BIO102-Foundations of Biology (I)

Enzyme kinetics

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K_{cat}

The actual meaning of K_m depends on specific aspects of the reaction mechanism such as the number and relative rates of the individual steps.

For reactions with two steps, $\mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E + P}$ $\qquad K_\mathrm{m} = \frac{k_2 + k_{-1}}{k_1}$

When k_2 is rate-limiting, $k_2 << k_{-1}$ and K_m reduces to k_{-1}/k_1, which is defined as the dissociation constant (K_d) of the ES complex. Where these conditions hold, K_m does represent a measure of the affinity of the enzyme for its substrate in the ES complex.

Sometimes $k_2 >> k_{-1}$, and then $K_m = k_2/k_1$. In other cases, k_2 and k_{-1} are comparable and K_m remains a more complex function of all three rate constants. The Michaelis-Menten equation and the characteristic saturation behavior of the enzyme still apply, but K_m cannot be considered a simple measure of substrate affinity.

K_{cat}

If an enzyme reacts by the two-step Michaelis-Menten mechanism, $V_{max} = k_2[E_t]$, where k_2 is rate-limiting. However, the number of reaction steps and the identity of the rate-limiting step(s) can vary from enzyme to enzyme.

For example, consider $\mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{EP} \overset{k_3}{\rightleftharpoons} \mathrm{E + P}$

In this case, EP → E + P is rate-limiting step, most of the enzyme is in the EP form at saturation, and $V_{max} = k_3[E_t]$.

Therefore, a more general rate constant, k_{cat}, is defined to describe the limiting rate of any enzyme-catalyzed reaction at saturation.

For the simple reaction two steps reactions, $\mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_2}{\rightleftharpoons} \mathrm{E + P}$, $k_{cat} = k_2$.

For the reaction $\mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{EP} \overset{k_3}{\rightleftharpoons} \mathrm{E + P}$, $k_{cat} = k_3$.

When several steps are partially rate-limiting, k_{cat} can become a complex function of several of the rate constants that define each individual reaction step.

Turnover number

In the Michaelis-Menten equation, $k_{cat} = V_{max}/[E_t]$, and equation becomes $V_0 = \frac{k_{\mathrm{cat}}[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$

The constant k_{cat} here is a first-order rate constant and hence has units of reciprocal time. It is also called the turnover number.

It is equivalent to the number of substrate molecules converted to product in a given unit of time on a single enzyme molecule when the enzyme is saturated with substrate.

Comparing Catalytic Mechanisms and Efficiencies

The parameters k_{cat} and K_m allow us to evaluate the kinetic efficiency of enzymes, but either parameter alone is insufficient for this task.

Two enzymes catalyzing different reactions may have the same k_{cat} (turnover number), yet the rates of the uncatalyzed reactions may be different and thus the rate enhancements brought about by the enzymes may differ greatly.

Experimentally, the K_m for an enzyme tends to be similar to the cellular concentration of its substrate. An enzyme that acts on a substrate present at a very low concentration in the cell usually has a lower K_m than an enzyme that acts on a substrate that is more abundant.

Comparing Catalytic Mechanisms and Efficiencies

To compare the catalytic efficiencies of different enzymes or the turnover of different substrates by the same enzyme we compare the ratio k_{cat}/K_m for the two reactions. This parameter, called the specificity constant, is the rate constant for the conversion of E + S → E + P.

When [S] << K_m, the equation $V_0 = \frac{k_{cat}[E_t][S]}{K_m + [S]}$ reduces to the form $V_0 = \frac{k_{cat}}{K_m}[E_t][S]$

V_0 in this case depends on the concentration of two reactants, $[E_t]$ and [S]; Therefore, this is a second-order rate equation and the constant k_{cat}/K_m is a second-order rate constant with units of $M^{-1}s^{-1}$. There is an upper limit to k_{cat}/K_m , imposed by the rate at which E and S can diffuse together in an aqueous solution. This diffusion controlled limit is 10^8 to 10^9 $M^{-1}s^{-1}$, and many enzymes have a k_{cat}/K_m near this range. Such enzymes are said to have achieved catalytic perfection.

Problems to solve

1. Which of the following effects would be brought about by any enzyme catalyzing the simple reaction

$$S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P \quad \text{where} \quad K'_{eq} = \frac{[P]}{[S]}?$$

(a) Decreased K_{eq} (b)Increased k_1

(c) Increased K_{eq} (d) Increased $\Delta G^{\ddagger}$

(e) Decreased $\Delta G^{\ddagger}$ (f) More negative $\Delta G'^{o}$

(g) Increased k_2

(b), (e), (g). Enzymes do not change a reaction's equilibrium constant and thus catalyze the reaction in both directions, making (b) and (g) correct. Enzymes increase the rate of a reaction by lowering the activation energy, hence (e) is correct.

Problems to solve

2. The enzyme urease enhances the rate of urea hydrolysis at pH 8.0 and 20°C by a factor of 10^{14}. If a given quantity of urease can completely hydrolyze a given quantity of urea in 5.0 min at 20°C and pH 8.0, how long would it take for this amount of urea to be hydrolyzed under the same conditions in the absence of urease? Assume that both reactions take place in sterile systems so that bacteria cannot attack the urea.

Time to hydrolyze urea

$$= \frac{(5.0 \text{ min})(10^{14})}{(60 \text{ min/hr})(24 \text{ hr/day})(365 \text{ days/yr})}$$

$$= 9.5 \times 10^{8} \text{ yr}$$

$$= 950 \text{ million years!}$$

Problems to solve

3. Determine the fraction of V_{max} that would be obtained at the following substrate concentrations [S]: ½K_m and 10K_m.

The Michaelis-Menten equation can be rearranged to

$$V_0/V_{max} = [S]/(K_m + [S])$$

Substituting $[S] = \frac{1}{2} K_m$ into the equation gives

$$V_0/V_{max} = 0.5\, K_m/1.5K_m = 0.33$$

And substituting $[S] = 10K_m$ gives

$$V_0/V_{max} = 0.91$$

Problems to solve

4. Neutral sphingomyelinase is an enzyme that converts sphingomyelin into ceramide and phosphocholine. A student studying the kinetics of this enzyme added 3 X 10-5 M of sphingomyelin to the reaction and observed an initial velocity of 6.0 µM/min. Calculate the KM for this reaction, provided the Vmax is 35 µM/min.

$[S] = 3 \times 10^{-5}$ M

$V_0 = 6.0$ µM/min

$V_{max} = 35$ µM/min

$V_0 = V_{max}\,[S] / (K_M + [S])$

$6 = (35\ \mu M/min \times 30\ \mu M) / (K_M + 30\ \mu M)$

$K_M = (1050 - 180)/6 = 145\ \mu M$