Chapter 8 - Enzymes

- Enzymes are biological catalysts
  - Most are proteins (Ribozymes = RNA catalysts)
  - May have multiple subunits
  - Often require additional components for activity (cofactors, coenzymes)
- Enzymes bind the substrate within an active site
  - Forms the Enzyme-substrate (ES) complex
  - Transition from reactant to product limited by energy barrier

1

- Enzymes increase reaction rates by lowering activation energy barriers
  - Binding energy, derived from numerous weak interactions between enzyme & substrate
- Binding energy allows an enzyme to discriminate between substrate & competing molecules
- Reaction equilibrium dependent on the difference in free energy of ground states of reactants/products
  - Equilibrium position not influenced by enzyme

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- Kinetics help us to understand the mechanisms of enzyme catalysis

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

E = enzyme S = substrate P = Product

ES = enzyme-substrate complex

Can be manipulated to give the Michaelis-Menten eqn.

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

  - M-M eqn describes relationship among initial velocity (V_o) and the maximal velocity (V_{max}) via the Michaelis Constant (K_m)
  - K_{cat} = turnover no. = number of substrate molecules converted to product when enzyme at V_{max}

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- K_m, V_{max} and k_{cat}/K_m all useful parameters for comparing enzyme activites
- Enzyme activity can be altered:
  - pH, temperature
  - Reversible inhibitors (competitive, uncompetitive, mixed)
  - Irreversible inhibitors ("suicide" substrates)
- Certain enzymes used to illustrate key concepts
  - Chymotrypsin - transition state stabilization
  - Hexokinase - induced fit
  - Enolase - metal ion catalysis

4

- Regulatory enzymes control overall rates of metabolic pathways
  - 2 major activation or inhibition mechanisms

(1) Reversible, noncovalent allosteric interactions

(2) Reversible, covalent modifications (phosyphorylation)

  - Irreversible activation also occurs (proteolytic cleavage)

5

Enzymes

Biological Catalysts

- Can increase reactions rates by 10^5 to 10^{17} X
- Often greater than synthetic or inorganic catalysts
- High specificity for substrate
- Function under very mild conditions

Much of the history of biochemistry is the study of enzymes

- Digestion of meat by stomach secretions (1700's)
- Conversion of starch to sugar (1800's)
- Conversion of sugar to alcohol (1800's)

6

Some enzymes require additional factors

table 8–1

Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Cofactors = ions

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Coenzymes = organic or metalloorganic molecules

table 8–2

Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups*

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

*The structure and mode of action of these coenzymes are described in Part III of this book.

Coenzymes act as carriers of functional groups

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Some enzymes require both a cofactor & coenzyme

Complete, catalytically active enzymes & cofactor/coenzyme = "holoenzyme"

Protein part of such enzymes = "apoenzyme" or "apoprotein"

9

Enzymes classified by reaction catalyzed

table 8–3

International Classification of Enzymes*

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group-transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

*Most enzymes catalyze the transfer of electrons, atoms, or functional groups. They are therefore classified, given code numbers, and assigned names according to the type of transfer reaction, the group donor, and the group acceptor.

6 major classes w/subclasses

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How enzymes work

Under biological conditions (37°C, pH 7.0) uncatalyzed reactions tend to be slow

Enzymes circumvent these problems by providing an environment which makes the reaction more favorable - the active site

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Surface of active site lined with αα residues - bind substrate & catalyze reaction

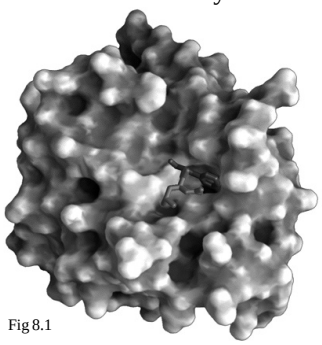

Fig 8.1

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Enzymes affect reaction rates, not equilibria

$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$

E = enzyme

S = substrate

P = product

ES, EP = transient complexes

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Any reaction (S ⇌ P) can be described by a reaction coordinate diagram

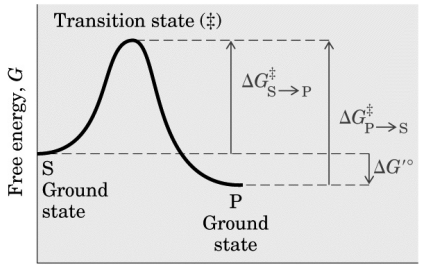

Fig 8.2

Plot of free energy (G) vs progress of reaction (reaction coordinate)

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To describe free energy changes, we need to define a standard state (G°)

- Temperature = 298 K (25 ° C)
- Each gas @ partial pressure = 1 atm
- Each solute @ concentration = 1 molar (M)

Biochemists require an additional condition

- pH = 7.0

Referred to as "Biochemical standard state" (G'°)

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Eq. Between S & P reflects difference in free energies of ground states

P < S, so ΔG'°< 0, Eq favors P

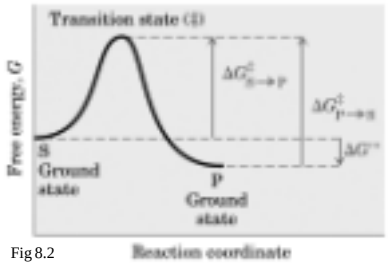

Fig 8.2

Doesn't mean S ⇌ P will occur at a detectable rate

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Enzymes accelerate interconversion between S & P

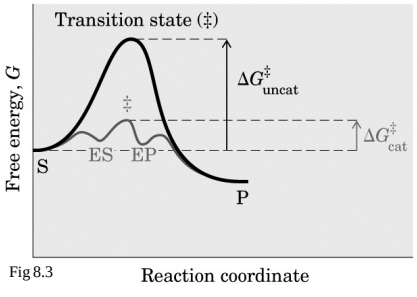

Fig 8.3

Overall rate determined by step with highest activation energy = rate limiting step

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Relationship b/w Eq constants & Free Energy

Reaction equilibria linked to ΔG'° (std. G change)

Reaction rates linked to ΔG‡ (activation energy)

For S ⇌ P

$K_{eq} = \frac{[P]}{[S]}$

From thermodynamics $\Delta G'^{\circ} = -RT \ln K'_{eq}$

R=gas constant (8.315 J/mol K)

T=Abs. Temp (298 K = 25°C)

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A positive K'$_{eq}$ ([P] > [S]) reflects a favorable (-) ▲G'°

table 8–4

Relationship between K'_{eq} and $\Delta G'°$ (see Eqn 8–3)

K'_{eq}	$\Delta G'°$ (kJ/mol)
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^{1}	−5.7
10^{2}	−11.4
10^{3}	−17.1

Larger K'$_{eq}$, more equilibrium favors products

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The rate of a reaction is determined by the concentration of reactant(s) and a rate constant (k)

for S ↔ P

Velocity (rate) of reaction = V = k[S]

1st order reaction (only [S])
units of k = s^{-1}

for 2nd order reaction: $S_1 + S_2 \leftrightarrow P$
$V = k[S_1][S_2]$
units of k = $M^{-1}s^{-1}$

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Expression derived from transition state theory which relates activation energy (▲G‡) to the rate constant (k)

$$k = \frac{\mathbf{k}T}{h} e^{-\Delta G‡/RT}$$

k = Boltzmann constant
h = Plank's constant

Important point: relationship between k and ▲G‡ in inverse and exponential

Lower ▲G‡ means higher k, *vice verse*

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Catalytic Power & Specificity of Enzymes

table 8–5

Some Rate Enhancements Produced by Enzymes

Cyclophilin	10^{5}
Carbonic anhydrase	10^{7}
Triose phosphate isomerase	10^{9}
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

Rate enhancements of enzymes 5-17 orders of magnitude over non-catalyzed reactions

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Where does energy come from to lower activation energy?

(1) Rearrangement of covalent bonds
- Transient bonds b/w catalytic groups & substrate
- Activates substrate for reaction

(2) Binding energy
- Weak, noncovalent interactions
- Each bond formation accompanied by release of free energy

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Weak interactions optimized in transition state

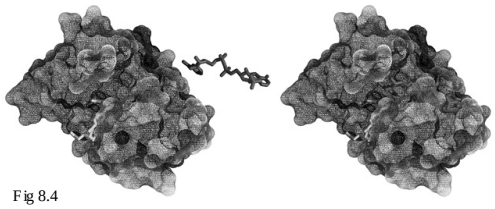

Fig 8.4

Substrates & binding site are imperfectly complementary

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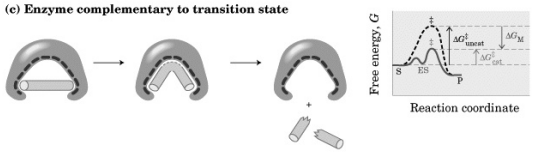
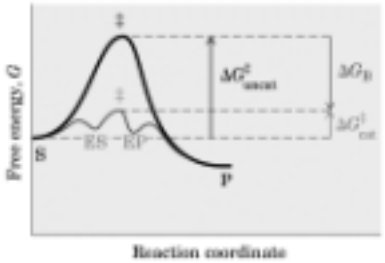
"Stickase" analogy - enzyme/transition state complementarity



Increase in free energy required for conversion "paid for" by binding energy in transition state

Binding energy translates into lower net activation energy & faster reaction rates



Requirement for multiple weak interactions one reason enzymes are large proteins

Binding energy contributes to reaction specificity & catalysis

- $\blacktriangle G^{\ddagger}$ needs to be lowered ~ 5.7 kJ/mol to accelerate a rxn 10-fold
- Energy available from the formation of a weak bond ~ 4-30 kJ/mol
- Multiple interactions may lower activation energies by 60-100 kJ/mol

Thermodynamic factors contributing to $\blacktriangle G^{\ddagger}$:

- Change in entropy (freedom of motion)
- Solvation shell around molecule
- Distortion during reaction
- Proper alignment for reaction

Binding energy can overcome all these barriers

- Holding substrates in place
- Weak interactions replacing solvation shell
- Precise alignment for reaction

Rate enhancement by entropy (S) reduction

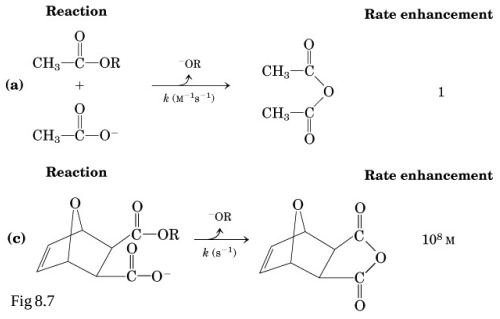


Fig 8.7

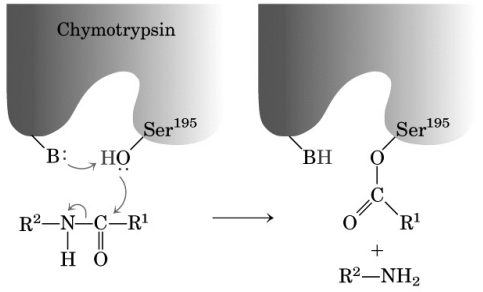
Specific groups contribute to catalysis

- Aid bond cleavage & formation
- Variety of mechanisms
  - Acid-base catalysis
  - Covalent catalysis
  - Metal ion catalysis
- Distinct from binding energy - generally form transient covalent interactions, or group transfer

Most common mechanism = <u>acid-base catalysis</u>

catalysis involving H_2O = <u>specific</u> acid-base catalysis

If proton transfer mediated by other acids/bases ($\alpha\alpha$ side chains) = <u>general</u> acid-base catalysis



Amino acids in general acid-base catalysis

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg	R—⁺NH (H) H	R—NH₂
Cys	R—SH	R—S⁻
His	R—C=CH, HN, NH, C, H	R—C=CH, HN, N:, C, H
Ser	R—OH	R—O⁻
Tyr	R— OH	R— O⁻

<u>Covalent Catalysis</u>

ex: Nucleophilic attack by "X" (R-COO^-, R-NH_2, cofactors, coenzymes, etc.) breaks bond:

$$A\text{-}B \xleftrightarrow{H_2O} A + B$$

$$A\text{-}B + X: \longleftrightarrow A\text{-}X + B \xleftrightarrow{H_2O} A{+}X: + B$$

<u>Metal ion catalysis</u>

- Metals can form weak ionic interactions w/substrate (Ca^{2+}, Zn^{2+}, etc.)
- Mediate oxidation-reduction reactions (Cu^{2+},Fe^{2+})

Nearly 1/3rd of all enzymes require metal ions for activity

Most enzymes use a combination of catalytic strategies

Enzyme kinetics help us to understand mechanisms

- What is the <u>rate</u> of a reaction?
- How does the rate change in response to changes in [substrate], temperature, pH, [activators], [inhibitors], etc?

Some terms

- V_o = Initial velocity = Reaction rate on enzyme catalysis
- V_{max} = maximal velocity
- [S] = substrate concentration
- K_m = michaelis constant

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One key factor that affects the rate (V_o) = [S]

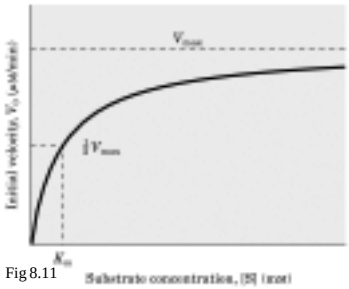

Fig 8.11

@ low [S], V_o increases in a linear fashion with [S]

@ high [S], a plateau in velocity is reached (V_{max})

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Theory of enzyme action

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

$$ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Because breakdown of ES to E & P is slower, overall reaction rate is proportional to [ES]

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@ low [S], most enzyme unbound (E vs. ES). Any increase in S pushes E + S $\rightleftharpoons$ ES to ES (linear increase)

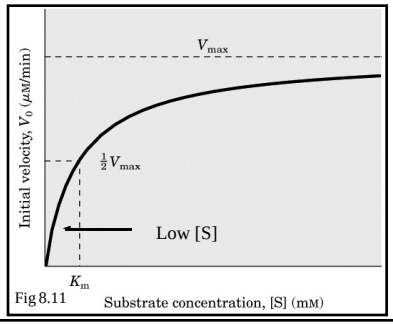

Fig 8.11

40

@ high [S],virtually all E already exists as ES - enzyme is saturated and any increase in [S] has little effect (plateau region)

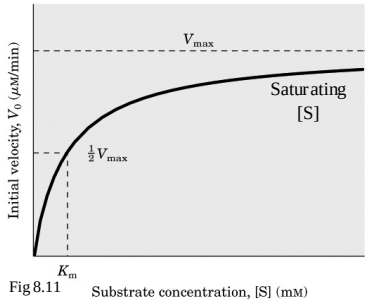

Fig 8.11

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Michaelis-Menten equation describes relationship between [S] and V_o

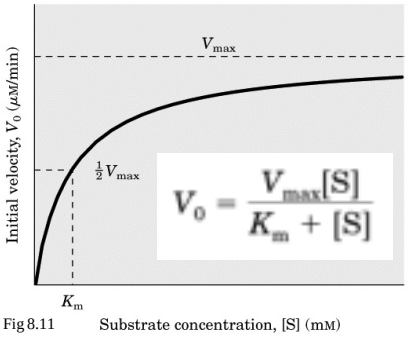

Fig 8.11

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Michaelis-Menten equation is a statement of the relationship between V_o, V_{max} and [S], all related via K_m (Michaelis constant)

Important derivation relates K_m and Velocity (V_o)

K_m = [S] velocity is half-maximal
(i.e. when V_o = 1/2 V_{max})

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Kinetic parameters used to compare enzyme activities

K_m can become complex, depending on <u>which step</u> is rate-limiting

$$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E+P$$

For our purposes defined as $K_m = \frac{k_2 + k_{-1}}{k_1}$

Depending on enzyme, could also be k_3, etc.

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Therefore, a more general rate constant = k_{cat}

k_{cat} describes the <u>limiting rate</u> of any enzyme-catalyzed reaction.

k_1, k_2, k_3, etc.

$$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E+P$$

45

K_{cat} is also called the <u>turnover number</u>

table 8–7

Turnover Numbers (k_{cat}) of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Turnover number = number of substrate molecules converted to product in a given unit of time <u>per</u> enzyme

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K_m & k_{cat} allow us to evaluate the kinetic efficiency of different enzymes

either alone is insufficient

e.g. 2 enzymes can have same k_{cat}, but <u>uncatalyzed</u> rates can be different - need term to compare

k_{cat}/K_m = <u>Specificity constant</u>

Upper limit of k_{cat}/K_m imposed by rate of diffusion of enzyme & substrate (~10^9 $M^{-1}s^{-1}$)

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Many enzymes have a k_{cat}/K_m near this range - "catalytic perfection"

table 8–8

Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-6}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8
Triose phosphate isomerase	Glyceraldehyde 3-phosphate	4.3×10^3	4.7×10^{-4}	2.4×10^8

Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W.H. Freeman and Company, New York.

Note - different values of k_{cat} & K_m can produce the maximum ratio of "catalytic perfection"

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Multiple substrate enzyme reactions

Many enzymes have 2 or more substrates

ATP + Glucose ↔ ADP + Glucose-6-P

Characteristic K_m for each substrate

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Multi-substrate reactions proceed by different paths

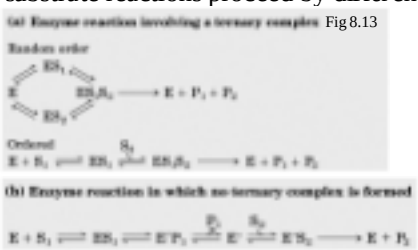
Fig 8.13

(1) Random or ordered: Enzyme & substrates form a ternary complex ES_1S_2

(2) Double displacement or "ping-pong": NO ternary complex formation - first substrate converted to product & released before 2nd substrate bound

50

Kinetics helps distinguish between these mechanisms

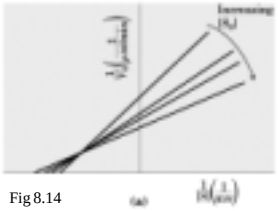
Fig 8.14

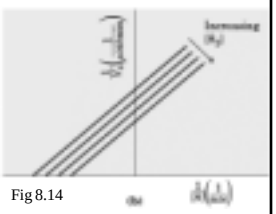
Fig 8.14

(a) intersecting lines indicate ternary complex

(b) parallel lines indicate "ping-pong" mechanism

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Enzyme Inhibitors

Molecules that interfere with catalysis - slowing or halting enzymatic reactions (powerful drugs)

Study of inhibitor action also provides insight into enzymatic mechanisms

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2 Broad classes of Inhibition:
(1) reversible (2) irreversible

Reversible inhibition:

- Competitive
- Uncompetitive
- Mixed

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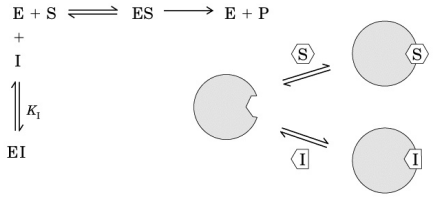
Competitive inhibition



(a) Competitive inhibition

- Competitive inhibitor competes w/substrate for active site
- Prevents binding of substrate
- Structure resembles substrate
- Forms EI (vs ES) complex, but w/o leading to catalysis

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Competitive inhibition can be analyzed quantitatively

In presence of inhibitor, M-M eqn. Becomes

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

where $\alpha = 1 + \frac{[I]}{K_I}$

and $K_I = \frac{[E][I]}{[EI]}$; $E + I \leftrightarrow EI$

αK_m = "apparent K_m"= K_m in presence of inhibitor

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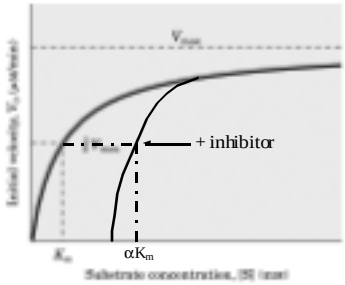
When [S]>>[I], probability of I binding E low, reaction exhibits normal V_{max}, but apparent K_m increases by α



Normal V_{max} combined with changed K_m in presence of inhibitor is diagnostic of competitive inhibition

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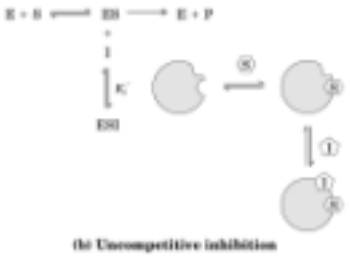
Reversible inhibition: Uncompetitive



- Enzymes with 2 or more substrates
- Uncompetitve inhibitors bind at sites distinct from substrate active site
- Bind only to ES complex; lowers K_{max} & V_{max}

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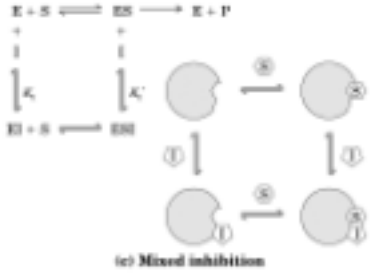
Reversible inhibition: Mixed



- Inhibitor also binds at site distinct from active site
- Inhibitor binds to either E or ES
- Usually affects both K_m and V_{max}

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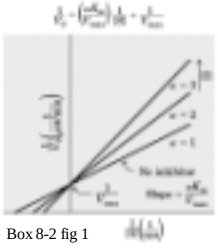
Kinetic tests used to determine Inhibition



Box 8-2 fig 1

Competitive inhibition

- Different slopes ($\alpha K_m/V_{max}$)
- Same y-intercept ($1/V_{max}$) at each [I] → V_{max} unchanged
- Different x-intercepts ($-1/K_m$) = different K_m's

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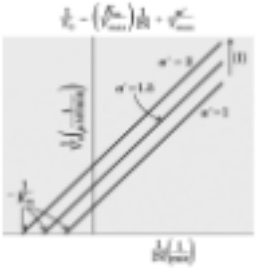
Uncompetitive inhibition



- Different y-intercepts ($1/V_{max}$) = different V_{max}
- Different x-intercepts ($-1/K_m$) = different K_m's
- Parallel slopes (K_m/V_{max}) = best diagnostic

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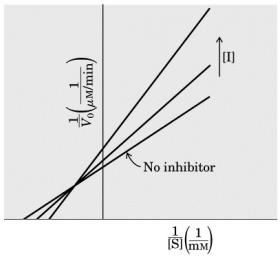
Mixed Inhibition

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



- Different slopes
- Different x-intercepts = different K_m's
- Different y-intercepts = different V_{max}'s

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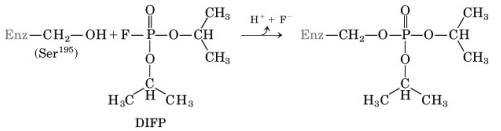
Enzyme Inhibition: Irreversible



- Inhibitors combine with or destroy functional group essential for activity
- Formation of covalent links common
- Special class = "suicide inhibitors"
  - Unreactive until bound to active site
  - Enzyme may carry out first few catalytic steps
  - Inhibitor converted to reactive compound
  - Compound combines irreversibly with active site

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Enzyme activity is affected by pH

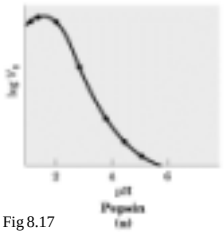

Fig 8.17

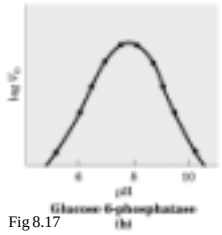

Fig 8.17

- Critical αα side chains act as acids/bases
  - Dependent on maintenance of certain state of ionization
  - Ionization state dependent on pH

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Examples of enzymatic reactions

Complete mechanism includes:

- ID of all substrates, cofactors, products, regulators
- Temporal sequence in which intermediates form
- Structure of each intermediate & transition state
- Rates of interconversion b/w intermediates
- Structural relationship of enzyme w/each intermediate
- Energy of all intermediate complexes & transition states

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Example Mx's: Chymotrypsin, Hexokinase, Enolase

Chymotrypsin

- Protease; catalyzes hydrolytic cleavage of peptide bonds
- Specific for cleavage of peptide bonds adjacent to aromatic residues (Tyr, Phe, Trp)
- Increases hydrolysis by factor of 10^9
- Good example of transition state stabilization, general acid-base catalysis & covalent catalysis

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2 major phases: Acylation & De-acylation

(1) Acylation - transient covalent acyl-enzyme intermediate formed

- Peptide bond cleaved
- Ester linkage formed b/w peptide carbonyl carbon and enzyme

(2) De-acylation

- Ester linkage hydrolyzed
- Nonacylated enzyme regenerated

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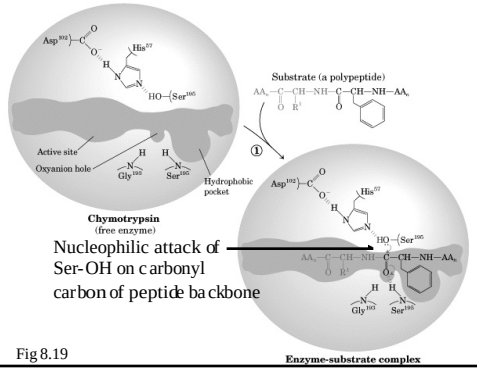
(1) binding of substrate & nucleophilic attack



Nucleophilic attack of Ser-OH on carbonyl carbon of peptide backbone

Fig 8.19

Formation of enzyme-substrate intermediate (acylation):

- (-) Charge forms in "oxyanion hole"
- Charge stabilized by H-bonds
- Example of bin ding energy

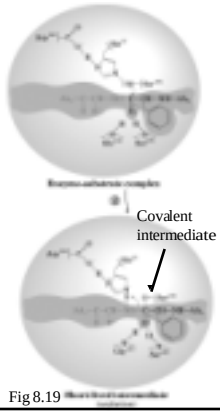


Fig 8.19

Acyl-enzyme intermediate formed

peptide cleaved

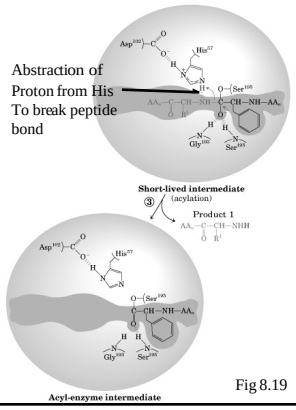


Fig 8.19

Water attacks carbonyl of acyl-enzyme ester linkage

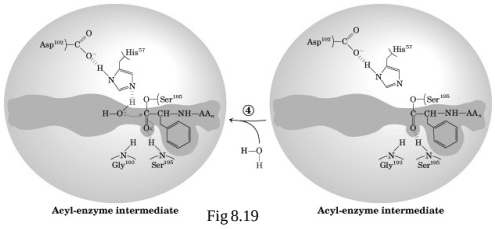
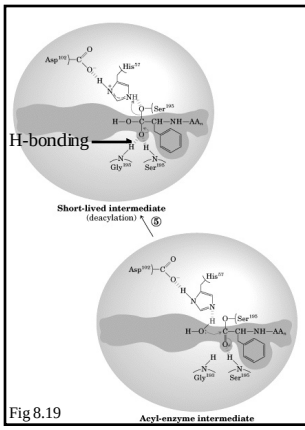


Fig 8.19

Abstraction of proton (H) from H_2O by His activates water for attack on carbonyl group



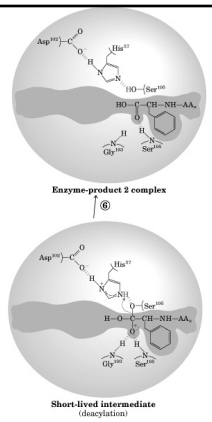
Short-lived intermediate formed:

- (-) charge on carbonyl carbon
- Stabilized by H-bonding in "oxyanion hole"

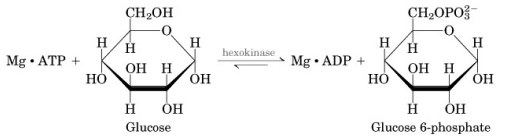
Fig 8.19

Deacylation

- His donates proton to Ser
- General acid-base catalysis
- New C-terminus on peptide
- Product 2 leaves
- Enzyme regenerated



Hexokinase - Induced Fit



Glucose -OH group similar in reactivity to H_2O, yet Hexokinase discriminates by a factor of 10^6

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When glucose absent, enzyme inactive

- Active site αα side chains out of position

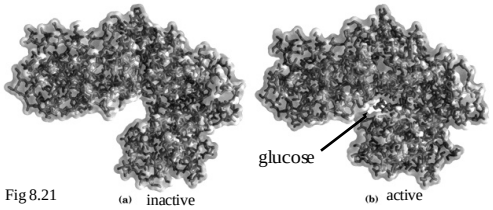

Fig 8.21

When glucose (not H_2O) binds, binding energy induces conformational change to active form

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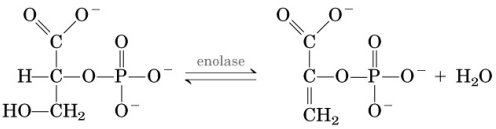
Enolase: Metal ion catalysis



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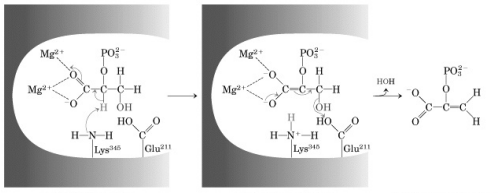

(1) carboxylate group (2PG) coordinated to Mg^{2+} ion

(2) Lys acts as general base to abstract proton from 2PG

(3) Glu acts as a general acid - donates proton to 2PG-OH leaving group to release H_2O and PEP

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Regulatory enzymes

groups of enzymes often work together to catalyze sequential reactions

Glucose → Glc-6-P → Fru-6-P → Fru-1,6-P → etc.

At least one enzyme in pathway will set rate for overall sequence - it catalyzes the slowest or rate-limiting reaction

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Regulatory enzymes exhibit increased or decreased activity in response to signals

allow cells to meet changing needs for energy & biomolecules

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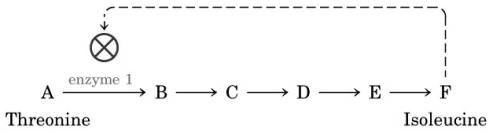
In most multienzyme pathways, the first enzyme is a regulatory enzyme



Prevents wasting energy & resources in catalyzing first few reactions

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Activities of regulatory enzymes modulated in a variety of ways

(1) reversible, noncovalent binding of regulatory compounds
- Often end products of pathways

(2) Reversible, covalent modification
- Phosphorylation, acetylation, etc.

(3) Binding of separate regulatory proteins
- RNA polymerase, gene expression, etc.

(4) Activation via proteolytic cleavage
- Removal of peptide segments
- irreversible

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2 major classes of regulatory enzymes

(1) Allosteric enzymes
- Reversible, noncovalent binding of allosteric modulators

(2) Reversible covalent modification

Both types tend to be multi-subunit proteins
- Active sites and regulatory sites sometimes on different subunits

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Allosteric enzymes undergo conformational changes in response to modulator binding

- Modulators convert less active to more active, vice verse
- Allosteric modulators can be either inhibitory or stimulatory
- When modulator = substrate - "homotropic"
- When modulator is different - "heterotropic"

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Allosteric enzymes generally have one or more regulatory (allosteric) sites for binding the modulator

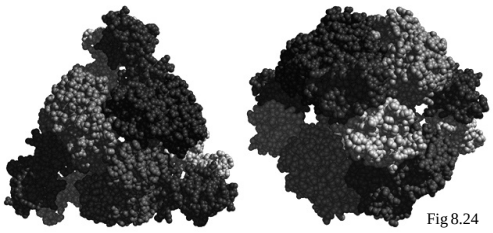

Fig 8.24

Allosteric enzymes generally larger, more complex

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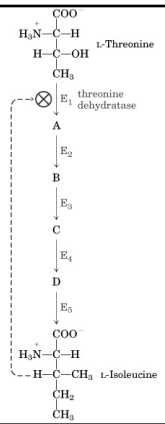
Feedback inhibition

Regulatory enzyme inhibited by end product of a pathway



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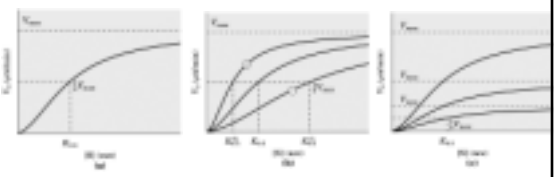
Kinetic properties of allosteric enzymes diverge from M-M behavior



Sigmoid saturation curves reflect cooperative interactions

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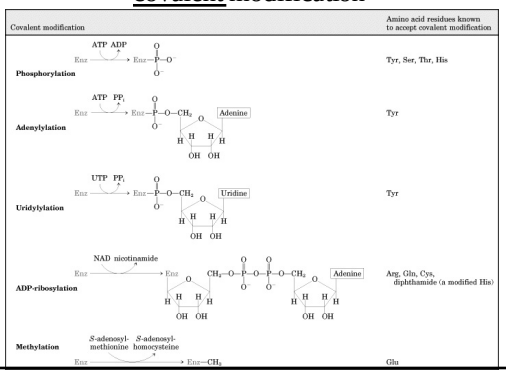
Some regulatory enzymes undergo <u>reversible covalent</u> modification



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Phosphoryl groups affect the structure & catalytic activity of proteins

often attatched to Ser-OH, Thr-OH, Try-OH groups

Introduces a bulky, charged group into a region that was only moderately polar

Protein <u>kinases</u> - <u>attach</u> phosphoryl groups
Protein <u>phosphotases</u> - <u>remove</u> phophoryl groups

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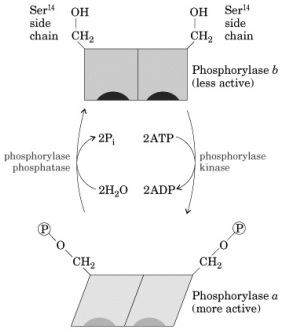
Example: glycogen phosphorylase



a & b forms differ in 2°, 3 ° and 4 °

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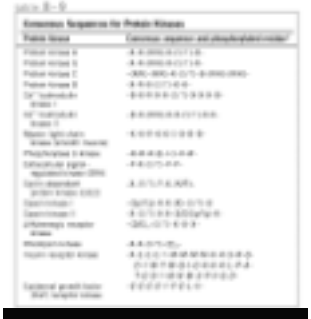
Phosphorylation consensus signals



Multiple phosphorylation signals provide potential for subtle modification - not simple "on/off" switch

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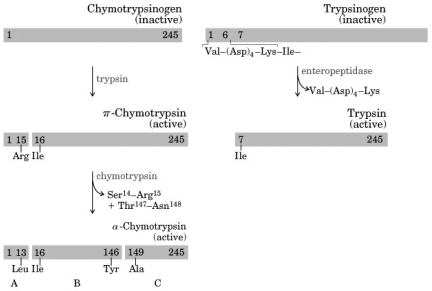
Some types of regulation require proteolytic cleavage of an enzyme precursor



Cleavage causes conformational change which exposes active site

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Chapter 8 Summary

- Virtually every cellular reaction is catalyzed by an enzyme - with few exceptions, enzymes are proteins
- Enzymes enhance reaction rates 10^5 to 10^{17} X
- Some enzymes require chemical cofactors (metal ions) or coenzymes (organic molecules)
- Enzymes are classified according to the reaction they catalyze (oxidation-reduction, etc.)

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- Energy for enzymatic rate enhancement derived from weak interactions w/substrate (binding energy
- Binding energy
  - Lowers substrate entropy (positions substrates)
  - Strains substrate for reaction
  - Induced fit (conformational change)
- Common catalytic mechanisms:
  - Specific acid-base catalysis (H_2O)
  - General acid-base catalysis (R-groups)
  - Metal ion catalysis
  - Covalent catalysis

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Kinetics

As [S] ↑, catalytic activity ↑ to approach a maximum rate (V_{max}), where all E in ES form

K_m = [S] where $V_o = V_{max}/2$

Michaelis-Menten equation relates velocity (V_o) to [S] and V_{max} through K_m

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

Both K_m and V_{max} can be measured experimentally

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- k_{cat}/K_m provides a good measure of catalytic activity
  - k_{cat} = turnover number
- Enzymes can be inactivated by reversible or irreversible modification (binding or covalent modification)
  - Competitive inhibitors (compete w/substrate)
  - Uncompetitive inhibitors (bind ES, separate site)
  - Mixed inhibitors (bind either E or ES, separate site)

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- Feedback inhibition: end product of a pathway inhibits first enzyme in pathway
  - Reduces wasted energy and resources
- Allosteric enzymes: activity adjusted by reversible binding of activators (+) or inhibitors (-)
- Other enzymes can be modulated by covalent modification (phosphorylation, adenylation, etc.)
- Many proteolytic enzymes activated by peptide cleavage.

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