How can we distinguish positive cooperativity from auto-catalysis in enzyme kinetics?

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Abstract

Different graphical plots involving the catalytic rate with the (initial) substrate concentration exist in the enzyme kinetics literature to estimate the reaction constants. But, none of these standard plots can unambiguously distinguish between the two important mechanisms of rate enhancement: positive cooperativity among the active sites of an oligomeric enzyme and auto-catalysis of the intermediate complex of an enzyme with a single active site. We achieve this distinction here by providing a nice linear plot for the latter. Importantly, to accomplish this task, no extra information other than the steady-state rate as a function of substrate concentration is required.

Keywords: Enzyme kinetics; Cooperativity; Auto-catalysis; Steady state

1 Introduction

Enzyme catalysis is a highly important biochemical reaction where specific substrates are efficiently converted into products [1]. Investigations on the catalytic mechanisms span over a century. The Michaelis-Menten (MM) scheme [2] is a cornerstone in the field of theoretical modelling of enzyme kinetic data [3]. The rate of product formation in MM scheme under steady-state approximation [4] of the intermediate complex plays the benchmark role in the analyses of kinetic constants. To determine the rate parameters, various ways of plotting the rate against (initial) substrate concentration are present in the literature with their respective advantages and disadvantages [3]. Some notable examples are the Lineweaver-Burk (LB), Eadie-Hofstee (EH) and Hanes-Woolf (HW) plots [1, 3].

The MM scheme represents the simplest model of enzyme catalysis. Naturally, the enzyme is considered to have a single substrate binding site or active
site. However, there are many enzymes in nature with multiple binding sites. Interactions among these sites leads to cooperativity [5]. As a result, one notices either an enhancement (positive cooperativity) or diminution (negative cooperativity) of the catalytic rate compared to that obtained from the MM scheme with equal number of binding sites acting independently, i.e., zero cooperativity [6]. A prime example of cooperative kinetics is the oxygen binding to hemoglobin [7]. Another possible way of rate enhancement is auto-catalysis [8] of the intermediate complex. This can also increase the rate compared to the MM case at similar substrate concentration even for an enzyme with a single active site. Auto-catalysis is thought to play crucial roles in the evolution of population [9] as well as gene [10]. A well-known signature of MM kinetics is the hyperbolic curve of rate against starting substrate concentration, \([S]_0\). Now, both positive cooperativity and auto-catalysis can show non-hyperbolic sigmoidal nature [11] of the rate against \([S]_0\). Thus, these rate-enhancing mechanisms can be identified, in principle, as non-MM ones. Then, the important question to ask is: How to distinguish between auto-catalysis and positive cooperativity from catalytic rate measurements?

In this work, we show that none of the standard plots can unambiguously discriminate auto-catalytic behaviour from positive cooperativity. As a remedy, we introduce here a graphical method that can do the job quite well. In what follows, cooperativity stands for positive cooperativity only.

2 Steady-state catalytic rates

We determine the rates of product formation in all the cases under the steady-state approximation (SSA) of the respective intermediate complexes. The calculations are based on the schemes shown in Fig. 1. We take \([E]_0 = 1 \mu M\) and \([S]_0 \geq 100 \mu M\). The validity of SSA is checked for two different choices of the set of rate constants. This also ensures \([S] \sim [S]_0\) in all the cases.

2.1 MM kinetics

The steady-state (SS) rate of product formation comes out as

\[
\mathcal{R} = \frac{k_2[E]_0[S]}{K_M + [S]} \tag{1}
\]
Figure 1: Reaction schemes for the various cases considered. MM: Michaelis-Menten; Auto: auto-catalysis (of the intermediate); 2-site: cooperative kinetics for an enzyme with two active sites; 3-site: cooperative kinetics for an enzyme with three active sites. The factors ‘2’ and ‘3’ account for the statistical weightage.

where $[X]_0$ is the starting concentration of the species $X$ and $[X]$ denotes SS concentration of $X$. The MM constant is $K_M = \frac{k_{-1} + k_2}{k_1}$. The conservation of enzyme and substrate concentrations are given by

$$[E]_0 = [E] + [ES] ; \quad [S]_0 = [S] + [ES] + [P].$$  \hspace{1cm} (2)

### 2.2 Auto-catalysis

In this case, the SS rate is given as

$$\overline{R} = \frac{k_2 [E]_0 [S]}{K_M + [S] - \frac{k_{-1} [E]_0 [S]}{k_1}}.$$  \hspace{1cm} (3)

The conservation conditions are the same as given in Eq.(2).
2.3 2-site cooperativity

The expression of SS rate for this scheme is

$$\bar{R} = \frac{2k_4[E]_0[S]}{K_M K_{M1} + 2K_{M1} [S] + [S]_2} \left( k_2 K_{M1} / k_4 + [S] \right). \quad (4)$$

Here, $K_{M1} = \frac{k_1 + k_3}{k_5}$ and $K_M$ is as defined above for the MM case. The conservation relations used are

$$[E]_0 = [E] + [ES]_1 + [ES]_2; \quad [S]_0 = [S] + [ES]_1 + 2[ES]_2 + [P]. \quad (5)$$

2.4 3-site cooperativity

This scheme has a SS rate of

$$\bar{R} = \frac{3k_6[E]_0[S]}{K_M K_{M1} K_{M2} + 3K_{M1} K_{M2} [S] + 3K_{M2} [S]^2 + [S]^3} \times$$

$$\left( \frac{k_2 K_{M1} K_{M2}}{k_6} + \frac{2k_4 K_{M2} [S]}{k_6} + [S]^2 \right). \quad (6)$$

Here, $K_{M2} = \frac{k_5 + k_8}{k_7}$ and $K_M, K_{M1}$ are as defined already. The conservation relations are obtained as natural extensions of Eq.(5)

$$[E]_0 = [E] + [ES]_1 + [ES]_2 + [ES]_3; \quad [S]_0 = [S] + [ES]_1 + 2[ES]_2 + 3[ES]_3 + [P]. \quad (7)$$

3 Non-MM behavior of cooperative and auto-catalytic kinetics

The nature of variation of the SS catalytic rate, $\bar{R}$, with $[S]_0$ can give a nice indication of non-MM behavior. The curve is hyperbolic for MM kinetics but generally sigmoidal for cooperativity and auto-catalysis. This is evident from Fig. 2 for two different choices of rate constants (see Table 3). The two sets of rate constants can result in up to 100-times difference in the respective SS rates. It is clear from Fig. 2 that cooperativity and auto-catalysis can not be distinguished from each other, as already mentioned in Section 1.
Table 1: Rate constants for the various cases considered in the main text. $k_1$, $k_3$, $k_5$ are in $(\mu M \cdot s)^{-1}$, $k_0$ is in $\mu M^{-2} \cdot s^{-1}$, $k_{-1}$, $k_2$, $k_{-3}$, $k_4$, $k_{-5}$, $k_6$ are in $s^{-1}$.

| Set | Case | $k_1$ | $k_{-1}$ | $k_2$ | $k_0$ | $k_3$ | $k_{-3}$ | $k_4$ | $k_5$ | $k_{-5}$ | $k_6$ |
|-----|------|-------|---------|-------|-------|-------|---------|-------|-------|---------|-------|
| 1   | MM   | 0.1   | 200     | 0.01  | -     | -     | -       | -     | -     | -       | -     |
|     | Auto | 0.01  | 500     | 2.5   | 0.1   | -     | -       | -     | -     | -       | -     |
|     | 2-site | 0.008 | 500    | 1.0   | -     | 0.05  | 100     | 1.1   | -     | -       | -     |
|     | 3-site | 0.006 | 500    | 1.0   | -     | 0.02  | 100     | 1.1   | 0.05  | 100     | 1.2   |
| 2   | MM   | 0.1   | 200     | 0.01  | -     | -     | -       | -     | -     | -       | -     |
|     | Auto | 0.1   | 200     | 0.04  | 0.5   | -     | -       | -     | -     | -       | -     |
|     | 2-site | 0.2   | 250    | 0.01  | -     | 0.25  | 200     | 0.0175| -     | -       | -     |
|     | 3-site | 0.2   | 350    | 0.01  | -     | 0.25  | 325     | 0.0125| 0.3   | 300     | 0.015 |

One important point to mention is that, if the degree of cooperativity or auto-catalysis is small, the sigmoidal nature of the curve may be difficult to identify. Hence, the curves for all the cases may appear similar, i.e., hyperbolic. In such cases, and also generally, plots like HW and LB may help to distinguish the non-MM behavior. For example, in Fig. 3 we show the HW plots for all the cases taking the two sets of rate constants. The MM case has a positive slope throughout whereas, rest of the schemes have negative slopes at lower range of $[S]_0$. But, even these plots can not unambiguously distinguish cooperativity from auto-catalysis. Similar observations are made with the LB and EH plots.

4 Method to discriminate auto-catalysis from cooperativity

The expression of rate under SSA for the auto-catalysis scheme can be rearranged to the form

$$\frac{(R_\infty - \bar{R})[\bar{S}]/R}{(R_\infty - \bar{R})} = \frac{k_0}{k_1k_2}(R_\infty - \bar{R})[\bar{S}]. \quad (8)$$

Here $R_\infty$ is the saturated catalytic rate obtained at large substrate concentration. In case of auto-catalysis, it is given by $R_\infty = k_2[E]_0$. It follows from Eq.(8) that a plot of $(R_\infty - \bar{R})[\bar{S}]/R$ against $(R_\infty - \bar{R})[\bar{S}]$ yields a straight line with negative slope. This is clearly shown in Fig. 4. However, for the
cooperative kinetics, the plots become non-linear. For example, for the 2-site cooperative scheme, we can write

\[ (R_\infty - \bar{R})[S]/\bar{R} = f([S])(R_\infty - \bar{R})[S]. \]  

Here,

\[ f([S]) = \frac{K_M K_{M1} + 2K_{M1}[S] + [S]^2}{R_\infty[S]} \left( \frac{k_2 K_{M1}}{k_4} + [S] \right)^{-1} \]  

and \( R_\infty = 2k_4[E]_0 \). The non-linear nature of the plot is depicted in Fig. 4. As the substrate is in excess, we replace \([S]\) by \([S]_0\) without introducing any significant error. For 3-site cooperativity, \( R_\infty = 3k_6[E]_0 \).
Figure 4: Plot of \((R_\infty - \overline{R})[S]/\overline{R}\) against \((R_\infty - \overline{R})[S]\) for all the schemes with the two different sets of rate constants shown in (A) and (B).

5 Conclusion

The standard graphical methods to characterize the nature of enzyme kinetics fail to distinguish auto-catalysis of the intermediate in an enzyme with a single active site from positive cooperativity among active sites in an oligomeric enzyme. Here, we introduce an approach to attain this discrimination between these rate-enhancing mechanisms. Our method should be accurate provided (i) the SSA holds for the intermediate(s) and (ii) \(R_\infty\) can be measured with reasonable accuracy. Condition (i) is expected to be maintained for any standard laboratory experiment and consequent modelling of enzyme kinetics. In fact, all the standard plots in literature are based on the validity of SSA. Condition (ii) also can be fulfilled either using direct measurement or extrapolation of rate data. Thus, we believe that, the graphical approach introduced here should be convenient to get a clear distinction between auto-catalysis and positive cooperativity.

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