Introduction to Enzyme kinetics

Why study kinetics?

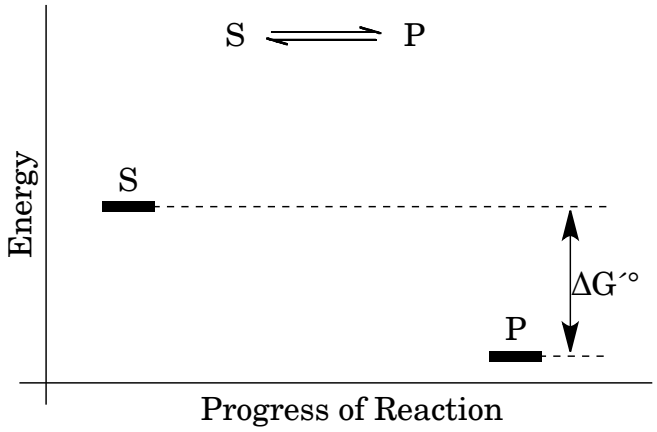
Kinetic information is useful for examining possible **mechanisms for the reaction**. This is true for all types of reactions; kinetic principles are used to understand both catalyzed and non-catalyzed reactions.

For enzymes, kinetic information is useful for understanding **how metabolism is regulated** and how it will occur under different conditions. For enzymes, kinetic information is useful for understanding **pathological states**. Diseases and disorders often involve alterations in enzymes or enzyme activities. Understanding the way that enzymes work is critical for understanding how drugs work, because **many drugs function by interacting with enzymes**. In addition, the more you know about an enzymatic reaction, the more information you will have for designing new drugs.

Finally, essentially all of biochemistry is based on enzymes. It is nearly impossible to understand biochemistry without understanding enzymes, and it is impossible to understand enzymes without understanding the kinetic principles of the reactions they mediate.

Thermodynamic considerations and transition state theory

Consider a simple system involving the conversion of one molecule, S into another molecule, P. The molecules S and P have different energies.



For this system, as for any other, $\Delta G'^\circ = -RT \ln K_{eq}$.

$\Delta G'^\circ$ is a measure of the relative energies of the two molecules, and thus gives the direction for the reaction when the concentrations of the two molecules are equal. For reactions involving multiple substrates and products, $\Delta G'^\circ$ gives the direction in which the reaction will proceed under standard conditions (*i.e.* when the concentration of all participating species is one molar, except protons and water: protons and water are exceptions, because $\Delta G'^\circ$ refers to pH 7.0, and includes the high concentration of water in the term as a constant).

Thermodynamics determines which direction is preferred; however, standard thermodynamics yields **no information** about the **rate** of the reaction. Because the tools and concepts of thermodynamics are very powerful, they were extended to allow an understanding of why reactions occur with different rates.

If a maximum energy state exists **intermediate** between S and P, and if this state is assumed to be in equilibrium with S and P, it is possible to apply the concepts of thermodynamics to the reaction process. This transient high energy intermediate is usually called a **transition state**, and will be abbreviated $X^{\ddagger}$ (the "‡" symbol is typically used to designate the transition state). The extension of thermodynamics to consider rates of reaction is called **transition state theory** to emphasize the importance of this concept.

$$S \rightleftharpoons X^{\ddagger} \rightleftharpoons P$$

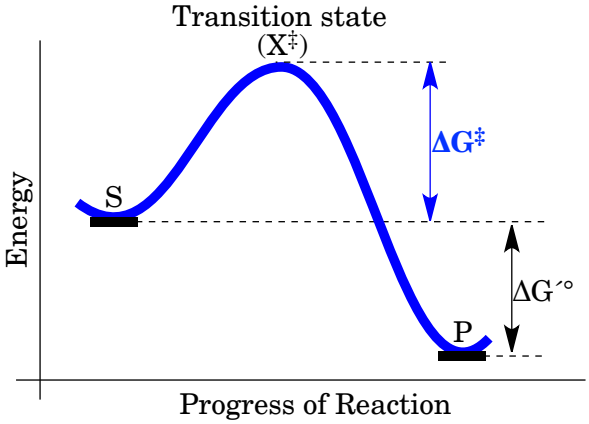
The equilibrium constant for the process of S going to the transition state is:

$$K_{eq}{}^{\ddagger} = \frac{[X^{\ddagger}]}{[S]}$$

(Note that "concentration of S" is usually abbreviated as "[S]"). This allows the calculation of the ΔG for the transition state.

$$\Delta G^{\ddagger} = -RT \ln K_{eq}{}^{\ddagger}$$

The $\Delta G^{\ddagger}$ is the free energy difference between S and the transition state. (Note that there is also a separate $\Delta G^{\ddagger}$ (in this case, a larger one) between P and the transition state). $\Delta G^{\ddagger}$ is often referred to as the activation energy; it is the energy that molecules of S must have in order to form molecules of P.



The **rate of a chemical reaction depends on the $\Delta G^{\ddagger}$ for the reaction**. Using the principles of transition state theory, J.H. van't Hoff and later Svante Arrhenius

derived an equation for the reaction rate constant:

$$k = Ae^{\frac{-\Delta G^{\ddagger}}{RT}}$$

In this, the **Arrhenius equation**, k is the rate constant for the reaction, and A is the Arrhenius constant for the reaction. The Arrhenius constant for a reaction is a measurable quantity. Transition state theory predicts that $A = k_B T/h$, where k_B is the Boltzmann constant (1.380649×10^{-23} J/K), T is absolute temperature, and h is Planck's constant (6.6261×10^{-34} J•sec); for real reactions the measured value of A tends to vary from this theoretical value.

The Arrhenius equation states that, if the $\Delta G^{\ddagger}$ decreases, the rate of the reaction will increase (note that it also states that the rate of the reaction will increase with increasing temperature; changing temperatures is rarely possible for biological processes, but control of temperature can be important for *in vitro* experiments).

Based on transition state theory, the rate of a reaction is dependent on the energy difference between the initial state and the highest energy transient state along the reaction pathway. Assuming that this is true, rate enhancements by enzymes must be mediated at least in part by a decrease in the energy of the highest energy transition state, although in some reactions, an enzyme may function in part by destabilizing the state that immediately precedes the transition state.

A decrease in the energy of the highest energy transition state can be accomplished in one of two main ways. Some enzymes use one of these ways, and some use both.
1) The **enzyme stabilizes the transition state**. The same transition state that would normally be present in the reaction pathway is also present in the enzyme-catalyzed pathway, but in the enzyme-catalyzed pathway, this state has a lower energy.
2) The **enzyme allows a different pathway** for the process. Without the enzyme, the reaction might proceed by some pathway. The enzyme allows a different series of reactions to occur that would otherwise be either of much higher energy or impossible. In the enzyme-catalyzed process, the highest energy transition state (which does not exist in the non-catalyzed process) has a lower energy than the energy of the transition state for the non-catalyzed process.

Let us consider the first of these possibilities. Consider a reaction in which S is converted to P, either as a simple chemical reaction or as an enzyme-catalyzed process. Instead of merely stating S→P, for transition state theory we need to add an additional term:

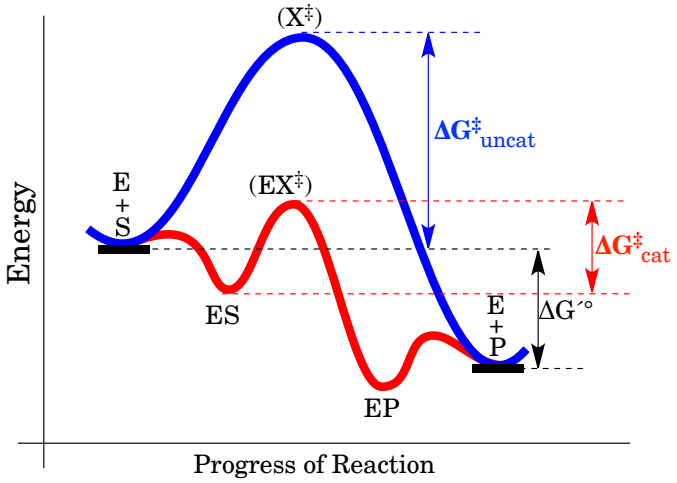
$$S \underset{}{\overset{k_{uncat}}{\rightleftharpoons}} X^{\ddagger} \rightleftharpoons P$$

$$E + S \rightleftharpoons ES \overset{k_{cat}}{\rightleftharpoons} EX^{\ddagger} \rightleftharpoons EP \rightleftharpoons E + P$$

In each case, the reaction pathway passes through an identical transition state, $X^{\ddagger}$ (in the uncatalyzed pathway, this species is not bound to the enzyme, but it is the same species in both cases). In each case in the scheme above, the rate constant

shown is the one for the slowest step in the forward direction reaction.

In the reaction diagram, we can show the binding of the substrate and dissociation of the product from the enzyme explicitly. Note that the ES complex is lower in energy than the free S, which is why this complex forms spontaneously.



Using the principles of transition state theory, we can derive an equation for the rate enhancement mediated by the enzyme. Recall that the rate constant is related to the difference in energy of between the initial state and the transition state.

Arrhenius equation: $$k = Ae^{\frac{-\Delta G^{\ddagger}}{RT}}$$

If we divide the rate constant for the catalyzed reaction by that of the uncatalyzed reaction:

$$\frac{k_{cat}}{k_{uncat}} = \frac{Ae^{\frac{-\Delta G^{\ddagger}_{cat}}{RT}}}{Ae^{\frac{-\Delta G^{\ddagger}_{uncat}}{RT}}}$$

which simplifies to:

$$\frac{k_{cat}}{k_{uncat}} = e^{\frac{\Delta G^{\ddagger}_{uncat} - \Delta G^{\ddagger}_{cat}}{RT}}$$

Recall that K_{eq} and $\Delta G°$ are related. Rearranging the standard equation for $\Delta G°$ to solve for K_{eq}, we obtain:

$$K_{eq} = e^{\frac{-\Delta G°}{RT}}$$

Using this, we see that:

$$\frac{k_{cat}}{k_{uncat}} = e^{\frac{\Delta G^{\ddagger}_{uncat} - \Delta G^{\ddagger}_{cat}}{RT}} = \frac{K^{\ddagger}_{eq}}{K_{eq}}$$

Thus, the ratio of the rate constant for the catalyzed reaction to that of the uncatalyzed reaction is equal to the ratio of the equilibrium constant for the binding of the enzyme to the transition state to that for the binding to the substrate.

$$E + S \overset{K_{eq}}{\rightleftharpoons} ES \qquad\qquad E + X^{\ddagger} \overset{K^{\ddagger}_{eq}}{\rightleftharpoons} EX^{\ddagger}$$

This deserves a few comments. The first is that while neither the $X^{\ddagger}$ nor the $EX^{\ddagger}$ are present in significant concentrations due to the transient nature of these species, it is, at least in principle, possible for the $X^{\ddagger}$ to bind to the free enzyme, or for the $EX^{\ddagger}$ complex to dissociate. The second is more important: the **interaction between the enzyme and the transition state exhibits much higher affinity than the interaction between the enzyme and the substrate**. The magnitude of this difference in affinity is proportional to the magnitude of the increase in rate constant.

The function of a catalyst is to lower the energy of the transition state for the reaction: in other words, enzymes change $\Delta G^{\ddagger}$. The most common method for allowing this is for ***the enzyme to bind the transition state more tightly than the substrate***. The additional energy obtained from this binding stabilizes the transition state and therefore accelerates the reaction. Note that enzymes do not change the energy of *S* and *P* (because the free *S* and *P* are not bound to the enzyme). As a result, **enzymes do not alter the $\Delta G'^{\circ}$ for the reaction**, but instead **only alter the rate** of the reaction, usually by altering $\Delta G^{\ddagger}$.

Chemical kinetics

Zeroth-order kinetics

Some reactions occur at rates that are independent of reactant concentration. In these cases, the rate of the reaction (often called the **velocity** of the reaction) is constant, regardless of the concentration of the participating compounds.

Consider a simple reaction: $S \rightleftharpoons P$

For this reaction the velocity of the reaction can be expressed as:

$$v = \frac{d[P]}{dt} = \frac{-d[S]}{dt} = k$$

These equations state that at any given instant, the velocity is equal to the *increase* in concentration of *P* divided by the time interval, and that the velocity is also equal to the *decrease* in concentration of *S* divided by the time interval (note the minus sign in the $-d[S]/dt$ expression). In this case, because the rate is independent of [*S*] and [*P*], the velocity = *k*, where *k* is the rate constant for the reaction. These (somewhat unusual) reactions are called **zeroth-order** reactions.

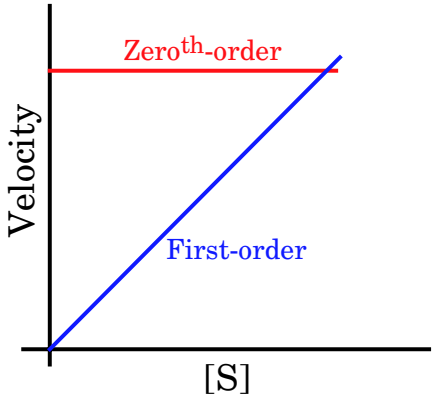
First-order kinetics

Again, consider a simple reaction, but now a **first-order reaction** in which the rate depends on the reactant concentration:

$$S \rightleftharpoons P \qquad\qquad v = \frac{d[P]}{dt} = \frac{-d[S]}{dt}$$

If you assume that the reverse reaction does not occur (which is a valid assumption if the initial concentration of P is zero), then **at any given time**, $v = k\,[S]$. For zeroth-order and first-order reactions, k is the rate constant for the reaction; k is a measure of how rapidly the reaction will occur at any concentration of S, and has the units of time^{-1} (usually seconds^{-1} or minutes^{-1}). In a first-order reaction, the rate depends on the concentration of a single species (in this case, the reactant S).

The equation $v = k\,[S]$ states that velocity is a linear function of S concentration. In the graph (below) the zeroth-order reaction has a constant velocity regardless of the concentration of S, while the first-order reaction velocity increases linearly with increasing S concentration.



Another way to look at the course of these types of reactions is to consider the concentration of S as a function of time. For a zeroth-order reaction:

$$[S]_t = [S]_0 - kt$$

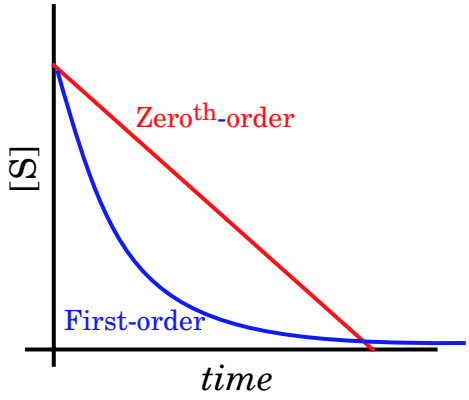
while for a first-order reaction:

$$[S]_t = [S]_0 e^{kt}$$

where $[S]_0$ is the concentration of S at the beginning of the reaction, and $[S]_t$ is the concentration of S at time t.

Plotting these equations reveals that S concentration decreases linearly with time for a zeroth-order reaction, and that S concentration decreases exponentially with time for a first-order reaction. These observations make sense: for a first-order reaction, the rate of the reaction decreases as S is used up, because the rate

depends on the S concentration.



(Note: the following, slightly simplified, derivation of the equation for the first-order reaction is included for completeness.)

The rate equation: $\frac{-d[S]}{dt} = k[S]$ rearranges to: $\frac{d[S]}{[S]} = -kdt$

Integrating both sides: $\int_{[S]_0}^{[S]} \frac{d[S]}{[S]} = \int_0^t -k\,dt$ gives: $\ln[S] - \ln[S]_0 = -kt$

Raising both sides to the ***e*** power and rearranging gives: $[S]_t = [S]_0 - kt$

Second-order kinetics

More complicated reactions can also occur: $S + R \rightleftharpoons P + Q$

For these reactions:

$$v = \frac{d[\mathrm{P}]}{dt} = \frac{d[\mathrm{Q}]}{dt} = \frac{-d[\mathrm{S}]}{dt} = \frac{-d[\mathrm{R}]}{dt}$$

and

$$v = k[\mathrm{S}][\mathrm{R}]$$

Reactions of this type are **second-order**, and k is a second-order rate constant, because the rate of the reaction depends on the product of [S] and [R]. If the reaction involved the collision of two molecules of S, the velocity equation would be:

$$v = k[\mathrm{S}][\mathrm{S}] = k[\mathrm{S}]^2$$

The order of the reaction comes from the exponent that describes the number of reactants. **Second-order rate constants have units of $\mathrm{M^{-1} \cdot sec^{-1}}$.**

Real chemical reactions rarely have more than two molecules interacting at one time, because the simultaneous collision of more than two molecules is unlikely.

(Note: there are a few examples of trimolecular reactions; in most reactions that appear to involve more than two reactants, two reactants form an intermediate, which then reacts with the other compound to form the final product.)

Pseudo-first-order kinetics

Studying second-order reactions is usually more difficult than studying first-order reactions. One way around this difficulty is to create "pseudo-first-order conditions". These are conditions in which the concentration of one compound is very high. If the concentration of R is very high compared to S, then the concentration of R will essentially be constant during the reaction. This allows the equation to be re-written:

$$v = k[\mathrm{S}][\mathrm{R}] = k_{\mathrm{pseudo}}[\mathrm{S}]$$

The term for concentration of R did not disappear; because the concentration of R is approximately a constant, it was merely incorporated into the k_{pseudo} first-order rate constant.

Rate-limiting steps

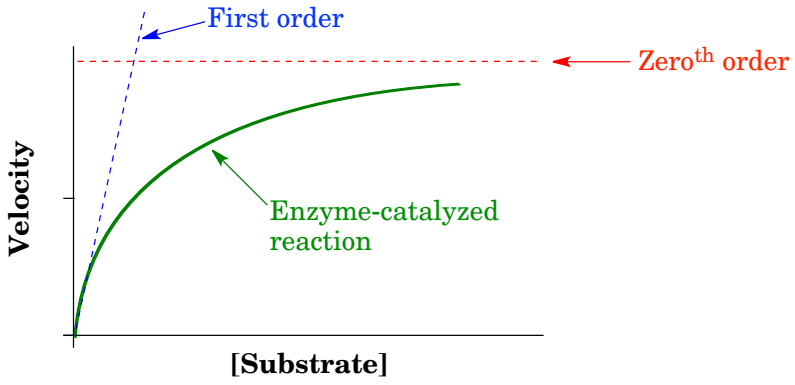
Many reaction pathways involve multiple steps. In most cases, one step will be appreciably slower than the others. This step is the **rate-limiting step**; it is the step upon which the rate of the overall reaction depends. Analysis for rate-limiting steps is important for understanding all types of reactions. (In biochemistry, analysis for rate-limiting steps in metabolic pathways is especially important, because these steps tend to be the ones that act as regulated control points for the pathway.)

Michaelis-Menten kinetics

Initially, it was assumed that a simple enzyme catalyzed reaction, such as S→P would be a first-order reaction:

$$\mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{P}$$

However, when the reaction data were analyzed, instead of a linear plot of velocity *versus* [S], the experiment yielded a hyperbolic curve.



Analysis of the data suggested that the reaction was first-order at *very* low concentrations of S (*i.e.* $v \approx k[S]$, where k is a rate constant appropriate for the conditions of the enzymatic reaction), and that as [S] increased, the reaction became progressively closer to zeroth-order (*i.e.* $v \approx k[E] = k_{cat}[E]$, where k_{cat} is the catalytic rate constant for the enzyme, and [E] is the enzyme concentration).

To explain this phenomenon, it was necessary to hypothesize the formation of a complex between the enzyme and S. This hypothesis means that, even for the simple conversion of S to P, the reaction was actually somewhat more complicated than the expected first-order process:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

The velocity for the reaction is then:

$$v = \frac{d[P]}{dt} = k_2[ES]$$

Note that this appears to be a first-order rate equation; however, the concentration of ES is not a linear function of [S]. Instead, the change in [ES] concentration as a function of time is more complex:

$$\frac{d[ES]}{dt} = k_1[E]_{free}[S] - k_{-1}[ES] - k_2[ES]$$

Note that the above equation consists of the rate of formation of the ES complex ($k_1[E]_{free}[S]$) minus the rates at which the ES complex disappears (both $k_{-1}[ES]$ and $k_2[ES]$ describe rates of ES complex disappearance). This equation is rather difficult to use unless some simplifying assumptions are made. This is true for several reasons; the most obvious reason is that neither [ES] nor $[E]_{free}$ (the concentration of enzyme unbound to S) can be calculated (or measured) accurately, because neither one is an independent variable.

Steady state assumption

Most enzyme kinetics derivations depend on several assumptions:

Assumption (1): $\frac{d[E]}{dt} = 0$

The total amount of enzyme does not change during measurement of the reaction. This is usually a good assumption, because enzymes are catalysts that are not consumed during the reaction.

Assumption (2): $k_2 << k_1$ and $k_2 << k_{-1}$

The rate constant for the reaction, k_2, is much smaller than the rate constants for

ES formation, k_1 and for ES dissociation, k_{-1}. In other words, **the rate-limiting step for the reaction is the actual catalytic step**, while the ES complex rapidly reaches an equilibrium concentration.

Assumption (3): $[E]_{total} << [S]$

The total concentration of enzyme is much less than the total concentration of S. Consequently, [S] remains approximately constant during the reaction. This assumption is very similar to that used in pseudo-first-order kinetics experiments, but it is a somewhat flawed assumption, because if [S] were not changing, no product would be formed.

Assumption (4) $[P]_{t=0} = 0$

The initial concentration of P is zero. This is implicit in the rate equation given above, because there is no term for formation of ES from P. In experimental systems, this assumption is usually valid, because the product can be omitted from the reaction vessel.

Leonor Michaelis and Maud Menten, and later G.E. Briggs and James B.S. Haldane made these assumptions in slightly different ways (the Briggs-Haldane assumptions probably more closely model reality, but because both methods result in the same equation, the majority of textbooks refer to Michaelis and Menten as the originators of the concepts). Using these assumptions, it is possible to derive equations that describe the behavior of enzyme-catalyzed reactions.

If the ES complex forms rapidly, and ***if*** the concentration of S does not change significantly during the reaction, then, for much of the reaction, the concentration of the ES complex will be constant. Mathematically, this concept is expressed as:

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}]_{free}[\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}] = 0$$

The equation above describes **steady state conditions**, and is critical to enzyme kinetics. (Note that the steady state assumption is an *approximation*; it is rarely absolutely correct, unless $k_2 = 0$ or in situations where the substrate is replenished and the product removed during the reaction.) The term "steady state" refers to the fact that, while the concentration of ES is constant, it is continuously being both formed and disrupted. Thus, substrate is flowing, via the enzyme, in a steady stream to form product.

Assuming that the amount of enzyme does not change during the reaction, the total enzyme concentration $[\mathrm{E}]_{total} = [\mathrm{E}]_{free} + [\mathrm{ES}]$. Using this observation and the steady state equation, Michaelis and Menten derived an equation for velocity as a function of [S]. This important equation is known as the **Michaelis-Menten equation**.

$$v = \frac{V_{max}[\mathrm{S}]}{\mathrm{K}_m + [\mathrm{S}]}$$

(Note: the derivation of the Michaelis-Menten equation is given in nearly all biochemistry textbooks, and it is unnecessary to reproduce it here.)

The Michaelis-Menten equation is applicable to most enzymes, and is critically important to understanding enzyme action in biological systems. At least for simple systems, the Michaelis-Menten equation describes the way that the reaction velocity depends on the substrate concentration.

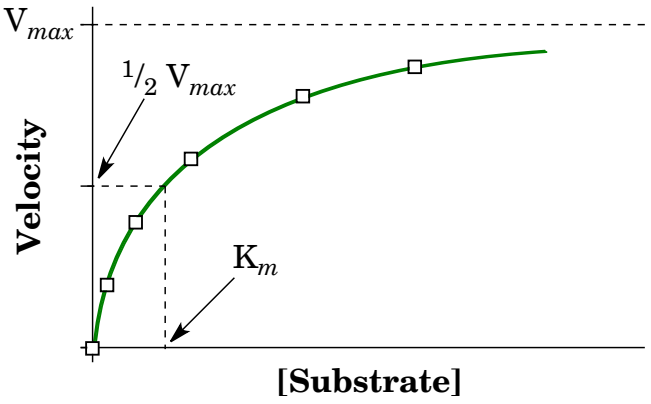
The parameters K_m and V_{max} cannot be determined from a single measurement; instead, they must be determined by measuring velocity at a variety of [S].

The meaning of V_{max} and K_m

V_{max} is the velocity observed when all of the enzyme present is fully saturated with substrate; in other words, when [ES] = $[E]_{total}$. This is only completely true if the concentration of S is infinitely high (which is obviously impossible in the real world).

For the simple reaction we have been discussing, $V_{max} = k_2[E]_{total}$. For more complex reactions, $V_{max} = k_{cat}[E]_{total}$, where k_{cat} is the rate constant for the slowest step of the reaction. Note that the ***V_{max} is not an intrinsic property of the enzyme***, because it is dependent on the enzyme concentration; the actual intrinsic property is the k_{cat}.

The Michaelis-Menten equation, and the definition of V_{max} have one major consequence for biological systems: the **velocity is directly proportional to the enzyme concentration**. This means that **one simple method for increasing the velocity is to synthesize more enzyme molecules**. Increased enzyme concentrations result in higher velocities at any substrate concentration.



In contrast to V_{max}, the parameter ***K_m is an intrinsic parameter of the enzyme***. When properly performed, measurements of K_m yield constant results, regardless of enzyme concentration. Note that if [S] = K_m, the Michaelis-Menten equation reduces to $v = {}^1/_2\ V_{max}$. Therefore, K_m is a measure of the ability of the substrate to interact with the enzyme. Altering K_m (either by having multiple isozymes with

different K_m values, or by having an enzyme with a K_m that can be regulated), also allows alteration in the velocity of a reaction.

Another, related, way of looking at K_m is to compare it to K_d, the equilibrium dissociation constant for formation of ES complex. The dissociation constant is a measure of affinity, with higher values indicating lower affinity.

$$K_m = \frac{k_{-1} + k_2}{k_1} \qquad\qquad K_d = \frac{k_{-1}}{k_1}$$

If $k_2 = 0$, then $K_m = K_d$. Because, for most enzymes, k_2 is relatively small compared to k_{-1}, the K_m value is often close to the K_d value.

Note that k_1 is a second-order rate constant, and has units of $M^{-1} \cdot sec^{-1}$. The other rate constants are first-order, and have units of sec^{-1}. This means that K_m has units of M; in other words, both K_m and K_d are expressed in concentration units.

Contemplation of the Michaelis-Menten equation suggests that a **low K_m means a high affinity**, and therefore, for a given substrate concentration, a high velocity. In contrast, a high K_m means low affinity, and therefore low velocity at any [S].

Uses of K_m

K_m can act as a measure of several useful properties of enzymes.

1) Measurement of K_m is used to determine the **substrate preferences** of an enzyme. If more than one endogenous compound can act as a substrate for an enzyme, the substrate with the lowest K_m is probably the preferred physiological substrate.

2) Measurement of K_m is used to **distinguish isozymes**. Isozymes often have different affinities for the same substrate.

3) Measurement of K_m is used to check for **abnormalities** in an enzyme: An altered K_m reflects some change in the way the enzyme binds the substrate. K_m is therefore sensitive to modifications to the enzyme; measurement of K_m can often reveal extremely useful information regarding mutations or other changes in the structure of an enzyme.

Determining V_{max} and K_m

A cursory examination of a velocity *versus* [S] plot (such as the one above) may suggest that the graph could be used to determine K_m and V_{max}. In practice, for any type of plot, accurately determining the values from a curve is difficult; for enzyme kinetics, it is especially difficult, because achieving $v = V_{max}$ is impossible, and because nothing about the curve states that: "the K_m is right *here*!"

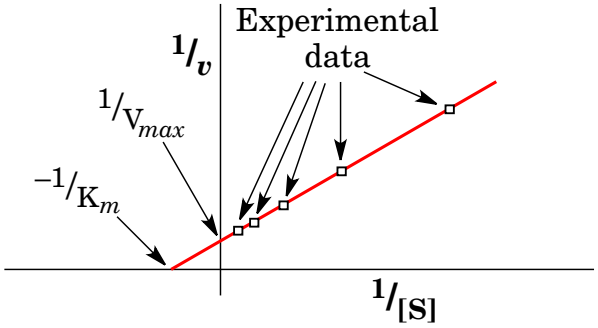
To avoid this problem, several scientists derived linear forms of the Michaelis-Menten equation. Hans Lineweaver and Dean Burk were the first to do one of these

derivations, and developed the double reciprocal plot (also called the Lineweaver-Burk plot) in 1934. This plot has its deficiencies, but it is still useful, and all biochemists must be able to understand the information it presents.

The equations below reveal that a plot of 1/v *versus* 1/[S] has a slope of K_m/V_{max} and a *y*-intercept of $1/V_{max}$. Further examination shows that *x*-intercept = $-1/K_m$.

$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

$$y = m \quad x \quad + \quad b$$



The Lineweaver-Burk plot is very useful for illustrative purposes, but tends to yield aberrant values of K_m and V_{max} unless the *v versus* [S] data fit the hyperbolic curve closely; in other words, for real-world data containing experimental errors, the **Lineweaver-Burk plot is frequently inaccurate**.

When the Lineweaver-Burk plot was invented, computers effectively did not exist, and linearization of the Michaelis-Menten equation allowed for a simple analysis of the data. These linearization routines remain popular because most calculators and all graphing software packages are capable of calculating a least-squares linear regression analysis exactly. In contrast, most non-linear equations cannot be solved exactly. However, current computer technology allows for rapid iteration and optimization of the K_m and V_{max} parameters that best fit a set of data. The Excel Solver package and many other optimization routines are available to allow analysis of the non-linear Michaelis-Menten equation. For analysis of actual experimental *v versus* [S] data, non-linear regression methods that fit the data to the unmodified Michaelis-Menten equation are ***far*** more likely to yield satisfactory values for K_m and V_{max}.

How fast is fast?

The collision of molecules is limited by diffusion. Diffusion limits[2] for proteins in aqueous solution are about 10^9 $M^{-1} \cdot sec^{-1}$. This is a measure of the maximum

[2] Diffusion depends on the viscosity of the medium and the size of the molecules involved. Because both of these terms vary, the maximum rate of diffusion-based collisions depends both on the specific enzyme-substrate pair, and on the properties of the solution in which the measurements are performed. The value of ~10^9 $M^{-1} \cdot sec^{-1}$ is based on average values for these parameters.

possible rate for any reaction involving proteins in aqueous solution. (Note that the diffusion limit is a second-order rate constant; it has a concentration term because collision between molecules is concentration dependent.)

k_{cat} is a term used for the rate constant for the overall reaction. In complex reactions with several steps, where the maximal catalytic rate depends on several rate constants, k_{cat} is the rate constant for the rate-limiting step.

k_{cat} = **turnover number** = the number of product molecules formed by one enzyme molecule in one second (or, for slow enzymes, in one minute).

k_{cat}/K_m is a measure of the catalytic efficiency of an enzyme; in effect, it takes into account both substrate binding and conversion to product. k_{cat}/K_m cannot be faster than the diffusion limit (E and S must collide in order to react).

Some enzymes operate at efficiencies approaching the diffusion limit.

Enzyme[3]	K_m **(M)**	k_{cat} **(sec^{-1})**	k_{cat}/K_m **(M^{-1}•sec^{-1})**
Acetylcholinesterase	1×10^{-4}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
Catalase	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Superoxide dismutase	3.6×10^{-4}	1.0×10^{6}	1.8×10^{9}
Ferredoxin reductase	1.0×10^{-7}	1.0×10^{1}	1.0×10^{8}

Specific activity is a term used when the molar enzyme concentration is not known. (If the moles of enzyme present is unknown, it is impossible to calculate k_{cat}.) Specific activity is similar to turnover number, but is usually given in terms of **velocity per milligram of total protein**.

Multisubstrate enzymes

So far we have been looking at the kinetics of the enzyme reaction with one substrate. However, many enzyme reactions involve two substrates, and some involve even more. Kinetic equations for multi-substrate reactions have been derived that are analogous to the Michaelis-Menten treatment for single substrate reactions.

[3] The data in the table come from a variety of sources. For enzymes with turnovers appreciably greater than 10^5 sec^{-1}, the values are largely estimates due to the difficulty of accurately measuring reactions of this rapidity, and different sources report different values.

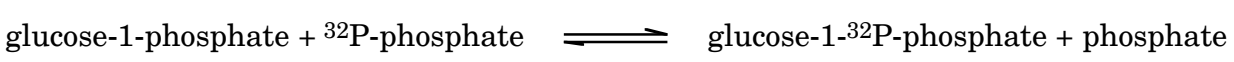
By keeping the concentration of one substrate constant, and varying the concentration of the other substrate, K_m and V_{max} values can be obtained for multisubstrate enzymes. These values will only approximate the true K_m and V_{max} values because the concentration of the constant substrate used will not fully saturate the enzyme. Because the values are approximate, the values obtained in this manner are usually referred to as "apparent" K_m and V_{max} values.

Mechanisms of multi-substrate reactions

It was initially assumed that two substrate reactions would involve a ternary complex: $[ES_1S_2]$. However, this is not necessarily true.

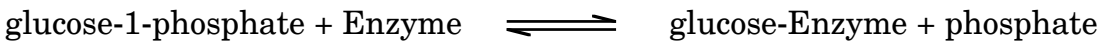
Consider the reaction catalyzed by sucrose phosphorylase:

In the absence of fructose, and in the presence of ^{32}P-labeled inorganic phosphate (note that ^{32}P is radioactive, and is therefore readily detected) the following reaction was observed:



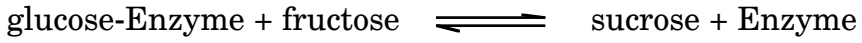
In other words, the enzyme catalyzed the exchange between phosphate covalently bound to the glucose and the phosphate in solution. This reaction was possible because the enzyme reacted with glucose (and fructose) in separate steps:

Step 1:



where "glucose-Enzyme" is a covalent reaction intermediate between glucose and sucrose phosphorylase.

Step 2:



There are two general types of 2-substrate reactions: **Double displacement** and **Sequential**.

Double displacement reactions (also called "ping-pong" reactions) include the reaction mechanism shown in the sucrose phosphorylase example. One substrate binds the enzyme, is modified, and is released. The other substrate has to wait until the first product is released before binding to the enzyme.

In contrast, **in sequential reactions all substrates bind to the enzyme before catalysis occurs**.

There are two types of sequential enzymes, ordered and random. For ordered sequential enzymes, the reactants combine in a specific order and products are released in a specific order.

For example, $substrate_1$ binds, then $substrate_2$ binds. The reaction occurs, and then $product_2$ is released followed by the release of $product_1$.

For random sequence enzymes, there are **no restrictions to the order of either substrate binding or product release**.

Careful analysis of kinetic data can be used to distinguish these mechanisms, but the methods involved are beyond the scope of this course.

Summary

For enzyme-catalyzed reactions, the velocity of product formation can be described by the equation: $v = k[ES]$. The reaction is therefore first-order in relation to the concentration of ES complex. However, the reaction is **not** first-order relative to the directly measurable substrate concentration. Instead, under steady state conditions where [ES] is effectively constant, the velocity of an enzyme-catalyzed reaction is a hyperbolic function of [S]:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

The Michaelis-Menten equation has two parameters, V_{max} and K_m.

$V_{max} = k_{cat}[E]_{total}$, and therefore is a function of both the total enzyme concentration and of the catalytic rate constant of the enzyme for the reaction; k_{cat} is an intrinsic property of an enzyme, while V_{max} is not.

K_m is an intrinsic property of an enzyme; it is a measure of the affinity of the enzyme for the substrate. Biological systems often vary velocity by altering V_{max} (either by increasing or decreasing the amount of enzyme present); K_m is a sensitive measure of changes in the enzyme (either modifications of one enzyme, or the presence of more than one isozyme).

All biochemists need to be able to interpret the information presented in the Lineweaver-Burk plot, and to understand the implications of k_{cat}, V_{max}, and K_m.

The Michaelis-Menten equation is somewhat difficult to analyze directly. One simple method for determining V_{max} and K_m from a set of velocity versus substrate data is to use the double reciprocal (Lineweaver-Burk) plot, a linear transformation of the Michaelis-Menten equation. A second method, which is preferable for any set of actual data, is to use non-linear regression techniques to directly fit experimental data to the Michaelis-Menten equation.