ENZYMES: Binding & Catalysis

A. Binding
B. Catalysis
C. Nomenclature
D. Catalysis
   1. Transition State Theory
   2. **Catalytic** strategies (**What**)
      a. Position (proximity), Polarization, Strain, desolvation
   3. **Mechanistic** strategies (**How**)
E. Quantifying the Catalytic Power: Kinetics
   1. Review of kinetics
   2. Enzyme Kinetics (M-M equation)
      a. M-M equation
      b. Lineweaver-Burk; double reciprocal
      c. Inhibition
         i. Competitive; like substrate; K_m affected by $(1 + [I]/K_I) = \alpha$
         ii. Uncompetitive; binds only ES; both K_m and V_{max} affected in opposite ways
         iii. Noncompetitive; binds both E & ES equally; V_{max} affected
         iv. Mixed inhibition if I binds E differently than it binds ES ; V_{max} affected, K_m affected
      a. Uses of Steady-state kinetics
      b. Active-site identification
         i. Determine mechanism-distinguish ping-pong *versus* sequential
         ii. pH studies; do ionizations match amino acid pK_a's when looking at pH *vs.* activity?
         iii. Protein modification; Irreversible
         iv. X-ray crystallography structure; cleft, complexes with ligands (inhibitors or substrates)
      c. Energetics of Catalysis
         i. $\Delta\Delta G^{\ddagger}$ is negative
         ii. $\Delta\Delta G^{\ddagger} = \Delta\Delta H^{\ddagger} - T\Delta\Delta S^{\ddagger}$; bonding effects & proximity/position effects
         iii. Rate dependent on $(kT/h)EXP(-\Delta G^{\ddagger}/RT)$
         iv. Example of enzyme; Proline Racemase
         v. HIV protease; tetrahedral t.s. seen in two nM inhibitors ($K_I < 1$ nM); bioavailability

Lecture 17 (10/22/25)

- Reading: Ch5; 157
  Ch12; 413-415

NEXT

- Reading: Ch5; 157-160
  Ch6; 213-220
- Homework #18

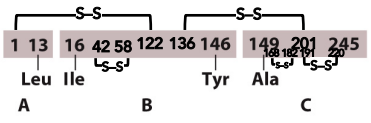
F. **Enzyme Mechanisms**
   1. Proteases
      a. Introduction; roles and types
         Serine, Thiol, Acid, Me
   2. Serine Proteases
      a. Reaction & specificity
      b. Activation (zymogens); Ile α-N
      c. Active Site Determination
         i. esterase activity
         ii. burst kinetics => two-steps (Ping-pong bi bi)
         iii. protein modification
         iv. pH studies
         v. X-ray crystallography
      d. Proposed mechanism
         i. Catalytic triad (Ser-His-Asp)
         ii. Mechanism;
      e. Specificity
         i. Chymotrypsin *versus* elastase
   3. Other protease mechanisms
G. **Enzyme Regulation**
   1. Introduction
   2. Strategies

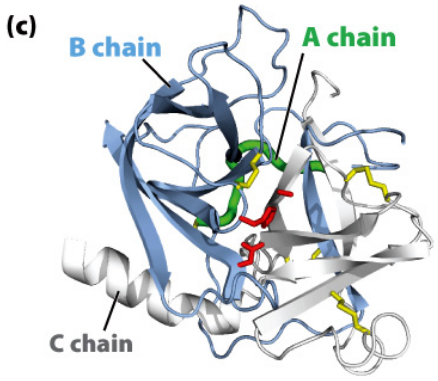
Enzyme Mechanism

- ***structural studies***

Chymotrypsin

- *X-ray crystallography*

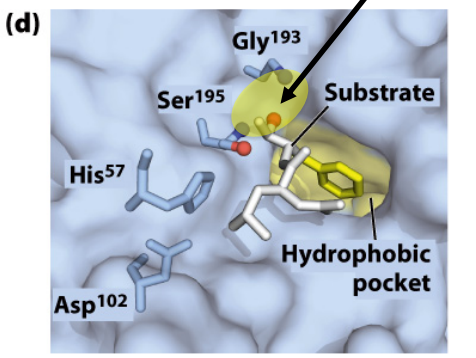




Chymotrypsin Uses Two of our the Mechanistic Strategies

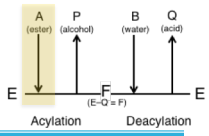
covalent
acid/base

Anion "Hole"



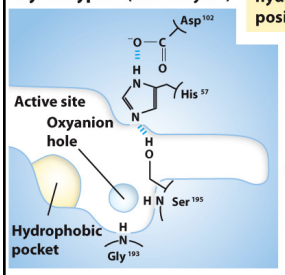
Chymotrypsin Mechanism

Step 1: Substrate Binding



When substrate binds, the side chain of the residue adjacent to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack.

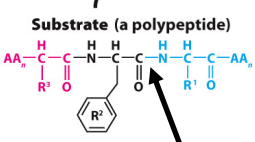
Chymotrypsin (free enzyme)



E

1

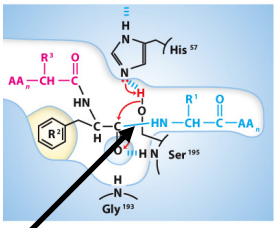
Substrate (a polypeptide)



S(A)

Sessile bond

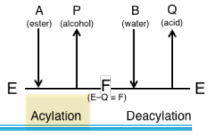
Enzyme-substrate complex



ES (EA)

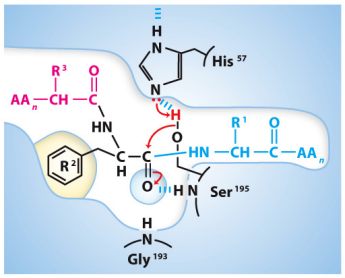
Chymotrypsin Mechanism

Step 2: Nucleophilic Attack



Interaction of Ser^{195} and His^{57} generates a strongly nucleophilic hydroxyl on Ser^{195}; which attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accompanied by formation of ashort-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.

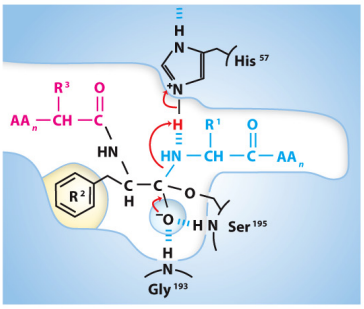
Enzyme-substrate complex



EA

2

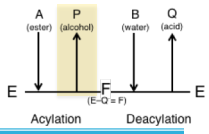
Short-lived intermediate* (acylation)



F-A

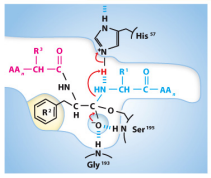
Chymotrypsin Mechanism

Step 3: Substrate Cleavage



Short-lived intermediate* (acylation)

Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His[57], facilitating its displacement.

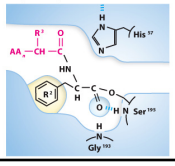


F-A⇌F-QP

Product 1

P

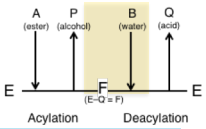
Acyl-enzyme intermediate



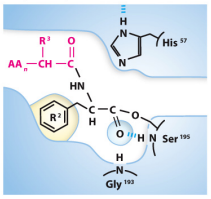
F-Q’

Chymotrypsin Mechanism

Step 4: Water Comes In



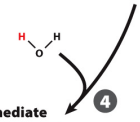
Acyl-enzyme intermediate



F-Q’

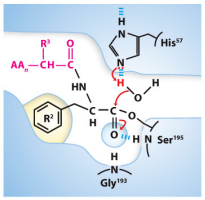
What if His: were protonated to His:H⁺?

Recall, the second half-reaction is slow



B

Acyl-enzyme intermediate

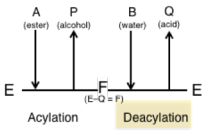


An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.

F-Q’•B

Chymotrypsin Mechanism

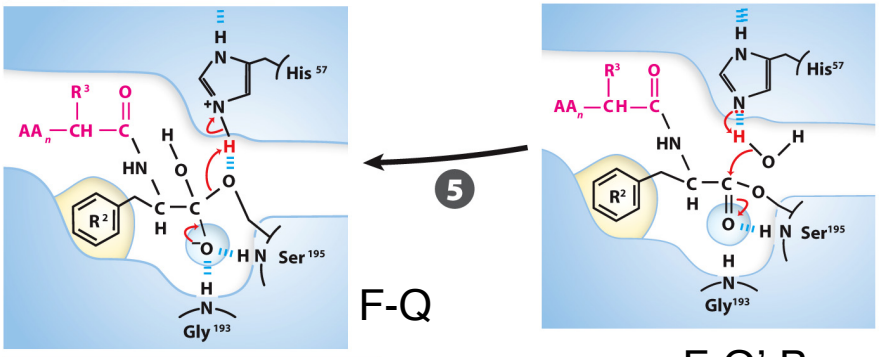
Step 5: Water Attacks



Short-lived intermediate (deacylation)

What if this His:H+ were deprotonated?

Acyl-enzyme intermediate



F-Q

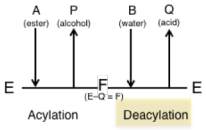
F-Q'-B⇌F-Q

F-Q'-B

Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser_{195}.

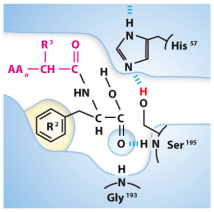
Chymotrypsin Mechanism

Step 6: Break-off from the Enzyme

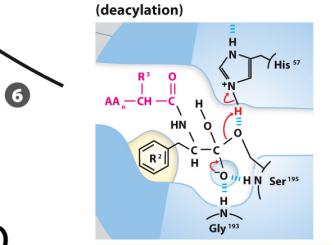


Enzyme-product 2 complex

E•Q



Short-lived intermediate * (deacylation)

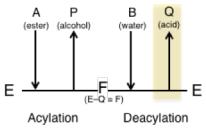


F-Q

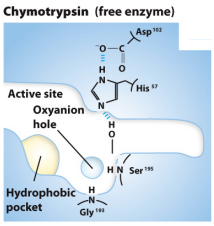
Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser_{195}.

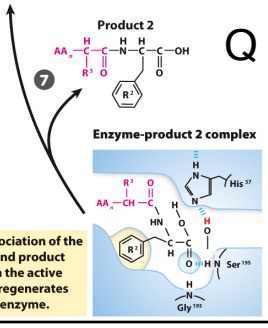
Chymotrypsin Mechanism

Step 7: Product Dissociates



E





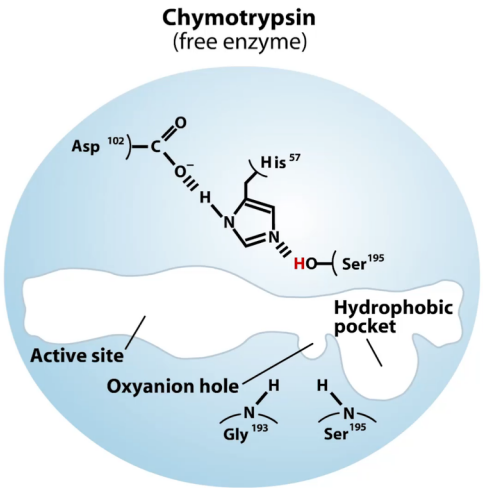
Q

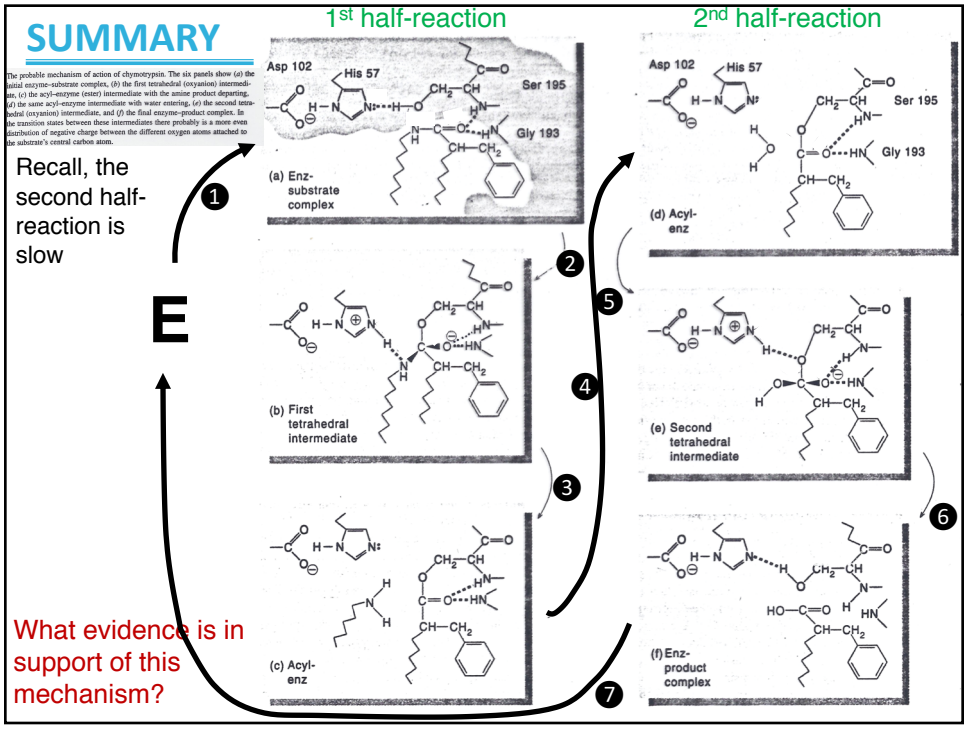
E•Q

Dissociation of the second product from the active site regenerates free enzyme.

Chymotrypsin Mechanism

Animation

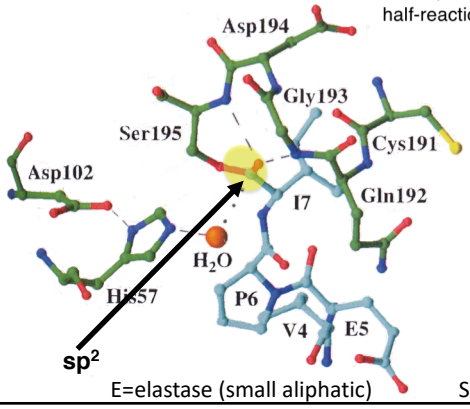




Chymotrypsin Mechanism

Recall, the second half-reaction is slow.
Perform X-ray crystallography but by trapping the enzyme in action!

If you protonate the His-57, it can't get beyond the acyl-enzyme intermediate.



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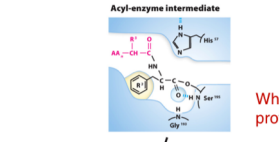
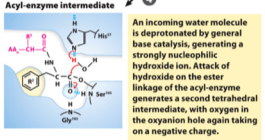


F-Q'

What if His: were protonated to His:H⁺?

B



F-Q'•B

E=elastase (small aliphatic)

S=YPFVEPI

Chymotrypsin Mechanism

Recall, the second half-reaction is slow.
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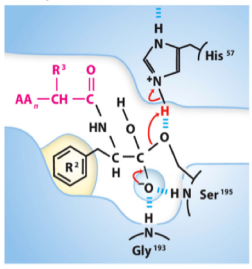
If you de-protonate during turnover and stop the reaction, its built up at the slowest step (the His-57 proton is titrated and can't participate in breakdown of the tetrahedral intermediate).

Short-lived intermediate (deacylation)

What if this His:H⁺ were deprotonated?

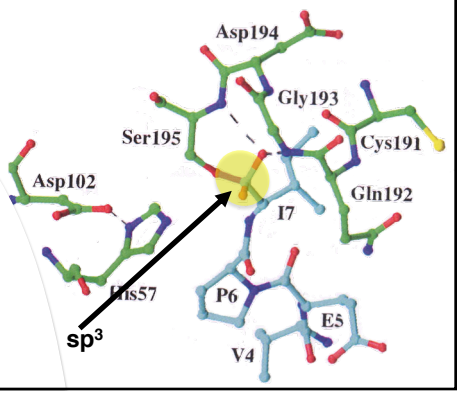


5

F-Q

F-Q'-B

Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser_{195}.



Chymotrypsin Mechanism

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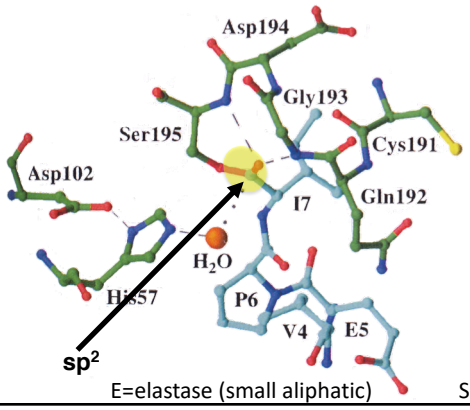
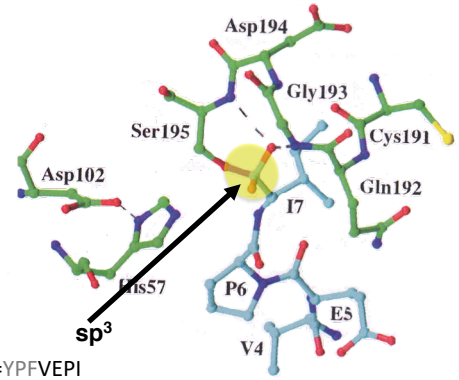


E=elastase (small aliphatic)



S=YPFVEPI

• *Enzyme mechanism and binding energy*

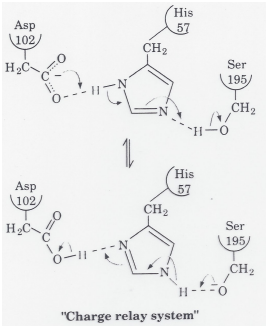
Chymotrypsin Mechanism

The "catalytic triad"

Asp102 – His57 – Ser195

The catalytic triad is found in all Serine Protease and Serine Esterases (e.g., acetylcholinesterase)

How does this work?



For this to work, all pK_a values should be similar. In other words, pK_a value of Asp slightly higher than that of Ser

	Asp102	His57	Ser195
pK_a values as amino acids are:	3.5	6.0	15.0
The measured pK_a values are:	7.0	7.0(12.0)*	15.0

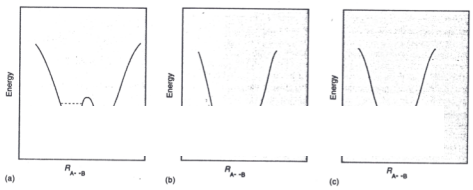
*only transiently

• *Enzyme mechanism and binding energy*

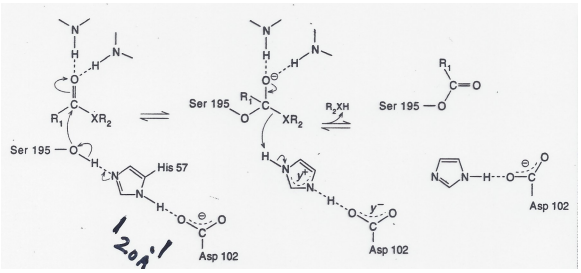
Chymotrypsin Mechanism

Low-barrier H-bond in the Transition State?

What is a Low-Barrier H-bond?



Where is the LBHB in the triad?

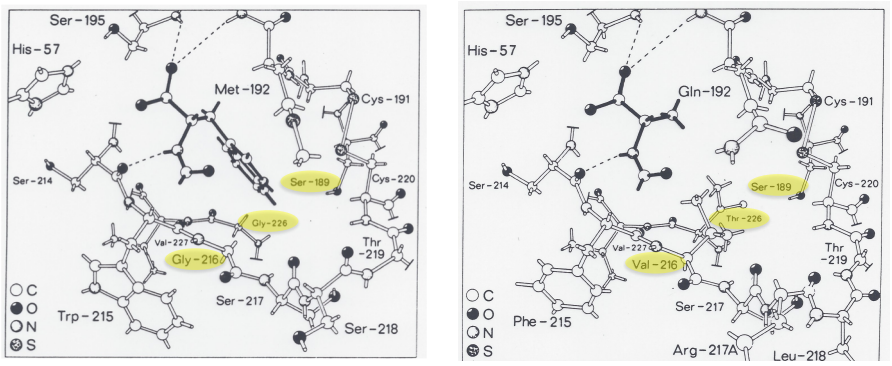


What is significant about LBHBs is that they are 4-5x stronger than normal 2.8 Å H-bonds (~20 kcal/mole)

Enzymes

Substrate Specificity

Chymotrypsin vs. Elastase

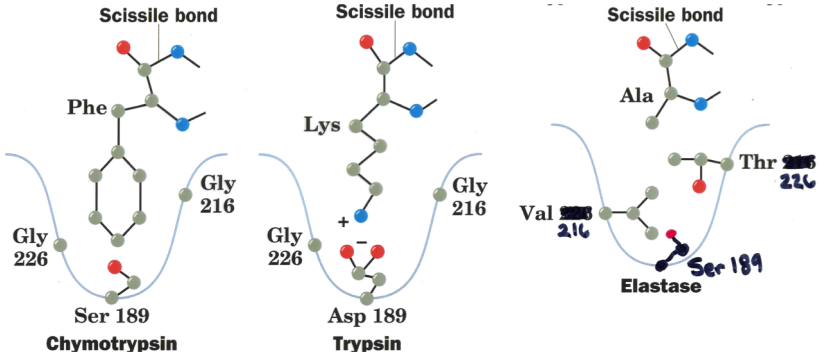


Trypsin is much like Chymotrypsin except for a Asp-189 instead of Ser-189.

Enzymes

Substrate Specificity

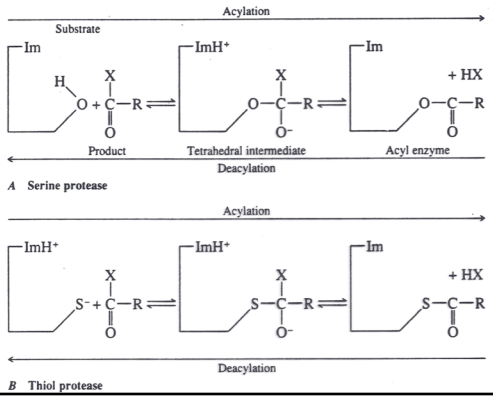
Chymotrypsin vs. Trypsin vs. Elastase



Enzymes

Class of Protease	Examples
Serine	Trypsin, Chymotrypsin, Elastase
Thiol	Papain, Cathepsin B, Caspases
Acid	HIV protease, Pepsin, Cathepsin D, Renin, Chymosin
Metal	Carboxylpeptidase A, Thermolysin

Very similar with acylation/deacylation half-reactions

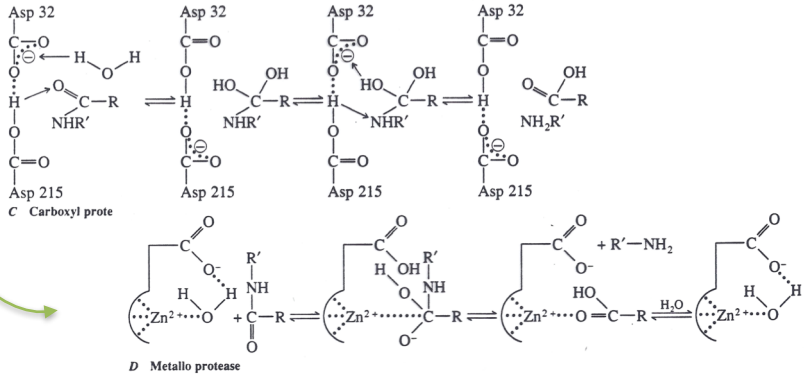


Serine Protease

Cysteine (Thiol) Protease

Enzymes

Class of Protease	Examples
Serine	Trypsin, Chymotrypsin, Elastase
Thiol	Papain, Cathepsin B, Caspases
Acid	HIV protease, Pepsin, Cathepsin D, Renin, Chymosin
Metal	Carboxylpeptidase A, Thermolysin



Enzyme Regulation

Enzyme Regulation

Recall that enzymes have 4 major attributes:

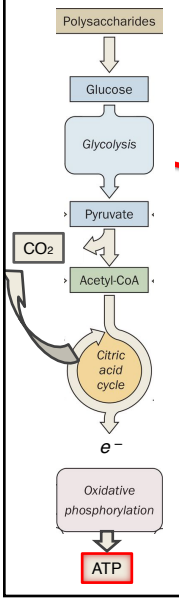
1. Increase rates of chemical reactions
2. Catalysis under mild conditions of temperature and pH
3. Very specific binding to substrates
4. Can regulate their activity

Control of Enzyme Activity

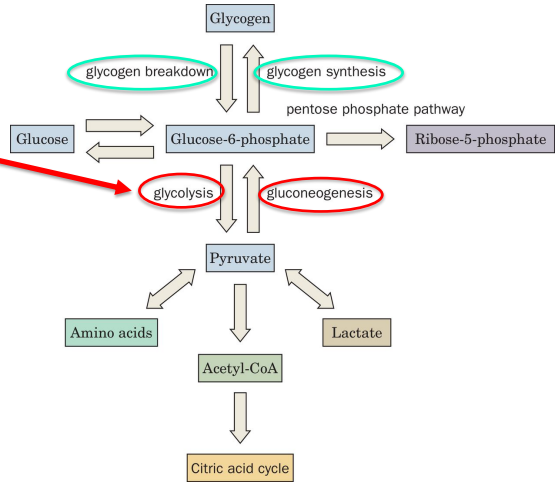
EXAMPLE:

Enzyme Regulation

Overview of Catabolism



Interconnected and opposing pathways:

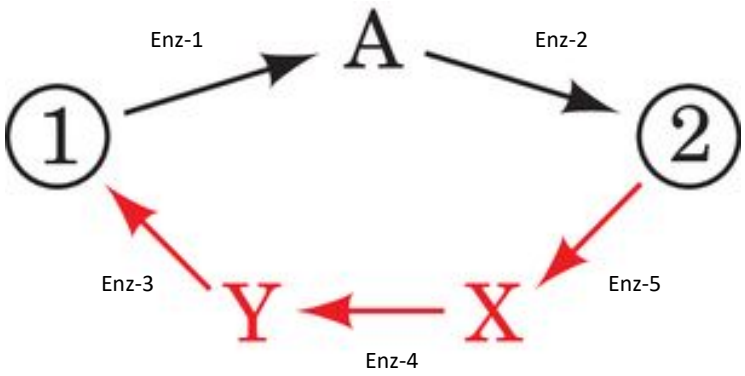


How are these opposing pathways regulated so that they are not working at the same time in a futile cycle?

EXAMPLE:

Enzyme Regulation

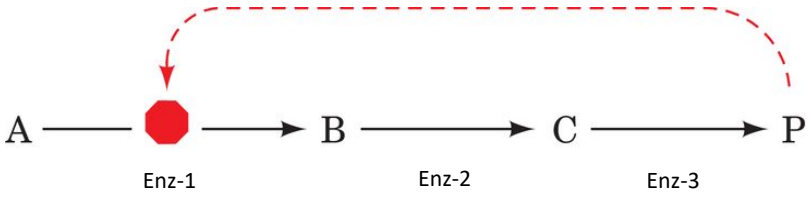
First: Catabolic & Anabolic Pathways Differ



Second: Give an enzyme specific for each pathway an on/off switch

EXAMPLE:

Enzyme Regulation



- **This on/off switch is normally at the start of a pathway so all the intermediates need not be made; i.e., at the committal step.**
- **This on/off switch is often controlled by a small molecule, often the product of the pathway; i.e., Negative Feedback Regulation**

Enzyme Regulation

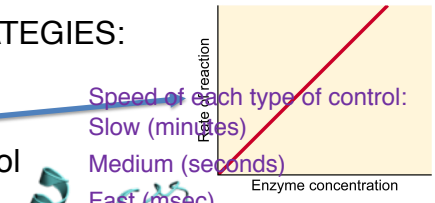
So, how are cellular processes controlled?

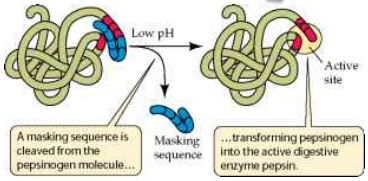
These controls are everywhere, from simple binding/release to embryological problems that set into motion all the processes to take a fertilized egg to an embryo.

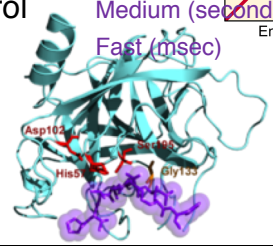
THERE ARE THREE BASIC STRATEGIES:

1. Genetic Control
2. Covalent-Modification Control
3. Allosteric Control

Speed of each type of control:
Slow (minutes)
Medium (seconds)
Fast (msec)







Enzyme Regulation

Control by Covalent Modification:

- Proteolytic activity
- Phosphorylation
- Adenylylation
- *etc.*
- Methylation

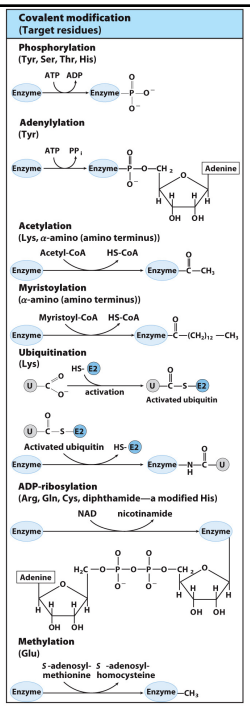


Figure 6-36

Enzyme Regulation

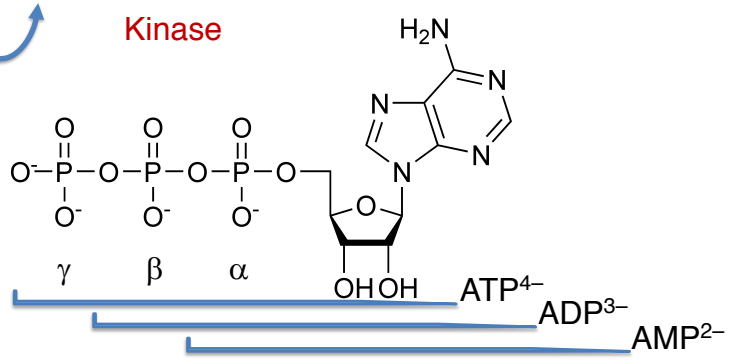
Control by Covalent Modification:

Phosphorylation

$$ATP^{4-} + R\text{-}OH \rightleftharpoons ADP^{3-} + R\text{-}O\text{-}PO_4^{2-} + H^+$$

Kinase

Now, if R = an enzyme



Enzyme Regulation

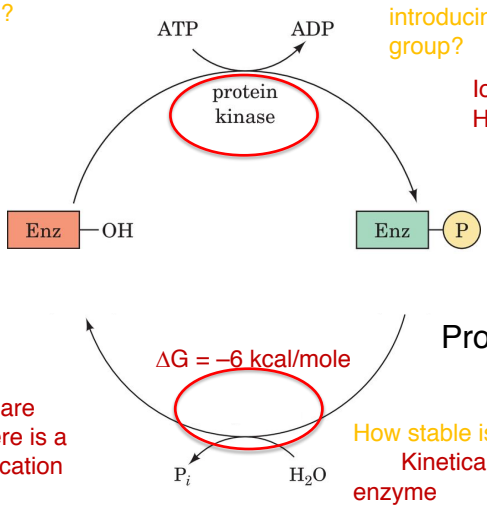
Control by Covalent Modification:

$ATP^{4-} + Protein\text{-}OH \rightleftharpoons ADP^{3-} + Protein\text{-}O\text{-}PO_4^{2-} + H^+$

Protein Kinase

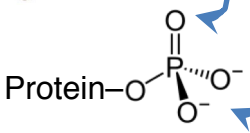
What R-groups?

Ser, Thr, Tyr



What are the effects of introducing a phosphoryl group?

Ionic environment
H-bond acceptors



Because enzymes are involved in this, there is a potential for amplification

How stable is this modification?

Kinetically stable; requires an enzyme