modifier reversibly acting on Michaelis-type enzymes can be regarded as a particular case of the general modifier mechanism of Botts and Morales, as is depicted in Fig. 1 (Botts and Morales, 1953). Many theoretical biologists have studied the steady state and transient phase kinetics of the general modifier mechanism (Segel, 1993; Fontes et al., 2000; Botts and Morales, 1953; Segel and Martin, 1988; Topham, 1990; Schmitz et al., 1991; Topham and Brocklehurst, 1992; Di Cera et al., 1996; Varó et al., 1999, 2002; Al-Shawi et al., 2003) and its particular cases, in which modifiers act on Michaelis-type enzymes as competitive inhibitors, uncompetitive inhibitors or pure non-competitive inhibitors (Laidler, 1983; Cornish-Bowden, 2004; Moruno-Dávila et al., 2001a,b).

Segel (1993) and Segel and Martin (1988) reported a steady state rate equation that is second degree in both substrate concentration [S] and modifier concentration [R]. They also found several conditions under which the rate equation can be reduced to one that is first degree in [S] and in [R]. Fontes et al., 2000 discussed the behavior of the modifier with the change of substrate concentration [S] under the assumption of rapid equilibrium. Laidler (1983) studied the behavior of the modifier with the change of modifier concentration [R] under some simplifying assumptions. He also suggested definitions of competitive, uncompetitive and noncompetitive activation, by analogy with the generally accepted definitions for inhibition.

The major differences among the contributions of these authors are the set of simplifying assumptions made about the steady state reached by the enzyme-catalyzed reaction system (Varón et al., 2005). However, to date, there is still a lack of a complete analysis about the steady state kinetics of the general modifier mechanism of Botts and Morales without any simplifying assumptions. The major difficulty lies in the fact that the

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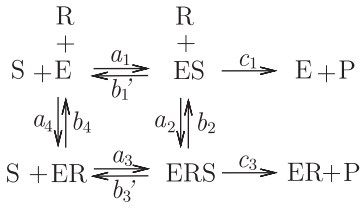

Fig. 1. General modifier mechanism of Botts and Morales. The symbols *E*, *S*, *R* and *P* stand for the enzyme molecule, the substrate molecule, the modifier molecule and the product molecule, respectively, while the composite symbols *ES*, *ER* and *ERS* represent the corresponding complexes. The symbols a_i, b_i' and c_i are rate constants.

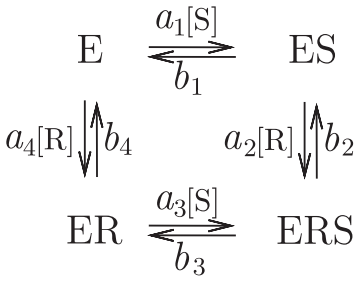

Fig. 2. Catalytic cycle of the general modifier mechanism depicted in Fig. 1. Since both reaction rates b_1' and c_1 (b_3' and c_3) in Fig. 1 contribute to the transition from *ES* (*ERS*) to *E* (*ER*), we use the symbol b_1 (b_3) to represent $b_1'+c_1$ ($b_3'+c_3$).

traditional approaches in solving this problem obscure the essence, which can hardly be realized without the concept of the net flux introduced in this article, of the enzyme system to some extent.

It is illuminating to point out that the modifier- and substrate-binding steps are not dead-end reactions in the enzyme system, and so they are not necessarily in equilibrium (Cornish-Bowden, 2004). We have good reasons to believe that biochemical systems in living cells generally operate in a state far from equilibrium. Whether the cyclic reaction mechanism in Fig. 1 satisfies the equilibrium assumption, i.e. detailed balance, depends on whether the system is closed or open. A closed system will finally approach an equilibrium steady state, whereas an open system, driven by an external source of energy, tend to reach a non-equilibrium steady state (NESS) (Hill, 1977; Wyman, 1975; Qian, 2007, 2008).

In this paper, we remove the equilibrium assumption and provide a general analysis of the kinetic behavior of the modifier in NESS. Enlightened by the circulation theory of Markov chains (Jiang et al., 2004), we introduce the net flux into discussion and acquire an expression of the rate of product formation in NESS, which has clear biophysical significance. The essence of the general modifier kinetics is then revealed to be the competition between the equilibrium and non-equilibrium effects.

So far, it is a general belief that a modifier acts as either an activator or an inhibitor for all its possible concentration values [*R*] when the substrate concentration [*S*] is fixed. However, we find that a modifier cannot be regarded as an overall activator or inhibitor when the reaction system is in NESS. According to our results, a particular modifier may convert from an activator to an inhibitor or vice versa with the change of [*R*]. More specifically, we classify the kinetic behavior of the modifier into three categories, which are named hyperbolic behavior, bell-shaped behavior, and switching behavior, respectively. The latter two kinds of behavior will never occur in an equilibrium steady state.

Incidentally, drugs are typical modifiers in pharmacology. The presence of the drug can activate or inhibit the enzyme activity. Experimental data show that a drug can always act as an activator regardless of its concentration, or first act as an activator then, from a certain concentration value, transit to be an inhibitor (Al-Shawi et al., 2003). Here the occurrence of switching phenomenon provides strong support for the argument presented in this paper.

2. Methods

2.1. Catalytic cycle

In this paper, the symbols *E*,*S*,*R* and *P* stand for the enzyme molecule, the substrate molecule, the modifier molecule, and the product molecule, respectively, while the composite symbols *ES*,*ER* and *ERS* represent the corresponding complexes. If there is only one enzyme molecule, it may convert among four states: the free (unbound) enzyme *E*, the complex *ES*, the complex *ERS* and the complex *ER*. Then from the perspective of the single enzyme molecule, the kinetics are stochastic and cyclic, as is shown in Fig. 2, with pseudo-first-order rate constants $a_1[S]$,$a_2[R]$,$a_3[S]$, and $a_4[R]$, and first-order rate constants $b_1=b_1'+c_1$,b_2,$b_3=b_3'+c_3$, and b_4. We add the rate constants b_1' and c_1, since there are two ways of transition from the complex *ES* to the free enzyme *E*. The rate constants b_3' and c_3 are added for the same reason.

2.2. Net flux

Based on the law of mass action, we have the following kinetics equations:

$$\begin{cases} \dfrac{d[E]}{dt} = -(a_1[S]+a_4[R])[E]+b_1[ES]+b_4[ER], \\ \dfrac{d[ES]}{dt} = a_1[S][E]-(b_1+a_2[R])[ES]+b_2[ERS], \\ \dfrac{d[ERS]}{dt} = a_2[R][ES]-(b_2+b_3)[ERS]+a_3[S][ER], \\ \dfrac{d[ER]}{dt} = a_4[R][E]+b_3[ERS]-(b_4+a_3[S])[ER], \end{cases} \tag{1}$$

where $b_1=b_1'+c_1$, $b_3=b_3'+c_3$. The above four equations constitute a system of linear equations with coefficient matrix

$$Q=\begin{pmatrix} -(a_1[S]+a_4[R]) & b_1 & 0 & b_4 \\ a_1[S] & -(b_1+a_2[R]) & b_2 & 0 \\ 0 & a_2[R] & -(b_2+b_3) & a_3[S] \\ a_4[R] & 0 & b_3 & -(b_4+a_3[S]) \end{pmatrix}. \tag{2}$$

Let $E_0=[E]+[ES]+[ER]+[ERS]$ be the total enzyme concentration. Then the quantities $\mu_E=[E]/E_0$,$\mu_{ES}=[ES]/E_0$,$\mu_{ERS}=[ERS]/E_0$, and $\mu_{ER}=[ER]/E_0$ represent the probability distribution of the four enzyme states, respectively. The whole setup is nothing but a continuous-time Markov chain with transition density matrix Q^t (the transpose of the matrix Q).

It should be indicated that the enzyme–modifier and enzyme–substrate interactions often involve rapid binding steps followed by a slow conformational change or chemical step (Zhao and Wang, 1996). Thus, the quasi-steady approximation can be applied based on the difference in timescales between the catalytic cycle kinetics and the overall rate of change of biochemical reactions (Beard and Qian, 2008). Assuming that the cycle kinetics represented in Fig. 2 are rapid and maintain the enzyme and the complexes in a rapid quasi-steady state, we can obtain the steady state rate v of product formation for the general modifier kinetics

$$v=\frac{d[P]}{dt}=c_1[ES]+c_3[ERS]=E_0(c_1\mu_{ES}+c_3\mu_{ERS}). \tag{3}$$