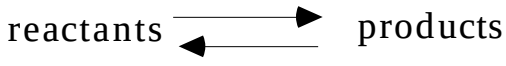
MIT Department of Biology
7.014 Introductory Biology, Spring 2005

7.014 Handout

Biochemistry III & IV

ΔG and K_{eq}

For the chemical equilibrium:



The equilibrium constant, K_{eq} is defined as: $K_{eq} = \frac{[\text{products}]}{[\text{reactants}]}$ at equilibrium.

Where [] symbolizes concentration, for example, [products] indicates concentration of products.

The equation that relates the free energy of the reaction, ΔG, to the standard free energy, $\Delta G^{o'}$ and the concentrations of reactants and products is:

$$\Delta G = \Delta G^{o'} + RT \ln \frac{[\text{products}]}{[\text{reactants}]} \quad \text{Equation (1)}$$

where: R= the universal gas constant
T= the temperature in ^{o}K

if T = 25 ˚C RT = 0.59 $\frac{\text{kcal}}{\text{mol}}$ if T = 37 ˚C RT = 0.61 $\frac{\text{kcal}}{\text{mol}}$

Under **Standard Conditions** where: [products] = 1 M and [reactants] = 1 M (M = moles / liter)

(Equation 1) becomes: $\Delta G = \Delta G^{o'}$

therefore, the $\Delta G^{o'}$ of the reaction is the free energy change (ΔG) under standard conditions.

At **equilibrium**, $\Delta G = 0$, and equation (1) becomes:

$$\Delta G^{o'} = -RT \ln \frac{[\text{products}]}{[\text{reactants}]} = -RT \ln\left(K_{eq}\right) \quad \text{or: } K_{eq} = e^{-\frac{\Delta G^{o'}}{RT}}$$

Significance of K_{eq}:

$K_{eq} > 1$ $\Delta G^{o'}$ has negative value, therefore reaction → is possible.

$K_{eq} < 1$ $\Delta G^{o'}$ has positive value, therefore reaction → is possible if [react] > [prod].

$K_{eq} >> 1$ $\Delta G^{o'}$ has large negative value, therefore reaction → is irreversible.

$K_{eq} << 1$ $\Delta G^{o'}$ has large positive value, therefore reaction → cannot occur.

Enzymes

(This handout is designed to supplement the coverage of enzymes in Purves *et al.*)

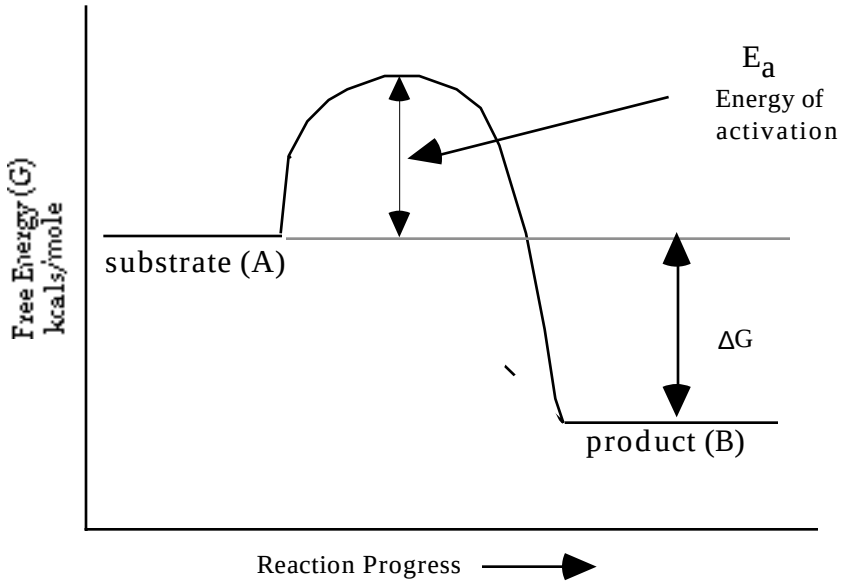
A catalyst is a molecule that increases the rate of a reaction but is not the substrate or product of that reaction.

Enzymes are usually proteins that catalyze chemical reactions in cells (the exception is catalytic RNA).

A substrate is a molecule upon which an enzyme acts to yield a product.

The part of the enzyme that binds substrate is called the **active site**.

Consider the reaction: A (substrate) ------> B (product)
A graph of the free energy against the reaction progress is shown below:



ΔG is negative overall for forward reaction. The free energy of this reaction is not changed by the presence of the enzyme, BUT the enzyme can speed up the reaction by lower the E_a.

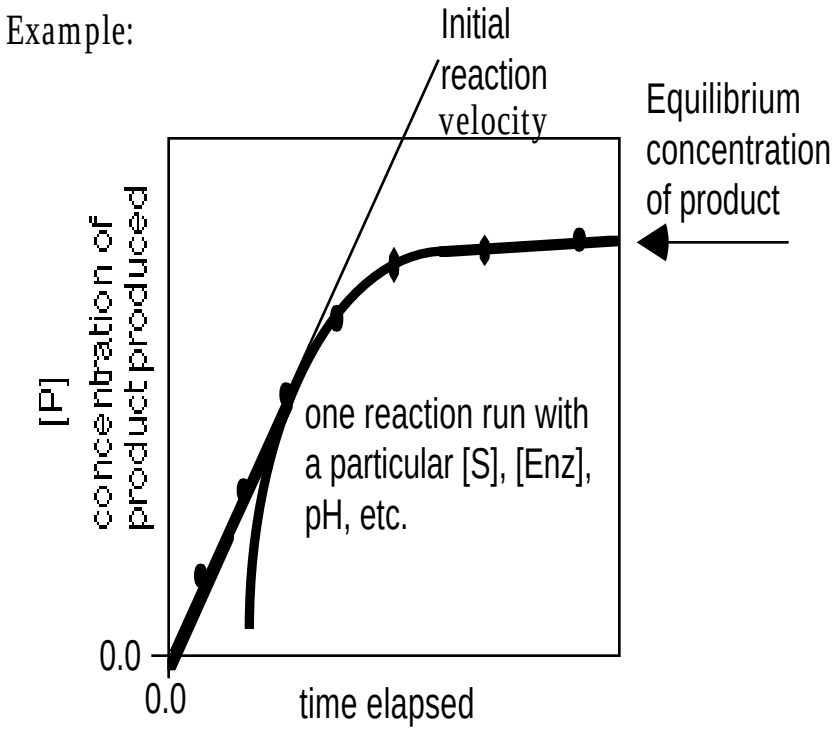
Enzyme Mechanisms

In a reaction like that above, E_a could represent an energetically unfavorable transitional state that the substrate must obtain before forming product. Enzymes can lower the E_a by stabilizing the substrate in that transitional state and thus speed up the reaction.

For a reaction involving two substrates: A + B ---> C,
the enzyme may increase the local concentration of A and B and drive the reaction forward.

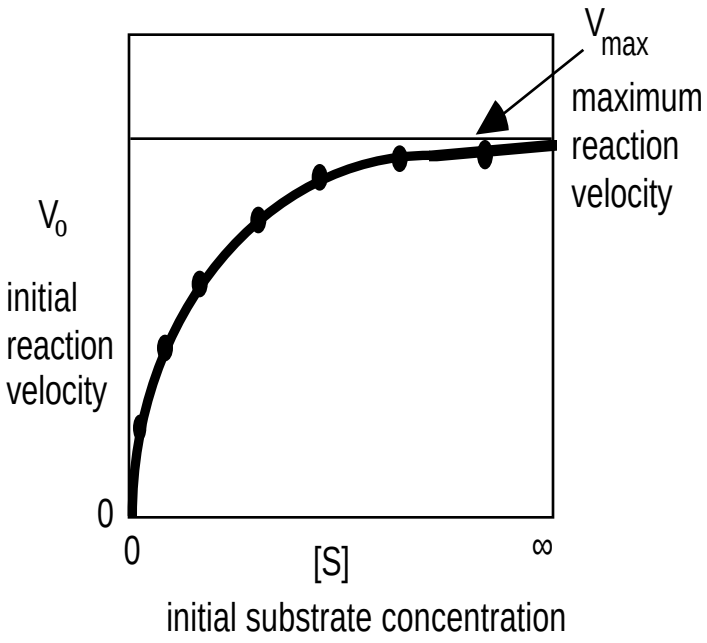
Kinetics of enzyme reactions

One way to study an enzyme is to measure the formation of the product. If you were to perform an experiment under defined conditions at a given concentration of substrate and enzyme you could plot a time course of the enzyme catalyzed reaction.



Enzymes catalyze both forward and reverse reactions. In the reaction S -->P, the enzyme converts substrate (S) to product (P). Initially, the concentration of P is small and the net reaction is S -->P. As [P] increases, the rate of the reaction S -->P decreases until the rate of the forward reaction equals the rate of the reverse reaction. At this point the reaction is in equilibrium.

To measure the kinetic properties of a given enzyme, you must perform many experiments like the one above, holding the enzyme concentration constant and varying the substrate concentrations. The initial reaction velocity at each substrate concentration is measured, and the data from all the experiments is used to plot the initial reaction velocity, V_o, as a function of substrate concentration [S]. An example is found below.


V_{max}
maximum
reaction
velocity
V_0
initial
reaction
velocity
0
0
[S]
∞
initial substrate concentration

A few things about the V vs. [S] graph:
Velocity is dependent on [S], but remember this graph was generated at **one** enzyme concentration. If more enzyme was added, the reaction velocity would increase.
The velocity asymptotically approaches a maximum as [S] --> ∞.

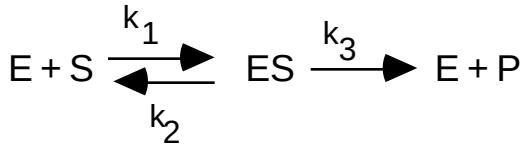
Why isn't the graph linear?
As [S] gets large, the enzyme becomes saturated. Every enzyme molecule is complexed with substrate and since the amount of enzyme used in this series of experiments is fixed, the rate asymptotically approaches a maximum.

NOTE: the two preceding graphs look very similar, but represent different things. It is important to understand the difference between an individual reaction: [P] vs. t (from which you get V_o) and a kinetic graph of many such reactions: V_o vs. [S].

Enzymes that display this kinetic behavior can be modeled mathematically as was first shown by Michaelis and Menten in 1913:

Michaelis-Menten Enzyme Kinetics

For an enzymatic reaction:



The symbols represent (in our example):

E	enzyme
S	substrate
ES	enzyme-substrate complex
P	product
k_1	the rate at which E combines with S to form ES
k_2	the rate at which ES can dissociate into E and S
k_3	the rate at which ES can proceed to form E and P

Michaelis and Menten modeled the catalytic rate of enzymes under steady-state conditions where the concentration of ES remains unchanged over time while the concentrations of starting materials and products are changing. At steady state the rate of ES formation equals the rate of ES breakdown. The following conditions are assumed in this model.

1) The uncatalyzed rate of S ---> P is negligible (the reaction does not go without enzyme).

2) Because the [S] is far greater than the [E], the amount of substrate bound by enzyme is negligible compare to the total [S]. Also assumed is that [S] does not vary with time (this reaction is under examination for a brief moment), and the reaction rate remains the same.

3) The reverse reaction (P ---> S) does not occur.

Given the above assumptions and approximations, the dependence of V_o on [S] can be derived mathematically. (For further information, see "Biochemistry" 4th edition by Stryer; pp 192-193)

$$V_o = k_3[E]_{tot}\left(\frac{[S]}{[S] + K_M}\right) \qquad (1)$$

Where: V_o = initial velocity of the reaction
$[E]_{tot}$ = total concentration of enzyme in reaction
[S] = substrate concentration
K_M = the Michaelis constant, $K_M = \frac{k_2 + k_3}{k_1}$

Three cases will illustrate how this equation behaves.

CASE 1: [S] very large ([S]>>K_M => Enzyme is saturated with substrate)

in this case [S] + $K_M \approx$ [S], so (1) becomes: $V_0 = k_3[E]_{tot}\left(\frac{[S]}{[S]}\right)$ or: $V_o = k_3[E]_{tot}$

This is the rate at a large substrate concentration - (the maximal rate) which is called $\mathbf{V_{max}}$

$$\mathbf{V_{max}} = k_3[E]_{tot}$$

Substituting into (1) gives the **Michaelis-Menten Equation** as it is normally shown:

$$V_0 = \frac{V_{max}[S]}{K_M + [S]} \qquad (2)$$

Remember, this equation is derived for V_o, when very little product has formed and the back-reaction can be ignored.

CASE 2: [S] small ([S]<<K_M => linear range)

in this case [S] + $K_M \approx K_M$ so (2) becomes: $V_0 = \frac{V_{max}[S]}{K_M}$ or: $V_0 \alpha [S]$

So, at low [S], V_o is linearly proportional to [S].

CASE 3: [S] = $\mathbf{K_M}$ (The definition of K_M)

$$V_0 = \frac{V_{max}[S]}{[S] + [S]}$$

or

$$V_o = \frac{V_{max}}{2}$$

K_M is defined as the [S] that results in half-maximal reaction rate.

SIGNIFICANCE OF K_M and V_{max}

V_{max} and K_M are the two parameters which define the kinetic behavior of an enzyme as a function of [S].

V_{max} is a **rate of reaction**. It will have units of: $\frac{\text{moles}}{\text{min}}$ or $\frac{\text{moles}}{\text{sec}}$ or $\frac{\mu\text{moles}}{\text{min}}$ etc.
V_{max} depends on the structure the enzyme itself and the concentration of enzyme present.

K_M is a the concentration substrate required to approach the maximum reaction velocity - if $[S] >> K_m$ then V_o will be close to V_{max}.

K_M is a **concentration**. It will have units of: $\frac{\text{moles}}{\text{liter}}$ (M),or $\frac{\mu\text{moles}}{\text{liter}}$ (μM),etc.
K_M depends only on the structure of the enzyme and is independent of enzyme concentration.

Measuring K_M and V_{max}

The quantities K_M and V_{max} are experimentally determined and different for each enzyme. Once you have an assay for enzyme activity, you can determine these parameters. You can estimate K_M and V_{max} from the graph of initial velocity versus [S].

1) Run a series of reactions each with a single $[E]_{tot}$, varying [S], and measure V_0.
2) Graph V_0 vs. [S].
3) Estimate V_{max} from asymptote.
4) Calculate $V_{max}/2$
5) read K_M from graph.