

Lecture 17 (10/21/20)

- Reading: Ch5; 166
Ch12; 443, 446-447
- Problems: none

NEXT

- Reading: Ch5; 166-167
Ch6; 225-232
- Problems: Ch5 (text); 5, 2
Ch6 (study guide-facts); 5, 6, 7, 14

Remember Tuesday at 7:30 in MORSE is the first MB lecture & quiz

Lecture 17 (10/21/20)

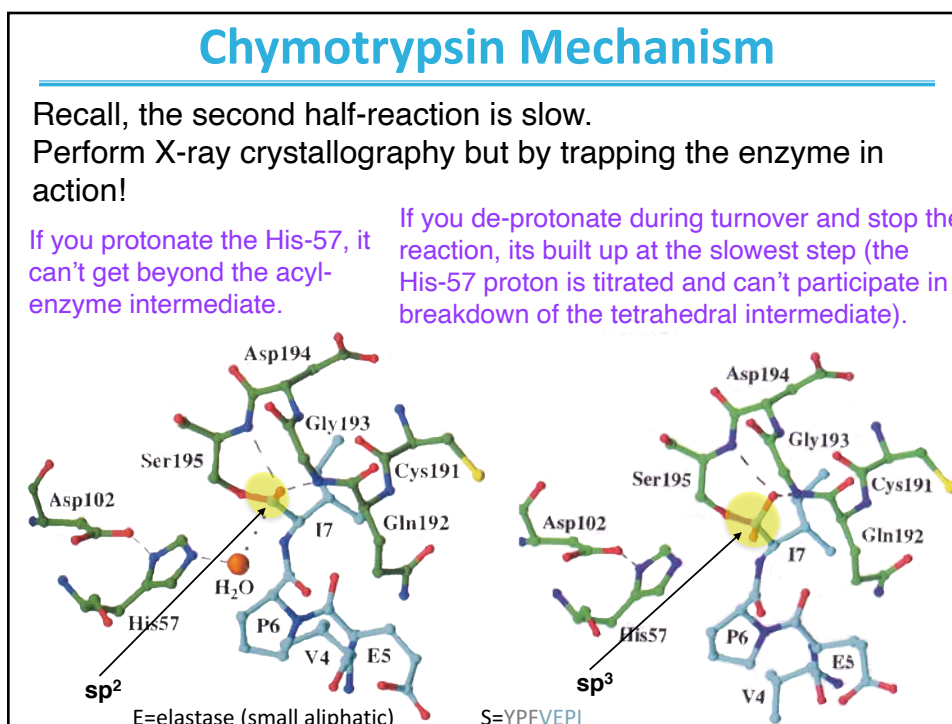
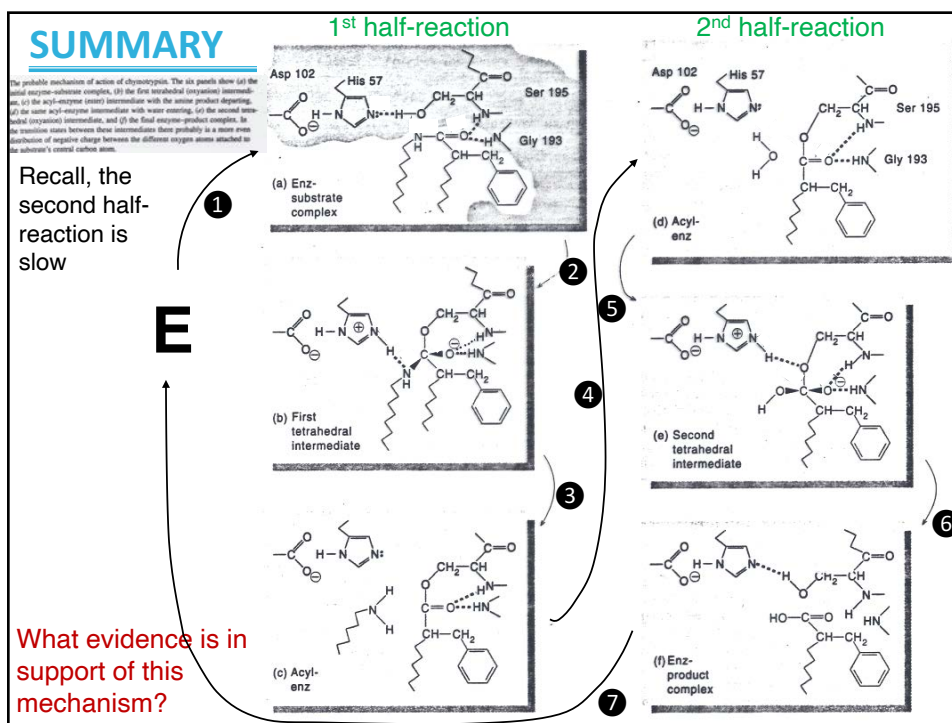
ENZYMES:

A. Enzyme Mechanisms

1. Serine Proteases
 - a. Proposed mechanism
 - i. Catalytic triad (Ser-His-Asp) – highly conserved
 - ii. Mechanism; tetrahedral intermediates and stabilize t.s. with oxy-anion hole
 - iii. Old; charge relay (but Ser-195 does not have the correct pK_a)
 - iv. Low-barrier Hydrogen bonds- Role for Asp
 - b. Specificity
 - i. Chymotrypsin *versus* elastase
2. Other protease mechanisms

B. Enzyme Regulation

1. Introduction
2. Strategies
 - a. Gene Regulation
 - b. Covalent Modification
 - c. Allosteric Control
3. Covalent Modification
 1. Proteolysis
 2. Protein modification
 - i. Phosphorylation
 - a) Kinases
 - b) Phosphatase



• Enzyme mechanism and binding energy

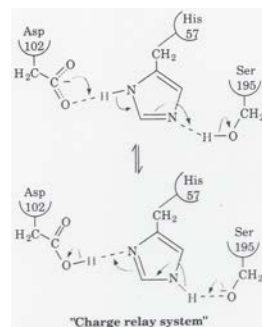
Chymotrypsin Mechanism

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.

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

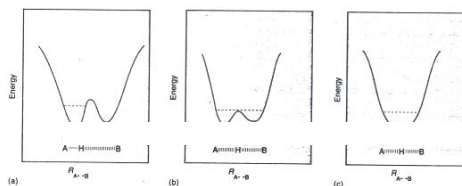
*only transiently

• Enzyme mechanism and binding energy

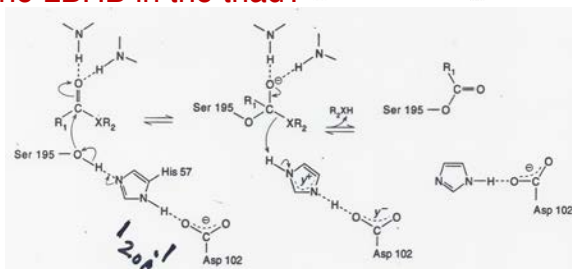
Chymotrypsin Mechanism

Low-barrier H-bond in the Transition State?

What is a Low-Barrier H-bond?



Where is the LBHB in the triad?

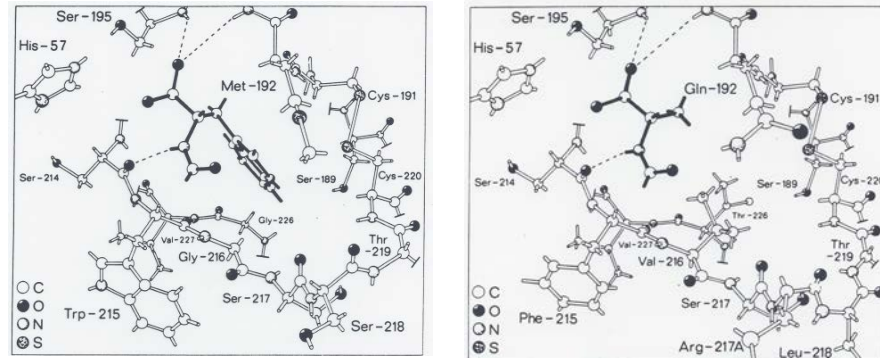


What is significant about LBHBs is that they are 4-5x stronger than normal 2.8 Å H-bonds (~20 kcal/mole)

Enzymes

Substrate Specificity

Chymotrypsin vs. Elastase

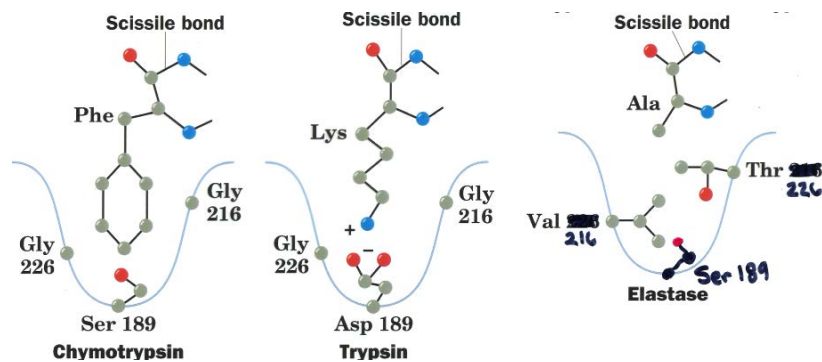


Trypsin is much like Chymotrypsin except for a Asp-189 instead of Ser-189.

Enzymes

Substrate Specificity

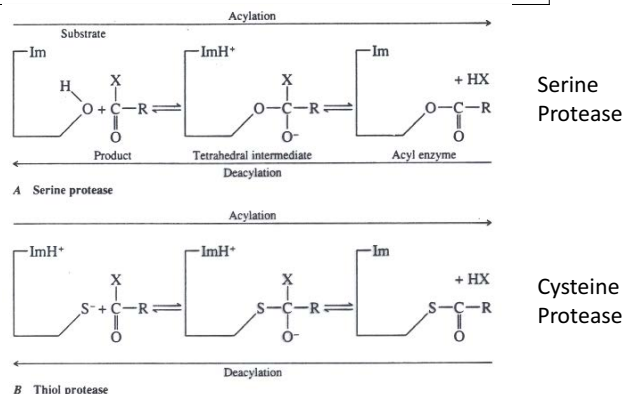
Chymotrypsin vs. Trypsin vs. Elastase



Enzymes

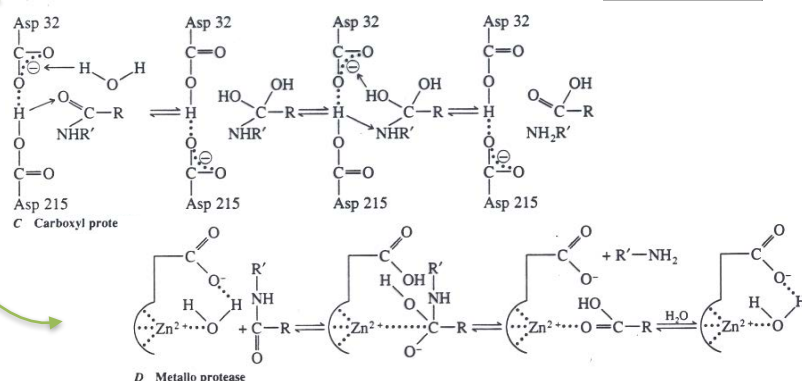
Class of Protease	Examples
Serine	Trypsin, Chymotrypsin, Elastase
Thiol	Papain, Cathepsin B, Caspases
Acid	HIV protease, Pepsin, Cathepsin D, Renin, Chymosin
Metal	Carboxypeptidase A, Thermolysin

Very similar with acylation/deacylation half-reactions



Enzymes

Class of Protease	Examples
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Enzyme Regulation

Enzyme Regulation

Recall that enzymes have 4 major attributes:

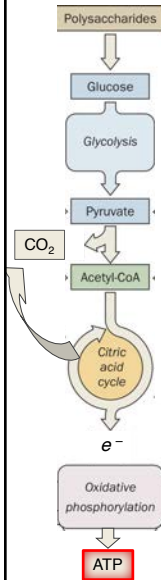
1. Increase rates of chemical reactions
2. Catalysis under mild conditions of temperature and pH
3. Very specific binding to substrates
4. Can regulate their activity

Control of Enzyme Activity

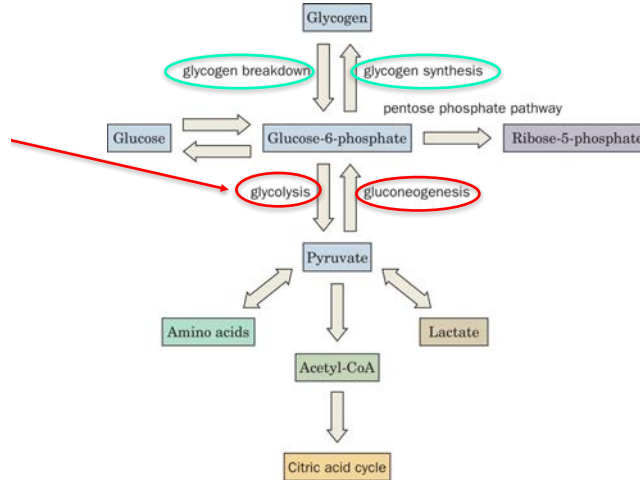
EXAMPLE:

Enzyme Regulation

Overview of Catabolism



Interconnected and opposing pathways:

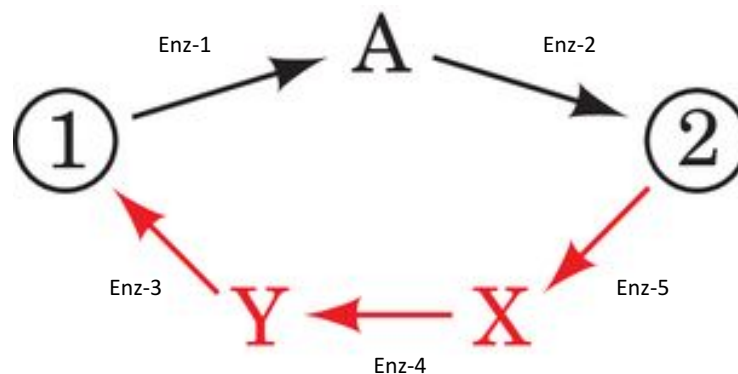


How are these opposing pathways regulated so that they are not working at the same time in a futile cycle?

EXAMPLE:

Enzyme Regulation

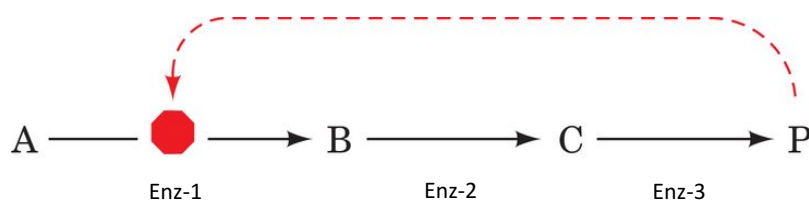
First: Catabolic & Anabolic Pathways Differ



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

EXAMPLE:

Enzyme Regulation

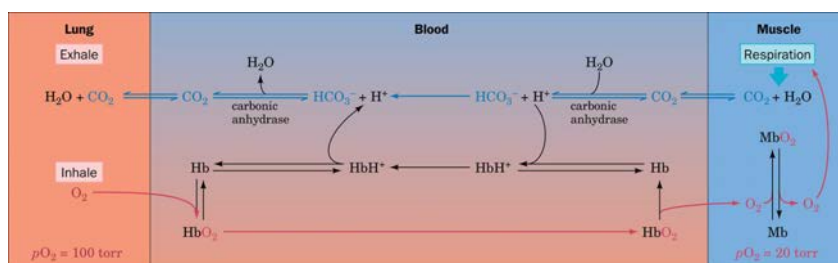


- This on/off switch is normally at the start of a pathway so all the intermediates need not be made; i.e., at the committal step.
- This on/off switch is often controlled by a small molecule, often the product of the pathway; i.e., Negative Feedback Regulation

Enzyme Regulation

Another Example:

Hemoglobin & Myoglobin
in O_2 & CO_2 Transport



Hb has high affinity = ON

Hb has low affinity = OFF

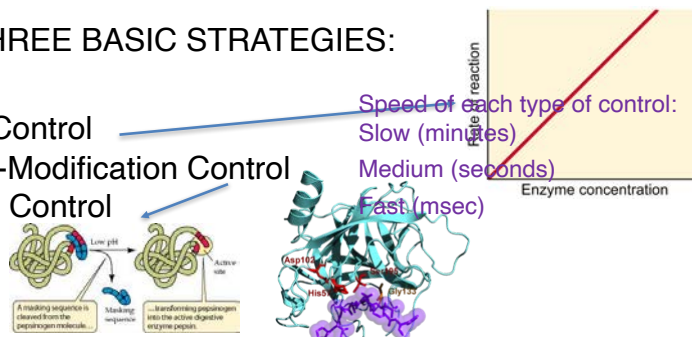
Enzyme Regulation

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



Enzyme Regulation

Control by Covalent Modification:

Proteolytic activity
Phosphorylation
Adenylation
etc.
Methylation

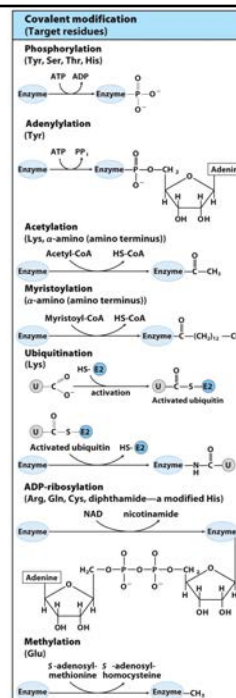


Figure 6-36

Enzyme Regulation

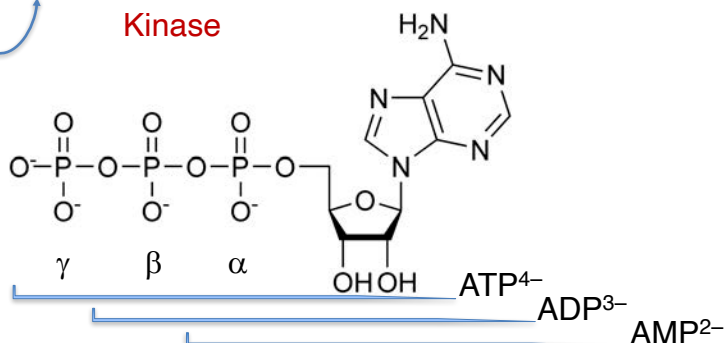
Control by Covalent Modification:

Phosphorylation



Kinase

Now, if R =
an enzyme



Enzyme Regulation

Control by Covalent Modification:



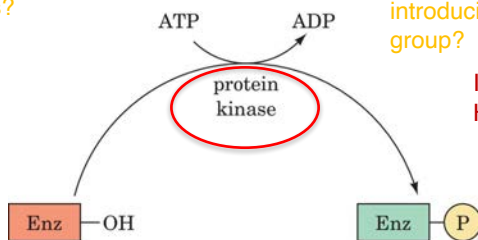
Protein Kinase

What R-groups?

Ser, Thr, Tyr

What are the effects of
introducing a phosphoryl
group?

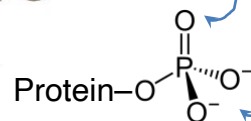
Ionic environment
H-bond acceptors



$\Delta G = -6 \text{ kcal/mole}$

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

How stable is this modification?
Kinetically stable; requires an
enzyme



Enzyme Regulation

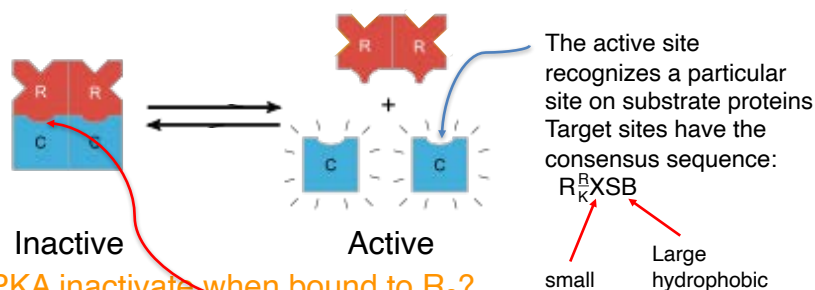
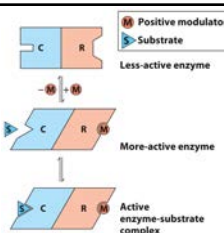
Control by Covalent Modification:

EXAMPLE: Protein Kinase A

$\alpha_2\beta_2$ (90 kDa & 40 kDa)

α is Regulatory (R)

β is Catalytic (C) and has the kinase activity



Why is PKA inactivate when bound to R_2 ?

R has a sequence: RRGAI that fits into the C active site!

How is this equilibrium shifted? **Cyclic-AMP (cAMP)**

Enzyme Regulation

Control by Allostery:

What is cAMP?

