

## Chapter 6: Outline-1

Properties of Enzymes  
Classification of Enzymes  
Enzyme Kinetics  
Michaelis-Menten Kinetics  
Lineweaver-Burke Plots  
Enzyme Inhibition  
Catalysis (**We are here.**)  
Catalytic Mechanisms  
Cofactors

6P2-1

## Chapter 6: Outline-2

Catalysis cont.  
Temperature and pH  
Detailed Mechanisms  
Genetic Control  
Enzyme Regulation  
Covalent Modification  
Allosteric Regulation  
Compartmentation

6P2-2

## Introduction

The previous presentation covered the introduction and Michaelis-Menten kinetics for nonallosteric enzymes.

This presentation covers catalytic mechanisms, cofactors, and enzyme regulation.

Remember, allosteric enzymes show cooperative binding. As the first substrate binds it influences subsequent binding.

6P2-3

## 6.4 Catalysis

### Proximity and Strain Effects

Substrate closely approaches the catalytic site with proper orientation. Enzyme conformation probably changes to give a strained E-S complex.

### Electrostatic Effects

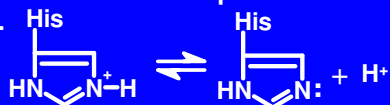
A hydrophobic pocket in the enzyme lowers the surrounding dielectric constant allowing electrostatic interaction between E and S.

6P2-4

## Catalytic Mechanisms-2

### Acid-Base catalysis

Enzyme side chains act as proton donors and acceptors.



### Covalent Catalysis

A nucleophilic side chain forms an unstable covalent bond to the substrate.



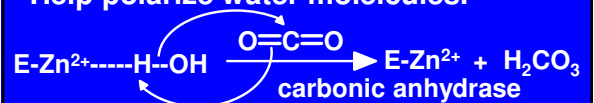
6P2-5

## Cofactors: Metals

Transition metals are often involved in catalysis, eg.  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $(\text{Zn}^{2+})$ .

They are useful because they:

- Have a high positive charge density.
- Act as Lewis acids (accept e pairs).
- Can mediate redox reactions. ( $\text{Fe}^{3+/2+}$ )
- Help polarize water molecules.



6P2-6

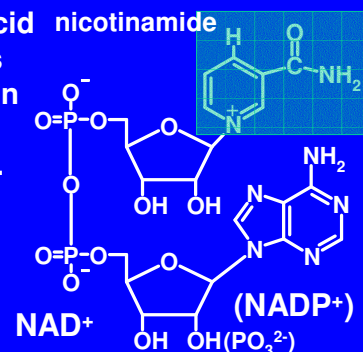
### Cofactors: Coenzymes

Coenzymes are organic molecules often derived from vitamins.

Vitamin	Coenzyme	Process
Thiamine(B <sub>1</sub> )	TTP	decarboxylation
Niacin	NAD(P) <sup>+</sup>	redox
Riboflavin(B <sub>2</sub> )	Pyridoxal P	amino group transfer
Folic acid	THF	one-carbon transfer
Vit A	retinal	vision, growth

### Cofactors: Coenzymes-2

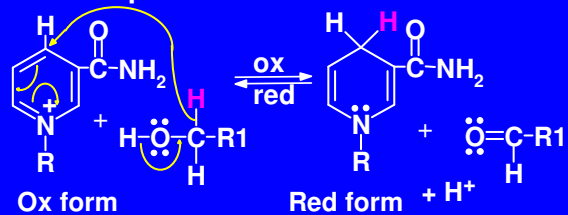
Nicotinic acid (niacin) is involved in redox reactions.



6P2-8

### Cofactors: Coenzymes-3

The nicotinamide part of NAD<sup>+</sup> accepts a hydride (H plus two electrons) from the alcohol to be oxidized. The alcohol loses a proton to the solvent.



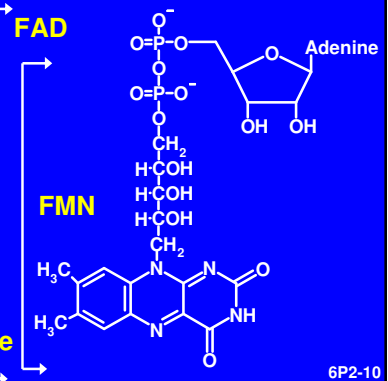
6P2-9

### Cofactors: Coenzymes-4

Flavin coenzymes also serve in redox reactions

Flavin adenine dinucleotide

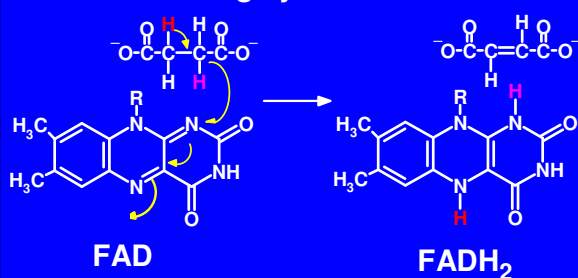
Flavin mononucleotide



6P2-10

### Cofactors: Coenzymes-5

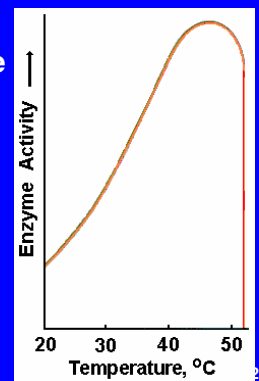
The flavin coenzymes accept electrons in the flavin ring system.



6P2-11

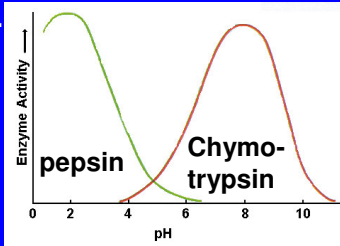
### Temperature and pH

An enzyme has an optimum temperature that is usually close to the temperature at which it normally works, ie. 37 °C for humans. Excessive heat can denature a protein.



## Temperature and pH-2

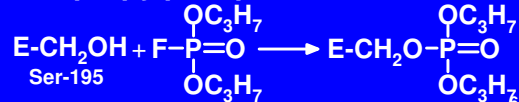
Enzymes work best at the correct physiological pH. Extreme pH changes will denature the enzyme. Pepsin (stomach) and chymotrypsin (small intestine) have different optimum pHs.



6P2-13

## Mechanism for Chymotrypsin

Chymotrypsin (CT) catalyzes the hydrolysis of peptide bonds next to aromatic side chains. The active site on CT involves the serine 195 residue. (CT is a serine protease.) This was determined by **labeling** the serine with diisopropylphosphofluoridate



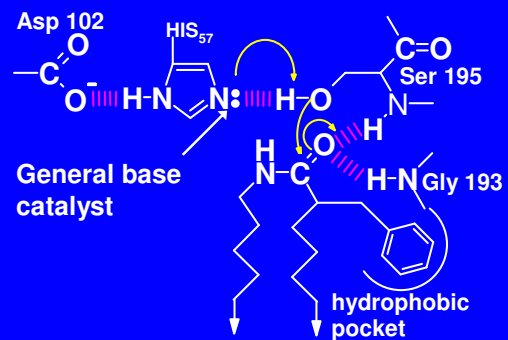
6P2-14

## Mechanism for Chymotrypsin-2

The active site on CT also involves the histidine 57 residue. Ser 195 and His 57 are close together in the active site of the enzyme. The histidine acts as a general base catalyst, converting the serine to its anion and making it a better nucleophile for attack at the carbonyl carbon to be hydrolyzed.

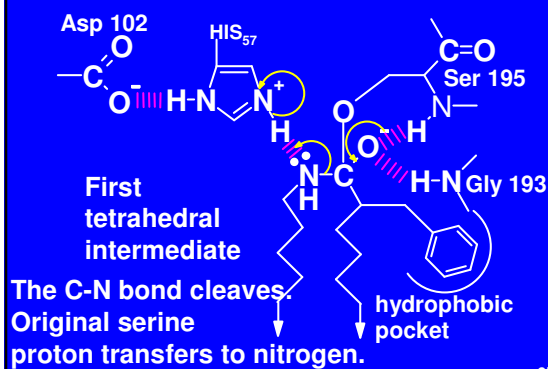
6P2-15

## Mechanism for Chymotrypsin-3



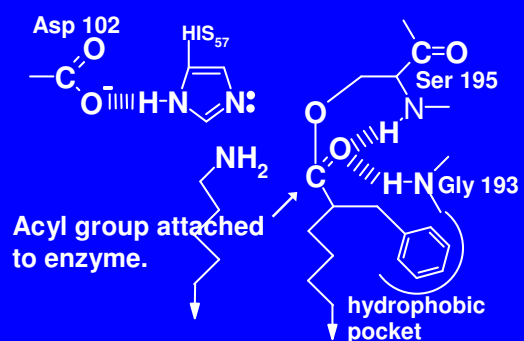
6P2-16

## Mechanism for Chymotrypsin-4



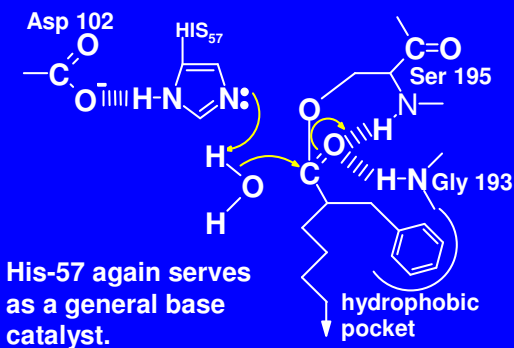
6P2-17

## Mechanism for Chymotrypsin-5



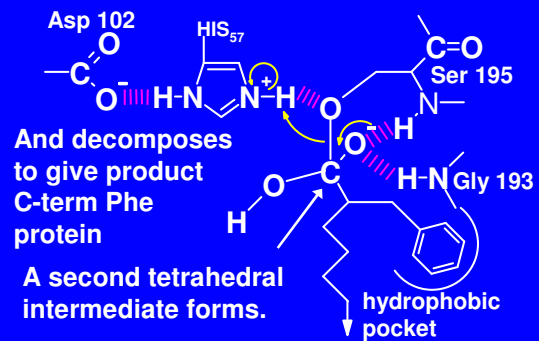
6P2-18

### Mechanism for Chymtrypsin-6



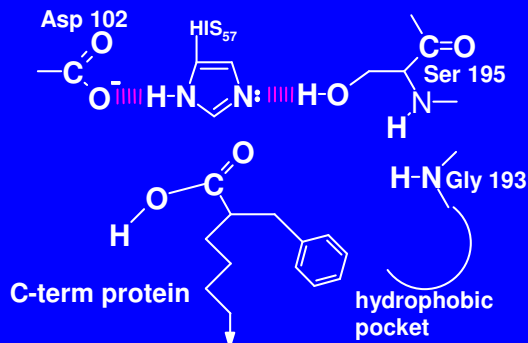
6P2-19

### Mechanism for Chymtrypsin-7



6P2-20

### Mechanism for Chymtrypsin-8



6P2-21

### Regulating Enzymes

Some methods that organisms use to regulate enzyme activity are:

1. Genetic control
2. Covalent modification
3. Allosteric regulation
4. Compartmentation

6P2-22

### 1. Genetic Control

An example of **enzyme induction** is when *E. coli* is induced to use lactose as an energy source in the absence of glucose. The enzyme for lactose use is turned on by genetic control.

In **enzyme repression**, the product of a biochemical pathway inhibits the functioning of a key enzyme of a previous step in the pathway.

6P2-23

### 2. Covalent Modification

Phosphorylation/dephosphorylation is a common way to control enzyme activity. Glycogen phosphorylase is a good example of an enzyme using this mechanism.

Methylation and acetylation are two other examples of covalent modification.

Conversion of zymogens (preenzymes) to active enzymes is another example.

6P2-24

### 3. Allosteric Regulation

Pacemaker (regulatory) enzymes usually catalyze the “committed” step in a series of biochemical reactions or a step where branching to two paths can occur.

Often these enzymes are **allosteric enzymes** (See Proteins II) and usually they are composed of several protomers.

6P2-25

### 3. Allosteric Regulation-2

Allosteric ligands (effectors) can be positive or negative.

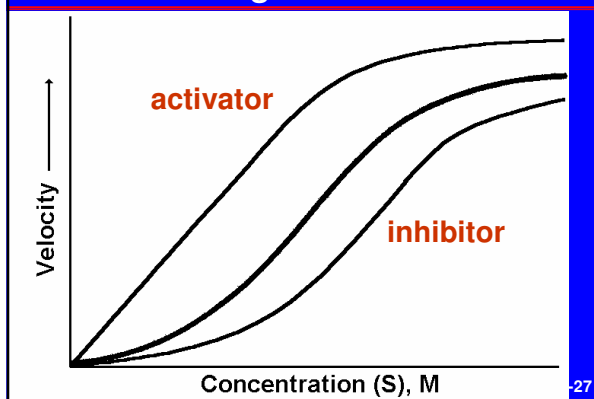
Eg CTP is an inhibitor of ATCase activity or a negative effector

ATP is an activator of ATCase or a positive effector.

The graph on Slide 28 shows the effect of positive and negative effectors on an allosteric enzyme.

6P2-26

### 3. Allosteric Regulation-3



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### Allosteric Models-1

The concerted model assumes the enzyme has two states: T(taut) and R (relaxed). Substrates and activators bind easily to the R form while inhibitors bind more easily to the T form.

The first effector to bind changes the conformation of all the protomers simultaneously thereby greatly promoting activation or inhibition.

6P2-28

### Allosteric Models-2

The sequential model is needed to explain negative cooperativity, a situation in which the binding of the first ligand **reduces** the affinity for for similar ligands.

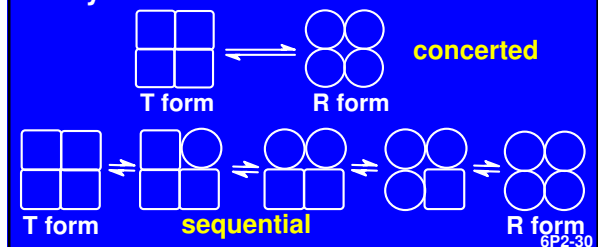
In this model, the first ligand is assumed to induce conformational changes that are transmitted sequentially to other protomers in the enzyme.

Neither model above fully explains all allosteric enzyme activity.

6P2-29

### Allosteric Models-3

The pictures below attempt to show the difference between the concerted and sequential models of allosteric enzyme behavior.



6P2-30

#### 4. Compartmentation

Eukaryotic cells are divided into organelles which often allows for separation of opposing processes.

Eg. Fatty acid oxidation occurs in the mitochondria while synthesis occurs in the cytosol.

Organelles also allow for concentration of specific reagents. Eg. Lysosomes require a low pH (~5) and their membrane keeps the high  $[H^+]$  inside.

6P2-31

#### Medical Apps: Diagnosis

To confirm a heart attack and monitor the treatment, doctors use creatine kinase (CK) and lactate dehydrogenase (LDH) which are found in blood serum.

Both enzymes exist in multiple forms called isozymes which have slightly different AA sequences.

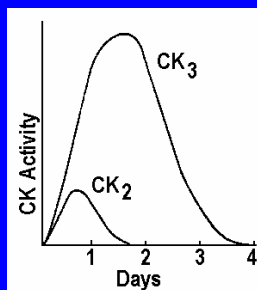
The forms are separable by electrophoresis which gives characteristic patterns after an infarct.

6P2-32

#### Medical Apps: Diagnosis-2

Dimeric CK has two types of protomers, muscle (M) and brain (B).

Heart muscle has  $CK_2$  (MB) and  $CK_3$  (MM). Only  $CK_2$  is found exclusively in heart muscle. (See graph.)



6P2-33

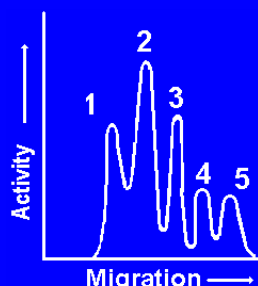
#### Medical Apps: Diagnosis-3

LDH is a tetramer composed of two protomers, heart (H) and muscle (M). Of the five LDH isozymes,  $LDH_1$  ( $H_4$ ) and  $LDH_2$  ( $H_3M$ ) are found only in heart muscle and red blood cells.

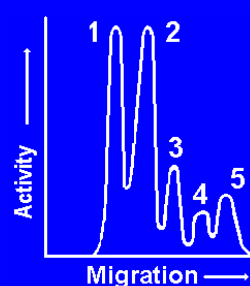
Again, electrophoresis patterns can be used to diagnose an infarct. The next slide shows normal and abnormal patterns for  $LDH_{1-5}$ .

6P2-34

#### Medical Apps: Diagnosis-4



Normal LDH electrophoresis pattern



LDH electrophoresis pattern after infarct

6P2-35

#### Medical Apps: Therapy

Streptokinase and human tissue plasminogen activator (tPA) are both used to treat heart attack because they dissolve blood clots

Asparaginase does not occur in human blood. Some cancer cells (eg some adult leukemias) cannot synthesize asparagine. Infusing the enzyme can cause cancer cell death due to lack of asparagine. Serious side effects can occur.

6P2-36