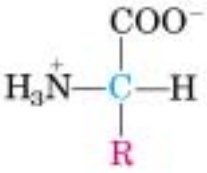
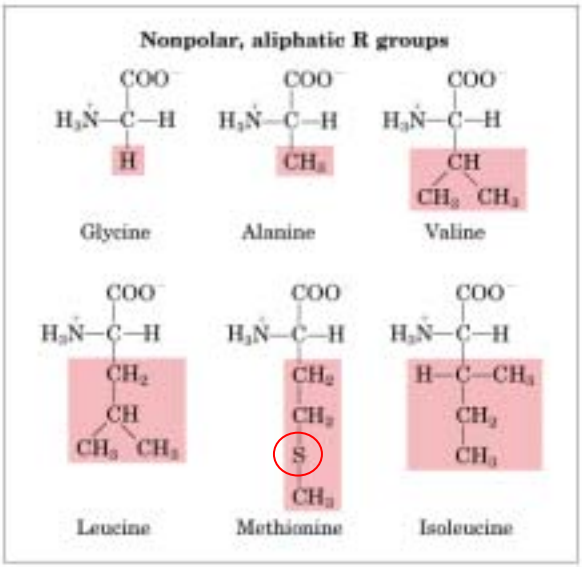
Part (I) Structure and Function of Proteins & Enzymes

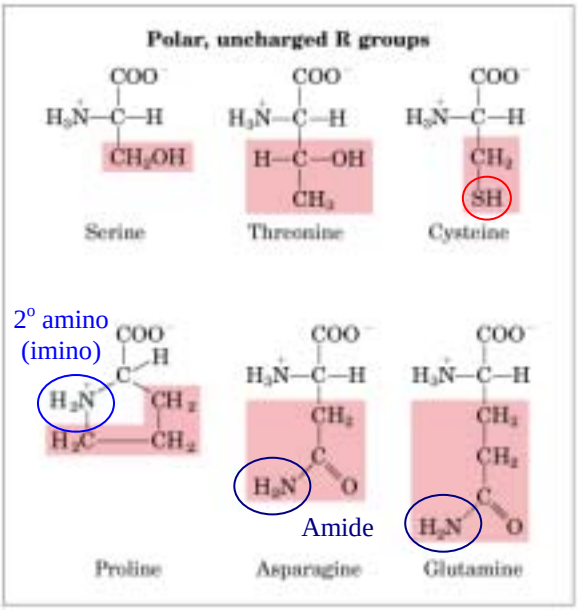
A. Amino acids, peptide, and protein

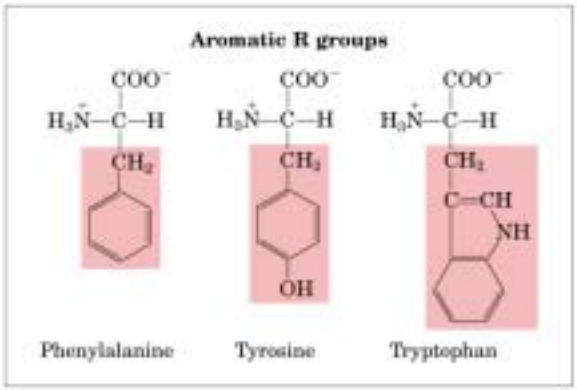
1. Basic amino acid structure and property:
   - C_α: (charial center): D-, L-form (stereoisomer, enantiomer)
     - D-form: found only in few small peptides of bacterial cell wall, antibiotics.
   - Amino group
   - Carboxyl group
   - Side chain (R-group)

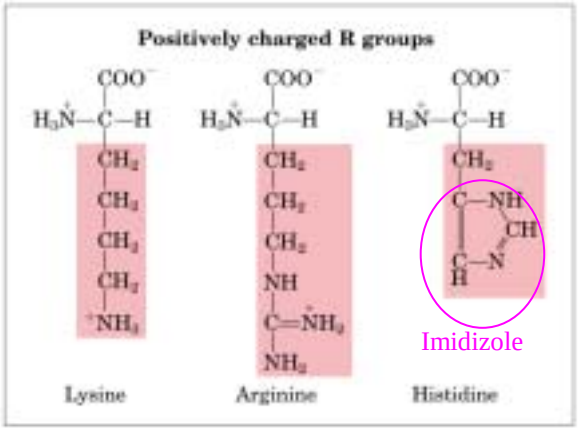


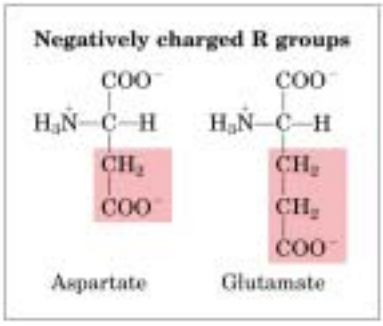
2. 20 standard amino acids:
   - BCAA: Val, Leu, Ile
   - Physical property: aromatic amino acids absorb UV light: $\varepsilon_{280\ nm}$: Trp > Tyr >> Phe





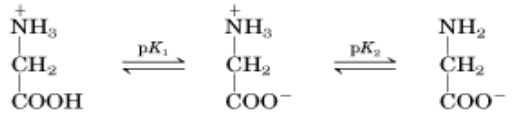




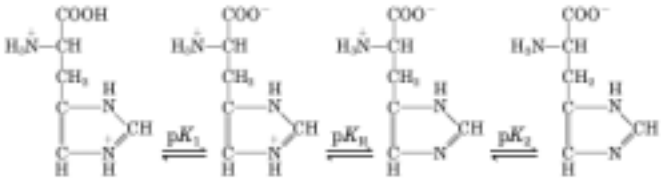


甘胺酸	Glycine	Gly	G	
丙胺酸	Alanine	Ala	A	
纈胺酸	Valine	Val	V	
白胺酸	Leucine	Leu	L	
異白胺酸	Isoleucine	Ile	I	
脯胺酸	Proline	Pro	P	
甲硫胺酸	Methionine	Met	M	
苯丙胺酸	Phenylalanine	Phe	**F**	"**F**enylalanine"
酪胺酸	Tyrosine	Tyr	Y	"t**Y**rosine"
色胺酸	Tryptophan	Trp	**W**	Double ring
絲胺酸	Serine	Ser	S	
酥胺酸	Threonine	Thr	T	
半胱胺酸	Cysteine	Cys	C	
天門冬醯胺	Asparagine	Asn	**N**	Contains **N**
麩胺醯胺	Glutamine	Gln	**Q**	"**Q**-tamine"
離胺酸	Lysine	Lys	**K**	Near L
精胺酸	Arginine	Arg	**R**	"a**R**ginine"
組織胺酸	Histidine	His	H	
天門冬酸	Aspartate	Asp	**D**	"aspar**D**ic"
麩胺酸	Glutamate	Glu	**E**	"glu**E**mate"

3. Chemical property:
   - HA = $H^+ + A^-$, $K_a = K_{eq} = [H^+][A^-]/[HA]$
     - $pK_a = \log (1/K_a) = - \log K_a$
     - $pH = pK_a + \log([A^-]/[HA])$
   - Gly: Two ionizable group: $-NH_3^+$, and $-COO^-$; $pI = ½ (pK_1 + pK_2)$



   - His: Plus an ionizable R group (imidizole), pK_R near 7; $pI = ½ (pK_2 + pK_R)$



4. Non-standard amino acids:
   - 4-hydroxyproline, 5-hydroxylysine: found in collagen.
   - 6-*N*-methyllysine: occur in muscle protein myosin.
   - γ-carboxyglutamate: found in prothrombin and certain Ca^{2+}-binding protein.
   - Desmosine (a derivative of four Lys residues): found in the fibrous protein elastin.
   - Selenocysteine: Selenium replaces sulfur in cysteine during amino acid synthesis (derived from serine).
   - Amino acids not as constituents of proteins, but play other cellular functions:
     - Ornithine, citrulline: key intermediates in the biosynthesis of arginine and urea cycle.

5. Peptide bond:
   - Two amino acids joined to form the **CO-NH** upon removal of one water molecule.
   - Chemical property: free N-terminal, free C-terminal, and all ionizable R-group
   - Biological peptide:
     - Aspartame = Aspartate + phenylalanine (restricted intake for PKU)
     - Glutathione (GSH, γ-glutamyl-cysteinyl-glycine), glutathione S-transferase (GST)
       - Maintain –SH and Fe^{2+} in reduced state.
       - As a reducing agent for glutaredoxin in deoxyribonucleotide synthesis.
       - Remove toxic peroxides formed under aerobic condition.
       - 2 GSH + R-O-O-H → GSSH + H_2O + R-OH, reaction catalyzed by glutatione peroxidase that contain selenocysteine.
     - Heptapeptide opioids dermorphin and deltophorin (South American tree frog skin) contain D-tyrosine and D-alanine.

6. Techniques often used in protein purification
   - Ammonium sulphate precipitation (salting out) and dialysis
   - Column chromatography (preparative use)
     - Gel filtration (Size exclusion) chromatography
     - Ion exchange chromatography
     - Affinity chromatography
   - Gel electrophoresis (analytical use)
     - SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis)
       - SDS: negatively charged detergent that denatures protein
       - Separate proteins according to their molecular weight
     - IEF (isoelectric focusing)
       - Separate proteins according to their pI
   - Functional assay: enzyme activity (unit, mole/min)
     - Specific Activity: unit/mg

7. Protein structure:
   - Primary structure: amino acid sequence (covalent structure)
     - N-terminal labeling free α-amino group of a peptide:
       - ✓ Sanger's method (1-fluoro-2,4-dinitrobenzene, FDNB)
       - ✓ Edman degradation (phenylisothiocyanate, PITC)
       - ✓ Dansyl chloride (fluorescent)
       - ✓ Dabsyl chloride (orange)
       - ✓ Ninhydrin (purple)
     - Mass spectrometry: MALDI-TOF, Tandem Mass (MS-MS)
       - ✓ Protease cleavage site:

Treatment	Cleavage points
Trypsin	Lys, Arg (C)
Chymotrypsin	Phe, Trp, Tyr (C)
Pepsin	Phe, Trp, Tyr (N)
Cyanogen bromide (CNBr)	Met (C)

     - Deduced from DNA sequence
   - Secondary structure: recurring structural pattern (restricted phi-Φ and psi-Ψ angle)
     - Circular dichroism (CD, 圓二色極化光譜儀) can estimate the 2^o structure content.
   - Tertiary structure: 3D folding → evolutionary relationship
     - X-ray crystallography = protein crystal + X-ray diffraction
     - Nuclear Magnetic Resonance (NMR)
   - Quaternary structure: Subunits arrangement within a protein

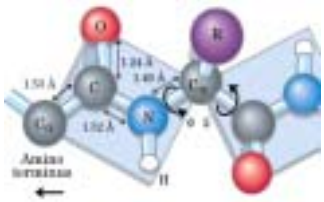
8. Proteomics (蛋白質體學):
   - Protein : Proteome : Proteomic *vs.* Gene : Genome : Genomic
   - DNA chip (DNA micro-array) and bioinformatics (*in silico*).
   - System biology and evolution

B. Protein structure and function

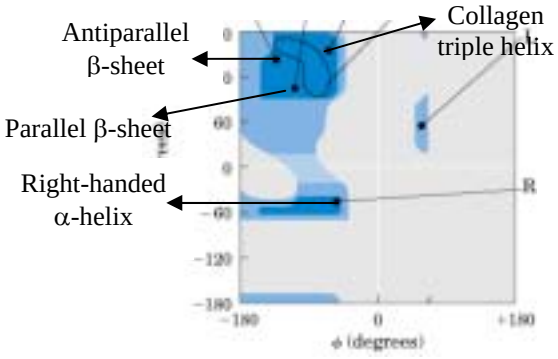
1. Protein conformation: native, folded, stable, lowest Gibbs free energy (G)

2. Maintained by disulfide bond (covalent) and "weak (non-covalent) interactions"
   - H-bond
   - Hydrophobic interaction
   - Ionic interaction
   - Van der Waals interaction

3. Planar and rigid peptide bond limit the possible ψ and ϕ (Ramachandran Plots)
   - Common secondary structure (local conformation, maintained by H-bond)
     - α-helix
       - right-handed, 3.6 a.a./turn, 5.4 Å/turn
       - ψ = -45 ^{o} ~ -50^{o}, ϕ : -60^{o}
       - Pro: helix breaker (ends a helix)
       - H-bond between backbone residues
         - -CO of residue$_i$ and the –NH of residue$_{i+3}$



     - β-conformation (sheet)
       - Parallel and antiparallel
     - β-turn
       - A 180^{o} turn involves 4 a.a.
       - Favor Gly and Pro
   - α-helix and β-sheet in transmembrane proteins



4. Structural motif (supersecondary structure), domain
   - DNA-binding motif:
     - Helix-loop-helix (HLH, c-Myc, dimmer)
     - Helix-turn-helix (HTH, homeobox domain)
     - Zinc finger (TFIIIA)
     - Leucine zipper (c-Jun/c-Fos dimmer)

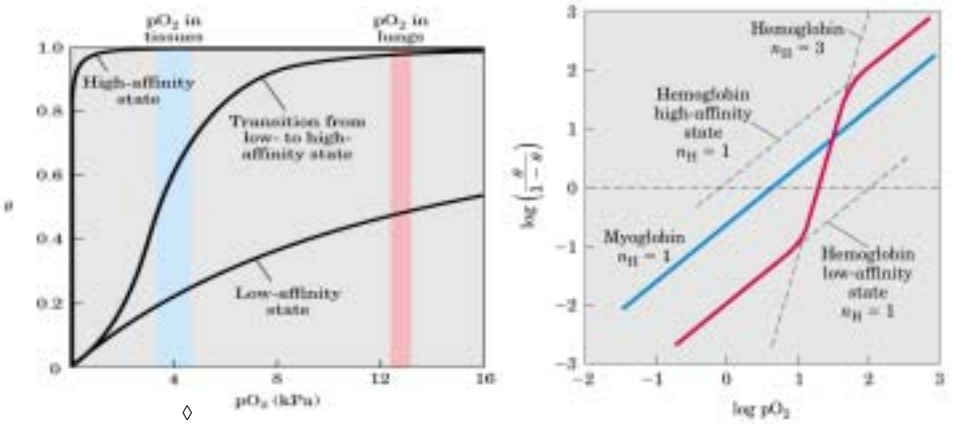
5. Structural protein: e.g. α-keratin, collagen, silk.
   - Collagen:
     - Hydroxy-proline and lysine: intrastrand H-bond (to stabilize cllagen triple helix)
       - Hydroxylation requires cofactor : ascorbic acid (Vit C)
       - Vit C is required for Fe^{2+} regeneration
       - Scurvy
     - Certain Lys are modified by lysyl oxidase (a copper-containing protein)
       - Menke's syndrome: a dietary deficiency of the copper

6. Protein folding
   - Weak interactions (non-covalent interactions):
   - The native conformation is thermodynamically favored.
   - Assisted folding: chaperones, chaperonins (heat shock protein)
   - Misfolded protein and disease:
     - The prion disease (hCJD, mad cow disease, etc) and prion protein (PrP^C, PrP^{SC})
     - Alzheimer's disease (β-amyloid)

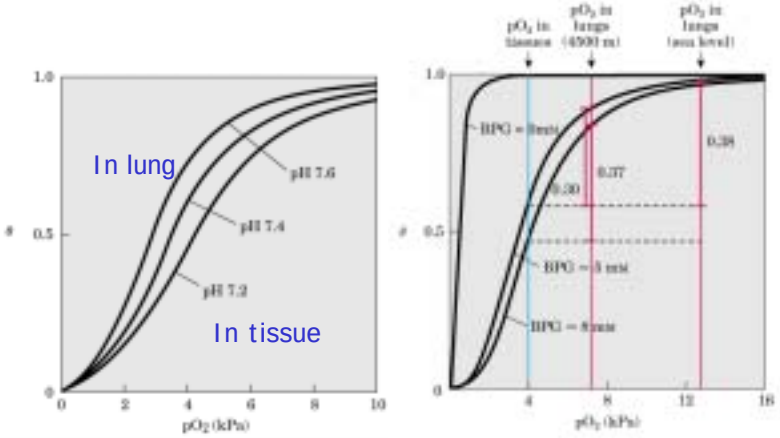
7. Globular protein: Myoglobin (Mb) and hemoglobin (Hb)
   - Heme = Fe^{2+} + porphyrin
     - Heme containing protein: Mb, Hb, cytochrome (Fe and Cu) chlorophyll (Mg)

   - Protein-ligand binding curve (O_2 binding curve)
     - K_d: binding affinity; dissociation constant; [L] at half-saturation. (PO_2)
     - Mb: hyperbolic, small K_d, high affinity.
     - Hb: sigmoid (S-shape), cooperative O_2 binding, subunit interaction and T-R transition.

   - Cooperativity
     - Hb is an allosteric protein (T – taut state, low affinity, R – relaxed state, high affinity), O_2 is a homotrophic effector.
       - ◊ Hill plot; Hill coefficient



   - Bohr effect: CO_2, H^+ also bind Hb and affect (reduce) O_2 affinity (heterotrophic effector)



   - BPG (2,3-bisphosphoglycerate) or DPG stabilized the T state, reduce O_2 affinity. Adaptation to high altitude.

- Hb isoforms:
  - The primary structures of the β, γ, and δ chains of human Hb are highly conserved.
  - HBA ($\alpha_2\beta_2$, normal adult Hb)
  - HbF ($\alpha_2\gamma_2$, fetal Hb)
  - HbS (α_2S_2, sickle cell Hb) → sickle cell anemia, sticky patch on HbS.
  - HbA2 ($\alpha_2\delta_2$, a minor adult Hb)

- Methemoglobin (MetHb, Fe^{3+})
  - Methemoglobin reductase can reduce Fe^{3+} to Fe^{2+} (MetHb → Hb).
  - Fe oxidation can be a side effect of sulfonamide, from hereditary HbM, or reduced activity of methemoglobin reductase.
  - Hemoglobin M (HisF8 replaced by Tyr, inhibited T-R transition):

- Biomedical implications
  - Myoglobinurea: Following massive crush injury, myoglobin released from damaged muscle fibers colors the urine dark red.
  - Anemia: reflect impaired synthesis of Hb (e.g. iron deficiency) or impaired production of erythrocyte (e.g. in folic acid or Vit B_{12} deficiency).
  - Thalassemias: result from the partial or total absence of one or more α or β chains of hemoglobin. Apart from marrow transplantation, treatment is symptomatic.
  - Glycosylated hemoglobin (HbA_{1c})
    - ✓ When blood glucose enters the erythrocytes it glycosylates the ε-amino group of lysine and the N-terminal of Hb. The fraction of Hb glycosylated, normally about 5%, is proportionate to blood glucose concentration. Since the half-life of an erythrocyte is typically 60 days, the level of glycosylated hemoglobin (HbA_{1c}) reflects the mean blood glucose concentration over the preceding 6-8 weeks. Measurement of HbA_{1c} therefore provides valuable information for management of diabetes mellitus.

C. Enzymes

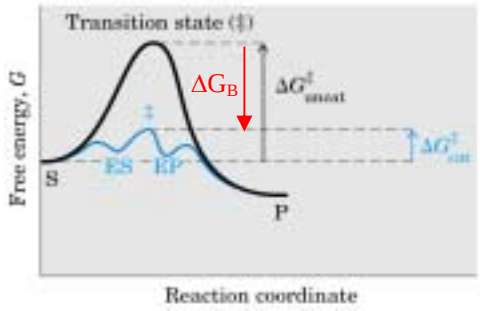
1. Enzyme as catalyst
   - Ribozyme (catalytic RNA)
     - RNA enzyme, production of tRNAs from pre-tRNAs catalyzed by ribonuclease P
   - Abzyme (catalytic antibody)
     - Ab as enzyme, induced by transition state analogue
   - Protein
     - Holoenzyme (holoprotein) = Prosthetic group + Apoenzyme (apoprotein)
     - Prosthetic group: coenzyme (organic molecule) or cofactor (metal ion).
     - Cofactor: metalloenzymes, often participate in redox reaction (table 8-1, below)

2. Many coenzymes and cofactors are derived from B vitamins
   - Coenzyme: pyridoxal phosphate (PLP), flavin mononucleotide (FMN), flavin dinucleotide (FAD), thiamin pyrophosphate, biotin.

table 8–1

Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

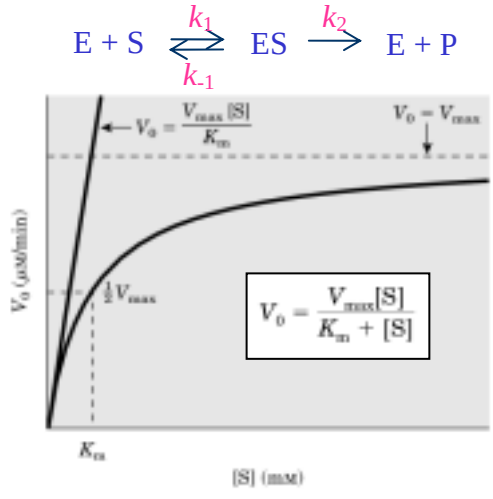


3. Energetics
   - Standard free energy change ←→ Equilibrium (Enz. only alters the reaction rate).
   - Enzyme facilitates the formation of transition state.
   - Activation energy = Binding energy

4. Enzyme classification

Oxidoreductases	Transfer electrons, catalyze oxidations and reductions. e.g. Dehydrogenases, oxidases, reductases, peroxidases, catalase, oxygenases, hydroxylases.
Transferase	Group transfer reaction e.g. transaldolase, transketolase, acyl-, methyl-, glocosyl-, and phosphoryl-transferase, kinases, phosphomutases
Hydrolyses	Hydrolysis reaction (transfer of functional group to H_2O), hydrolytic cleavage of C-C, C-O, C-N, P-O and other bonds. e.g. esterases, glycosidases, peptidases, phosphatases, thiolases, phospholipases, amidases, deaminases, ribonucleases.
Lyases	Addition of groups to double bonds or formation of double bonds by group removal e.g. decarboxylases, aldolases, hydratases, dehydratases, synthases, lyases.
Isomerase	Transfer group within molecules to yield isoform e.g. racemases, epimerases, isomerases
Ligase (Synthase)	Formation of C-C, C-S, C-O, C-N by condensation coupled to ATP cleavage (catalyze the joining together of two molecules). e.g. synthetases, carboxylases.

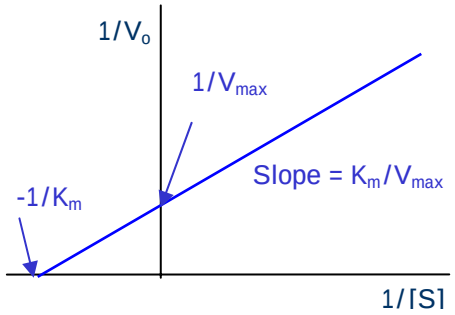
5. Assumptions of Mechaelis-Menten equation
   - V_o is determined when [S] >>[E];
   - V_o is determined only at the beginning of the reaction when the [P] is infinitely small and the back reaction (S ← P) can be ignored.
   - k_{-1} << k_2. Under this condition the rate-limiting step (the slowest step), is the conversion of ES complex to free enzyme and product.
   - Hyperbolic plot:
     - V_o vs. [S]; Km = [S], when V_o = ½ Vmax.



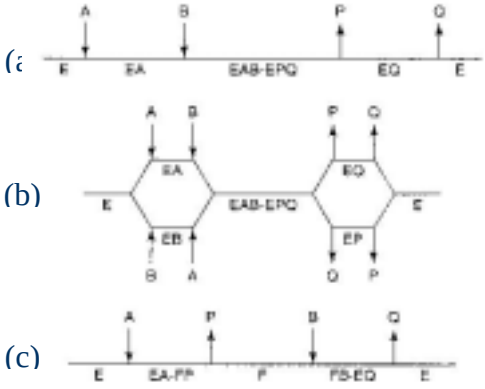
6. Catalytic efficiency
   - K_m: a character of the enzyme, rate of the catalytic process.
   - K_{cat}: turnover number = V_{max} / [E_T], the number of S → P in a given unit of time when the E is saturated with S.
   - K_{cat}/K_m: the specificity constant, enzyme efficiency

7. Lineweaver-Burk plot, double reciprocal, $1/V_o$ vs. $1/[S]$
   - X-intercept: $1/V_{max}$
   - Y-intercept: $-1/K_m$

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



8. Bi-substrate and Bi-product reaction (Bi-Bi reaction)
   - A + B ↔ P + Q, catalyzed by enzyme E
   - Sequential displacement (form ternary complex)
     - Compulsory order (ordered Bi-Bi)
       (a) LDH (pyruvate + NADH → lactate + NAD^+)
     - Random order (random Bi-Bi)
       (b) Creatine kinase (ATP + Cr → PCr + ADP)
   - Ping-Pong reaction (double displacement reaction, no ternary complex)
       (c) Aminotransferases

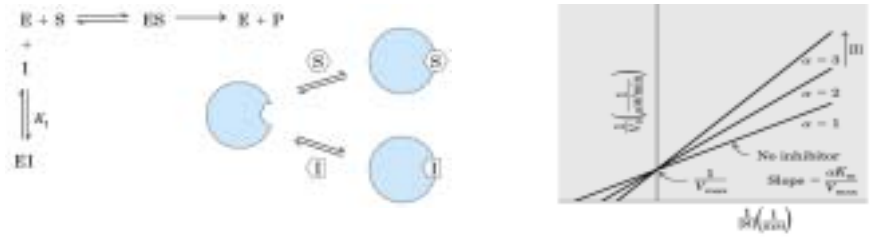


9. Irreversible inhibitor (destroy the active site):
   - Group specific reagent: react with specific R-group of a.a.
     - DIFP (diisopropylphosphofluoridate) inhibits chymotrypsin (serine protease), and acetylcholinesterase.
     - Iodoacetamide → modify –SH of cysteine residue.
   - Substrate analogs (affinity label)
     - TPCK (tosyl-L-phenylalanine chloromethyl ketone) → chymotrypsin
       ◊ TPCK binds chymotrypsin at the active site and actis with His irreversibly.
     - 3-bromoacetol → triose phosphate isomerase (TIM)
       ◊ Normal substrate: dihydroxyl-acetone phosphate
   - Suicide inhibitor (mechanism-based)
     - Penicillin →acts by covalently modifying transpeptidase (suicide inhibitor)
     - N, N-dimethylpropargylamine → monoamine oxidase (MAO) + coenzyme (flavin)
       ◊ MAO catalyze the deamination to form dopamine, serotonin.
       ◊ (-) Deprenyl to treat Parkinson's disease.

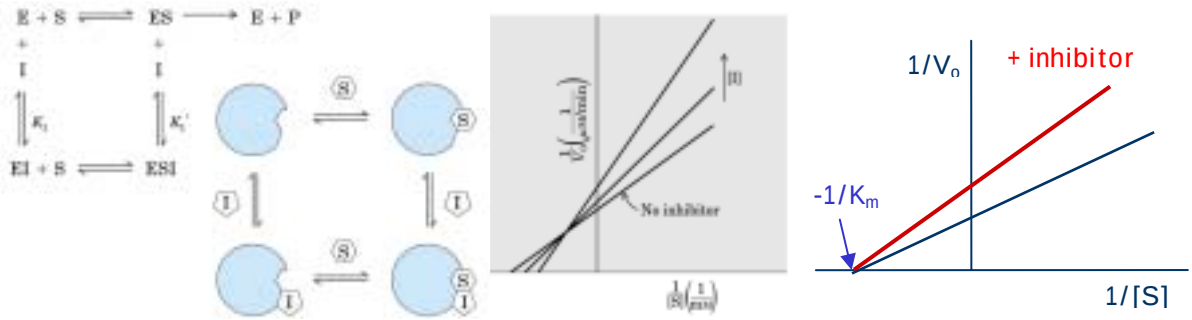
10. Reversible inhibitor

Inhibitor type	Binding site on enzyme	Kinetic effect
Competitive	Specifically at the catalytic (active site), inhibitor has a similar structure as the substrate. Inhibition is reversed by substrate	V_{max} unchanged, K_m increased
Noncompetitive (mixed type)	I binds E or ES complex other than the active site. Inhibition can not be reversed by substrate.	V_{max} decreased, K_m unchanged
Uncompetitive	I binds only to ES complex other than the active site. Inhibition can not be reversed by substrate.	V_{max} decreased, K_m decreased

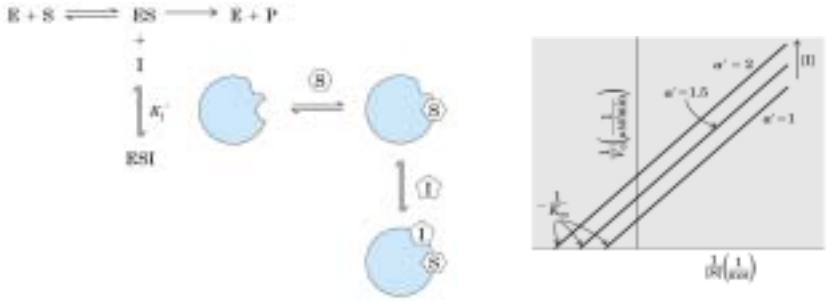
- Competitive:
  - Methanol vs. ethanol and alcohol dehydrogenase
  - CO vs. O_2



- Mixed (non-competitive, a special case):
  - Heavy metal ion Hg^{2+}, Pb^{2+}, which bind to strategically positioned sulphydryl groups and modulate the conformation of the enzyme.

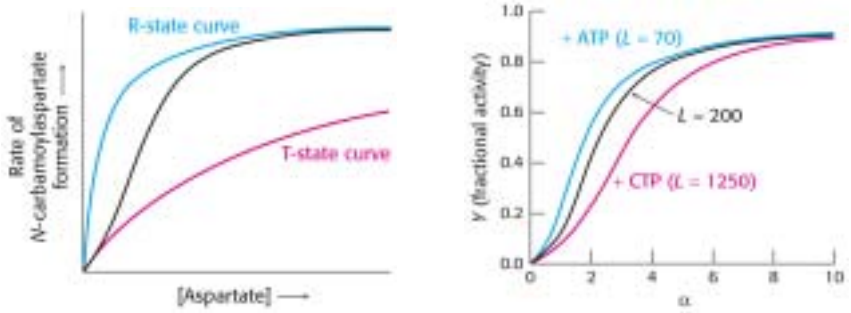


- Uncompetitive:



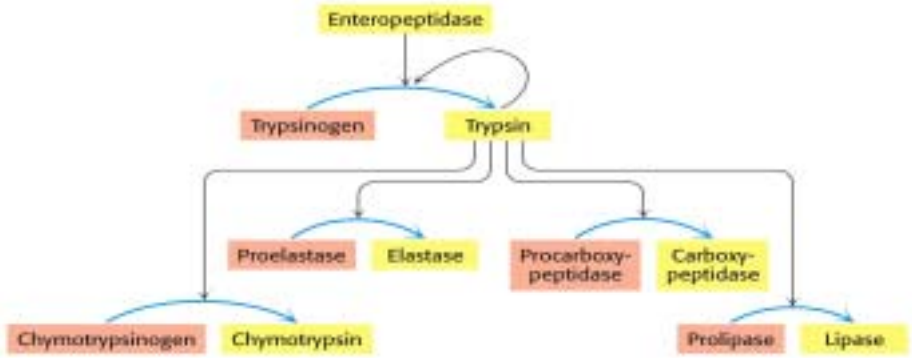
11. Regulation of enzyme activity
    - Regulation level
      - DNA level: transcription rate, inducer/suppressor
      - Protein level:
        - ◊ Translation rate
        - ◊ Degradation rate
        - ◊ Post-translational modification
          - ♦ Covalent
          - ♦ Non-covalent: allosteric, pH, temperature, coenzyme/cofactor
        - ◊ Substrate-product level (feedback inhibition)

- Regulation type:
  - Allosteric (non-covalent)
    - ◊ Hemoglobin
    - ◊ Aspartate transcamoylase (ATCase)
      - ♦ 1st step in pyrimidine synthesis
      - ♦ T (less active, favored by CTP binding)←→ R (more active, favored by substrate binding)
      - ♦ Concerted mechanism (all-or-none)
      - ♦ Heterotrphic: ATP, CTP (- feedback)



  - Covalent modification
    - ◊ Phosphorylation-dephosphorylation (kinase-phosphatase)
    - ◊ Methylation, etc.

  - Peptide bond cleavage (proteolytic cleavage)
    - ◊ Zymogen, proenzyme, proprotein (inactive) → active enzyme
    - ◊ Digestive enzymes (proteases)
      - ♦ Trypsin formed by enteropeptidase (master activation step)
        - Coordinated control of digestive enzyme:



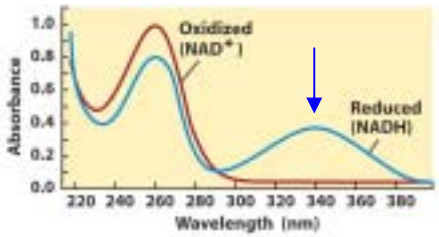
      - ♦ Protease inhibitor
        - ◊ Pancreatic trypsin inhibitor
        - ◊ α_1-antitrypsin (α_1-antiprotease) inhibits elastase (secreted by neutrophils)
          - Cigarette smoking and emphysema (destructive lung disease)
          - Smoke oxidize Met358 of the inhibitor, essential in binding to elastase.

    - ◊ Procaspase → caspase, (programmed cell death, apoptosis)
    - ◊ Proinsulin → insulin (hormone)
    - ◊ Procollagen → collagen
      - Procollagenase → collagenase (timed tissue remodeling in development)
        - Metamorphosis of a tadpole into a frog
        - Mammalian uterus after delivery

- ◊ Proteins in the blood-clotting cascade
  - ♦ Properties
    - ◊ *Serine proteases*: enzymes has *serine* in the active site
    - ◊ *Zymogen:* factors circulate in blood as *inactive form*
    - ◊ *Cascade reaction:* 1st factor (small amount) → end reaction (large amount) → Rapid response → limiting blood loss
  - ♦ Prevention of clotting
    - ◊ Slow: activated factors are removed by liver
    - ◊ Fast: antithrombin III (enz. inhibitor), Heparin
    - ◊ Drug: dicoumarol (Vit. K analog), Aspirin
  - ♦ Inherited defects in clotting
    - ◊ Hemophilia A
      - ■ Deficiency of factor VIII
      - ■ X-linked recessive gene
    - ◊ Hemophilia B (*Christmas disease*)
      - ■ Deficiency of factor IX
      - ■ Autosomal recessive trait
    - ◊ Von Willibrand's disease
      - ■ Deficiency in platelet adherence
      - ■ Defect in factor VIII
      - ■ Prolonged clotting time

- Isoenzyme, or isozyme (caused by gene duplication)
  - ◊ Properties:
    - ♦ Enzyme catalyze the same reaction
    - ♦ Different kinetic property: K_m, V_{max}
    - ♦ Different physical property: molecular weight
    - ♦ Different chemical property: amino acid sequence
    - ♦ Different tissue specificity: location
  - ◊ L-lactate dehydrogenase: lactate + NADH → pyruvate + NAD^+
    - ♦ H, heart and M, muscle
    - ♦ LDH (a tetramer): HHHH, HHHM, HHMM, HMMM, MMMM
  - ◊ Creatine kinase (creatine phosphate kinase): Cr + ATP → CrP (PCr) + ADP
    - ♦ CK_1: BB (brain and colon)
    - ♦ CK_2: BM (heart muscle)
    - ♦ CK_3: MM (skeletal muscle)
    - ♦ Mit: MtMt (muscle, brain and colon)

12. Enzymes facilitate diagnosis disease:
    - Polymerase chain reaction (PCR)
    - Restriction fragment length polymorphisms (RFLPs) facilitates prenatal detection of hereditary disorders (restriction endonucleases).
    - Enzyme-linked immunoassays (ELISAs):
      - ◊ Ab + a "reporter enzyme" → readily detectable product.
    - Colored product:
      - ◊ NADH and NADPH (340 nm)
      - ◊ Substrate for phosphatase: *p*-nitrophenyl phosphate (pNP) absorb 419 nm (405 nm).



13. How to study the mechanism of an enzyme:
    - Amino acid modification with irreversible inhibitor
    - Site-directed mutagenesis → functional assay