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A kinetic model for analysis of physical tunnels in sequentially acting enzymes with direct proximity channeling

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ABSTRACT

Direct channeling is a well-known process in which intermediates are funneled between enzyme active sites through a physical tunnel and can be a potential way to enhance the biocatalytic efficiency for cascading bioreactions. However, the exact mechanism of the substrate channeling remains unclear. In this work, we used mathematical models to describe the mass transfer in the physical tunnels and to gain further understanding of direct proximity channeling. Simulation with a diffusion-reaction model showed that the reduction of the diffusion distance of intermediates could not cause proximity channeling. A second kinetic model, which considered the physical tunnel as a small sphere capable of preventing diffusion of the intermediate into the bulk, was then constructed. It was used to show that the maximum channeling degree in branched pathways depends on the strength of the side reactions, suggesting that proximity channeling in a physical tunnel is more suitable for a pathway with strong side reactions. On the other hand, for a linear pathway, proximity channeling is more beneficial when the constituting enzymes have relatively low activities and expression levels. Our kinetic model provides a theoretical basis for engineering proximity channeling between sequentially acting enzymes in microbial cell factories and enzyme engineering.

1. Introduction

In cascading enzymatic reactions, substrate channeling is a well-known phenomenon in which the product of an enzyme passes directly into the active site of the subsequent enzyme without diffusing away [1–3]. This phenomenon normally results in an accelerated reaction rate. As substrate channeling can confine a metabolic intermediate to a small region and reduce its concentration in the bulk, engineering of substrate channeling has potential uses in metabolic engineering, multi-enzyme-mediated biocatalysis, and cell-free biosynthetic systems, by avoiding the toxicity of intermediates, side reactions, allosteric regulation and the escape of the intermediates into the bulk [3–5].

Substrate channeling generally occurs when sequentially acting enzymes are positioned close together, which is called 'proximity channeling' [1–3]. Some researchers have suggested that proximity channeling occurs by reducing the diffusion distance of the intermediates, therefore enabling the intermediates to be consumed immediately before diffusing away [6–8]. However, proximity channeling is only likely to occur when the two active sites of sequential enzymes are aligned to each other and are as close as 1 nm apart [9]. However, the large size of proteins means that it is sterically quite difficult to obtain such spatial proximity of active sites [10]. Direct substrate channeling, in which enzymes are funneled between enzyme active sites through a physical tunnel, was then proposed, and it has been proved to be a possible mechanism of proximity channeling [1,11,12]. Another possible mechanism is that the sequential enzymes are assembled to form a large agglomerate, which restricts the reaction to a diffusion-limited regime; this is known as agglomerate channeling [1,4].

Several previous attempts to understand substrate channeling have used diffusion-reaction models to explain the transport of intermediates between sequential enzymes. To illustrate the mechanism of agglomerate channeling, Castellana et al. divided the cell cytoplasm into several basins, with each basin containing an enzyme cluster and its surrounding volume. Diffusion-reaction equations and boundary conditions were then used to describe the mass transfer of the substrates and intermediates in the enzyme cluster [1]. Castellana et al. used this model to show that the optimal

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spacing between clusters, namely that which would maximize the metabolic efficiency of the agglomerate channeling, was 6.5 μm. Such a large spacing implied that agglomerate channeling could only be possible in large cells such as human cells. In the case of enzymes that have high catalytic constants, for example, a catalytic efficiency of more than 10^7 M^{-1} s^{-1}, agglomerate channeling could also occur in small cells, and this was confirmed by an engineered CarB–PyrB enzyme cluster in *Escherichia coli* [1]. However, in terms of direct substrate channeling, although there is evidence supporting the existence of physical tunnels that are favorable for substrate transport [2,13,14], there were few studies to elucidate the transport of intermediates through these tunnels. Bauler et al. used Brownian dynamics to simulate the transport of intermediates between the two active sites of sequential enzymes and found that, in order to maximize the catalytic efficiency, the two active sites should be aligned to each other very closely [9]. Eun et al. combined the concentration field and electrostatic field to study the transfer of the intermediate between two consecutive active sites in a diffusion limited system, and found that attractive electrostatic interactions could confine the intermediate to the vicinity of the enzymes and thus elevate its local concentration [15]. However, there has been no study that used mathematical models to explain the process of mass transfer in physical tunnel-based proximity channeling, and the exact mechanisms still remain unclear.

In this study, we consider fusion enzymes as a strategy to obtain a closer distance between two consecutive active sites, with the assumption that the fusion of the enzymes does not significantly affect their activity. We first establish a diffusion-reaction model based on a two-step pathway with a branch point, in order to test the effect of the diffusion distance on proximity channeling. We then present a kinetic model that ignores the diffusion rates and introduces a small volume to represent the physical tunnel and we use this model to elucidate the process of substrate channeling with a physical tunnel. Finally, we apply the kinetic model to gain an understanding of the role of proximity channeling in branched pathways and linear pathways. Our approach provides a clear explanation of how physical tunnel-based proximity channeling occurs, and can potentially be a useful tool for guiding protein engineering with a view to optimizing substrate channeling between sequentially acting enzymes.

2. Model development

2.1. Diffusion-reaction model

Here, we consider a two-step metabolic pathway with a branch point as a model system to study substrate channeling between two sequential enzymes (Fig. 1A). Such a model could be used to describe pathways with side reactions, unstable intermediates and reversible reactions.

As shown in Fig. 1B and C, to simplify the system, the cell was divided into many spherical basins [1], where each basin contains an E_1 enzyme located at its center and is delimited by a thin boundary layer. The radius of the basin is determined by the concentration of E_1. As the concentration of overexpressed proteins in *E. coli* is approximately 2000 enzymes per cell [1], equal to 0.5 μM, each E_1 occupies a volume with a radius of about 100 nm (R in Figs. 1 and 3 A). The second enzyme, E_2, is placed in the center of the basin for the E_1–E_2 fusion protein system and in the thin boundary layer of the basin for individual enzymes. Es, which catalyzes the side reaction, is placed in the thin boundary layer of the basin (Fig. 1B and C). The diffusion-reaction equations presented below were used to describe the transfer of the intermediate M [1,16].

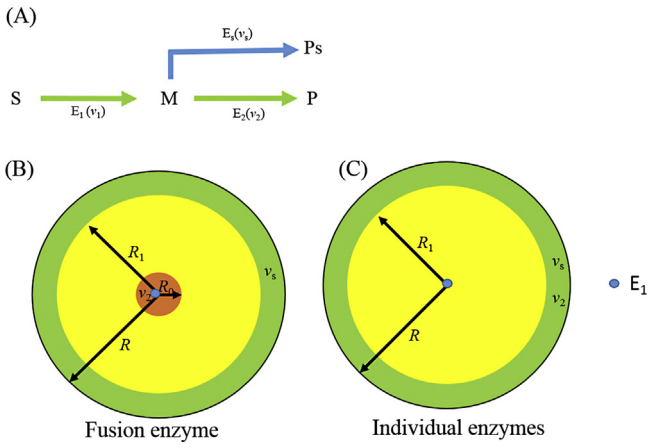

Fig. 1. The models for proximity channeling. (A) A metabolic pathway with a branch point was chosen as the model system. The geometry for (B) the fusion enzyme and (C) individual enzymes considered in this paper. Two fused active sites are placed in the orange circle to describe their close proximity, and the active site for side reactions is placed in the green region. The symbols 'S', 'M' and 'P' represent 'substrate', 'intermediate' and 'product', respectively, and 'Ps' is the final product of the side reactions. E_1 and E_2 are two enzymes involved in the coupled reaction and Es is the enzyme for the side reaction that competes with E_2 for M. R_0 is the radius of the region where E_2 consumes the intermediate when fused with E_1. R is the radius of the basin. v_2 and v_s are the reaction rates of E_2 and Es, respectively.

For the fusion enzyme:

$$\begin{cases} -4\pi r^2 D\dfrac{\partial C}{\partial r} = N_1 & r \to 0 \\ \dfrac{\partial C}{\partial t} = D[\dfrac{1}{r^2}\dfrac{\partial}{\partial r}(r^2\dfrac{\partial c}{\partial r})] - v_2 & 0 < r < R_0 \\ \dfrac{\partial C}{\partial r}|_{r\to R_1^+} = \dfrac{\partial C}{\partial r}|_{r\to R_1^-} & r = R_0 \\ \dfrac{\partial C}{\partial t} = D\left[\dfrac{1}{r^2}\dfrac{\partial}{\partial r}\left(r^2\dfrac{\partial C}{\partial r}\right)\right] & R_0 < r < R_1 \\ \dfrac{\partial C}{\partial r}|_{r\to R_2^+} = \dfrac{\partial C}{\partial r}|_{r\to R_2^-} & r = R_1 \\ \dfrac{\partial C}{\partial t} = D\left[\dfrac{1}{r^2}\dfrac{\partial}{\partial r}\left(r^2\dfrac{\partial C}{\partial r}\right)\right] - v_s & R_1 < r < R \\ \dfrac{\partial C}{\partial r}|_{r=R} = 0 & r = R \end{cases} \quad (1)$$

For individual enzymes:

$$\begin{cases} -4\pi r^2 D\dfrac{\partial C}{\partial r} = N_1 & r \to 0 \\ \dfrac{\partial C}{\partial t} = D[\dfrac{1}{r^2}\dfrac{\partial}{\partial r}(r^2\dfrac{\partial C}{\partial r})] & 0 < r < R_0 \\ \dfrac{\partial C}{\partial r}|_{r\to R_2^+} = \dfrac{\partial C}{\partial r}|_{r\to R_2^-} & r = R_1 \\ \dfrac{\partial C}{\partial t} = D\left[\dfrac{1}{r^2}\dfrac{\partial}{\partial r}\left(r^2\dfrac{\partial C}{\partial r}\right)\right] - v_2 - v_s & R_1 < r < R \\ \dfrac{\partial C}{\partial r}|_{r=R} = 0 & r = R \end{cases} \quad (2)$$

In these equations, N_1 is the rate of the reaction catalyzed by the first enzyme and D is the diffusion coefficient of the intermediate. In our model, we assume that the substrate of the first enzyme is present in excess, thus N_1 remains nearly unchanged with time. v_2 and v_s are the rates of the reactions catalyzed by E_2 and E_s, respectively. The basin has spherical symmetry, that is, the concentration of the intermediates depends only on the distance r. The no-flux bound-