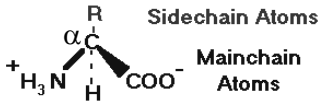
Protein function and Enzyme kinetics

Lecture 5

Proteins and Enzymes

The structure of proteins
How proteins functions
Proteins as enzymes

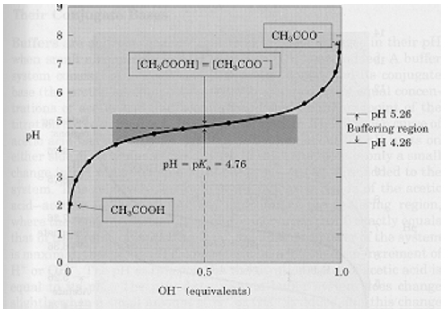
The R group gives and amino acid its unique character



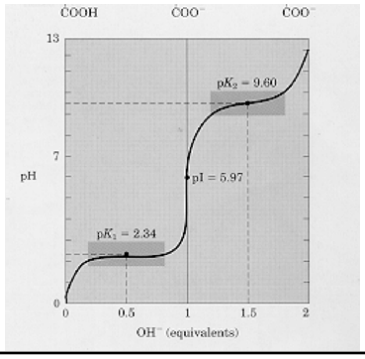
Dissociation constants

$$HA \rightleftharpoons H^+ + A^-$$

Titration curve of a weak acid



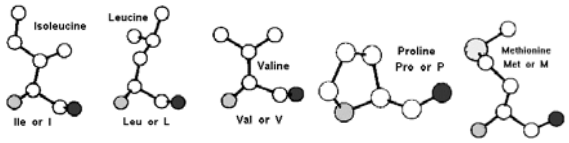
Titration curve of glycine



Properties of Amino Acids

Alaphatic amino acids

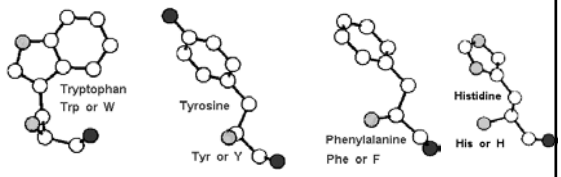
only carbon and hydrogen in side group



Honorary member

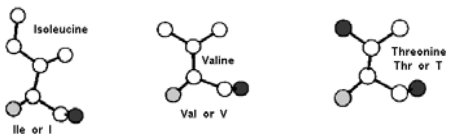
Strictly speaking, aliphatic implies that the protein side chain contains only carbon or hydrogen atoms. However, it is convenient to consider Methionine in this category. Although its side-chain contains a sulphur atom, it is largely non-reactive, meaning that Methionine effectively substitutes well with the true aliphatic amino acids.

Aromatic Amino Acids



A side chain is aromatic when it contains an aromatic ring system. The strict definition has to do with the number of electrons contained within the ring. Generally, aromatic ring systems are planar, and electons are shared over the whole ring structure.

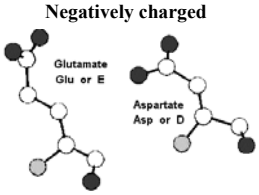
Amino acids with C-beta branching



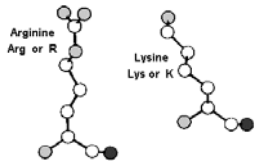
Whereas most amino acids contain only one non-hydrogen substituent attached to their C-beta carbon, C-beta branched amino acids contain two (two carbons in Valine or Isoleucine; one carbon and one oxygen in Theronine) . This means that there is a lot more bulkiness near to the protein backbone, and thus means that these amino acids are more restricted in the conformations the main-chain can adopt. Perhaps the most pronounced effect of this is that it is more difficult for these amino acids to adopt an alpha-helical conformation, though it is easy and even preferred for them to lie within beta-sheets.

Charged Amino Acids

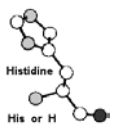
Negatively charged



Positively charged

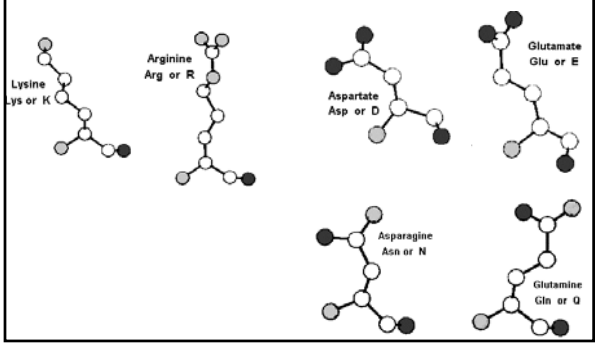


It is false to presume that Histidine is always protonated at typical pHs. The side chain has a pKa of approximately 6.5, which means that only about 10% of of the species will be protonated. Of course, the precise pKa of an amino acid depends on the local environment.

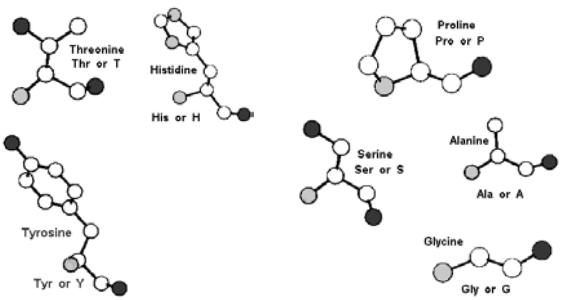


Partial positive charge

Polar amino acids

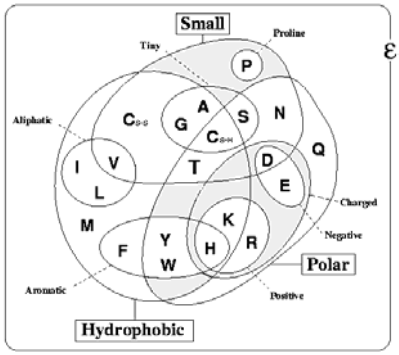


Somewhat polar amino acids



Polar amino acids are those with side-chains that prefer to reside in an aqueous (i.e. water) environment. For this reason, one generally finds these amino acids exposed on the surface of a protein.

Amino acids overlap in properties



How to think about amino acids

- *Substitutions*: Alanine generally prefers to substitute with other small amino acid, Pro, Gly, Ser.
- *Role in structure*: Alanine is arguably the most boring amino acid. It is not particularly hydrophobic and is non-polar. However, it contains a normal C-beta carbon, meaning that it is generally as hindered as other amino acids with respect to the conformations that the backbone can adopt. For this reason, it is not surprising to see Alanine present in just about all non-critical protein contexts.
- *Role in function*: The Alanine side chain is very non-reactive, and is thus rarely directly involved in protein function. However it can play a role in substrate recognition or specificity, particularly in interactions with other non-reactive atoms such as carbon.

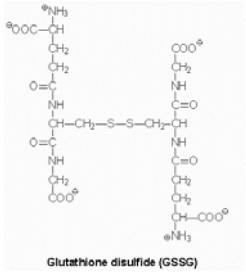
Tyrosine

- *Substitutions*: As Tyrosine is an aromatic, partially hydrophobic, amino acid, it prefers substitution with other amino acids of the same type (see above). It particularly prefers to exchange with Phenylalanine, which differs only in that it lacks the hydroxyl group in the ortho position on the benzene ring.
- *Role in function:* Unlike the very similar Phenylalanine, Tyrosine contains a reactive hydroxyl group, thus making it much more likely to be involved in interactions with non protein atoms. Like other aromatic amino acids, Tyrosine can be involved in interactions with non-protein ligands that themselves contain aromatic groups via stacking interactions.
- A common role for Tyrosines (and Serines and Threonines) within intracellular proteins is phosphorylation. Protein kinases frequently attach phosphates to Tyrosines in order to fascilitate the signal transduction process. Note that in this context, Tyrosine will rarely substitute for Serine or Threonine, since the enzymes that catalyse the reactions (i.e. the protein kinases) are highly specific (i.e. Tyrosine kinases generally do not work on Serines/Threonines and vice versa)

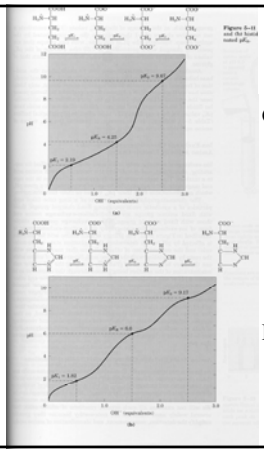
Cysteine

- *Substitutions*: Cysteine shows no preference generally for substituting with any other amino acid, though it can tolerate substitutions with other small amino acids. Largely the above preferences can be accounted for by the extremely varied roles that Cysteines play in proteins (see below). The substitutions preferences shown above are derived by analysis of all Cysteines, in all contexts, meaning that what are really quite varied preferences are averaged and blurred; the result being quite meaningless.
- *Role in structure*: The role of Cysteines in structure is very dependent on the cellular location of the protein in which they are contained. Within extracellular proteins, cysteines are frequently involved in disulphide bonds, where pairs of cysteines are oxidised to form a covalent bond. These bonds serve mostly to stabilise the protein structure, and the structure of many extracellular proteins is almost entirely determined by the topology of multiple disulphide bonds

Cystine andGlutathione



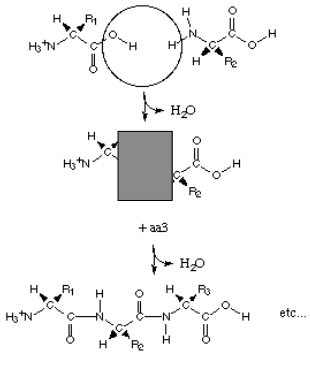
Glutathione (GSH) is a tripeptide composed of g-glutamate, cysteine and glycine. The sulfhydryl side chains of the cysteine residues of two glutathione molecules form a disulfide bond (GSSG) during the course of being oxidized in reactions with various oxides and peroxides in cells. Reduction of GSSG to two moles of GSH is the function of glutathione reductase, an enzyme that requires coupled oxidation of NADPH.



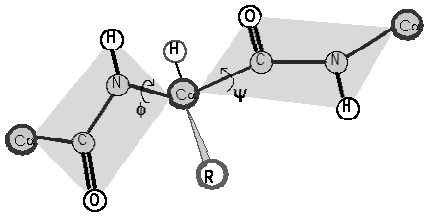
Glutamic acid

Histidine

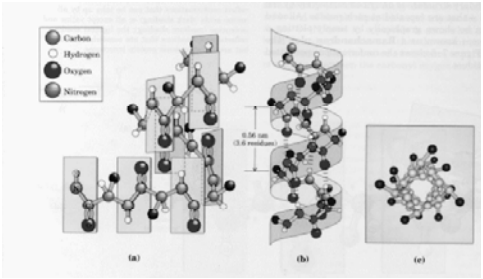
The peptide bond



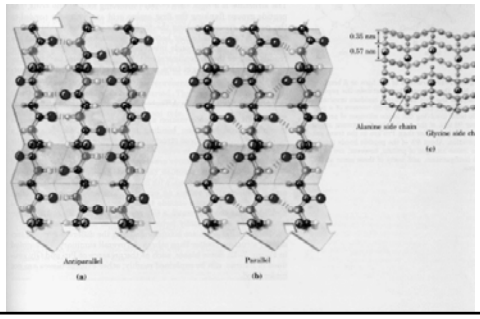
There is free rotation about the peptide bond



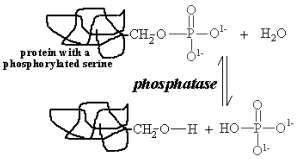
Proteins secondary structure, alpha helix

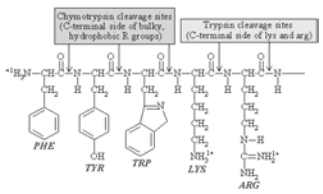


Secondary structure, beta pleated sheet

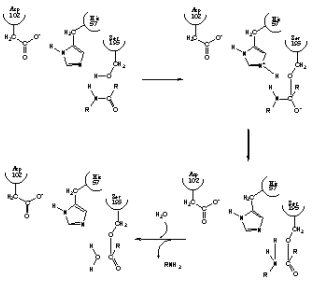


How enzymes work

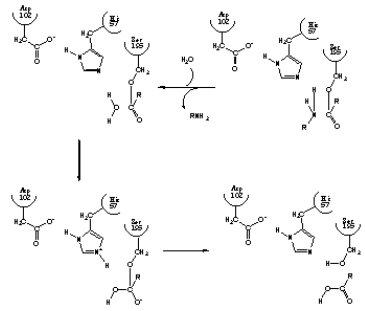




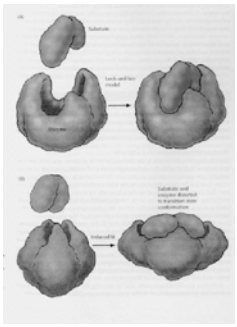
Catalytic Mechanism of Serine Proteases - 1



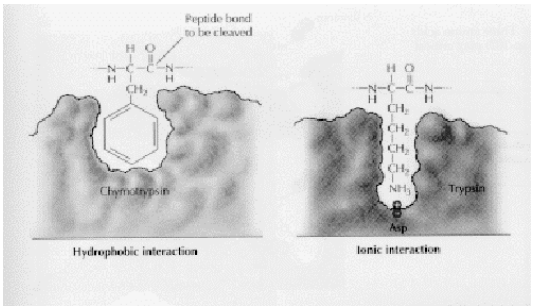
Catalytic Mechanism of Serine Proteases - 2



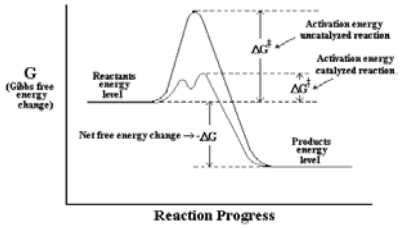
Lock and key



Specific interactions at active site



Enzymes lower the energy of activation



Transition State Stabilization

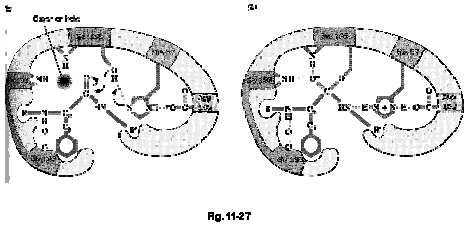
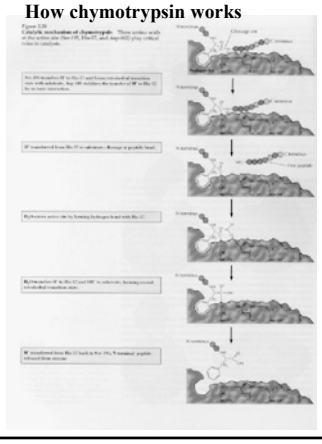


Fig. 11-27

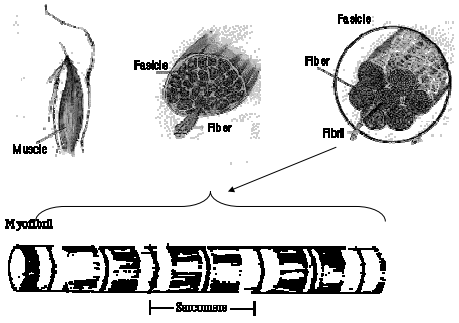
How chymotrypsin works



How do proteins function?

- Structural: Actin is an example it is a major component of the cells architecture as well as the contractile apparatus
- Carriers: Hemoglobin is an example. It functions to carry O_2 to tissue and eliminate CO_2
- Regulatory: Transcription factors bind to DNA a control the level of mRNA that is produced
- Transport: EGFR-epithelial growth factor receptor. Binds EGF and signals for cell growth.
- Binders: Immunoglobulin proteins or antibodies-bind to foreign proteins and destroy infectious agents.

Actin and myosin: the contractile apparatus



Skeletal Muscle Cells

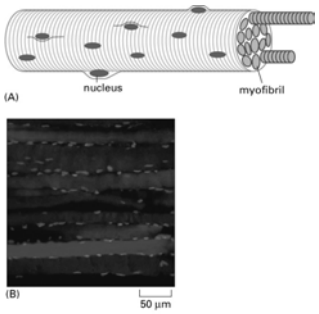
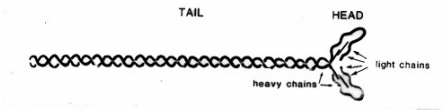


Figure 16–68. Molecular Biology of the Cell, 4th Edition.

Skeletal Muscle Structure

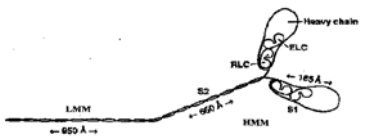
- Muscle cells are formed by fusion of myoblasts
- Myofibrils are parallel arrays of long cylinders packed in the muscle cell
- Sarcomeres are symmetric repeating units from z-line to z-line in the myofibril
- Thick filaments are myosin filaments
- Thin filaments are actin filaments

Structure of Myosin



Myosin is a large asymmetric molecule, it has a long tail and two globular heads (Fig. M1). The tail is about 1,600 Å long and 20 Å wide. Each head is about 165 Å long, 65 Å wide and 40 Å deep at its thickest part. The molecular weight of myosin is about 500,000. In strong denaturing solutions, such as 5 M guanidine-HCl or 8 M urea, myosin dissociates into six polypeptide chains: two heavy chains (molecular weight of each heavy chain about 200,000) and four light chains (two with a molecular weight of 20,000, one with 15,000 and another with 25,000). The two heavy chains are wound around each other to form a double helical structure. At one end both chains are folded into separate globular structures to form the two heads. In the muscle, the long tail portion forms the backbone of the thick filament and the heads protrude as crossbridges toward the thin filament. Each head contains two light chains.

More myosin structure

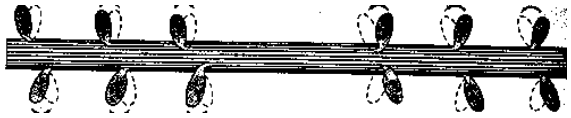


More details of the myosin structure. When myosin is exposed to the proteolytic enzyme trypsin, fragmentation occurs in the middle of the tail yielding heavy meromyosin (HMM, molecular weight about 350,000) and light meromyosin (LMM, molecular weight about 150,000) HMM containing the head and a short tail can be further split by proteolytic enzymes, such as papain, into subfragment 1 (S1, molecular weight about 110,000) and subfragment 2 (S2). The regions of proteolytic fragmentation may serve as hinges. HMM and S1 bind actin, hydrolyze ATP and are water-soluble. LMM has no sites for actin or ATP binding, but inherits the solubility of myosin in 0.6 M KCl and the self-assembling property of myosin in 0.03 M KCl. S2 is water-soluble. Myosin and its proteolytic fragments can be visualized by electron microscopy

Arrangement of Myosin Molecules in Thick Filaments

- bipolar polymer of myosin
- myosin tails align and point to center of sarcomere
- myosin heads arranged in a helical pattern pointing away from center
- myosin heads reach out from the thick filaments to contact the actin filaments
- contain ~300 molecules of myosin

Myosin filament



Thin Filaments

- actin filaments in the sarcomere are of fixed length
- actin filaments are cross-linked by α-actinin at Z-line
- both ends of actin filaments are capped
- barbed ends are embedded at the Z-line
- tropomyosin and troponins bind along each filament

Structure of actin filament

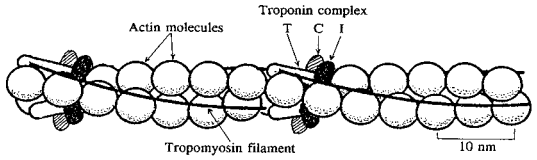
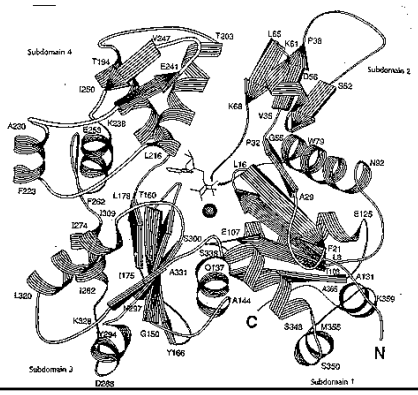


Figure 11.9 Schematic drawing of part of an actin filament showing the relationship between successive pairs of actin molecules, thin filaments of tropomyosin, and the three types of troponin. Another depiction of a (nonmuscle) actin filament is given in Figure 2.4A.

Actin in detail



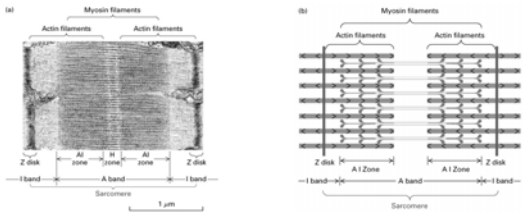
Actin structure

- Folding of the actin molecule is represented by ribbon tracing of the a-carbon atoms. N and C correspond to the amino- and carboxyl-terminals, respectively. The letters followed by numbers represent amino acids in the polypeptide chain. A hypothetical vertical line divides the actin molecule into two domains "large", left side, and "small", right side. ATP and Ca2+ are located between the two domains. These two domains can be subdivided further into two subdomains each, the small domain being composed of subdomains 1 and 2, and the 2 has significantly less mass than the other three subdomains and this is the reason of dividing actin into small and large domains). The four subdomains are held together and stabilized mainly by salt bridges and hydrogen bonds to the phosphate groups of the bound ATP and to its associated Ca2+ localized in the center of the molecule.

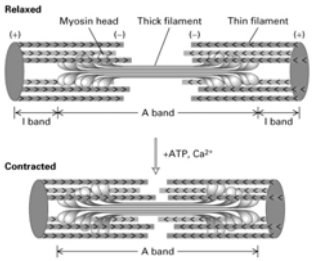
Actin domains

- 1. Where does it polymerize with actin?
- 2. Where does it interact with troponin and tropomyosin?
- 3. Where does it interact with myosin?
- 4. How could we answer this question?

Structure of a Sarcomere

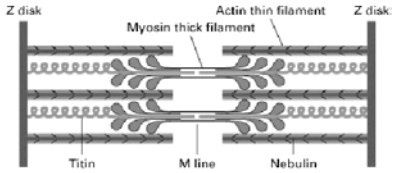


Muscle Contraction

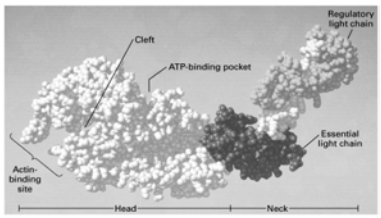


Neither thick or thin filaments change length during muscle contraction, only the overlap between them changes, leading to changes of sarcomere length (z- to z distance)

Stabilization of the Alignment of Thick and Thin Filaments



Crystal Structure of Myosin Head and Lever Arm



Regulation of Non-muscle Myosin II Assembly

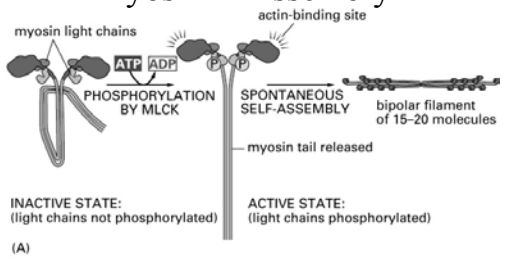
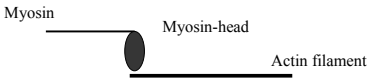


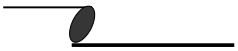
Figure 16–67 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Muscle continue



Muscle continue

Muscle continue



Muscle continue

Muscle continue

Muscle continue

Muscle continue

Muscle continue

Muscle continue

Myosin Superfamily

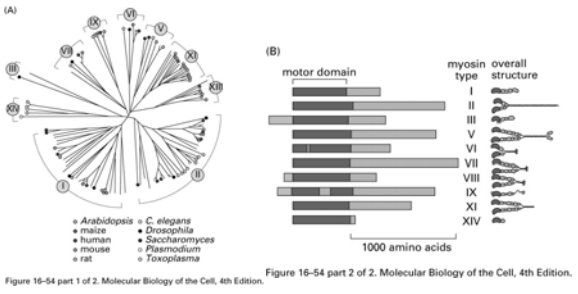
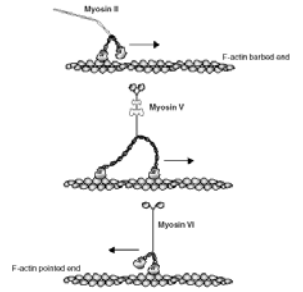


Figure 16–54 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 16–54 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Three examples of the diverse structures of members of the myosin superfamily



Three examples of the diverse structures of members of the myosin superfamily.

In vitro Motility Assay

1. Attach myosin S1 on the cover slip
2. Add fluorescently tagged actin filament
3. Addition of ATP initiates the movement of the filaments
4. Also done by coating cover slip with actin filaments and use fluorescently tagged myosin motor domain

In vitro motility assay

Proteins as enzymes

There are 6 major classes of enzymes:

1. Oxidoreductases, which are involved in oxidation, reduction, and electron or proton transfer reactions;
2. Transferases, catalyzing reactions in which groups are transferred;
3. Hydrolases that cleave various covalent bonds by hydrolysis;
4. Lyases catalyze reactions forming or breaking double bonds;
5. Isomerases catalyze isomerization reactions;
6. Ligases join constituents together covalently.

Enzymes fall into classes based on function

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Enzyme Kinetics

- Enzymes are protein catalysts that, like all catalysts, speed up the rate of a chemical reaction without being used up in the process.

Enzyme reaction rates are determined by several factors.

- the concentration of substrate molecules (the more of them available, the quicker the enzyme molecules collide and bind with them). The concentration of substrate is designated [S] and is expressed in unit of molarity.
- the temperature. As the temperature rises, molecular motion - and hence collisions between enzyme and substrate - speed up. But as enzymes are proteins, there is an upper limit beyond which the enzyme becomes denatured and ineffective.

Enzymes cont.

- the presence of inhibitors.
  - **competitive inhibitors** are molecules that bind to the same site as the substrate - preventing the substrate from binding as they do so - but are not changed by the enzyme.
  - **noncompetitive inhibitors** are molecules that bind to some other site on the enzyme reducing its catalytic power.
- pH. The conformation of a protein is influenced by pH and as enzyme activity is crucially dependent on its conformation, its activity is likewise affected.

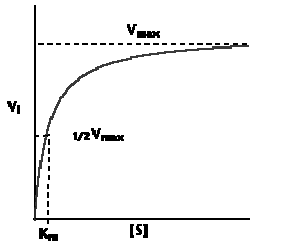
How we determine how fast an enzyme works

- We set up a series of tubes containing graded concentrations of substrate, [S] . At time zero, we add a fixed amount of the enzyme preparation.
- Over the next few minutes, we measure the concentration of product formed. If the product absorbs light, we can easily do this in a spectrophotometer.
- Early in the run, when the amount of substrate is in substantial excess to the amount of enzyme, the rate we observe is the initial velocity of Vi.

Mechaelis Menton kinetics

- Plotting Vi as a function of [S], we find that
- At low values of [S], the initial velocity,Vi, rises almost linearly with increasing [S].
- But as [S] increases, the gains in Vi level off (forming a rectangular hyperbola).
- The asymptote represents the maximum velocity of the reaction, designated Vmax
- The substrate concentration that produces a Vi that is one-half of Vmax is designated the Michaelis-Menten constant, Km(named after the scientists who developed the study of enzyme kinetics).
- Km is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and its substrate. The lower the Km, the greater the affinity (so the lower the concentration of substrate needed to achieve a given rate).

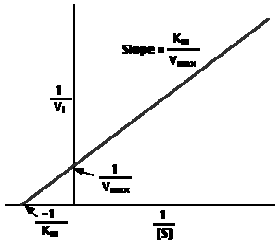
Plotting out our data it might look like this.



V_i = initial velocity (moles/time)
[S] = substrate concentration (molar)
V_{max} = maximum velocity
K_m = substrate concentration when V_i is one-half V_{max} (Michaelis-Menton constant)

Lineweaver-Burke plot

Plotting the reciprocals of the same data points yields a "double-reciprocal" or Lineweaver-Burk plot. This provides a more precise way to determine Vmax and Km. Vmax is determined by the point where the line crosses the 1/Vi = 0 axis (so the [S] is infinite). Note that the magnitude represented by the data points in this plot decrease from lower left to upper right. Km equals Vmax times the slope of line. This is easily determined from the intercept on the X axis.



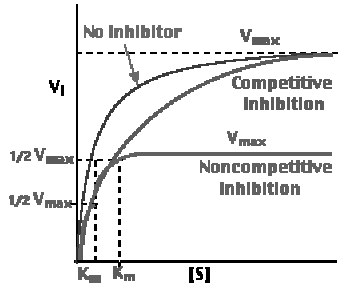
Competitive inhibitors

- Enzymes can be inhibited competitively, when the substrate and inhibitor compete for binding to the same active site or noncompetitively, when the inhibitor binds somewhere else on the enzyme molecule reducing its efficiency.
- The distinction can be determined by plotting enzyme activity with and without the inhibitor present.
- Competitive Inhibition
- In the presence of a competitive inhibitor, it takes a higher substrate concentration to achieve the same velocities that
- were reached in its absence. So while Vmax can still be reached if sufficient substrate is available, one-half Vmax requires a higher [S] than before and thus Km is larger.

Non-competitive inhibitor

- With noncompetitive inhibition, enzyme molecules that have been bound by the inhibitor are taken out of the game so enzyme rate (velocity) is reduced for all values of [S], including Vmax and one-half Vmax but
- Km remains unchanged because the active site of those enzyme molecules that have not been inhibited is unchanged.

Competitive/noncompetitive inhibitor



Effect of inhibitors