Opinion TRENDS in Biotechnology Vol.25 No.6

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

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The ratio k_{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_{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-sate conditions, both *in vivo* and *in vitro*, the relevant kinetic parameters of an enzyme that are determined are k_{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_{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_{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_{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_{cat} becomes the parameter that best describes the better substrate. This dichotomy is resolved by using k_{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):

$$\frac{v_x}{v_y} = \frac{(k^x_{cat}/K^x_M)\,[S_x]}{(k^y_{cat}/K^y_M)\,[S_y]} \qquad \text{[Equation 1]}$$

Cornish-Bowden [1] derives this relationship from the rate equations for the pair of reactions, where each substrate acts as a competitive inhibitor of the other and does so with a K_i value equal to the value of its K_M Equations (2,3):

$$v_x = \frac{k^x_{cat}E_0[S_x]}{K^x_M\left(1+\frac{[S_y]}{K^y_M}\right)+[S_x]} \qquad \text{[Equation 2]}$$

$$v_y = \frac{k^y_{cat}E_0[S_y]}{K^y_M\left(1+\frac{[S_x]}{K^x_M}\right)+[S_y]} \qquad \text{[Equation 3]}$$

Fersht [2] obtains the same relationship from a non-operational form of the Michaelis–Menten equation Equation (4):

$$v = (k_{cat}/K_M)[E][S] \qquad \text{[Equation 4]}$$

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_{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_{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_{cat}/K_M is variously called 'catalytic

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Available online 12 April 2007.

 0167-7799/$ – see front matter doi:10.1016/j.tibtech.2007.03.010

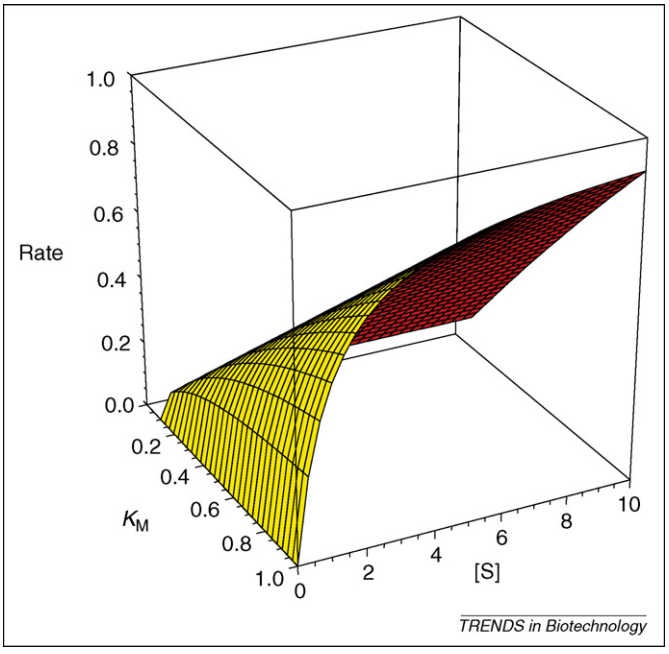

Figure 1. The k_{cat}/K_M surface showing the effect of altering K_M of an enzyme on the rate at varying substrate concentrations. All points on the surface have a constant k_{cat}/K_M value (=1). The surface was generated from the Michaelis–Menten equation in the form rate = $(k_{cat}/K_M)E_0[S]/(1+[S]/K_M)$ at $E_0 = 1$ (arbitrary units), setting rate as dependent and K_M and [S] as independent variables and varying K_M from 0.1–10, and [S] from 0–10 (arbitrary units).

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_{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_{cat} or K_M values separately, as explained above.

This is because the ratio of rates obtained by two different enzymes having identical k_{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_{cat}/K_M surface for a series of reactions catalyzed by enzymes with constant k_{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_{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_{cat}/K_M constant, k_{cat} also has to increase, and the change in k_{cat} has a more profound effect on the velocity than a change in K_M.

A graphical illustration of the pitfalls when using k_{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

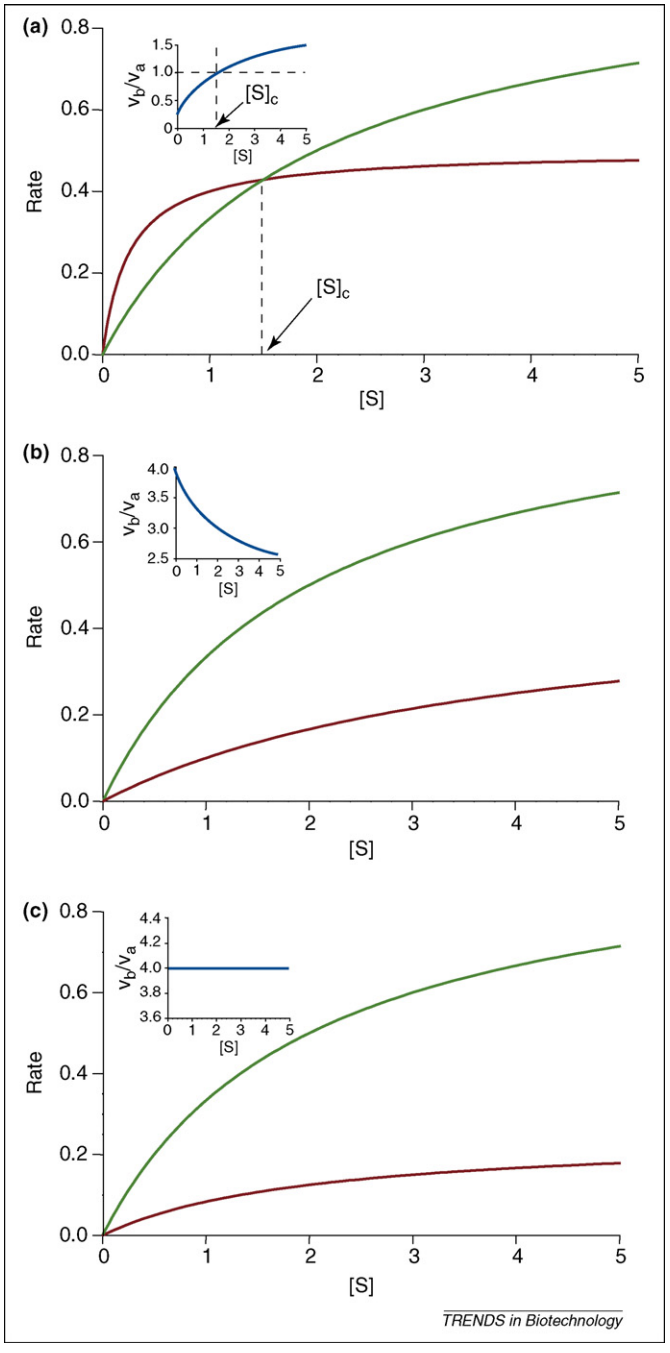

Figure 2. Velocity–substrate profiles for two enzymes, A (red) and B (green) with different k_{cat}/K_M values, where $E_0 = 1.0$ (arbitrary units):

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

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) $k_{cat}^a = 0.5, K_M^a = 0.25, k_{cat}^b = 1.0, K_M^b = 2.0; (k_{cat}^a/K_M^a)/(k_{cat}^b/K_M^b) = 4.0$
(b) $k_{cat}^a = 0.5, K_M^a = 4.0, k_{cat}^b = 1.0, K_M^b = 2.0; (k_{cat}^b/K_M^b)/(k_{cat}^a/K_M^a) = 4.0$
(c) $k_{cat}^a = 0.25, K_M^a = 2.0, k_{cat}^b = 1.0, K_M^b = 2.0; (k_{cat}^b/K_M^b)/(k_{cat}^a/K_M^a) = 4.0$

Insets show the ratio of the rates of the two reactions (v_b/v_a) 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:

$$[S]_c = \frac{k^a_{cat}.K^b_M - k^b_{cat}.K^a_M}{k^b_{cat} - k^a_{cat}} \quad \text{[Equation 5]}$$

Equation 5 is derived from the Michaelis–Menten equations for enzymes A and B Equations (6,7) by setting $E^a_0 = E^b_0$, $v_a = v_b$, and solving for [S].

$$v_a = k^a_{cat}E^a_0[S]/(K^a_M + [S]) \quad \text{[Equation 6]}$$

$$v_b = k^b_{cat}E^b_0[S]/(K^b_M + [S]) \quad \text{[Equation 7]}$$

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 $K^b_M > (k^b_{cat}/k^a_{cat})K^a_M$ is satisfied. The ratio of rates varies from $(k^b_{cat}/K^b_M)/(k^a_{cat}/K^a_M)$ at $[S]/K_M \approx 0$ to k^b_{cat}/k^a_{cat} as $[S]/K_M$ approaches infinity (Figure 2a inset).

In Figure 2b, $K^b_M < (k^b_{cat}/k^a_{cat})K^a_M$. The curves do not cross in physically meaningful space, but the ratio of rates is not a constant and, again, varies from $(k^b_{cat}/K^b_M)/(k^a_{cat}/K^a_M)$ at $[S]/K_M \approx 0$ to k^b_{cat}/k^a_{cat} as $[S]/K_M$ approaches infinity (Figure 2b inset). Only for the special case shown in Figure 2c, where $K^a_M = K^b_M$, 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.

Acknowledgements

We thank Athel Cornish-Bowden and Nick Price for helpful advice and discussions. Work in the authors' laboratory is supported by the Royal Society and the Biotechnology and Biological Sciences Research Council (UK).

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