

ENZYMES (DR. NUGENT)

I. INTRODUCTION TO ENZYMES/CATALYSTS

- a. Definition: enzyme = biological catalyst (catalyst = speeds rate of reaction)
- b. Features of enzymes:
 - i. Enzymes speeds up the rate of a reaction 1000000 fold, up to 10^{12} → reaction can now fulfill a need
 - ii. enzymes are **specific** – to certain substrates or to class of compounds (this is different from a general catalyst such as Ni or Pt)
 - iii. enzymes put compounds in an environment where certain reactions are now accelerated (don't want to change environment of entire system, ie body ph, so need active site of enzyme to create micro environment)
 - iv. enzymes CANNOT:
 1. change equilibrium of a reaction
 2. be consumed or changed (they are always **regenerated**)

II. THERMODYNAMICS OF CHEMICAL REACTIONS

a. Terms and Equations

- i. G = free energy; $\Delta G^\circ = G_{\text{products}} - G_{\text{reactants}}$; if $\Delta G^\circ < 0$ then reaction is spontaneous
- ii. $K_{\text{eq}} = \text{Product/Reactant}$; if $K_{\text{eq}} > 1$ then reaction is spontaneous (there are more prod than reactants at equilibrium)
- iii. $\Delta G^\circ = RT \ln K_{\text{eq}}$; R = constant and T = temp

b. Interpretation of G :

- *relative terms {
- i. **High** free energy means thermodynamically **unstable**
 - ii. **Low** free energy means thermodynamically **stable** (high probability that compound will stay in this state)
 - iii. ΔG° for hydrolysis of glucose = -686 kcal/mol → means glucose is very unstable BUT **kinetically stable** (refers to time frame)...

c. Activation energy

- i. Activation energy (ΔG^\ddagger) is the barrier the reaction must get past
- ii. transition state = least stable conformation of reactant (state in which all C-C bonds are half-broken, half-formed) → very unlikely reactant will stay in this state
- iii. The rate of the reaction is defined by the activation energy:
 1. $\Delta G^\ddagger = G_{\text{transition state}} - G_{\text{ground}}$ $\Delta G^\ddagger > 0$
 2. A catalyst lowers activation energy (ΔG^\ddagger)
- iv. Ways enzymes reduce ΔG^\ddagger :
 1. By altering the course of a reaction: many enzymes work together in a pathway ("staircase analogy")
 2. By destabilizing the ground state → higher G^0 ; smaller ΔG
 3. By stabilizing the transition state → lower G_{trans} , smaller ΔG

III. RATE THEORY

a. Equations

- i. $\Delta G^\ddagger = -RT \ln K^\ddagger = G_{\text{trans}} - G_{\text{ground}}$
- ii. $K^\ddagger = [T]/[\text{ground}] \ll 1$ (small fraction because T is very unstable)
- iii. $A \rightarrow P$ is an irreversible reaction, and velocity is not constant (A gets used up) so to find velocity: $d[P]/dt = V = k[A]$ M/sec $k = \text{rate constant}$
*this is a first order reaction

b. Derivation of relationship of ΔG^\ddagger to k

- i. $d[P]/dt = k[A]$
- ii. $k[A] = v[A^\ddagger]$ (rate constant relates to activation energy)
- iii. $\Delta G^\ddagger = -RT \ln K^\ddagger = -RT \ln [A^\ddagger]/[A]$
- iv. $\Delta G^\ddagger = 17.4 - 1.36 \log k$
 1. So ΔG^\ddagger relates to log of the rate constant k
 2. if ΔG^\ddagger decreases, then k increases exponentially:

$v = 6.2 \times 10^{12} / \text{sec}$
Bolt's constant, the theoretical frequency of how fast things change if there is no barrier

ΔG^\ddagger	k
17.4	1
13.4	1000

c. Reverse reaction

- i. $d[P]/dt = k_f[A] - k_r[P] = 0$ at equilibrium
- ii. $K_{\text{eq}} = [P]/[A] = k_f/k_r$
- iii. Tells us relative speeds: if $K_{\text{eq}} > 0$, then $k_f > k_r$
- iv. *enzymes cannot change preference: a catalyst that speeds up k_f speeds up k_r to an equal magnitude (eg enzyme that accelerates phosphorylation also accelerates dephosphorylation)

IV. ENZYME CATALYSIS IN PHYSIOLOGY AND MEDICINE

a. The Blood Clotting Cascade - Introduction

- i. The circulatory system is a closed system. A cut violates this closed system \rightarrow body needs to stop bleeding without changing circulation everywhere else \rightarrow need to change liquid to solid (clot) in one area
- ii. Biochemistry has certain time constraints \rightarrow need enzymes to speed a reaction exponentially (1 enzyme \rightarrow 1,000,000 enzymes \rightarrow 1,000,000,000,000)
- iii. Enzymes can not only speed up a reaction; they can also speed up the reactions of other enzymes.
- iv. **Proteases** change circulating proteins; some change activity of other enzymes

b. Blood Clotting Cascade – Mechanism

- i. **Fibrinogen** – a protein in the blood that is soluble (has hydrophilic residues)
- ii. **Thrombin (IIa)** – a protease
 1. clips off “hairs” on fibrinogen
 2. hydrophilic region is exposed
 3. fibrin self-associates to form **fibrin clot** (precipitates)
- iii. Thrombin is not floating in blood; it is made by activating **prothrombin** (made in liver)

- iv. **Factor Xa** (made from X, which is in blood) clips prothrombin to make thrombin
- v. **Factor IXa** activates factor X
- vi. **Factor VIII** activates IX

So: there are multiple ways to activate enzymes, and the clotting can be controlled at various points in cascade.

c. Control of Blood Clotting

i. Vitamin K

- 1. injected in newborns to prevent hemorrhagic disease
- 2. collaborates with γ glutamyl carboxylate in liver to activate prothrombin (also factor X, others)
- 3. takes days-weeks to build up clotting capabilities with vitamin K

ii. Blood clotting inhibitors:

1. Warfarin

- a. acts at beginning of cascade
- b. Acts in liver: antagonizes vitamin K
- c. prevents prothrombin (II) and factor X from being sufficiently carboxylated - can't be active
- d. inhibits clotting
- e. Examples = d-Con, Coumadin

2. **Antithrombin III** = natural inhibitor

- a. acts at end of cascade
- b. inactivates thrombin(IIa) – forms a covalent cross-link and kills it
- c. antithrombin III binds to a healthy surface on endothelium, changes into more active conformation
- d. **heparin sulfate** (linear polysaccharide) = compound expressed by endothelium
- e. *Heparin can be isolated and used as an anticoagulant

3. Mechanism for eliminating a clot:

- a. **T-PA (tissue plasminogen activator)** - enzyme that activates **plasminogen** into **plasmin** (a protease)
- b. Plasmin “chews up” fibrin (insoluble) into soluble products*
*not reusable – blood clotting is only for “emergencies” because it is “expensive”; entire system requires resynthesis
- c. can't administer plasmin (would need too much!) but can administer t-PA, which then activates exponentially more plasmin.
- d. Returns system back to normal

Q: How would you treat a Coumadin overdose? A: With vitamin K

V. ENZYME MECHANISMS

Enzymes can reduce ΔG^\ddagger in a number of ways:

- a. Alter course of reaction – break an unlikely event into multiple steps
 - i. ΔG^\ddagger is log related to K – drop ΔG^\ddagger by a little \rightarrow increase K exponentially
- b. Destabilization of substrate/Stabilization of transition state
 - i. Geometric – twisting, turning, pulling (ie tetrahedral conformation into planar – induces formation of double bonds)
 - ii. Electrostatic – put compound in a different environment \rightarrow raise free energy
 - iii. Desolvation – make compound no longer soluble (ie by excluding water in active site)
- c. Two models of enzyme specificity
 - i. Lock and Key (older hypothesis)
 1. Enzymes are geometrically (or chemically) oriented so they have an active site that is complementary - a mirror image - of substrate binding site
 2. Meeting of two chemical groups \rightarrow reaction is favored
 3. Suggests that enzymes evolved to be perfect fits for substrates
 4. Formal definition of **substrate binding site**= region of enzyme that makes noncovalent contact with substrate such that it can hold the substrate in place
 5. **Active site** = components on the enzyme that are actively involved in chemical catalysis (doesn't have to be same as substrate binding site)
 - ii. Induced fit (newer – addresses destabilization)
 1. Evolutionary pressure is for enzyme to be complementary to the transition state so “if it ever found transition state around it would bind very nicely”
 2. BUT enzyme never finds transition state (unlikely conformation because unstable) \rightarrow enzyme tries to find substrate that somewhat resembles, and then changes
 3. Both substrate and enzyme are strained a little to destabilize ground state, make transition state a little more likely \rightarrow more likely to react

VI. ENZYME KINETICS - Michaelis-Menten Equation

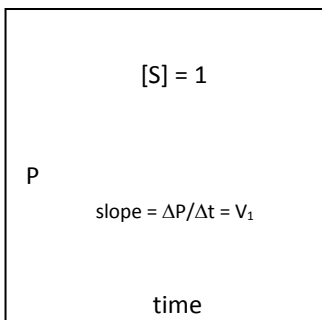
a. Reaction Model



ii. Initial Rate approximates linear generation (given rate of reaction)

iii. Assumptions:

1. No reverse reaction occurring
2. Neglect Product Inhibition (Feedback inhibition)
3. $[S]$ is constant (1 million – 1 is still roughly 1 million)



4. Enzyme activity is constant (in reality, enzyme is subject to side reactions, and it loses its reactivity)

b. Relationship of V to [S]

- i. V is dependent on [S] – more input → more output, but not linear (eventually not enough enzyme to go around)

ii. Describe V using **Michaelis-Menten Equation** – derivation:

1. $E_{total} = E_{free} + E \bullet S$ (1)

2. $V = k_{cat} [E \bullet S]$ (2)

a. 1st order reaction, units are /sec

b. k_{cat} = catalysis constant, or “turnover number” – defines rate at which enzyme can turn over

3. $V_{max} = k_{cat} [E_{total}]$ (3)

a. Tells how many products enzyme can make per second, if S is unlimited

b. But this still doesn't tell us how much S we need → K_m is concentration of substrate needed to drive reaction to half its potential (units = M)

4. When $[S] = K_m$, reaction runs at half potential: $E = E \bullet S = \frac{1}{2} E_{total}$, $V = \frac{1}{2} V_{max}$

a. Substitute for (3) and get: $V = k_{cat} [E \bullet S]$

b. Plug in derivative: $d [E \bullet S] / dt = 0$ (steady state approximation)

c. Substitute ks for all reactions and get $[S] = K_m = (k_{-1} + k_{cat} / k_1)$

d. Solve for $[E \bullet S]$ and get:

$$V = \frac{k_{cat} [E_{tot}] [S]}{K_m + [S]}$$

Michaelis-Menten Equation

- * Velocity relates to how much enzyme and how much substrate, defined by these parameters
- * An enzyme is defined by these parameters → if you know K_m and k_{cat} , you can tell how much product it will make per unit time at any given [S]
- * Also can use equivalent equation:

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

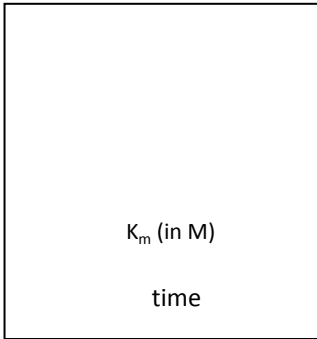
c. Applications of Michaelis-Menten Equation

i. When $[S] \gg K_M \rightarrow K_M + [S] \approx [S] \rightarrow V = \frac{V_{max} [S]}{[S]} \approx V_{max}$

ii. When $[S] = K_M \rightarrow K_M + [S] \approx 2 K_M \rightarrow V = \frac{V_{max} [S]}{2 [S]} \approx \frac{1}{2} V_{max}$

iii. When $[S] \ll K_M \rightarrow K_M + [S] \approx K_M \rightarrow V = \frac{V_{max} [S]}{K_M} \approx V_{max} [S] / K_M$

* for low values of [S], slope = V_{max} / K_M (linear)



- d. Catalytic efficiency: ratio of k_{cat} to K_m
 - i. describes how well enzyme reacts with small $[S]$
 - ii. Units are $M^{-1} sec^{-1}$
 - iii. Velocity is most sensitive to $[S]$ at low $[S]$ – velocity is linear: k_{cat}/K_m
- e. “Perfect Catalyst”
 - i. $k_{cat}/K_m = k_{cat} / ((k_{cat} + k_{-1}) / 1) = k_1$
 - ii. k_1 is as big as it can be = “diffusion control” - on the order of 10^{10} , 10^{12}
 - iii. means every time enzyme encounters substrate, it binds
- f. Binding constant
 - i. Pseudo-equilibrium between $E+S$ and $E\bullet S$
 - ii. Can be described as K_s (equilibrium dissociation binding constant) = k_{-1}/k_1
 - iii. By convention, described in reverse direction: $K_s = [E][S]/[E\bullet S]$
- g. Lineweaver-Burk Plot
 - i. take reciprocals: $1/[S]$ and $1/V = (K_m + [S]) / (V_{max}[S]) = (K_m/V_m)(1/[S]) + 1/V_{max}$
 - ii. data can now be related in a linear fashion

VII. ENZYME INHIBITION

- a. Reversible Inhibition
 - i. Competitive
 1. Inhibitor and substrate compete for same binding site
 2. Reaction model: $E + I \rightleftharpoons E\bullet I$
 3. Equation: $K_i = [E][I]/[E\bullet I] = M$
 - a. If $K_i = [I] \rightarrow E = [E\bullet I] \rightarrow$ ½ available enzyme will be occupied
 4. Parameters
 - a. more $[S]$ added \rightarrow greater probability that S will bind $\rightarrow V_{max}$ does not change
 - b. Apparently reduces K_m
 - c. So catalytic efficiency is decreases
 5. Relationship of V to $[S]$ and $[I]$
 - a. $E_{tot} = E + ES + EI$ $K_i = [E][I]/[EI]$ $K_{M_i} = K_M (1 + [I]/K_i)$
 - b. **$V = V_m[S] / (K_m(1 + [I]/K_i) + [S])$**
 - *The lower the K_i , the more potent the inhibitor (binds better)
 - ii. Noncompetitive
 1. Inhibitor binds at different site from binding site
 2. $K_i = [E][I]/[EI] = [ES][I]/[ESI]$ – sum of $[EI]$ and $[ESI]$ is a constant at a given $[I]$ – doesn’t matter if I is bound to E or $E+S$ – independent of $[S]$
 3. Parameters
 - a. K_M stays the same (same amt S to occupy available enzyme)
 - b. V_{max} decreases (no amt of S can overcome I)
 - c. Apparently reduces k_{cat} : $k_{cat\ app} = k_{cat} / (1 + [I]/K_i)$
 - d. So catalytic efficiency decreases

$$4. \quad V = \frac{K_{cat} [(1+[I]/K_i) [E_{tot}] [S]}{(K_M+[S])}$$

iii. Uncompetitive

1. Inhibitor is dependent on substrate – cannot bind to enzyme in unbound state because binding site is unavailable
2. $K_i = [ES][I]/[ESI]$
3. Parameters
 - a. Apparently reduces k_{cat} : $k_{cat\ app} = k_{cat}/(1+[I]/K_i)$
 - b. Apparently reduces K_m : $K_m\ app = K_m/(1+[I]/K_i)$
 - c. Since these both change, catalytic eff is the same

$$4. \quad \frac{K_{cat} [E_{tot}] [S]}{(K_M+[S] (1+[I]/K_i))}$$

5. With very low [S] I has little to no effect; with high [S], I has more effect

Summary

	K_m	k_{cat}	k_{cat}/K_m
Competitive	Increases	No change	Decreases
Noncompetitive	No change	Decreases	Decreases
uncompetitive	decreases	Decreases	No change

iv. What do good inhibitors look like?

1. Substrate analog
2. Transition state analog (like trans. except stable) – even better

b. Irreversible Inhibition

- i. Inactivators –forms a covalent bond with some component of the active site → result in a permanent loss of enzyme activity
- ii. No effect on K_m or V_{max} – just eliminates activity
- iii. Example: TPCK – inactivates chymotrypsin

VIII. Proteases

i. Example: Chymotrypsin

1. Protease – catalytic cleavage of peptide bonds (ex in intestines – digestive proteins)
2. Site of cleavage is next to bulky residues (Tyr, Trp, Phe) – substrate binding site (hydrophobic) holds residue, positions aa bond to be cleaved

ii. Experiment with chymotrypsin (Hartly and Kilby 1953)

1. PNPA: pseudosubstrate that is clear, yellow after cleaved to PNP-
2. Graph showed “initial burst” – stoichiometrically equal to amount of enzyme present
3. Conclusion: catalysis is 2 steps:
 1. Fast – formation of intermediate
 2. Slow – hydrolysis

4. Catalytic triad – if these three aa's aren't present, enzyme doesn't act
- iii. Summary of proteases
1. Substrate binding site is a hydrophobic pocket
 2. His 57 acts as a general acid/base – donates/accepts H⁺ during catalysis
 3. Ser 195 is the “reactive nucleophile” involved in forming the covalent intermediate with substrate during catalysis
 4. Asp102 donates negative charge through His57 to make Ser195 a more reactive nucleophile
 5. The catalytic process is comprised of 2 steps:
 - a. Acyl-intermediate formation
 - b. Acyl-intermediate hydrolysis

IX. ENZYME REGULATION

- a. Gene Control-expression
- b. Proteolytic activation – one way – blood clotting, digestion
- c. Inhibitors/Activators/Inactivators
- d. Reversible Covalent Modification
 - i. can activate/inactivate/change enzyme's preference for substrates
 - ii. ex: phosphorylation
 1. Ser, Thr, Tyr – all have –OH group → can be phos.
 2. Replace –OH with phosphate group (charged)
 3. Protein kinase couples sum of two processes:
 - a. $ATP \rightarrow ADP + P_i$
 - b. $E-OH + P_i \rightarrow E \bullet P_i$
 4. Protein phosphatase catalyzes reverse: $H_2O + E \bullet P_i \rightarrow E + P_i$
- e. Allosteric Control – ability of compounds to regulate enzymatic activity through multiple subunits – bind and change quaternary structure – NOT Mich-Menten enzymes
 - i. Types: homotropic/heterotropic
 - ii. Examples
 1. Hemoglobin
 2. Aspartate Transcarbamylase
 - a. Can be regulated both negatively and positively
 - b. First step of pyrimidine biosynthesis pathway
 - c. Regulatory units (dimers) have two conformations:
 - i. Relaxed = active – assumes when ATP binds
 - ii. Tense = inactive – assumes when CTP (in a final step) builds up and binds
 - iii. ATP and CTP bind competitively to regulatory domain
 3. Protein kinase A
 - a. 2 regular domains (dimer)
 - b. Hormones → prod of cAMP → binds to dimer → catalytic subunits released
 - c. cAMP falls off → goes back to dimer