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The Application and Usefulness of the Ratio k_{cat}/K_M

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The ratio of two important constituents in enzyme action, k_{cat} and K_M, has become of value and provides insight into enzymatic mechanisms and the functional effects of enzyme mutations. It is timely to examine how this ratio is used and where it can be effectively applied. It has been called on some occasions the "specificity constant" and on other occasions, the "performance constant," and it is of interest to examine which then is the most accurate and useful way to utilize this ratio.

INTRODUCTION

In Michaelis–Menten terms, where the enzymatic reaction is as shown in Eq. [1]

$$\begin{aligned} E + S &\rightleftarrows ES \\ ES &\rightarrow E + P \end{aligned} \qquad [1]$$

the constants k_{cat} and K_M are equal to k_2 and $(k_{-1} + k_2)/k_1$, respectively. Thus k_{cat}/K_M is $k_2k_1/(k_{-1} + k_2)$. Under these circumstances, it is clear that k_{cat}/K_M will equal (a) k_1 when $k_{-1} \ll k_2$ and (b) $k_2\, k_1/k_{-1}$ when $k_2 \ll k_{-1}$.

THE SPECIFICITY CONSTANT

The ratio k_2/K_M has been referred to as "the specificity constant" (*1*) because the relative velocities of two substrates in competition for a single enzyme (v_1/v_2) will be given by $([s_1]k_{cat1}/K_{M1})([s_2]K_{cat2}/k_{M2})$. However, the term "specificity constant" seems broad and can be deceptive. The specificity of an enzyme often is identified in textbooks with the differences between general chemical structures such as proteins vs carbohydrates or between stereoisomers, e.g., "proteases are specific for hydrolyzing peptide bonds or chymotrypsin is specific for l rather than *d* isomers" (*3*). One would expect that a parameter identified as "specificity constant" would provide a means of contrasting the specificities of different enzymes towards their substrates. That is not the case, however, for the ratio k_{cat}/K_M (a carbohydrate substrate, *S*, for a kinase could in fact have exactly the same k_{cat}/K_M as peptide substrate, (S_2), for a protease).

In fact, the use of the term specificity constant may have led some authors (*1*) to

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use it to evaluate mechanisms of specificity such as the induced fit theory, an analysis that went far astray. The induced fit theory was postulated in the era before crystallography to explain why some smaller substrates fail to react even though they would be expected to according to the template lock and key theory (*3*). A classic example involved the need to explain why H_20 was a poor substrate for hexokinase whereas glucose was a good substrate. The induced fit theory (*3a*) postulated that the good substrate (glucose) had to induce a conformational change to bring the catalytic groups into alignment whereas the poor substrate (H_20) could not. The X-ray evidence of Steitz (*4*) that glucose induced such a conformational change and H_20 did not was one of the first pieces of x-ray evidence that the induced fit theory was correct. The crystallography for many enzymes and the kinetic evidence is now so large that there is little doubt of its correctness. Using (k_{cat}/K_M) as a criterion of specificity led the authors to postulate that induced fit could not contribute to specificity when simple common sense and data like that for hexokinase clearly shows the opposite. The authors made other errors such as assuming that the conformations induced by both the poor substrate and the good substrate were the same (*1*) (an assumption contrary to any logic of induced fit and one that made their erroneous conclusions foreordained). My guess is that the use of the term "specificity constant" for (k_{cat}/K_M) led them to a series of incorrect assumptions that managed to hide the simple common sense error of their conclusion. (Conformational change can be part of k_{cat} and/or K_M, but k_{cat}/K_M does not evaluate either conformational change or the ability to discriminate between substrate structures.)

THE PERFORMANCE CONSTANT

In studies on the specificity of isocitrate dehydrogenase the k_{cat}/K_Ms of a series of modified malate derivations (isocitrate, malate, methyl malate, etc.) were compared and described as the "performance constants" (*2*). As described in the derivation above, k_{cat}/K_M will sometimes be equal to k_2K_a (where K_a is the association constant) when $k_2 \ll k_1$, which it was for isocitrate dehydrogenase. The association constant cannot always be assumed to be equal to the reciprocal of K_M but in any case $1/K_M$ is usually a rough indication of the affinity. Hence, k_{cat}/K_M will be a combination that will go up as the enzyme shows higher affinity for the substrate and higher catalytic rates. Thus, in general, the higher k_{cat}/K_M, the better the enzymatic performance, whether the change results from a mutation or a change of substrate.

For isocitrate dehydrogenase, Doyle *et al.* (5) studied the effect of mutations on k_{cat} and K_M separately. Sometimes a mutation raised k_{cat} (the catalytic power) and also decreased K_M (increasing the affinity of the enzyme), both changes that increased the performance of the enzyme. On other occasions, an improvement in k_{cat} was accompanied by a decrease in affinity. The separate analyses for k_{cat} and K_M are useful but the combined term k_{cat}/K_M would summarize the net advantage or disadvantage of a mutation to the enzyme in one constant. The term "performance constant" seems to be a more accurate and descriptive designation for this concept than the "specificity constant."

Another term, the "enzymatic proficiency," has been suggested by Wolfenden (*6*) and Lee and Houk (*7*) to relate the catalytic power of an enzyme to the rate of noncatalyzed reaction. Therefore it would seem more appropriate to use the term

"performance constant" for k_{cat}/K_M, and "enzymatic proficiency for $k_{enzyme}/k_{nonenzyme}$, and save the name "specificity constant" for some system, yet to be discovered, that can quantify differences in chemical structures so that the specificity gap between, for example, a protease and a carbohydrase can be quantified.

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