Enzyme Kinetics I

10/15/2009

Enzyme Kinetics

Rates of Enzyme Reactions

Thermodynamics says I know the difference between state 1 and state 2 and $\Delta G = (G_f - G_i)$

But

Changes in reaction rates in response to differing conditions is related to path followed by the reaction

and

is indicative of the reaction mechanism!!

Enzyme kinetics are important

1. Substrate binding constants can be measured as well as inhibitor strengths and maximum catalytic rates.

2. Kinetics alone will not give a chemical mechanism but combined with chemical and structural data mechanisms can be elucidated.

3. Kinetics help understand the enzymes role in metabolic pathways.

4. Under "proper" conditions rates are proportional to enzyme concentrations and these can be determine " metabolic problems".

Chemical kinetics and Elementary Reactions

Rate Equations

Consider aA + bB + • • • + zZ. The rate of a reaction is proportional to the frequency with which the reacting molecules simultaneously bump into each other

$$\text{Rate} = k[A]^a[B]^b \bullet\bullet\bullet[Z]^z$$

The order of a reaction = the sum of exponents

Generally, the order means how many molecules have to bump into each other at one time for a reaction to occur.

A first order reaction one molecule changes to another

$A \rightarrow B$

A second order reaction two molecules react

$A + B \rightarrow P + Q$

or

$2A \rightarrow P$

3rd order rates A + B + C → P + Q + R rarely occur

and higher orders are unknown.

Let us look at a first order rate

$A \rightarrow B$

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

v = velocity of the reaction in Molar per min. or moles per min per volume

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

k = the rate constant of the reaction

Instantaneous rate: the rate of reaction at any specified time point that is the definition of the derivative.

We can predict the shape of the curve if we know the order of the reaction.

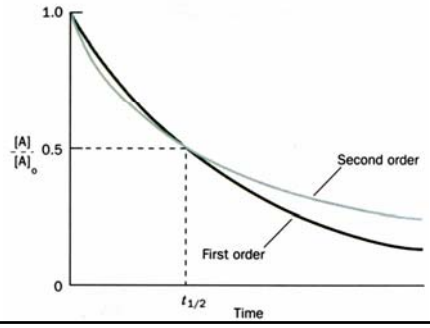
A second order reaction: $2A \rightarrow P$

$$v = -\frac{d[A]}{dt} = k[A]^2$$

Or for $A + B \rightarrow P + Q$

$$v = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k[A][B]$$

Percent change in A (ratio) versus time in first and second order reactions



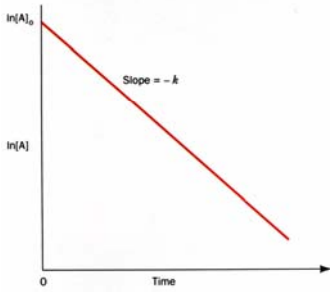
It is difficult to determine if the reaction is either first or second order by directly plotting changes in concentration.

$$-\frac{d[A]}{dt} = k[A] \qquad \frac{d[A]}{[A]} = -k\,dt$$

$$\int_{A_o}^{A} \frac{dA}{A} = -k\int_{0}^{t} dt \qquad \ln[A] = \ln[A]_o - kt$$

$$[A] = [A]_o e^{-kt}$$

However, the natural log of the concentration is directly proportional to the time.
- for a first order reaction-



The rate constant for the first order reaction has units of s^{-1} or min^{-1} since velocity = molar/sec

and v = k[A] : k = v/[A]

Gather your data and plot ln[A] vs time.

The half-life of a first order reaction

$[A] = \frac{[A]_o}{2}$ Plugging in to rate equation $\ln\left(\frac{[A]_o}{\frac{2}{[A]}}\right) = -kt_{\frac{1}{2}}$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

The half-life of a first order reaction is the time for half of the reactant which is initially present to decompose or react.

^{32}P, a common radioactive isotope, emits an energetic β particle and has a half-life of 14 days. ^{14}C has a half life of 5715 years.

A second order reaction such like $2A \rightarrow P$

$$\int_{[A]o}^{[A]} -\frac{d[A]_o}{[A]^2} = k\int_{0}^{t} dt$$

$$\frac{1}{[A]} = \frac{1}{[A]_o} + kt$$

When the reciprocal of the concentration is plotted verses time a second order reaction is characteristic of a straight line.

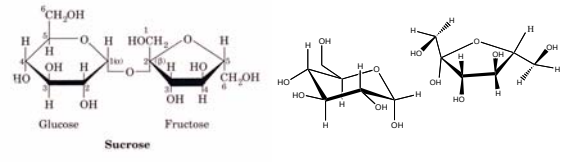
The half-life of a second order reaction is and shows a dependents on the initial concentration $t_{\frac{1}{2}} = \frac{1}{k[A]_o}$

Kinetics of Enzymes

Enzymes follow zero order kinetics when substrate concentrations are high. Zero order means there is no increase in the rate of the reaction when more substrate is added.

Given the following breakdown of sucrose to glucose and fructose

Sucrose + H_20 ⟶ Glucose + Fructose



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

E = Enzyme S = Substrate P = Product

ES = Enzyme-Substrate complex

k_1 rate constant for the forward reaction

k_{-1} = rate constant for the breakdown of the ES to substrate

k_2 = rate constant for the formation of the products

When the substrate concentration becomes large enough to force the equilibrium to form completely all ES the second step in the reaction becomes rate limiting because no more ES can be made and the enzyme-substrate complex is at its maximum value.

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

[ES] is the difference between the rates of ES formation minus the rates of its disappearance.

1

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

Assumption of equilibrium

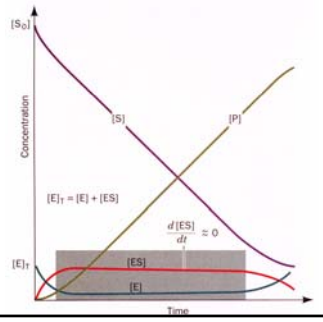
$k_{-1} >> k_2$ the formation of product is so much slower than the formation of the ES complex. That we can assume:

$$K_s = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

K_s is the dissociation constant for the ES complex.

Assumption of steady state

Transient phase where in the course of a reaction the concentration of ES does not change



2

$$\frac{d[ES]}{dt} = 0$$

$$[E]_T = [E] + [ES]$$

3

Combining 1 + 2 + 3

$$k_1([E]_T - [ES])[S] = (k_{-1} + k_2)[ES]$$

rearranging

$$[ES](k_{-1} + k_2 + k_1[S]) = k_1[E]_T[S]$$

Divide by k_1 and solve for [ES]

$$[ES] = \frac{[E]_T[S]}{K_M + [S]}$$

Where

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

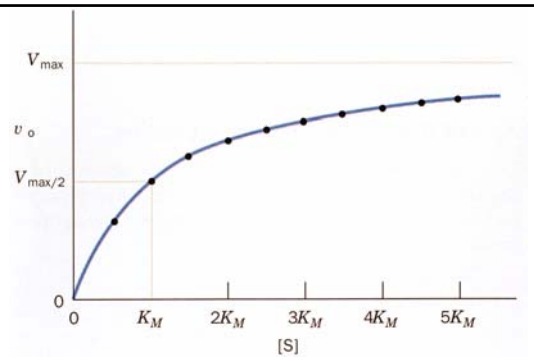
$$v_o = \left(\frac{d[P]}{dt}\right)_{t=0} = k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]}$$

v_o is the initial velocity when the reaction is just starting out.

And $V_{max} = k_2[E]_T$ is the maximum velocity

$$v_o = \frac{V_{max}[S]}{K_M + [S]}$$

The Michaelis - Menten equation



The K_m is the substrate concentration where v_o equals one-half V_{max}

The K_M widely varies among different enzymes

The K_M

can be expressed as: $K_M = \frac{k_{-1}}{k_1} + \frac{k_2}{k_1} = K_s + \frac{k_2}{k_1}$

As Ks decreases, the affinity for the substrate increases. The K_M can be a measure for substrate affinity if $\mathbf{k_2 < k_{-1}}$

TABLE 13-1. THE VALUES OF K_M, K_{cat}, AND K_{cat}/K_M FOR SOME ENZYMES AND SUBSTRATES

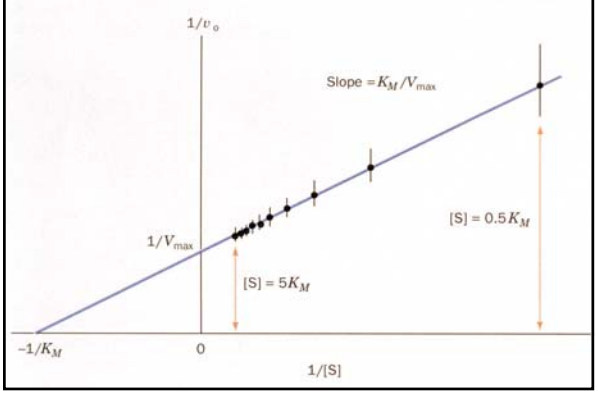
Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}\,s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^4	1.5×10^8
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^6	8.3×10^7
	HCO_3^-	2.6×10^{-2}	4.0×10^5	1.5×10^7
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^7	4.0×10^8
Chymotrypsin	*N*-Acetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^2	2.9×10^5
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^2	1.6×10^8
	Malate	2.5×10^{-5}	9.0×10^2	3.6×10^7
Urease	Urea	2.5×10^{-2}	1.0×10^4	4.0×10^5

There are a wide range of K_M, Vmax , and efficiency seen in enzymes

But how do we analyze kinetic data?

The double reciprocal plot

$$\frac{1}{v_o} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



Lineweaver-Burk plot: slope = K_M/V_{max},

$1/v_o$ intercept is equal to $1/V_{max}$

the extrapolated x intercept is equal to $-1/K_M$

For small errors in at low [S] leads to large errors in $1/v_o$

$$k_{cat} = \frac{V_{max}}{[E]_T}$$

k_{cat} is how many reactions an enzyme can catalyze per second

The turnover number

For Michaelis -Menton kinetics $k_2 = k_{cat}$

When $[S] << K_M$ very little ES is formed and $[E] = [E]_T$

and

$$v_o \approx \frac{k_2}{K_M}[E]_T[S] \approx \frac{k_{cat}}{K_M}[E][S]$$

K_{cat}/K_M is a measure of catalytic efficiency

What is catalytic perfection?

When $k_2 >> k_{-1}$ or the ratio $\frac{k_1 k_2}{k_{-1} + k_2}$ is maximum

Then $\frac{k_{cat}}{K_M} = k_1$

Or when every substrate that hits the enzyme causes a reaction to take place. This is catalytic perfection.

Diffusion-controlled limit- diffusion rate of a substrate is in the range of 10^8 to 10^9 $M^{-1}s^{-1}$. An enzyme lowers the transition state so there is no activation energy and the catalyzed rate is as fast as molecules collide.

Lecture 16 – Dr. Legge
Thursday 10/15/09
Enzyme Kinetics II