Chapter 6 Enzymes

Problems - 1, 3, 4, 8, 9, 12, 15, 18 Additional homework available for extra credit

6.0 Intro

Enzymes - protein catalysts -without life would grind to stop quickly. Lets see how they work

6.1 An introduction to enzymes

late 1700's literature on digestion of meat by stomach juice 1850's Louis Pasteur something in yeast that ferments things 1897 Buchner proved that yeast extracts, not live yeast could ferment sugar term Enzyme coined by Fredrick Kuhne 1st isolated was urease by Sumner 1926 - proved were protein not until 1930's that widely accepted

A. Most enzymes are proteins

only a few catalytic RNA's are known, will ignore until chapter 26 catalytic activity depends on integrity of protein 3-D structure Hydrolyze a protein it dies Undergo mild denaturation it dies Allow to renature, regains activity Simply break multimers apart activity can change dramatically MW 12,000 to millions Some do all by themselves Some required additional chemicals

-Cofactors inorganic ions Table 6-1

-**coenzyme** complex organic or metallo-organic group Table 6-2

Cofactor or coenzyme that is very tightly bound or covalently bound called a **prosthetic** group -

Protein & coenzyme or cofactor called a **holoenzyme** Protein sans coenzyme or cofactor called **apoprotein** or **apoenzyme**

May also contain covalent modification of groups Often involved with regulation & control

B. Enzymes classified by reactions they catalyze

take name of substrate or reaction and add -ase

Urease works on urea

Oxidoreductase does a redox reaction

Enzyme frequently have many different, sometimes conflicting names base on forward and reverse reaction (will see some of this confusion second semester)

So instead evolved a Enzyme Commission to clearly denote name EC 2.7.1.1

Each digit more completely describes reacion, complete description beyond scope of class

6.2 How Enzymes work

Chemical that enzyme acts upon called **substrate** Substrate is bound by enzyme in an **active site** active site lined with AA to catalyze reaction

<u>A. Enzymes affect rate not equilibria</u> Figure 6-2

Energy released from reaction

ΔG°'

' denotes biochemical standard state pH7 Enzyme does not effect size of change Only effects hump in middle

Hill in middle is called the transition state

Difference between ground state and transition state is the transition energy

Catalysts work by lowering the transition energy

Little valleys called reaction intermediates

Step with highest activation E is slowest step From Gen Chem this is your **rate limiting step**

rule 1 to remember, can only speed up a reaction can't shift favorable or unfavorable equilibrium

Simple enzyme reaction $E + S \neq ES \neq EP \neq E + P$ Let's look at reaction coordinate Figure 6-3 Starting point - ground state- E + P

K_{eq} = [Products]/[reactants]

And ΔG° =-RTIn K'_{eq} R = 8.315J/mol·K

So can convert between K and free E

What about rate of reaction? Assume 1st order Rate =V = k[S]

> 2nd order V = k [S1][S2]

In transition state theory find that above k

 $k = KT/h e^{-\Delta G_{\ddagger}/RT}$

K = Boltzman Constant
T = Temp
H = Planck's constant
ΔG‡ transition state energy

So can tie transition state E to kinetics

C. Principles of enzyme catalysis

 10^5 to 10^{17} rate enhancements with enzymes Will see details in a bit but for now... 2 main area where help

I. Rearrangement of covalent bonds

Bonds may be made transiently between enzyme and substrate

Groups may move transiently for substrate onto enzyme Reaction occurs in active site

Via low energy reaction path

II. Noncovalent interactions make ES complex

H bonds, Charge-charge, hydrophobic

Each bond releases small E from complex Total is called the **binding energy** Site may be complementary not to substrate, but to transition state Enzyme pulls into transition state to encourage

reaction

D. Weak interaction optimized in transition state

Figure 6-5

explain why complementary to transition state

Bottom line weak bonding interaction between substrate and enzyme are a major force

May be extensive interaction , not just restricted to area around bond made or broken

E. Binding E contributes to specificity and catalysis

 Δ G[‡] must decrease by 5.7 kJ to get a 10X rate enhancement a single weak interaction may provide between 4 and 30 kJ so if have several, can get up to 60-100 kJ lower which is enough

Binding also explains specificity

How enzyme can distinguish between substrates If don't have all the correct interaction won't bind as well

Let's look at specifics on how binding affect catalysis

Binding E dominant force in many enzymes can be only force in some several facets to binding E in lowering ΔG_{\pm}^{\pm}

I. Entropy reduction

In 2 substrate rxn in solution both bouncing and need to collide In enzyme grab and hold so right next to each other

Also hold to remove rotation so in right orientation Illustrated in non-enzymatic case in figure 6-7

II. Desolvation

In aqueous solution each substrate caries water around in reaction this must be penetrated In enzyme remove water so can be more efficient

III. Correct geometry/ distribution

Enzyme not only stabilizes transition state structure, but can use charged groups to encourage proper movement of electrons in reaction

IV. Induced fit

Binding of substrate often make protein itself change structure

(Induced fit) this change further stabilizes transition state or even products

F. Specific Catalytic Groups

Another theme - once substrate positions, groups in enzyme or prosthetic group makes transient covalent bonds with substrate to make a reaction go

3 main types of covalent catalysis

Acid-base catalysis Covalent catalysis Metal ion catalysis

I. Acid-base catalysis

Many reaction involve formation of unstable charged intermediates Want to collapse back to starting material Want to stabilize so last longer

Better yet stabilize in a way that encourages breakdown to desired product

Frequently use acids and bases to do this (proton donor and acceptor)

Figure 6-8

In noncatalyzed reaction get intermediate that is not very stable

A. Specific acid/base

use H_3O^+ or OH^- from water to stabilize intermediate

B. General acid/base

use acid and base groups in protein to stabilize

In example base (proton acceptor) pulls proton off (-OH-)⁺ to remove charge stabilize intermediate The use acid to put charge on at a different place in this new place again not stabilize, but now as collapses goes to product rather than starting material Groups frequently used figure 6-9

II. Covalent catalysis

Transient covalent bond formed between substrate and enzyme A-B \neg A + B

Enz-Z:

Electrons from Z: attack A B pulls away

Enz-Z:A +B⁻

Enz releases A Enz:: + A

frequently combined with Acid base see to resolve charge issues

III. Metal lon catalysis

lonic interaction of metal can be used to orient intermediate and stabilize charge metal covalent bonds can be used at weak bonds also can be used for redox reaction about 1/3 of all enzyme used metals in some way

6.3 Enzyme Kinetic and Mechanism

Want to know blow -by-blow how enzyme works, which residue, how they interact and move

Have seen that 3D structure tells part of the story usually start an finish, but nothing in between can get clues with chemistry and site directed mutagenesis

But always need enzyme kinetics to pick mechanism apart **Enzyme kinetics** -determination of rate of reaction and see how it responds to changes in experimental parameters

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Had intro to kinetic in Gen chem 1 technique method of initial rate or initial velocity Why Changes on [S] are negligible and can be ignored No [P] to make back reaction so it can be ignored Will add a second wrinkle, [E]<<[S] What would expect for V_o vs [S] for first order reaction? (Linear plot) Second order (Exponential) Zeros order(flat) Typical V_o vs [S] for enzyme rxn shown figure 6-11 Doesn't match does it? At low [S] V_0 almost linear with [S] so looks like 1^{st} order But then levels off approaches a maximum velocity V_{max} (Note: have seen this kind of plot before, it the hyperbolic we saw for Mb!)

This hyperbolic behavior was first interpreted into a theory by Lenoir Michaelis and Maud Menten. Essentially they hypothesized that the V_{max} was caused by the fact the enzyme was saturated, that is it was working as fast as it could and increasing [S] doesn't make it go any faster. They developed a set of equation to describe this system that we will study in the next section, and we use the term Michaelis-Menten to describe enzymes that fit this system

The M-M theory was improved on my Briggs and Haldane in 1925 and a few assumption were cleared up and a deeper understanding of the behavior uncovered.. Lets' look at the equations

B. Michaelis-Menten kinetics

We have see that Enzyme kinetics follow a hyperbolic function that is it has the shape of figure 6-11. It look like it has a maximum rate when [S] is low, and the rate approaches a limiting value as [S] gets high

we will now use simple kinetic analysis to reveal why this

$$E + S \xrightarrow{\stackrel{k_1}{\longleftarrow}} ES \xrightarrow{\stackrel{k_2}{\longleftarrow}} E + P$$

Since we are studying initial reaction, the [P] is negligible so the reaction k_{2} can be ignored and

Lets look at the rate equation that gives us V₀

 $V_{o}=k_{2}[ES]$

Now to solve this equation we need to know how fast [ES] is formed, so let's look at the rate equations that deal with [ES]

ES is being formed by the reaction formation [ES]= k_1 [E][S] but it is disappearing through the reactions: k_2 [ES] and k_{-1} [ES] Destruction [ES]= k_2 [ES] + k_{-1} [ES] I want to get [E] out of the equation so I will rely on the mass balance equation

 $[E_T] = enz total = [E] + [ES]; [E]=[E_t]-[ES]$

so rate of formation of [ES] =

k₁([E_t]-[ES])[S]

Now I will make an important assumption (this was Briggs and Haldane's contribution)

As the reaction occurs, the [ES] will first increase, as you make it, and then it will level off as the two reactions that use up [ES] cut in. Eventually the rate of formation equals the rate of destruction and we reach what is called the **steady state**

mathematically

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rate formation [ES] = rate destruction [ES]

k_1([E_t]-[ES])[S] = k_2[ES] + k_{-1} [ES]

k_1([E_t]-[ES])[S] = (k_2 + k_{-1}) [ES]

K_1[E_t][S] - k_1[ES][S] = (k_2 + k_{-1}) [ES]

K_1[E_t][S] = k_1[ES][S] + (k_2 + k_{-1}) [ES]

K_1[E_t][S] = [k_1[S] + (k_2 + k_{-1})] [ES]

K_1[E_t][S]/[k_1[S] + (k_2 + k_{-1})] = [ES]

[E_t][S]/[[S] + (k_2 + k_{-1})/k_1] = [ES]

And plugging this back into our expression for V<sub>0</sub>
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$$V_o = k_2[ES]$$

 $V_o = k_2 [E_t][S]/[[S]+ (k_2+k_1)/k_1]$

$$V_o = \frac{k_2 [E_t][S]}{[S] + \frac{k_2 + k_{-1}}{k_1}}$$

Since $k_1 k_{-1} k_2$ are all constant, we can lump them together into a special constant called the Michaelis-Menten constant K_M

so

 $K_{M} = (k_{2} + k_{-1})/k_{1}$

And $V_o = k_2[E_t][S]/(K_m+[S])$

And this is exactly the kind of hyperbolic function we were expecting. Now lets see if we can use it to explain how enzyme kinetic works

Assume [S] is high, and in fact, higher than (k_2+k_1/k_1)

then $V_0 = k_2[E_t][S]/[S]$ $= k_2[E_t]$

This is a constant, so this represents that maximum value of rate Why is the rate limited? It is limited by the inherent rate of the reaction, and the total enzyme concentration

We call this constant, $V_{max} = k_2[E_t]$

and we have a final, simple equation

$$V_o = V_{max}[S]/(K_m+[S])$$

Figure 6-12

We just saw how the maximum rate fo the reaction is the limiting value of V as [S] gets very large. How would this show up an a plot? So from graphic analysis we van derive V_{max} We can also derive K_m

Look what happens to the equation when V we look at the value that is at $\frac{1}{2}\,V_{\text{max}}$

$$\frac{1}{2} V_{max} = V_{max}[S]/(K_m + [S])$$

 $\begin{array}{l} \frac{1}{2} = [S]/(K_m + [S]) \\ K_m + [S] = 2[S] \\ K_m = [S] \\ & \text{So can get } K_m \text{ by reading off } [S] \text{ when } \frac{1}{2} V_{max} \end{array}$

As it stands, the Michaelis-Menten equation explains enzyme kinetics, but , because it is nonlinear, is a little hard to deal with when you have real practical data

Most common transform is the Lineweaver-Burk plot or double reciprocal plot.

Take inverse of both sides of the equation

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

 $1/V_{o} = (K_{m}+[S])/V_{max}[S]$

 $=K_m/V_{max}[S] + [S]/V_{max}[S]$

So if we plot $1/V_{o}$ vs 1/[S] we get a nice straight line

The slope = K_m/V_{max} and the intercept = $1/V_{max}$

We will use this kind of data analysis later to look at how different kinds of inhibitors effect enzyme kinetics, so give a problem like this or two a try.

C. Kinetic Parameters used to compare enzyme activities

 K_{m}

many enzyme follow M-M kinetics however, that does NOT mean they follow the simplistic mechanism originally proposed by M-M

So Enz kinetics actually much more complex

However, since so many do display the MM kinetics under steady state conditions, the standard MM parameters are used to compare enzymes

K_m

$$K_{m} = (k_{2} + k_{-1})/k_{1}$$

IF k_2 is rate limiting, and $k_2 << k_1$ then reduces

To k_{-1}/k_1 = dissociation constant K_d of enzyme substrate complex [E][S]/[ES]

Thus often used to compare association or dissociation of substrate to for complex and evaluate affinity of enzyme for substrate

Done often in literature although is not technically correct until you have proved k_2 rate limiting and << k_{-1}

 $K_{\mbox{\tiny m}}$ oftens tend to be about the same as the cellular concentration of the substrate

 V_{\max}

We have seen that $V_{max} = k_2[E_t]$ For enzymes with more complicated mechanisms this is not always true (there may be $k_3 k_4$... and different intermediates)

 $\mathsf{k}_{\mathsf{cat}}$

we often to use the term $\boldsymbol{k}_{\text{cat}}$ to describe the limiting rate of a saturated enzyme

For an enzyme that follows M-M kinetics,

 $k_{cat} = V_{max}/[E_t] = K_2$ for simple M-M kinetics

It is a first order rate constant, so has unit of 1/t It is also called the turnover number because it tells you the number of substrate that are 'turned over' (reacted) in a unit of time Table 6-7

k_{cat}/K_m

one other number we tend to look at is k_{cat}/K_m Gives more information that looking at two parameters separately this is a measure for how efficient an enzyme is

It is called the **specificity constant**=k_{cat}/K_m

This is a second order rate constant so it depends on how fast two species can diffuse

Diffusion rate limits upper end of specificity constant to 10⁸-10⁹

Can see in table 6-8 that some enzymes are near this limit

This mean that as soon as 2 substrate diffuse into enzyme, the enzyme is near 100% efficiency for making the reaction go

C. Enzymes that catalyze reaction with 2 or more substrates

Started by looking at simple reaction with only 1 substrate, now look at multiple substrates

For instance ATP + glucose → ADP + glu-6-p

Reaction like this usually involve transfer of a group form one substrate to another

Can come up with different mechanisms for this

Figure 6-13

B is called ping pong

There is a whole range of equations and plots to pick these kinds of enzymes apart, but we won't get into them

D. Pre-Steady state kinetic

up to now talked about steady state kinetics tells us Km, Vmax, Kcat and Kcat/Km for multi substrate can even tell us if ping pong or ternary complex

Does not do a whole lot for picking apart individual steps of a complicated reaction

for this need pre-steady state kinetics

need to study rates of each reaction as it first turns over in a reaction much more complicated and difficult beyond the scope of this course needs high tech equipment to follow a singe turnover even at sub millisec speeds

E. Enzyme inhibition

enzyme inhibitors - substances that inhibit enzymatic reactions

Can slow or halt catalysis

Important to [pharmaceutical industry, because can target enzymes that want to shut down to help alleviate a disease state

Two broad classes of inhibitors Reversible (can be undone) Irreversible (permanent)

I. reversible inhibitors

Competitive- inhibitor competes with substrate for active site

Figure 6-15a

often substance that look like substrate so bind to same site If run through MM kinetics find effect in Km.

$$V_{o} = V_{max}[S]/(\alpha K_{m} + [S])$$

α=1 + [I]/K₁

 $K_{I} = [E][I]/[EI]$

Since is competitive binding, as increase [S] it competes better, so effect goes away. The way to diagnose using double reciprocal plot (figure 1 box 6-2) gives common point at X=0

Uncompetitive inhibitor

Binds at a site other than active site, but only binds in ES state so prevents reaction from going to completion 6-15-b, box 6-2-2 diagnose with parallel line on double reciprocal

Mixed inhibitor binds at site other than active site,

but binds to both E and es

figure 6-15-c

Affect both V_{max} and $K_{m_{\!_{\!\!\!\!\!\!}}}$ see both slope and intercept change in double reciprocal plot Box 6-2-3

Special case when K_1 same for both E and ES was called a **noncompetitive** inhibitor, anc was characterized a common point at Y =0 on double reciprocal plot

II. Irreversible inhibition

Compounds that combine with or destroy some part of the so enzyme is incapacitated

Since often an AA in active site, can be useful to study enzyme mech

Figure 6-16 DIFP commonly used against Ser active sites

Special class called suicide activator Unreactive compound Enzyme activates, then kills enzyme

Ideal drug candidates because non toxic until they hit their target

<u>F. Enzyme activity affected by pH</u> All enzymes have an optimum pH where have best activity

Figure 6-17

Often reason for curve is are titration groups in active site So is another clue to enzyme activity

Also note pH optimum is also usually matched to the solution that the enzyme sits in

6.4 Examples of Enzymatic Reactions

complete mechanism complicated

need all substrate, cofactors, products, regulators need temporal sequence of every enzyme bound intermediate structure of each intermediate and transitions state rates of interconversions between intermediates structural relationship between enzyme and intermediate knowledge of the E of interaction between enzyme and each intermediate

look at 2 mechanisms to illustrate general principles

A. Chymotrypsin

25,000 MW see figure 6-18 for structure cleaves peptide bond adjacent to aromatic residues increases rate of reacion by 10⁹ works through covalent intermediate (figure 6-19)

Step 1 substrate binds need large hydrophobic pocket for aromatic side

chain

Catalytic triad of asp 102 his 57 and ser 195 His 57 acts as general base to remove proton from ser Ser now very nucleophillic so attacks peptide bond nucleophillic attack on C=O of substrate to make a acyl-enzyme intermediate

Tetrahedral oxyanion is stabilized by H bond to Gly 193 and ser 195 backbones

At this point have acyl enzyme intermediate

His 57 (through catalytic triad)now , want to donate its H back to peptide, so his now acting as general acid to donate H, this allows peptide bond to break and 1st half of substrate become a leaving group and float off water now penetrates

His to regains its proton by removing from water (acts as a base again) Now water oxygen is activated to attack acyl enzyme to form tetrahedral intermediate

His now acts as acid to donate its H back to acyl to make it uncouple from enzyme

Evidence for acyl enzyme intermediate found in reaction is using presteady state kinetics (figure 6-19) if you use an analog you see enzyme rapidly making colored paranitorphenol, but then slows down because it takes time to deacylate

B. Hexokinase

bisubstrate enzyme Mw 100,000 Reaction shown top page 212 binding of ADP and ATP required Mg²⁼ Structure shown in figure 6-22 note in structure much more open in unbound state when glucose + Mg-ATP enters, get induced fit, enzyme closes End becomes active End transfers P

OH of glucose to which P is attached is about as reactive as water water and ATP can also enter active site so why Don't water and ATP react to give ADP and Pi?

Water doesn't trigger induced fit to end remains inactive

If use ATP and xylose (a 5 C sugar) Do get induced fit Enzyme becomes active Now get ATP to ADP + pi, but sugar not phosphorylated

C. Enolase

does reaction shown on left column 213 96,000 MW, 436 AA's/ subunit it is a dimer Active site and mech shown figure 6-23 illustration of metal ion catalysis, general acid/base and transition state stabilization

Lys 345 acts as general base to remove H from substrate This proton not very acidic, so that is why base is needed to kick the reaction

create intermediate stabilized by Mg

lonic interacts with Mg also make H in previous reaction more acidic

Use of coenzyme (vitamins not discussed here wait for part III essentially give the enzyme other functional groups to plays with.

D. Lysozyme - Skip

E. Enzyme mechanism and Medicine

Many drugs are enzyme inhibitors, and they work by inhibiting specific enzyme reactions in your body. Let's explore one example

Penicillin

Discovered 1928 by Alexander Fleming

15 years before was understood enough to use as a drug Interferes with synthesis of peptidoglycan (polymer of peptide and

carbohydrate- Chapter 20) Part of rigid cell wall of bacteria Specifically transpeptidase reaction Figure 6-26 where one peptide-

carbohydrate is cross-linked to another

The penicillin(and related antibiotics) binds to enzyme, mimics substrate, and gets covalently linked to enzyme Figure 6-27

Once enzyme dead, can't make rigid cell wall, bacteria easily killed by osmotic shock

What kind of an inhibitor is this? (Suicide inhibitor)

What about drug resistance?

These are bacteria that have β -lactamases, recognize and open up penicillin ring before it gets to active site Figure 6-28a

Genes for these lactamases are quickly spread among bacteria under the positive selection of use or over use or improper use of these kinds of antibiotics We have overcome drug resistance in this case by adding Clavulanic acid It acts as a suicide inhibit of the β -lactamase (figure 6-28b)

Combination of Amoxicillin and Clavulanic acid sold under trade name Augmentin

HIV protease inhibitors

Book discusses these, but I don't think we have the time. Still a good story, read it on your own.

6.5 Regulatory enzymes

making an enzyme work is one thing, now lets move onto a second important aspect, regulating the enzyme so it works in proper balance with all the other 1000's of enzymes to keep the organism in the proper dynamic steady state

Note: this is NOT controlling the enzyme through inhibition as we have seen above, this is and entirely new and important subject an should not be confused with inhibition

in a metabolic pathway, only 1 or 2 key enzymes will be **regulatory enzymes** - Enzymes that exhibit increased or deceased catalytic activity in response to different signal event

usually the first enzyme in a pathway can efficiently turn pathway on and off

two major classes of regulatory enzymes

1. Allosteric enzymes - modulated by reversible, noncovalent binding of regulatory compounds called **allosteric modulator** (or effectors or **modulator**)

2. Regulation by reversible covalent modification (No special name)

Regulatory enzymes tend to be multi subunit proteins regulatory site and active site can be on different subunits

Other control mechanisms control by binding a regulatory protein control by proteolytic cleavage - irreversible - used in digestion, blood clotting, hormone action, vision

A. Allosteric enzymes - control by conformational change on binding a modulator

induced fit interaction when a modulator binds can be used to increase or decrease an enzymes activity

Modulator can be the substrate itself (useful to turn on a pathway when excess of starting material is sensed)

Called **homotropic** when activator and substrate are identical Called **heterotropic** when activator and substrate are different Can you think of example of homotropic we have already looked at?

(Hemoglobin)

Not the same as uncompetitive and mixed inhibitors

These inhibitor do bind at sites other than active sites, but they do not always mediate conformational changes between active and inactive forms, so kinetics are different

Properties of allosteric enzymes different from non-regulated enzymes In addition to active site have one or more regulatory site Regulatory site specific for modulator

In homotropic enzyme active site and regulatory site are the same

Allosteric enzymes generally larger and more complex Allosteric generally have 2 or more peptide chains or subunits

See of example aspartate transcarbamoylase

Figure 6-32

Ist step of pyrimidine nucleotide synthesis

12 chains

Catalytic and regulatory subunits

Binding to reg units makes large change in structure and activity

B. Feedback inhibition

see figure 6-33

One common theme in allosteric control is feedback inhibition Use final output of a pathway to shut down first step in pathway This should prevent buildup of excess product

C. Allosteric enzymes do not follow MM kinetics

Do saturate at high [S] but plot of V vs [S] is sigmoidal (figure 6-34) Cannot refer to [S] at $\frac{1}{2} V_{max}$ as K_m use [S]_{0.5} or K_{0.5} sigmoid shape explained by either sequential or concerted mech we had back in Chapter 5 Homotropic enzymes 6-34a

Allosteric effector is one of the substrates of enzyme Generally multiple subunits

Sigmoid shape due to cooperative binding interaction between subunits

Small change in S binds produce large changes in activity Heterotropic enzyme

Allosteric modulator is not a substrate of the enzyme Harder to make generalizations Can effect both Km and Vmax Can be + or -

D. Reversible covalent modifications

modifying groups:

Phosphoryl, adenyl, uridyl, adenosinediphosphate ribosyl, methyl adds and removed by separate regulatory enzymes

Phosporylation is major one

1/3 of proteins in eukaryotic cell are phosphorylated may be a single or many sites for phosphorylation since used in a large number of enzymes will study on y this one in detail

E. Phosphoryl groups affect structure and catalytic activity

attachment of phosphorous catalyzed by **protein kinases** removal of phosphate catalyzed by **protein phophatases** usually added to Ser, Thr, or Tyr (so OH) Changing moderately polar OH to large bulky double negatively charged group

O's can make multiple H bonds

Double negative repel and negative in area (Glu or Asp)

Attracts any positives in area (Lys or Arg or his)

If located in key structural area can have dramatic structural effects

One example glycogen phophorylase of muscle & liver

94,500 MW - dimer

Rxn glycogen + Pi →glycogen-1 + glu-1-P

So liberates stored glucose for metabolism

Phosphorylase *a* more active Ser 12 is phosphorylated

Phosphorylase *b* less active ser 12 is non-phosphorylated

Activation of b with ATP done by phosphorylase kinase Deactivation of a to b+ Pi done by phosphorylase phophatase

Figure 6-36

A and b differ in 2,3,and 4 structure See changes in structure and reactivity F. Multiply phosphorylation allow regulatory control

the ser, thr, and tyr sites of phospho regulated enzyme are often in common structural motifs that are recognized by specific protein kinases see table 6-10

kinase sites more that given sequence

3D structure must allow kinase access to site same kind of story for phosphatases, but generally less specific

Can be very complicated

Some protein have sites recognized by several kinases and phophatases

Sometime kinase action regulated by phosphorous on nearby residue

G. Proteolytic activation

some enzyme synthesized in inactive form called **zymogen** Activated by enzymatic cleave event

used in many proteolytic enzymes of stomach and pancreas Chymotrypsin & chymotrypsinogen

Trypsin & trypsinogen

See figure 6-38

Cleavage usually causes structural change to expose active site irreversible

further control by cosynthesis of inhibitor proteins - pancreatic trypsin inhibitor

Cleave event also used in synthesis of many other proteins proproteins or proenzymes even preproenzymes!

H. Multiple regulatory mechanisms

Many enzymes use multiple mechanisms just talked about regulation of glycogenphosphorylase by adding/removing phosphorous also has allosteric control by AMP activator and several inhibitors

Multiple control frequently found at key metabolic crossroads