Mathematical basis of organelle's preference and selectivity on chemical reactions

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Abstract

Chemical reactions are mostly considered to happen in an ideal homogeneous mixture, in which, the reactants could diffuse freely and react with specific chemicals. In the synthetic biology, the same concept were also used in the most of modelings, based on the ideal of parts' modularity. However, non-specific interactions, artifacts, and underground reactions usually happen in the reality. One solution of how the nature solve this problem is by building organelle to host the main reaction and decouple them away from unwanted substrate. Though the intuition is obvious, mathematically, it was still elusive how it works, especially when we count into the complex facts that most of the enzymes, substrates, reactants come from outside of the organelle, and still may react in the cytoplasm. In this article, we exemplified that even when the reactants are synthesized in the cytoplasm and later recruited by the organelle, the organelle could still, not only enhance the desired reaction, but also abolish the underground reaction completely, by its Michaelis-Menten response to the reaction rate constant.

1 Introduction

1.1 Motivation

Our interest in this article is in comparing chemical reactions between in a homogeneous mixture and when the cell has a specific organelle for the reaction, and the sensitivity of this difference to the change of different parameters, i.e. the reaction rates.

Although our study is specifically on general organelles, we are motivated by the experimental design we have on synthetic RNA-repeat based organelle. We created this design experimentally to face the challenge in the complicated pathway designs in synthetic biology. Enzymes, substrates and other cellular components usually have underground interactions and reactions. After all, synthetic or exogenous components are not optimized for the host context. To optimize the whole system, to harness the power insider underground reactions instead of remove them, is fairly difficult and requires long-term directed evolution, although it has been done in very a few bioengineering cases and in the nature such as glycolysis.

A more common strategy seems to build spatial subcellular structures to host the certain reactions, i.e. organelles and its analogs. In fact, the biological system is usually so complicated that even natural enzymes are not optimal enough – absolute specificity is not common. To enhance the desired the reaction and remove the unwanted, biological components and small molecular substrates are distributed specifically according to the spatial structure.

In RNA organelle project,we successfully created a synthetic membrane-less organelle by RNA constructs consisting of triple-nucleotide repeats. The assembly of RNA organelle we build could be controlled by the repeat numbers in the construct, monovalent environment and the expression of small RNA with competing reverse complementary sequences; and we could also control its recruiting rate constant that RNA enriches the wanted enzymes by RNA aptamer engineering. Therefore, the insights we get from this mathematical analysis will also serves for the further exploration of the design principle for the synthetic organelle. Besides, some similar features shared by organelles, cells and higher level individuals may allow us to generalize our conclusion to more common circumstances.

Because the cell is highly dynamic and many enzymatic reactions happen very fast in nanoseconds to seconds, the concentration of reactant species participating may be at or near steady state instantaneously. Therefore, by analyzing the steady-state production rate, we could understand the difference between an organelle from the homogeneous mixture.

1.2 Main results

In the following mathematical description and analysis, we use the simples composition reaction which contains only two species, but also representative and complicated enough by putting them into a growth cell – an unstable, non-thermo-equilibrium condition. We compared the production rates of the system in either homogeneous mixture, or when the organelle is present to concentrate one of the reactants. The main results here we showed that given proper parameters – which is usually reasonable and true in biological realities – organelles could almost always enhance the specific reactions (when the reaction rate constant is hight) and abolish the non-specific reactions (when the reaction rate constant is low).

2 Mathematical description and proofs on organelle's sensitivity

2.1 Mass-action description of chemical reactions in homogeneous mixture

Here we use the simplest two-species reaction as an representative example and solve their kinetics according to the law of mass action. The results we acquired from this model, will be used as the control reference when we analyze the performance of organelles. The chemical reactions in the system includes:

$$0 \underset{\alpha_{\mathrm{A}}}{\overset{\beta_{\mathrm{A}}}{\rightleftharpoons}} \mathrm{A}; 0 \underset{\alpha_{\mathrm{B}}}{\overset{\beta_{\mathrm{B}}}{\rightleftharpoons}} \mathrm{B}; \mathrm{A}+\mathrm{B} \xrightarrow{k} \mathrm{P} \tag{R1}$$

where A and B are two reactants, and P is the product of composition reaction. Reactions including "0" represent the open-system feature of the growing cells. β, α, and k are synthesis, degradation or dilution, and reaction rate constant. Its deterministic model is as stated below:

$$\frac{\partial[\mathrm{A}]}{\partial t} = \beta_A - \alpha_A[\mathrm{A}] - k[\mathrm{A}][\mathrm{B}]$$

$$\frac{\partial[\mathrm{B}]}{\partial t} = \beta_B - \alpha_B[\mathrm{B}] - k[\mathrm{A}][\mathrm{B}]$$

$$\frac{\partial[\mathrm{P}]}{\partial t} = k[\mathrm{A}][\mathrm{B}]$$

Obviously, the production rate of this system will finally reach a linearly stable, positive steady state, if $\alpha_A\alpha_B \neq 0$:

$$v_{homo} = \frac{\partial[\mathrm{P}]}{\partial t}\Big|_{ss} = \frac{1}{2}\left(\frac{\alpha_A\alpha_B}{k} + \beta_A + \beta_B\right) - \frac{1}{2}\sqrt{\left(\frac{\alpha_A\alpha_B}{k} + \beta_A + \beta_B\right)^2 - 4\beta_A\beta_B} \tag{1}$$

When $\beta_A = \beta_B = \beta/2, \alpha_A = \alpha_B = \alpha$, the systems is optimized for cell to produce P with the least cost and the equations are degenerated, with the steady state:

$$[\mathrm{A}]_{ss} = [\mathrm{B}]_{ss} = \frac{-\alpha + \sqrt{\alpha^2 + 2k\beta}}{2k} = \frac{\beta}{\alpha + \sqrt{\alpha^2 + 2k\beta}} \overset{\text{if } k \to 0}{\approx} \frac{\beta}{2\alpha}$$

$$v_{homo} = \frac{1}{2}k\left(\frac{\beta}{\alpha + \sqrt{\alpha^2 + 2k\beta}}\right)^2 = \frac{1}{2}\left(\frac{\beta}{\sqrt{\alpha^2/k} + \sqrt{\alpha^2/k + 2\beta}}\right)^2$$

WARNING!: for numerical plotting of $[\mathrm{A}]_{ss}$ *or* v_{homo} *versus* k*, you had better use the formula that has* k *only in its denominator. Otherwise, because of the small error from the floating point operation, the result may explode when* $k \to 0$*.*

2.2 Reaction-diffusion description of chemical reactions hosted by organelle

In previous simulation works on phase transition, we showed that the RNA organelle assembles extraordinarily fast and stay stable. As a result, we could ignore the impact from undesigned spatial structure, and consider the reaction outside the organelles still as the mass-action kinetics. If both component are recruited by the organelle simultaneously, it is obvious that the reaction rate constant will be altered. It is easy to get any predefined steady-state production rate as:

$$\frac{\partial[\mathrm{P}]}{\partial t}\Big|_{ss} = k(\phi_c[\mathrm{A}][\mathrm{B}] + \phi_\mathrm{o}[\mathrm{A}]_\mathrm{o}[\mathrm{B}]_\mathrm{o}) \tag{2}$$

where ϕ_c and ϕ_o are the volume percentages, and $[\mathrm{A}][\mathrm{B}]$ and $[\mathrm{A}]_\mathrm{o}[\mathrm{B}]_\mathrm{o}$ are the reactant concentrations of cytoplasm and organelles. Technically, by refining the organelle's affinities to two reactant, we could explore any output as we want.

It is more interesting to study the asymmetric scenario when only one reactant (let it be A) is recruited into the organelle. It is also important to give insights for bioengineering and synthetic organelle design. Hereafter, we consider the situation that only reactant A will be specifically recruited by the organelle. A may represents a organelle-associated macromolecule, like proteins, or RNAs in the real cases, and B could be either small substrate or large molecules. Organelles concentrate reactant A by specific receptors – in our case, RNA aptamers. Therefore, the chemical reaction network consists of both described R1 and following reactions:

$$\mathrm{A} + \mathrm{A_nR} \underset{\gamma' \ll \gamma}{\overset{\gamma}{\rightleftharpoons}} \mathrm{A_{n+1}R}; \tag{R2}$$

$$\mathrm{B} + \mathrm{R} \xrightarrow{k} \mathrm{P} + \mathrm{R}; \tag{R3}$$

where R or AR represents the RNA organelle with (multiple) reactant A inside, γ and γ' are the respective flux of capture and dissociation of reactant A.

In the previous study, RNAs aggregate to form organelle rapidly and stay stable. The total number of RNAs per cell does not change significantly versus time, and also the surface size grows very slow given that the organelles are usually spherical and have a size grow rate constant proportional to $1/radius^3$. Thus we considered the RNA organelle shape and structure thermostatic. As a result, the recruitment and dissociation of reactant A is a linear function of concentration of A. Also, because RNA organelle contains full of receptors of A, on the surface of organelle, the equilibrium will be established very fast but until the organelle is saturated by A, the net dissociation is negligible. Thus, in a unit space, the distribution of A is:

$$\frac{\partial[\mathrm{A}]}{\partial t} = \beta_A - \alpha_A[\mathrm{A}] - k[\mathrm{A}][\mathrm{B}] - \gamma[\mathrm{A}]/\phi_c$$

$$[\mathrm{A}]_\mathrm{o} = \frac{\gamma}{\gamma'}[\mathrm{A}] = K[\mathrm{A}]$$

We idealize how organelles react with B by modifying equations derived from Fick's Law of diffusion [1]. Consider a spherical organelle with radius r, with reactant A available on the surface. Let A have radius s, and total number $N = 4\pi a^2[\mathrm{A}]_\mathrm{o}$. The flux of R3 per unit space is:

$$\begin{aligned} J([\mathrm{A}]_\mathrm{R}, s, r) &= \frac{3k}{4\pi r^3}\oint_V D\nabla^2[\mathrm{B}] \\ &= \frac{3k}{4\pi r^3}(4D\pi a[\mathrm{B}]\frac{Ns}{\pi a + Ns}) \\ &= \frac{3k}{r}D\frac{[\mathrm{A}]_\mathrm{o}}{1/4 + [\mathrm{A}]_\mathrm{o}}[\mathrm{B}] \end{aligned} \tag{3}$$

Then we could describe the whole system as:

$$\frac{\partial[\mathrm{A}]}{\partial t} = \beta_A/\phi_c - \alpha_A[\mathrm{A}] - k[\mathrm{A}][\mathrm{B}] - \gamma[\mathrm{A}]/\phi_c$$

$$\frac{\partial[\mathrm{B}]}{\partial t} = \beta_B/\phi_c - \alpha_B[\mathrm{B}] - k[\mathrm{A}][\mathrm{B}] - J\phi_o/\phi_c$$

Given the fact that $\gamma/\phi_c \gg k[\mathrm{B}]$, the system has a simple solution at the steady state given by:

$$v_{org} = \frac{\partial \mathrm{P}}{\partial t}\Big|_{ss} = \frac{\beta_B k f}{\alpha_B + kf} \tag{4}$$

where

$$f(\beta_A, \alpha_A; \phi_o, \phi_c, D, K) = [\mathrm{A}] + \frac{3\phi_o DK[\mathrm{A}]}{\phi_c r(K[\mathrm{A}] + 1/4)}$$

$$[\mathrm{A}] = \frac{\beta_A}{\phi_c \alpha_A + \gamma}$$

Remarkably, despite the fact that mechanisms underlying chemical reactions R2 and R3 are so different from mass action, it has almost the same topology that we usually used to describe the function of enzyme if we replace R to an enzyme. It is not surprising to expect that the organelle may have a similar mathematical description as an enzyme. Indeed, many large biomolecule complexes are usually considered as non-membrane organelles. Mathematically there may be no fine line between enzymatic components and more complex cellular compartments.

2.3 Organelle's preference on high rate constant catalysis

To simplify the situation, we assume $\beta_A = \beta_B = \beta/2$ and $\alpha_A = \alpha_B = \alpha$, which is optimal for cell to produce P with the least cost. Then we have:

$$v_{homo} = \frac{1}{2}\left(\frac{\beta}{\sqrt{\alpha^2/k} + \sqrt{\alpha^2/k + 2\beta}}\right)^2 \tag{5}$$

$$v_{org} = \frac{1}{2}\frac{\beta k f}{\alpha + kf} \tag{6}$$

There is a trivial solutions of $v_{homo} = v_{org}$ for k where no reaction occurs at all:

$$\lim_{k\to 0^+} v_{homo} = \lim_{k\to 0^+} v_{org} = 0 \tag{7}$$

If the reaction rate constant is high enough, both organelles and homogeneous mixtures will reach the maximal rate constant of the system:

$$\lim_{k\to\infty} v_{homo} = \frac{\beta}{4} < \lim_{k\to\infty} v_{org} = \frac{\beta}{2} \tag{8}$$

Notice that Eq.5 and Eq.6 are both monotonically increasing by calculating the derivative (not shown) – which is obvious in the physical chemistry situation that when k increases the production rate also increases and at the end reaches a plateau due to the limit of reactant inputs. Besides,a positive solution of Eq.5 equal to Eq.6 exists, if $\beta > 4\alpha f$, denoted as k^*:

$$k^* = \frac{2\alpha^2 f + \alpha\beta - 2\alpha\sqrt{\alpha^2 f^2 + 2\alpha\beta f}}{\beta f} \tag{9}$$

Now consider the constrain $\beta > 4\alpha f$, it holds when:

$$\frac{1}{4\alpha} > \frac{1}{\phi_c\alpha + \gamma} + \frac{3\phi_o DK}{\phi_c r(4\beta K + \phi_c\alpha + \gamma)} \tag{10}$$

The inequality will hold, when the affinity of organelle to reactant A (γ) is high, the diffusion of reactant B (D) is slow enough, the organelle size (r) is large but the fraction of organelle in the space is small enough implying a bigger cell. When InEq.10 holds, combining Eq.8 and Eq.9, we know:

$$v_{homo} > v_{org}, k < k^* \tag{11}$$

$$v_{homo} > v_{org}, k > k^* \tag{12}$$

In conclusion, the organelle has a selectivity over the reaction rate constant and prefers the chemical reactions with high rates: when the reaction rate constant is low, the organelle makes the whole-cell production rate lower, and vice versa.

References

[1] Berg, H. C., & Purcell, E. M. (1977). Physics of chemoreception. *Biophysical journal, 20(2)*, 193-219.