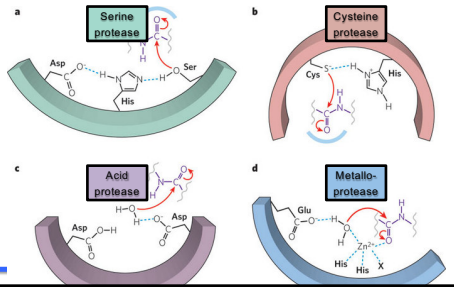
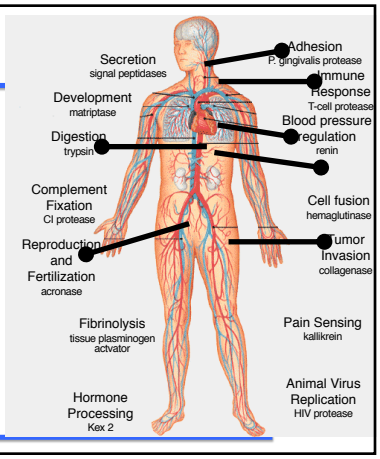
Covalent Cats - Proteases

4 classes of proteases: Serine, Thiol (Cys), Acid (Aspartyl), & Metal (Zn)



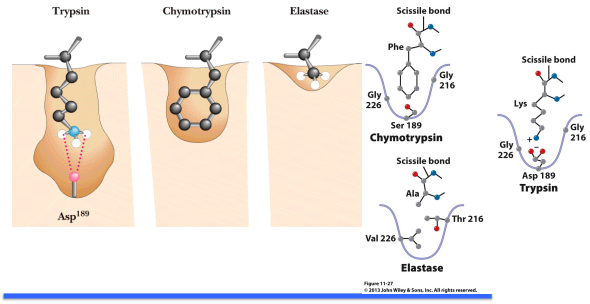
Roles

Function	Protease ex.
Nutrition	trypsin, subtilisin, α-lytic protease
Invasion	matrixmetalloproteases
Evasion	IgA protease
Adhesion	ADAM (a disintegrain and metalloproteinase)
Processing	signal peptidase, viral proteases, proteasome
Signaling	caspases, granzymes



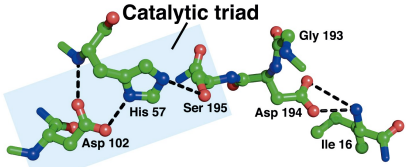
Substrate Specificity

Binding pocket is responsible for affinity



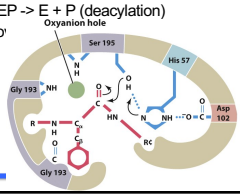
Serine Proteases

Ser, His and Asp in active site



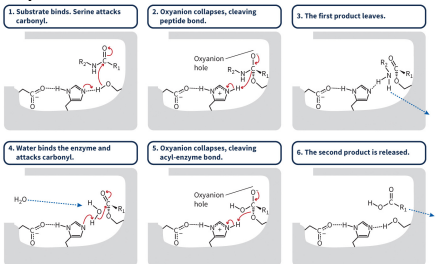
Ser Proteases Multiple Mechanism

- Serine not generally an active amino acid for acid/base catalysis
- Catalytic triad of serine, histadine and aspartate responsible for the reactivity of serine in this active site
- Covalent catalysis, Acid/base Catalysis, transition state bindingand Proximity mechanisms are used
- Two phase reaction when an ester is used
  - burst phase - E +S initial reactions
  - steady state phase - EP -> E + P (deacylation)
  - The first step is the co e actually is bound to the enzyme itself



Chymotrypsin Mechanism

Nucleophilic attack



Mechanism of chymotrypsin.

What Factors Influence Enzymatic Activity?

Principle means of regulating enzyme activity

- Reversible, non-covalent (allosteric and simple-MM) – typically small molecules
- Reversible, covalent
- Protein-Protein interactions
- Zymogen activation
- Protein expression and degradation
- Availability (both of enzyme and substrate)

Reversible Noncovalent

Simple activation and inhibition by small molecules – substrate, natural regulators of enzymes

MM kinetics Km, Vmax – competitive, non competitive…

Substrate inhibition or activation

The availability of substrates and cofactors usually determines how fast the reaction goes
As product accumulates, the apparent rate of the enzymatic reaction will decrease

Reversible Noncovalent: Allosteric

Action at "another site"

Enzymes situated at key steps in metabolic pathways are modulated by allosteric effectors

These effectors are usually produced elsewhere in the pathway

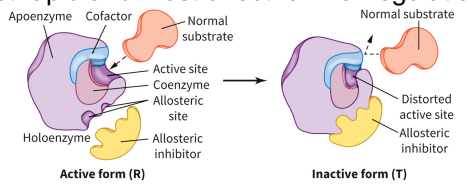
Effectors may be feed-forward activators or feedback inhibitors

Kinetics are sigmoid ("S-shaped")

Allosteric Regulation

Increases or decreases the enzymatic activity by binding at a site other than the active site

Most rapid and most direct form of regulation



(Black, *Microbiology: Principles and Explorations*, 8e, copyright 2012, John Wiley & Sons. This material is reproduced with permission of John Wiley & Sons, Inc.).

Figure 4.23 Allosteric regulation.

Allosteric Regulation Binding Curves

Exhibit sigmoidal activity curves

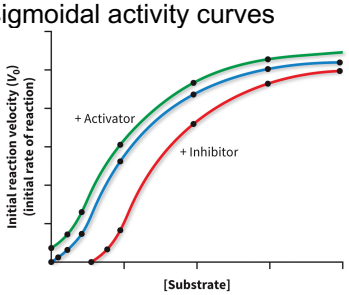
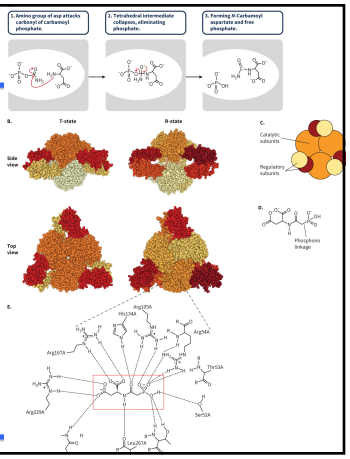


Figure 4.24 Allosteric enzymes have sigmoidal kinetics.

Aspartate Transcarbamolyase: Allosteric Regulation



The rate-limiting step in pyrimidine biosynthesis

ATCase regulation- An example of feedback control

Regulators are ATP, CTP, and UTP

Allosteric Regulators on ATCase

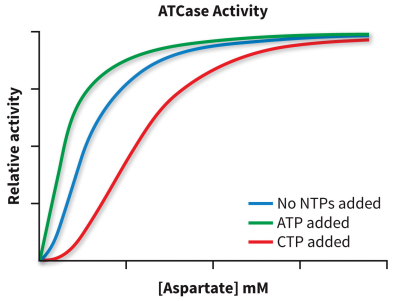
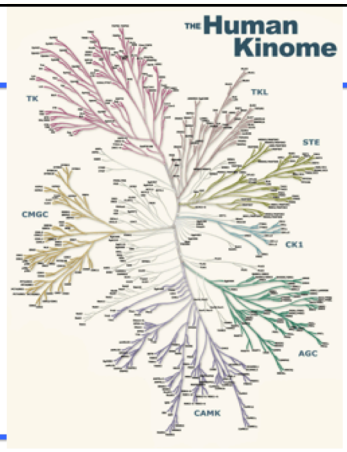


Figure 4.26 Effect of allosteric regulators on ATCase.

Reversible Covalent

Examples include: phosphorylation - dephosphorylaton, lipid modification.

This method is important because it does not alter the total amount of protein and it is easily reversed depending on cellular needs



Covalent modification - Protein kinases

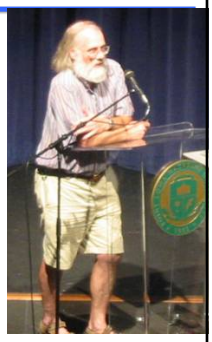
Phosphorylation/dephosphorylation

Most common method of reversible modification
- activation and localization

Up one-third of all cellular proteins are phosphorylated (so since there are ~30,000 genes, that would be ~10,000 phosphoproteins/organism, and perhaps a third of that in any given cell type, not including alternatively spliced isoforms).

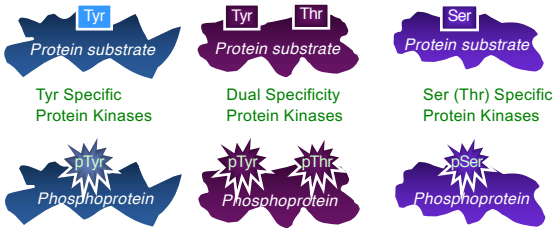
Leads to a very fast response to cellular stress, hormonal changes, learning processes transcription regulation ....

Tony Hunter (Salk Institute) '87 predicted 1001 protein kinases - more like 2000, but what is 999 proteins among friends?



Protein Kinases and Signal Transduction Pathways

Specificity in protein phosphorylation and biological diversity



Over-expression and constitutive activation of protein kinases exist in many human cancers; >80% of the proto-oncogenes and oncogenes identified encode protein tyrosine kinases.

What Kinds of Covalent Modification Regulate the Activity of Enzymes?

Protein kinases phosphorylate Ser, Thr, and Tyr residues in target proteins

Kinases typically recognize specific amino acid sequences in their targets

In spite of this specificity, all kinases share a common catalytic mechanism based on a conserved core kinase domain of about 260 residues

Kinases are often regulated by **intrasteric control**, in which a regulatory subunit (or domain) has a **pseudosubstrate sequence** that mimics the target sequence, minus the phosphorylatable residue

Covalent modification - Protein kinases

Regulation of protein phosphorylation varies depending on protein

- some turned on or off
- most kinases are regulated
- phosphatases generally not regulated
- can lead to large amplification of original signal

Four general classes of protein kinases, based on substrate (both sequence and amino acid phosphorylated), homology and regulation mechanisms (thousands of kinases)

Protein-Protein Interaction

Based on interface between two proteins

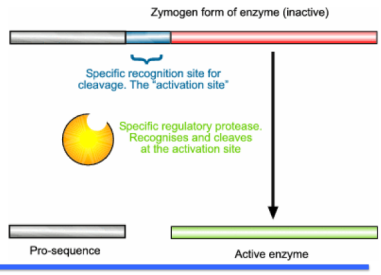
- can cause allosteric regulation but this style of interaction is different than between enzyme subunits

Protein binding can alter structure of second protein – one of the pairs are often regulated by a small molecule or covalent regulation

GTP Binding Proteins as an example

Zymogen Activity

Zymogens are inactive precursors of enzymes. Typically, proteolytic cleavage produces the active enzyme.



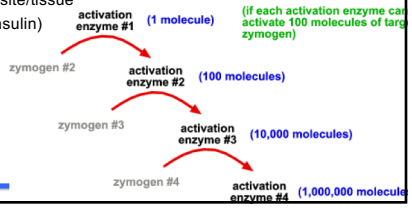
Zymogen Activation

Zymogen / proteolytic activation - this is an irreversible mechanism and must have tight control for the activation.

- most digestive enzymes such as trypsin and chymotrypsin
- blood clotting proteins are also commonly activated by this means. Why is this important?

Irreversible activation by cleavage of one or more peptide bonds. Usually protein is made in one organ and secreted in the inactive form and then made active at a distal site/tissue

- some hormones (insulin)
- digestive enzymes



Protein Concentration

Protein lifetime in cells is not indefinite

- Proteases – regulated activity within cells
- Proteins are usually **tagged** for **selective** destruction in proteolytic complexes called **proteasomes** by covalent attachment of **ubiquitin**, a small, compact protein that is highly conserved.

N-end rule: On average, a protein's **half-life** correlates with its **N-terminal residue**.

- Proteins with N-terminal Met, Ser, Ala, Thr, Val, or Gly have half lives greater than 20 hours.
- Proteins with N-terminal Phe, Leu, Asp, Lys, or Arg have half lives of 3 min or less.

PEST proteins, rich in Pro (P), Glu (E), Ser (S) and Thr (T), are more rapidly degraded than other proteins.

Protein expression – RNA levels influence protein production

Total levels of protein is a balance of both degradation and production

Availability

Availability - there are several means by which the cell controls metabolism this way.

- altering the physical location of the enzyme with or away from the substrate obviously controls the activity. Translocation of proteins from one organelle to another is the mode of operation.
- Sequestering or controlling the enzyme from it's substrate (glucose-6 phosphate is in the cytosol whereas the enzyme glucose 6 phosphatase is in the inside of the endoplasmic reticulum. The substrate is transported across the ER membrane when the reaction is needed)

University of San Diego

Availability

- Turnover - proteins generally have a defined half-life in the cell. Proteins are regularly being made and degraded. Altering either of these processes changes the total concentration of enzyme in the cell available for metabolism. The genetic control or rate of protein expression will play an important role in this regulation.
- Various pathways can be differentially regulated by the use of Isozymes - Enzymes that catalyze the same reaction but are different kinetic properties and regulation