Catalytic Strategies

- Covalent catalysis: temporary covalent modification of reactive group on enzyme active site
- Acid-Base catalysis: A molecule other than water is proton donor or acceptor (nucleophilic or electrophilic attack)
- Metal ion catalysis: Involvement of metal ion in catalysis. A metal ion is an electrophile and (i) may stabilize a negative charge on an intermediate; (ii) by attracting electrons from water, renders water more acidic (prone to loose a proton); (iii) may bind to substrate and reduce activation energy
- Catalysis by approximation: In reactions requiring more than one substrate, the enzyme facilitates their interaction by serving as an adapter that increases proximity of the substrates to each other

Chymotrypsin: A serine protease

- Hydrolyzes peptide bonds on the carboxyl side of Tyr, Phe, Trp, Met, Leu
- Since peptide bond is highly unreactive, a strong nucleophile is required for its hydrolysis
- Catalytic strategy is covalent modification and acid-base catalysis
- Contains catalytic triad of Ser, His and Asp. Ser is a nucleophile and participates in covalent modification, His is a proton acceptor (base), Asp stabilizes His (and active site) by electrostatic interactions

Reaction Mechanism

- Hydrolysis by chymotrypsin is a 2-step process
- Step 1: serine reacts with substrate to form covalent ES complex
- Step 2: release of products from ES complex and regeneration of enzyme
- Step 1 is faster than step 2 resulting in initial burst of product followed by steady-state rate of reaction
- In the lab, chymotrypsin catalysis is studied using a synthetic substrate N-acetyl-L-phenylalanine p-nitrophenyl ester. Formation of yellow colored p-nitrophenolate is measured spectrophotometrically

Step-wise reaction

- Enzyme active site is stabilized by ionic interactions between Asp and His and H-bond between His and Ser.
- In the presence of a substrate, His accepts a proton from Ser, Ser makes a nucleophilic attack on the peptide's carbonyl C converting its geometry to tetrahedral.
- The oxyanion is stabilized by H-bonds with peptide.
- The tetrahedral structure eventually collapses, the oxyanion reverts back to a carbonyl, the peptide bond is broken and a stable acyl-enzyme complex is formed.

Step-wise reaction (continued)

- Positively charged His transfers a proton to the amine group which is now free and is replaced by water.
- The carbonly is attacked by H_2O to form a tetrahedral intermediate, His accepts a proton and the carboxylic acid product is released.
- Interactions between the catalytic triad are reformed.

Proteases

- Most serine proteases have similar catalytic triads and mechanism of action
- A deep, hydrophobic pocket near the active site of chymotrypsin dictates its specificity for aa with large hydrophobic side chains
- Trypsin and elastase are homologs of trypsin with 40 % sequence identity. The variable regions account for different specificities
- Site-directed mutagenesis used to map the aa in the active site of proteases and other enzymes.

Regulatory Strategies for Enzymes and Proteins

- **Allosteric control**: Binding to distinct allosteric sites triggers structural and functional changes in active site. Exhibit cooperativity and sigmoidal kinetics. (Eg: Hb)
- **Isoenzymes**: Slightly variable forms of the same enzyme in different tissues; each with a unique K_M and V_{max}.
- **Reversible covalent modification** serves as an on/off switch for enzyme activity.
- **Proteolytic cleavage** of one or more aa leads to activation of enzyme from its zymogen or proenzyme form

Isoenzymes

- Isoenzymes have slightly different structures but catalyze the same reaction
- They are encoded by different genes which evolve through duplication and adaptation
- Oligomeric isoenzymes may have variable subunit composition
- Isoenzymes often found in different tissues or different stages of development
- Isoenzymes have very different kinetic properties: different K_M, V_{max} and specific inhibitors.
- Isoenzymes allow for regulation of metabolic activity according to necessity in different tissues

Covalent Modification

- Covalent attachment of another molecule to specific aa of enzymes and proteins can alter their activity
- Most common modifications are acetylation and phosphorylation. Other modifications include sugars, fatty acids, ubiquitin.
- Most regulatory modifications are reversible and serve as a on-off switch
- Covalent modification is used for regulation of transcription, signal transduction, metabolism, blood clotting, protein degradation

TABLE 10.1 Common covalent modifications of protein activity

Modification	Donor molecule	Example of modified protein	Protein function
Phosphorylation	ATP	Glycogen phosphorylase	Glucose homeostasis; energy transduction
Acetylation	Acetyl CoA	Histones	DNA packing; transcription
Myristoylation	Myristoyl CoA	Src	Signal transduction
ADP-ribosylation	NAD	RNA polymerase	Transcription
Farnesylation	Farnesyl pyrophosphate	Ras	Signal transduction
γ-Carboxylation	HCO_3^-	Thrombin	Blood clotting
Sulfation	3′-Phosphoadenosine-5′-phosphosulfate	Fibrinogen	Blood-clot formation
Ubiquitination	Ubiquitin	Cyclin	Control of cell cycle

Protein Phosphorylation

- Protein phosphorylation regulates metabolism and signal transduction
- The γ phosphoryl group of ATP is transferred to specific Ser /Thr or Tyr acceptors
- Protein kinases catalyze phosphorylation reactions and phosphatases catalyze dephosphorylation
- Eg: protein kinase A, protein kinase C, growth factor receptor tyrosine kinases
- Usually a sequential pathway of kinases involved
- The initial signal for phosphorylation is received through second messengers via endocrine or nutritional regulation

TABLE 10.2 Examples of serine and threonine kinases and their activating signals

Signal	Enzyme
Cyclic nucleotides	Cyclic AMP-dependent protein kinase
	Cyclic GMP-dependent protein kinase
Ca^{2+} and calmodulin	Ca^{2+}-calmodulin protein kinase
	Phosphorylase kinase or glycogen synthase kinase 2
AMP	AMP-activated kinase
Diacylglycerol	Protein kinase C
Metabolic intermediates and other "local" effectors	Many target specific enzymes, such as pyruvate dehydrogenase kinase and branched-chain ketoacid dehydrogenase kinase

Source: After D. Fell, *Understanding the Control of Metabolism* (Portland Press, 1997), Table 7.2.

Phosphorylation regulates metabolism

- Many metabolic enzymes are regulated by phosphorylation. Eg: Pyruvate dehydrogenase, glycogen synthase, glycogen phosphorylase
- In general, anabolic enzymes are inactivated by phosphorylation while catabolic enzymes are activated by phosphorylation
- Glycogen synthase is inactivated by phosphorylation, glycogen phosphorylase is activated by phosphorylation

Phosphorylation: mechanism of regulation

- Phosphorylation alters protein structure significantly
- Introduction of negative charge facilitates ionic interactions
- The H on phosphate participate in H-bonds
- A phosphate bond is a high energy bond, it stored energy changes the equilibrium between functional states of the protein by a factor of 10^4.
- The requirement for ATP allows tailoring of metabolic regulation to metabolic requirement
- Kinetics of phophorylation / dephosphorylation are adjustable according to metabolic needs
- Because of the cascading pathway of activation, regulation by phosphorylation is highly amplified

Zymogens

- Zymogens are inactive precursors to enzymes. They have an aa or peptide ‘cap’ that inhibits enzyme activity
- Zymogens are converted to the active enzymes by the hydrolysis of one or more peptide bonds to ‘expose’ the active site or allow folding of the enzyme in the active conformation.
- Zymogen activation is irreversible, but subject to specific inhibitors.
- Zymogens are most common with digestive proteolytic enzymes. Eg: pepsinogen/pepsin , trypsinogen/trypsin, plasminogen/plasmin, blood clotting proteases
- Proteins synthesized with a cap that needs to be hydrolyzed for protein activity are called proproteins. Eg: Proinsulin, proapoAI, procollagen, caspases, etc.