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Optimal Yield Rates in Enzymatic Reactions with Undesirable Intermediate States

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Abstract—**Optimizing the yield rate of product in reactions that are catalyzed by enzymes is of fundamental biological and technological importance. In many instances these reactions could have undesirable intermediate states distinct from the transition state. In the presence of a mechanism to non-specifically release all intermediate states to the reactant state, this paper shows the existence of maxima in the product yield rate at specific optimal values of the release rate. The existence of these optimal rates hold significance for both *in vivo* and engineered systems.**

Keywords: Enzymes, Catalysis, Optimal Yield, Nonlinear Dynamics

1 Introduction

Enzymes play a crucial role in all living systems by serving as catalysts for a variety of important reactions [1]. Understanding the kinetics of reactions that are catalyzed by enzymes is therefore of fundamental importance. The traditional type of process that is considered is one where the substrate A and enzyme/catalyst B come together to form an intermediate complex which then dissociates into product C and the enzyme. The catalyst is unaffected by the reaction and its main role is to lower the free energy barrier separating the reactant and product [2]. However the free energy landscape separating the reactant and product can be quite complex especially with large biomolecules. In this paper we consider the situation where an undesirable intermediate state exists that can be readily accessed from the normal transition state of the complex [3, 4]. With only a finite amount of enzyme available, this raises the possibility that the product yield rate could be badly impacted. We then consider a subsidiary energy consuming process that could rescue the situation by nonspecifically releasing both the transition state and the undesirable state at a rate k_{rel} into reactant and enzyme. This raises an interesting optimization problem i.e. what is the optimal release rate that maximizes the product yield rate? Too small a rate will have no effect, while too large a rate will also adversely affect the yield rate by not allowing the reaction to proceed to completion. We model the kinetics of the process using mass action kinetics and solve the resulting system of nonlinear equations to derive the yield rates.

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2 Model Description

To begin we start with the canonical catalytic reaction. To describe the kinetics we consider the following reaction [5].

$$A + B \overset{k_{\pm 1}}{\rightleftharpoons} [AB] \overset{k_2}{\to} C + B \tag{1}$$

Here the k's represent the rate constants for the reactions indicated. We can describe the rate of the change of the concentrations of each participant of the reaction by

$$\begin{cases} \dot{A} &= -k_1 AB + k_{-1} X \\ \dot{B} &= -k_1 AB + (k_{-1} + k_2) X \\ \dot{X} &= k_1 AB - (k_{-1} + k_2) X \\ \dot{C} &= k_2 X \end{cases}$$

where the concentration of the intermediate $[AB] = X$. Since B is a catalyst, the total amount of B remains constant during the process or

$$B_0 = B + X = const. \tag{2}$$

This simplifies our system to

$$\begin{cases} \dot{A} &= -k_1 A(B_0 - X) + k_{-1} X \\ \dot{X} &= k_1 A(B_0 - X) - (k_{-1} + k_2) X \\ \dot{C} &= k_2 X \end{cases}$$

This is an inherently nonlinear dynamical system. We are interested in the yield rate i.e. the rate of production of C at steady state. At steady state the time derivatives of the various concentrations are identically zero.

$$\dot{A}|_{X=X^*, A=A^*} = \dot{X}|_{X=X^*, A=A^*} = 0.$$

This allows us to solve the above system of equations for the steady state values of the various concentrations. In

particular we have

$$X^* = \frac{k_1 B_0 A^*}{k_1 A^* + (k_{-1} + k_2)}.$$

Since $\dot{C} = k_2 X$ this directly gives us an expression for the production rate v of C

$$v = \dot{C} = \frac{k_1 k_2 B_0 A^*}{k_1 A^* + (k_{-1} + k_2)} \tag{3}$$

The above equation is well known Michaelis-Menten equation [1] that is widely used to describe enzyme kinetics and is usually written in the form

$$v = \frac{v_{max} A^*}{k_m + A^*} \tag{4}$$

The maximum value of the yield rate is $v_{max} = k_2 B_0$.

We now consider the situation where we have an additional undesirable state that is readily accessed from the transition state. Both these states can further be dissociated into substrate and enzyme at a rate k_{rel}.

$$\begin{array}{ccccc} & & A+B & & \\ & & \uparrow{\scriptstyle k_{rel}} & & \\ A+B & \xrightarrow{k_{\pm 1}} & [AB] & \xrightarrow{k_3} & C+B \\ & & \downarrow{\scriptstyle k_{\pm 2}} & & \\ & & [A^*B] & & \\ & & \downarrow{\scriptstyle k_{rel}} & & \\ & & A+B & & \end{array}$$

We can now write down the system of kinetic equations that describe the above system.

$$\left\{ \begin{array}{rcl} \dot{A} &=& -k_1 AB + (k_{rel} + k_{-1})X + k_{rel} Y \\ \dot{B} &=& -k_1 AB + (k_{rel} + k_{-1} + k_5)X + k_{rel} Y \\ \dot{X} &=& k_1 AB + k_{-2} Y - (k_{rel} + k_{-1} + k_2 + k_5)X \\ \dot{Y} &=& k_2 X - (k_{-2} + k_{rel})Y \\ \dot{C} &=& k_5 X \end{array} \right.$$

Here $[AB] = X$ and $[A^*B] = Y$. Again we have the constrains on the catalyst $B_0 = B + X + Y$. This allows us to write down the following relations for the steady state concentrations of X and Y.

$$\left\{ \begin{array}{rcl} 0 &=& k_1 A(B_0 - Y) + k_{-2} Y - \alpha X \\ 0 &=& k_2 X - (k_{-2} + k_{rel})Y \end{array} \right.$$

Here we introduced the parameter

$$\alpha = k_{rel} + k_{-1} + k_2 + k_5 + k_1 A.$$

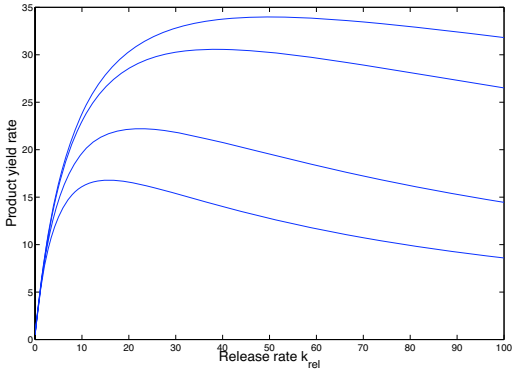

Figure 1: Maxima in yield rates. The curves are plotted for substrate concentration A=5,10,30 and 50 (bottom to top). The other parameters are $B = 5, k_1 = 5, k_{-1} = 1, k_2 = 10, k_{-2} = 0.1, k_3 = 5$.

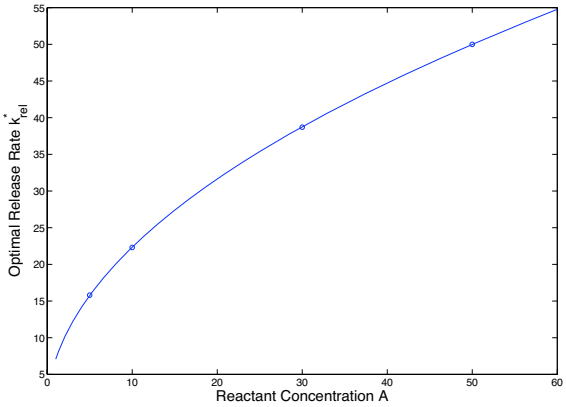

Figure 2: Optimal release rate as a function of reactant concentration. The points are maxima in fig.1 and the solid line is given by $k^*_{rel} = k_{-2} + \sqrt{k_2 \beta}$

This allows us to solve for the steady state concentration of the transition complex

$$X^* = \frac{k_1(k_{-2} + k_{rel})AB_0}{\alpha\gamma + \beta k_2}, \tag{5}$$

$$\beta = -k_{-2} + k_1 A, \ \gamma = k_{-2} + k_{rel}$$

The product yield rate at steady state is given by $\dot{C} = k_3 X^*$ which gives

$$v = \frac{k_3 k_1(k_{-2} + k_{rel})AB_0}{\alpha\gamma + \beta k_2}, \tag{6}$$

Fig 1 shows a plot of the yield rate as a function of the release rate for various values of the substrate concentration. We clearly see that there are maxima in the yield rate at specific optimal values of k_{rel}. We also see that the release rate can give orders of magnitude improvement in the yield rates over the value for zero release rate. The yield rate is small for zero release rates and decays to zero for very large release rates corresponding to the transition states being instantaneously converted back to substrate and enzyme. We can use our explicit expression for the yield rate to compute the optimal release rate giving $k^*_{rel} = k_{-2} \pm \sqrt{k_2\beta}$. Fig 2 shows the values of the optimal release rates obtained from fig 1 (circles) plotted against the substrate concentration. Also shown is the analytical result for $k^*_{rel} = k_{-2} \pm \sqrt{k_2\beta}$ (solid line) which shows the anticipated agreement. It is also important to realize that the optimum does not exist for all parameter values. $k^*_{rel} = k_{-2} \pm \sqrt{k_2\beta}$ does not have real roots when $k_1 A < k_{-2}$. Thus if the substrate concentration is too low then getting the transition state is a rare occurrence and releasing at any rate is detrimental to the overall yield rate. Our results indicating the existence of an optimal release rate could have significant implications for the functioning of enzymes *in vivo*.

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