Learning Objectives

Describe the general properties of enzymes using correct scientific vocabulary.

Examine the rates of enzymatically catalyzed reactions mathematically.

Construct reaction mechanisms for enzymatically catalyzed reactions based on experimental data.

Compare the different means by which enzymes are regulated.

Virtually All Reactions in Cells Are Mediated by Enzymes

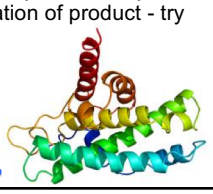
- Enzymes catalyze thermodynamically favorable reactions, causing them to proceed at extraordinarily rapid rates
- Enzymes provide cells with the ability to exert *kinetic control over thermodynamic potentiality*
- Living systems use enzymes to accelerate and control the rates of vitally important biochemical reactions
- Enzymes are the agents of metabolic function

Enzymatic catalysis

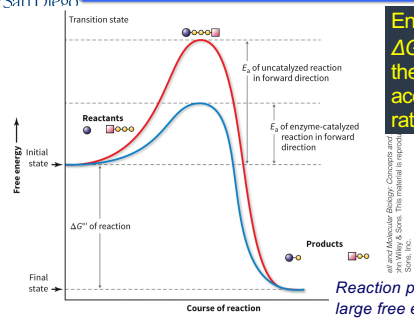
Role of enzymes

- serve the same role as any other catalyst in chemistry
- act with a higher specificity
- acid and base catalyst possible due to proximal locations of amino acids
- alter the structure location of quantity of the enzyme and you have regulated the formation of product - try that in organic chemistry !

- Don't lose sight of what enzymes do. catalyze reactions – nothing more reactants -> products.



What do Enzyme *ACTUALLY* do?



Enzymes lower $\Delta G^{\ddagger}$, (aka Ea) thereby accelerating rate.

Reaction profile showing the large free energy of activation for glucose oxidation.

Naming Enzymes

- Enzyme commission for each enzyme based on the type of reactions. Kind of like IUPAC
- Yeah right - the mother of invention – he/she who finds/discovers/purifies/clones the enzyme, names if. Trivial names rule…

First recognition of an enzyme and naming – 1833: alcohol precipitate of malt extract converted starch into sugar – called diatase (first use of suffix –ase)

Proteases traditionally are the only enzymes which don't end in 'ase' and use '-in'

Enzyme is coined after the Greek for "in yeast"

1050s started need to use a standardized naming: Otto Hofmann-Ostenhof and Dixon & Webb – common, non-systematic names still rule.

Enzyme Nomenclature Provides a Systematic Way of Naming Metabolic Reactions

Oh To Have Lived In Lisbon

Class	General reaction	
1. Oxidoreductases	$A_{Red} + B_{Ox} \leftrightarrows A_{Ox} + B_{Red}$	Redox – gain or loss of e-
2. Transferases	A-B + C → A + C-B	Transfers a group to other
3. Hydrolases	A-B + H_2O → A-H + B-OH	Cuts bond with water, reverse reaction is dehydration or condensation – resulting in water
4. Lyases ("synthases")	A-B ⇆ A + B (reverse reaction: synthases)	Breaking double bond without redox or water
5. Isomerases	A-B-C ⇆ A-C-B	Isomerize – rearrange bonds (e.g. enol-keto)
6. Ligases ("synthetases")	A + B + ATP → A-B + ADP + P_i	Join two molecules – often but not only using ATP

General Information on Enzymology

Important Enzyme Nomenclature

- Active site
- Substrate vs. reactant
- Prosthetic groups, coenzyme and cofactors
- Activity vs. reaction - BINDING is NOT activity – two events!
- Suffix –ase

Another important reminder - not all activity is on or off. Many times the enzyme has a low or constitutive activity that can be increased many times.

It is a common mistake to think of enzymes being on or off.

Substrate Defined

Substrate is a molecule (or molecules) that acts as the reactant in a enzymatically catalyzed reaction.

– More than 60% of biochemical reactions use multiple substrates.

– Binds to the enzyme at the active site

– When the enzyme binds to the substrate, product(s) is/are formed.

Active Site Defined

Active site is the location where the enzyme binds to the substrate and catalysis occurs.

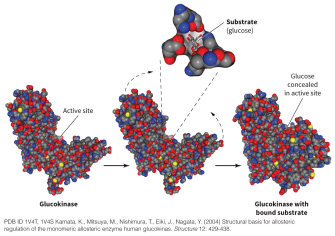
– Located on the surface of the enzyme

– Is often in a cleft, pocket, or trench

– Generally formed by residues on turns or coils

Enzymes are flexible

Lock and Key vs Induced Fit -> always flexible



PDB ID 1V4T. 1V4S Kamata, K., Mitsuya, M., Nishimura, T., Eiki, J., Nagata, Y. (2004) Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase. *Structure* 12: 429-438.

- What makes a substrate bind?
- What keeps the "key" in place?

Enzyme specificity

- One of the most powerful actions of enzymes.

– Group complementation - the ability to recognize specific regions of the substrate to align reactants with catalytic site. ***(geometric and electronic complementation)***

– Based on non-covalent molecular interactions. Lock and key vs. induced fit - both occur. Induced fit takes place when binding of one part of the substrate to the enzyme alters the conformation of the enzyme to make a true "fit"

– Binding does not mean activity

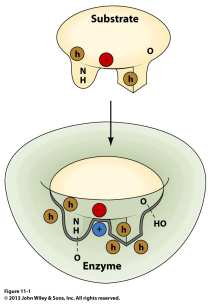


Figure 11-1
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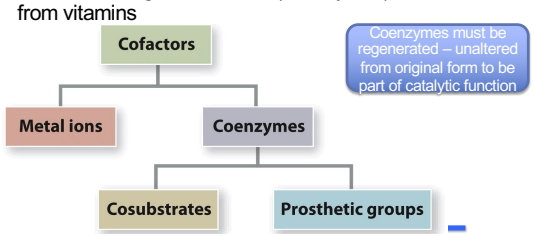
Cofactors

Group transfer, redox and some acid base reactions need additional components, aa alone is not sufficient for reaction

- Cofactors – metals (Cu, Fe, Zn),
- Cofactors – organics molecs (coenzymes) often converted from vitamins



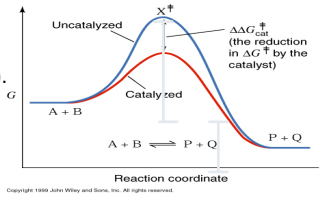
Job of Enzyme Catalysts

Specifically bind substrate(s) (reactants), decrease transition state energy to increase the rate (NOT thermodynamics) of a favorable reaction:

- High specificity
- Low side reactions
- Ability to be regulated: Active site – amino acids and cofactors binding and "doing" the reaction, are regulated by the rest of the protein

How do enzymes work?

- By reducing the energy of activation
- This happens because **the transition state is stabilized** by a number of mechanisms involving the enzyme/protein
- Without enzymes reactions occur by collisions between reactants or addition of various organic catalysts
- The energy barrier between the reactants and product, is the free energy of activation (Ea).
- The free energy (ΔG) of the reaction is what determines if a reaction is spontaneous.



Enzyme Kinetics

- Kinetics is the branch of science concerned with the rates of reactions
- Enzyme kinetics seeks to determine the maximum reaction velocity that enzymes can attain and the binding affinities for substrates and inhibitors
- Analysis of enzyme rates yields insights into enzyme mechanisms and metabolic pathways
- This information can be exploited to control and manipulate the course of metabolic events

Rates of reactions

For a simple unimolecular reaction

A -> B

The question is if this is a 1st order or 2nd order reaction?

1st Order Reaction:

$$\text{Rate} = d[B]/dt = k[A]$$

– *The rate of the reaction is directly related to the concentration of reactant A*

2nd Order Reaction:

$$\text{Rate} = d[B]/dt = k[A]^2$$

– *The rate varies with a square of [A] meaning when you double the conc of A, the reaction goes 4X faster*

Gen Chem – Rates of Reaction…

Quick Review – MOST enzymes catalyze reactions in a first or second order reaction.

- Less about collisions of freely moving molecules than binding and reacting on a surface of a catalyst (enzyme active site):

For single/uni-molecularity reactions –

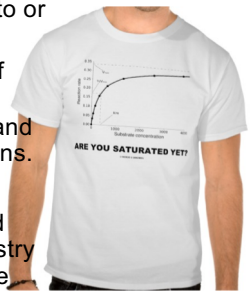
1st order A->P

The rate is proportional to the concentration of substrate

Enzyme Kinetics

Kinetic defn.: Of or relating to or pruduced by motion.

Kinetics defn.: the branch of chemistry or biochemistry concerned with measuring and studying the rates of reactions.



Enzyme kinetics are defined differently than basic chemistry kinetics because of the large… catalyst…

Measuring Rates of Enzyme Catalyzed Reactions

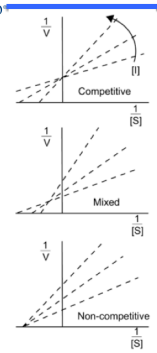
Louis Michaelis and Maud Menten's theory

S $\xrightarrow{E}$ P aka $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [ES] \xrightarrow{k_2} E + P$

Describes the rate of reaction as a function of substrate concentration!

- assumes the formation of an enzyme-substrate complex is first order *(or pseudo-first order)*
- assumes that the ES complex is in rapid equilibrium with free enzyme
- Assumes synthesis of ES is equal to rate of loss of ES to P allows [ES] to be steady state and constant value

Enzyme Kinetics…



How Inhibited are You?

Psuedo First Order Kinetics

For the most part enzyme reactions are treated as if there is only one substrate and one product.

If there are two substrates, one of them is held at a high concentration (0 order) and the other substrate is studied at a lower concentration

- so that for that substrate, it is a first order reaction.

This leads us to the M and M equation.

Two (three) Assumptions…

Because the rate law of binding is not easy to integrate – Michaelis and Menten made two assumptions to derive a simple way to measure how fast a reaction would go based on amount (concentration) of a reactant

To do this, two assumptions had to be made

1) Binding is faster than reaction

$$K_s = \frac{K_{-1}}{K_1} = \frac{[E]\,[S]}{[ES]}$$

Ks is the dissociation constant – a measure of binding and release of substrate to enzymes aka **Michaelis complex**
Equilibrium - ES is unchanging

$K_{-1} >> K_2$

Dissociation constant of first step

Two (three) Assumptions…

2) Steady state - the enzyme substrate complex ES is at a constant value.

That is the ES is formed as fast as the enzyme releases the product. For this to happen the concentration of substrate has to be much higher than the enzyme concentration. That is why we only study the initial velocity. Later in the reaction the substrate concentration is relatively lower and the rate of product starts to be limited by diffusion and not the mechanism of the enzyme.

For this to happen (unchanging [ES]), then [S] >> [E]

•Mathematical model of the representation of the M&M eq. -

For the reaction:

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

1) The Michaelis constant Km is:

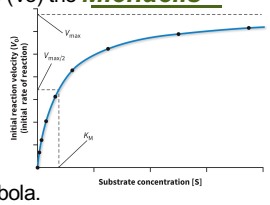
$$K_m = \frac{K_{-1} + K_2}{K_1}$$

Think of what this means in terms of the equilibrium.

Large vs. a small Km

2) When investigating the initial rate (Vo) the ***Michaelis-Menten equation*** is:

$$v_0 = \frac{V_{max}[S]}{K_M + [S]}$$



Graphical representation is a hyperbola.

When [S] << Km, the velocity is dependent on [S]
- When [S] >> Km, the initial velocity is independent of [S]
- When [S] = Km, then Vo = 1/2 V_{max}

Understanding V_{max}

The theoretical maximal velocity

V_{max} is a constant

V_{max} is the theoretical maximal rate of the reaction - but it is NEVER achieved in reality

To reach V_{max} would require that ALL enzyme molecules are tightly bound with substrate

V_{max} is asymptotically approached as substrate is increased

Understanding K_m

The "kinetic activator constant"

K_m is a constant

K_m is a constant derived from rate constants

K_m is, under true Michaelis-Menten conditions, an estimate of the dissociation constant of E from S

- *that is an equilibria constant of E, S and ES*

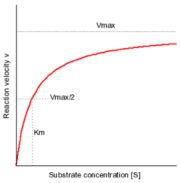
Small K_m means tight binding; high K_m means weak binding

Remember K_2 is speed of reaction…

Determining Km and Vmax

- Must be done experimentally/empirically
- Measure rate of enzyme vs different concentrations of substrate

[S]	V (µmol/ml/sec)
0.00	0.5
0.010	12
0.025	45
0.040	122



- ***BUT YOU CAN'T GET THERE FROM HERE***

Create a secondary plot to determine kinetic constants

Linear Plots Can Be Derived from the Michaelis-Menten EquationLineweaver-Burk:

Begin with $v = V_{max}[S]/(K_m + [S])$ and take the reciprocal of both sides

Rearrange to obtain the Lineweaver-Burk equation:

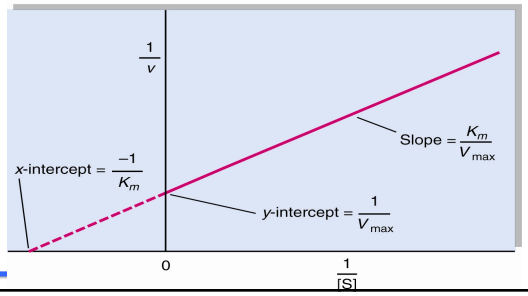
$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}} \qquad \text{Slope} = \frac{K_M}{V_{max}}$$

A plot of 1/v versus 1/[S] should yield a straight line

Linear Plots Can Be Derived from the Michaelis-Menten Equation

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

The Lineweaver-Burk aka double-reciprocal plot.



Hanes-Woolf:

Begin with Lineweaver-Burk and multiply both sides by [S] to obtain:

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

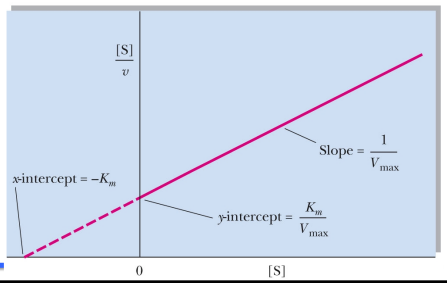
Hanes-Woolf is best - why?

Because Hanes-Woolf has smaller and more consistent errors across the plot

A Hanes-Woolf plot of

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

[S]/*v* versus [S]



Other uses of Km

The Turnover Number (k_{cat}) Defines the Activity of One Enzyme Molecule

A measure of catalytic activity – the number of reactions that the enzyme can catalyze per time

k_{cat}, the turnover number, aka k_2, is the number of substrate molecules converted to product per enzyme molecule per unit of time, when E is saturated with substrate.

If the M-M model fits, $k_2 = k_{cat} = V_{max}/E_t$

Values of k_{cat} range from less than 1/sec to many millions per sec

The Ratio k_{cat}/K_m Defines the Catalytic Efficiency of an Enzyme

The catalytic efficiency: k_{cat}/K_m

An estimate of "how perfect" the enzyme is

Used when less than saturating S (free ES exists)

k_{cat}/K_m is an apparent second-order rate constant
It measures how well the enzyme performs when S is low – i.e. how often a reaction occurs for every encounter of E and S.

The upper limit for k_{cat}/K_m is the diffusion limit - the rate at which E and S diffuse together

So What? Or WDIC?

Km

- K_m - relates to affinity ; V_{max} relates to efficiency
- K_m tell how much substrate to use in an assay
- If more than one enzyme share the same substrate, K_M also will determine how to decide which pathway the substrate will take

V_{max} tells about pathways

- Rate limiting enzyme in pathway
- K_m and V_{max} can be used to determine effectiveness of inhibitors and activators for enzyme studies and clinical applications