Catalytic efficiency and $k_{\text{cat}}/K_M$: a useful comparator?

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The ratio $k_{\text{cat}}/K_M$ – often referred to as the ‘specificity constant’ – is a useful index for comparing the relative rates of an enzyme acting on alternative, competing substrates. However, an alternative description, ‘catalytic efficiency’, is frequently used, and on occasions misused, to compare the reactivity of two enzymes acting on the same substrate. Here, we highlight the pitfalls in using $k_{\text{cat}}/K_M$ to compare the catalytic effectiveness of enzymes.

Introduction

The power provided by recombinant DNA techniques (e.g. site-directed mutagenesis and directed evolution) has enabled the production of enzymes ‘engineered’ to fit the requirements of technological applications. When considering the catalytic rate exhibited by a library of enzymes catalyzing the same reaction, the question arises as to which is the ‘better’ enzyme (often expressed by terms such as catalytic proficiency), and whether it is possible to describe relative catalytic effectiveness in quantitative terms. Such considerations also concern aspects of enzyme evolution, in the Darwinian sense.

Accepting that enzymes operate mostly under steady-state conditions, both in vivo and in vitro, the relevant kinetic parameters of an enzyme that are determined are $k_{\text{cat}}$ (the catalytic constant for the conversion of substrate to product) and $K_M$ (the Michaelis constant, which is defined, operationally, as the substrate concentration at which the initial rate is one-half of the maximum velocity). In fact, it is the ratio of these two parameters, $k_{\text{cat}}/K_M$, that is often used when comparing enzymes. In this article we explore this approach and suggest that, in general, when comparing enzymes catalyzing the same reaction, the use of $k_{\text{cat}}/K_M$ as a quantitative index of catalytic power is at best misleading and at worst invalid.

One enzyme, two substrates

The term $k_{\text{cat}}/K_M$ is often used as a specificity constant to compare the relative rates of reaction of each of a pair of substrates, when each is catalytically transformed by an enzyme. This is because, if $K_M$ is used on its own as the indicator of specificity, the effect of the ‘better’ substrate will be strongly manifested mainly at values of $[S]/K_M << 1$. As $[S]/K_M$ increases above this value, $k_{\text{cat}}$ becomes the parameter that best describes the better substrate. This dichotomy is resolved by using $k_{\text{cat}}/K_M$ as a specificity constant. In general, for an enzyme acting simultaneously on two substrates, $S_X$ and $S_Y$, at rates $v_x$ and $v_y$, Equation (1):

$$
v_x = \frac{k_{\text{cat}}^x[E][S_x]}{K_M^x(1 + \frac{[S_x]}{K_M^x}) + [S_x]}
$$

$$
v_y = \frac{k_{\text{cat}}^y[E][S_y]}{K_M^y(1 + \frac{[S_y]}{K_M^y}) + [S_y]}
$$

Fersht\textsuperscript{2} obtains the same relationship from a non-operational form of the Michaelis–Menten equation Equation (4):

$$
v = (k_{\text{cat}}/K_M)[E][S]
$$

where $[E]$ is free enzyme concentration. Equation 1 holds for any substrate concentration, and is particularly useful because the constituent parameters can be obtained from steady-state kinetic measurements using each substrate separately. It also has the attraction of reflecting the actual situation occurring in a biological system. Furthermore, in an in vitro biotransformation, for example, if $[S_X] = [S_Y]$ the $[S]$ terms in Equation 1 cancel, and the ratio of the $k_{\text{cat}}/K_M$ values for each substrate is equal to the relative rates at which the enzyme catalyzes the transformation of each substrate when both are present at equal concentrations with the enzyme in the same ‘pot’.

Comparing two enzymes

However, throughout the past two decades a regrettable extension of the use of $k_{\text{cat}}/K_M$ has been the increasingly prevalent use of this ratio as an index for comparing different enzymes. This is particularly commonplace in reports dealing with enzymes that are mutated (or engineered) with a view to altering the steady-state kinetic parameters to produce more powerful catalysts. When used in this context, $k_{\text{cat}}/K_M$ is variously called ‘catalytic
efficiency’, ‘catalytic potential’, ‘performance constant’ or some such term. Koshland [3] has addressed the problem of which term is the most appropriate to use in different circumstances.

Unfortunately, the use of $k_{\text{cat}}/K_M$ as a comparative index for the catalytic effectiveness of different enzymes suffers from a far more serious disadvantage than mere terminology. We show here that, as an index to describe which of two enzymes is the better catalyst when acting on the same substrate at a given concentration and at equal concentrations of active sites, it is as flawed as trying to describe substrate specificity solely in terms of $k_{\text{cat}}$ or $K_M$ values separately, as explained above.

This is because the ratio of rates obtained by two different enzymes having identical $k_{\text{cat}}/K_M$ values acting on the same substrate (at the same concentration of active sites) will depend on the ratio of $[S]/K_M$. Figure 1 shows the $k_{\text{cat}}/K_M$ surface for a series of reactions catalyzed by enzymes with constant $k_{\text{cat}}/K_M$ but varying values of $K_M$. Every point on the surface represents a rate obtained at different values of substrate concentration $[S]$ and $K_M$, at constant $k_{\text{cat}}/K_M$ as $K_M$ and/or $[S]$ is varied. Note that (perhaps counter-intuitively) the rate increases as $K_M$ increases for a given value of $[S]$. This is because, to maintain $k_{\text{cat}}/K_M$ constant, $k_{\text{cat}}$ also has to increase, and the change in $k_{\text{cat}}$ has a more profound effect on the velocity than a change in $K_M$.

A graphical illustration of the pitfalls when using $k_{\text{cat}}/K_M$ to compare two enzymes, A and B, is shown in Figure 2a, where the rate–substrate profiles cross at a particular substrate concentration $[S]_c$. The general equation for $[S]_c$, the substrate concentration at which the two reactions have the same rate at equal enzyme concentrations, is

$$[S]_c = \frac{K_M}{1 + (K_M/[S])}$$

The two curves in each figure were generated using the Michaelis–Menten equation for enzymes A and B, as described in the text. Values used are:

(a) $K_M^A > (k_{\text{cat}}^B/k_{\text{cat}}^A)K_M^B$
(b) $K_M^A < (k_{\text{cat}}^B/k_{\text{cat}}^A)K_M^B$
(c) $K_M^A = K_M^B$

Insets show the ratio of the rates of the two reactions ($v_A/v_B$) as a function of the substrate concentration. The dashed horizontal line in the inset for Figure 2a shows that the ratio switches from $>1$ to $<1$ at $[S] = [S]_c$, the substrate concentration at which the two reactions have the same rate at equal enzyme concentrations.
concentrations, is shown in Equation 5:

$$[S]_c = \frac{k_{cat}^a \cdot K_M^b - k_{cat}^b \cdot K_M^a}{k_{cat}^b - k_{cat}^a} \quad \text{[Equation 5]}$$

Equation 5 is derived from the Michaelis–Menten equations for enzymes A and B Equations (6, 7) by setting $E_0^a = E_0^b$, $v_a = v_b$, and solving for $[S]$.

$$v_a = k_{cat}^a E_0^a [S] / (K_M^a + [S]) \quad \text{[Equation 6]}$$

$$v_b = k_{cat}^b E_0^b [S] / (K_M^b + [S]) \quad \text{[Equation 7]}$$

In the case shown in Figure 2a, the ratio of rates ($v_b/v_a$) inverts at a particular substrate concentration, $[S]_c$, varying from <1 below $[S]_c$ to >1 above $[S]_c$, and the crossover occurs because the condition $K_M^a > (k_{cat}^b/k_{cat}^a)K_M^b$ is satisfied. The ratio of rates varies from $(k_{cat}^b/k_{cat}^a)/(k_{cat}^a/k_{cat}^b)$ at $[S]/K_M \approx 0$ to $k_{cat}^b/k_{cat}^a$ as $[S]/K_M$ approaches infinity (Figure 2a inset).

In Figure 2b, $K_M^b < (k_{cat}^b/k_{cat}^a)K_M^a$. The curves do not cross in physically meaningful space, but the ratio of rates is not a constant and, again, varies from $(k_{cat}^b/k_{cat}^a)/(k_{cat}^a/k_{cat}^b)$ at $[S]/K_M \approx 0$ to $k_{cat}^b/k_{cat}^a$ as $[S]/K_M$ approaches infinity (Figure 2b inset). Only for the special case shown in Figure 2c, where $K_M^a = K_M^b$, will the ratio of rates be constant at any value of $[S]$, and be equal to the ratio of the $k_{cat}/K_M$ values (Figure 2c inset). Note that in all the cases described, the ratios of the $k_{cat}/K_M$ values for the two enzymes being compared are identical.

**Recommended use of $k_{cat}/K_M$**

We have demonstrated that in a general case an enzyme having a higher catalytic efficiency (i.e. $k_{cat}/K_M$ value) can, at certain substrate concentrations, actually catalyze an identical reaction at lower rates than one having a lower catalytic efficiency. Even where the enzyme with the higher $k_{cat}/K_M$ catalyzes a reaction faster than one with a lower $k_{cat}/K_M$, the ratio of the two reaction rates is not a constant, but depends on the value of $[S]/K_M$ (except in the special case where the $K_M$ values of the two enzymes are identical). Thus, using $k_{cat}/K_M$ as an index for comparing the catalytic effectiveness of enzymes is not only incorrect, it is also misleading. Therefore, we recommend that the use of $k_{cat}/K_M$ as an index for comparing enzymes as catalysts, or as a generality without carefully specifying limitations, should be abandoned.

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