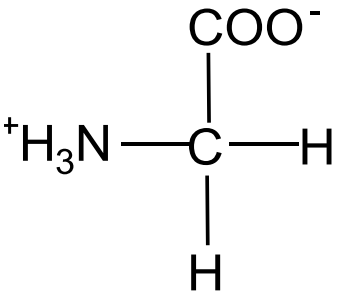
Amino Acids: An Introduction to Their Structure, Functions and Biochemical Properties

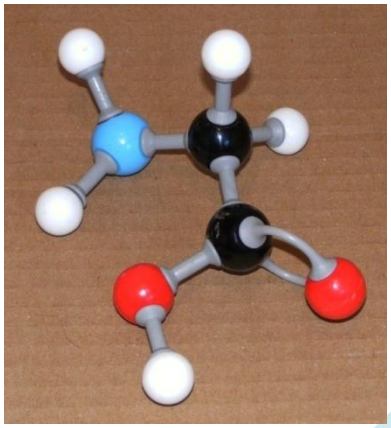
Introduction

Any time one deals with anything in Biology, one must also contend with amino acids and proteins: the products of gene activation. To understand proteins, it is necessary to understand amino acids, to learn their structures and to learn a few of the functions and essentiality of the amino acids. There are 20 **a**mino acids and 1 **i**mino acid we will study:

glycine (gly)	cysteine (cys)
alanine (ala)	cystine (cys-cys)
valine (val)	threonine (thr)
leucine (leu)	methionine (met)
isoleucine (ile or ileu)	aspartic acid (aspartate; asp)
proline (pro) ← imino acid	asparagine (asn)
	glutamic acid (glutamate; glu)
phenylalanine (phe)	glutamine (gln)
tyrosine (tyr)	histidine (his)
tryptophan (trp)	lysine (lys)
serine (ser)	arginine (arg)

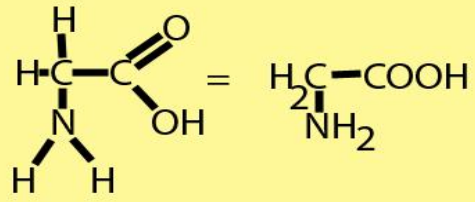


The simplest amino acid is glycine (gly). It consists of two carbon atoms covalently bonded to each other (Image at right). To one carbon atom, two oxygen atoms are bonded; to the other carbon atom, an amino group (NH_3^+) and 2 hydrogen atoms are bonded. The carbon that is directly attached to the CO_2^- is called the α-carbon. It is this carbon that makes all amino acids used by man the α -amino acids. When the carboxyl group is deprotonated to form a carboxylate group and when the amine is protonated to form an ammonium group, this form is called a zwitterion: a double ion. This ionization is due to water and/or buffer solvation/ionization. Both positive (+) and negative (-) charges exist per molecule which is normal under physiological conditions.



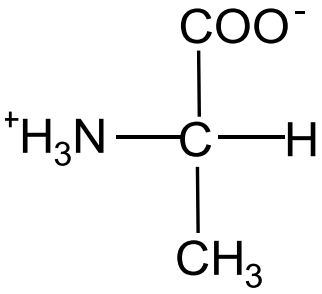
Gly (stick-n-ball model at right: remember that black = C; red = O; white = H; blue = N) is typically found in proteins where there are turns in the amino acid sequence, as it is very small and has a small "R" group (a hydrogen). R groups are radical groups, representative groups or reactive groups. In this case, and for

the case of all the amino acids, we will use the second definition of R group to mean the rest of the amino acid molecule beyond the 2d carbon in the back bone of the amino acid. Gly is an amino acid with an uncharged polar R group.

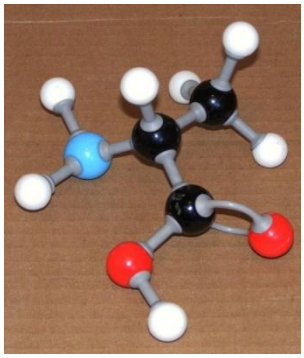


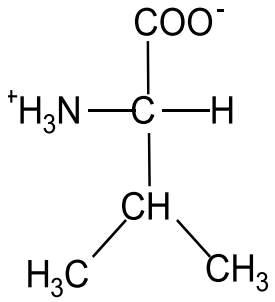
Another way to look at Glycine is illustrated in the graphic at top right. This method uses lines to represent bonds and either illustrates each atom or utilizes a condensed structure form. The same manner may be used with the other 19 amino acids.

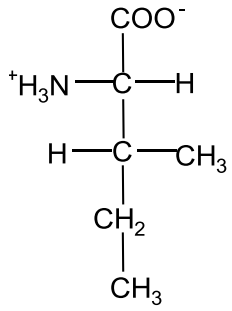
Amino Acids with Hydrophobic R Groups

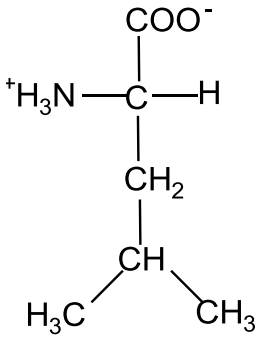


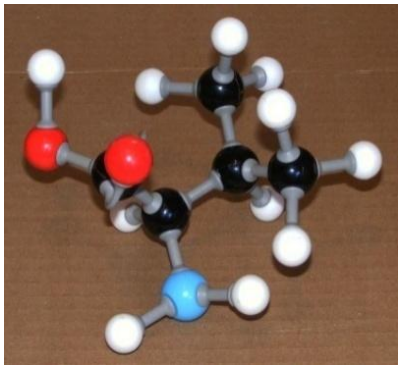
The next simplest amino acid is alanine. The difference between ala and gly is that the H in gly has been replaced by a CH_3 in ala (Images at left and right). Ala is a small amino acid, especially suited for diffusing from muscle cells into the blood to be transported by the blood to the liver for utilization in gluconeogenesis.

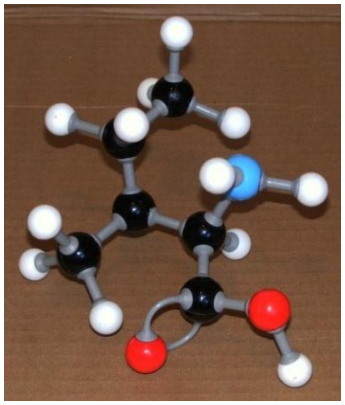


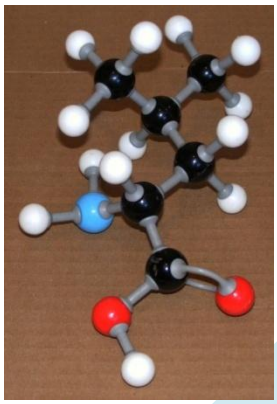




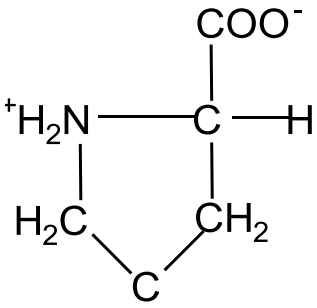




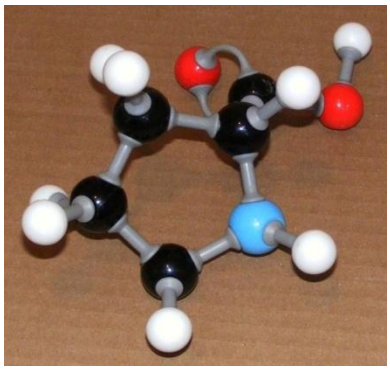


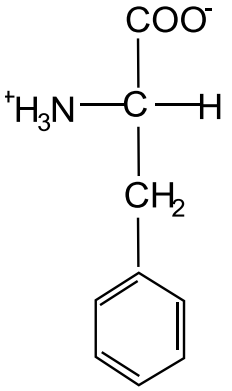


Valine (val), leucine (leu) and isoleucine (ile or ileu) are the next three simplest amino acids (line structures and stick-n-ball models, respectively, bottom of above page). These three amino acids are called branched chain amino acids (BCAA's) and are utilized for the synthesis of substrates for gluconeogenesis and for ketogenesis. Leu is the only purely ketogenic amino acid. Ketones are usually associated with someone who has diabetes mellitus and who is in diabetic coma. It is the ketones, or ketone bodies, that give the patient the sweet, fruity smelling breath of diabetic co ma.

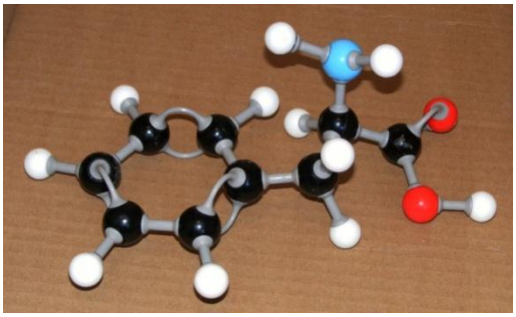


Proline is actually an *imino* acid (Images left and right). Note that it is a closed ring amino acid. Pro, like gly, is usually found in proteins where turns are required. A derivative of proline, hydroxyproline is found in connective tissue and helps make the tissue stronger.

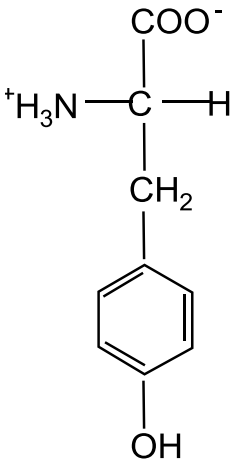


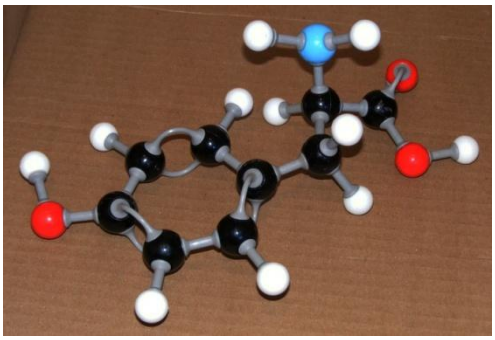


Phenylalanine is alanine with a benzene ring attached to it (C_6H_6) (Images left and right). Phenylalanine is necessary for the synthesis of the catecholamines dopa, dopamine, norepinephrine and epinephrine.

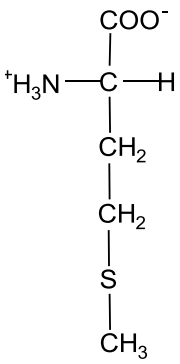


Some people are born lacking an enzyme that regulates the catabolism of phe. When this happens, a metabolite of phe, phenylpyruvic acid, builds up in nervous tissue and causes severe mental retardation. This condition is known as phenylketonuria, or PKU.

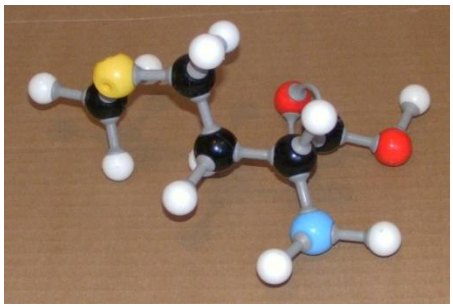




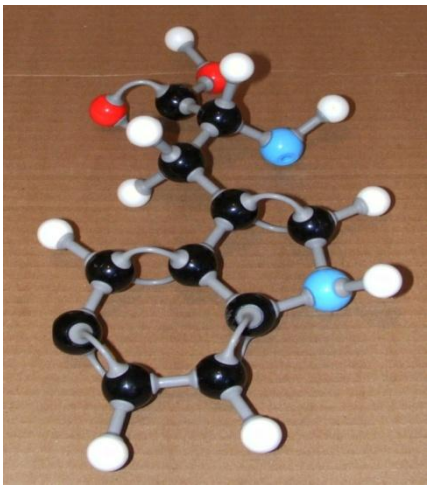
People who have PKU are generally blonde, blue-eyed and fair complected. The reason for this is that phe is also necessary for the synthesis of a pigment called melanin that contributes to eye, hair and skin color. People who have PKU must eat a diet low in phe the rest of their life. Since phe

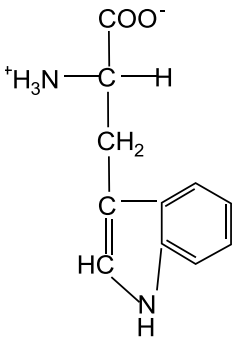


is required by the body to initiate the synthesis of the catecholamines for neurotransmitter and hormonal functions, people who have PKU must add tyrosine (left and right images at bottom of previous page) to their diet -- the product of the hydroxylation of phe that doesn't occur in PKU.



Methionine (Images left and right top this page) is a sulfur containing amino acid. Its necessity is to provide the methyl group (CH_3) to acceptor molecules in one-carbon metabolism. One-carbon metabolism is important in the production of red blood cells, white blood cells and platelets.

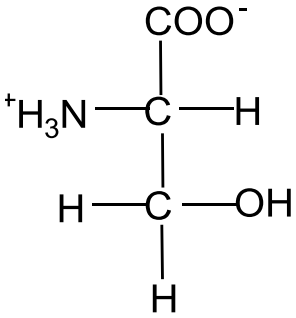




Tryptophan (trp; images immediately left and right) is the last of the amino acids with hydrophobic R groups. Trp is the precursor for the synthesis of serotonin (aka "nature's downer"). Serotonin from the health food store will NOT cross the blood brain barrier; trp is required for this to occur.

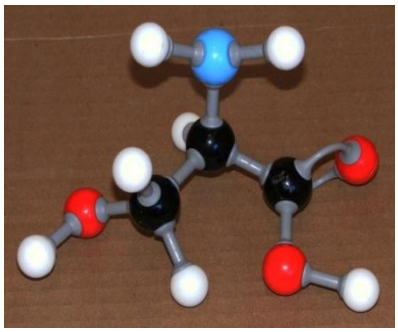
Turkey and milk have high levels of trp. There seems to be some controversy as to whether or not there is enough trp in milk (especially warm milk) to render a person drowsy so that they will fall asleep when it is difficult for them to do so without assistance. In recent times, *selective serotonin reuptake inhibitors* have seen use in depression, eating disorders, obsessive compulsive disorder, to name a few, e.g. prozac, celexa.

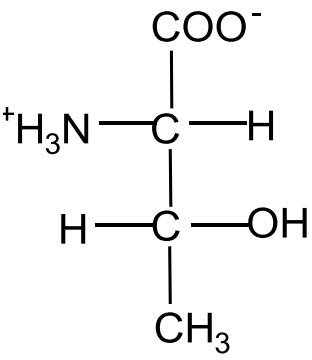
Amino Acids with Uncharged Polar R Groups



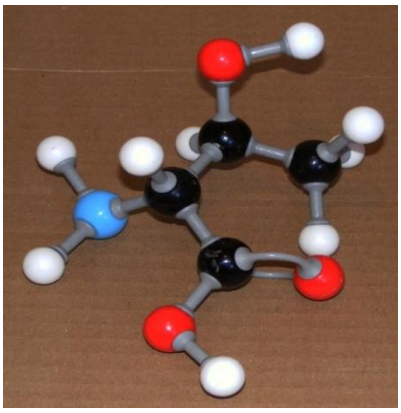
At physiologic pH, the R groups are not ionized as are the amino and carboxyl groups of the amino acids.

Glycine has already been mentioned. It was the first amino acid we examined. Since it's the simplest, that's where we started.

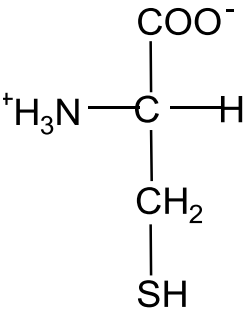




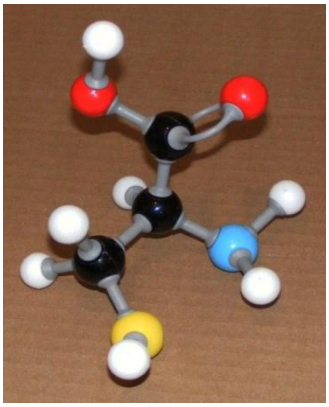
Serine (ser) is alanine with an -OH group replacing a –H (Images at bottom left and right of previous page). As a rule, ser has a function similar to that of threonine (thr; images left and right), another hydroxylated amino acid: it serves as an activation site in enzymes, i.e., when it is phosphorylated or dephosphorylated, the enzyme is turned on or off.



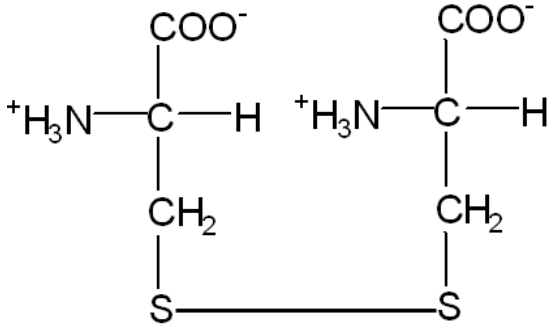
The last hydroxylated amino acid is tyrosine (tyr – with phe discussion). It is, simply, p-hydroxy phenylalanine, with the -OH group straight across the benzene ring from the alanine moiety. Tyr has been discussed, previously, as well, in the phenylalanine discussion.

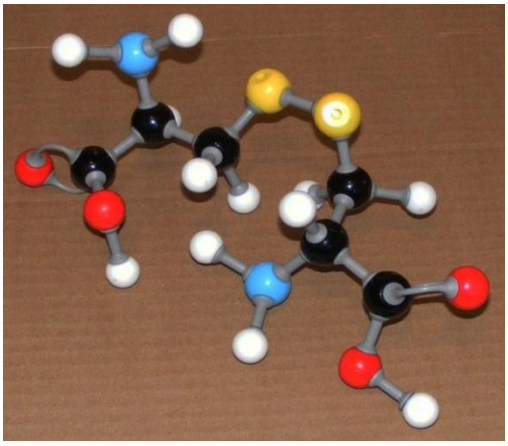


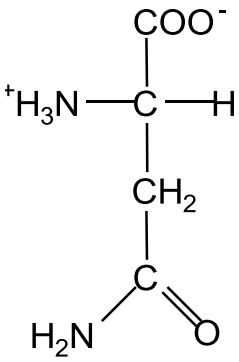
Cysteine (cys; images left and right) is a sulfur containing amino acid. It is found in most connective tissues. The most often thought about site of cys, though, is the hair.

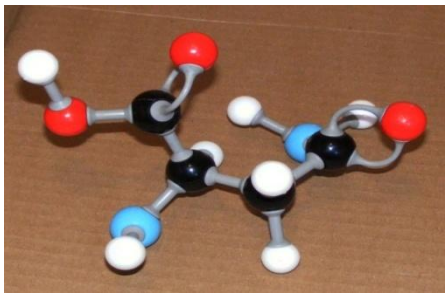


Hair maintains its shape by the presence of disulfide bonds (-S-S-). The disulfide bonds come from the loss of -H from the -SH group of two cys molecules in the hair which then bond to hold the hair in its appropriate shape to form cystIne (cys-cys; Images bottom left and right; remember yellow = S). Cosmetologists, beauticians utilize this property every day when they give perms. They first reduce the natural disulfide bonds in hair, then place the hair in the shape the customer asks, then finish the job with an oxidizing agent that forces the formation of the disulfide bonds and, voila!, a new style comes out from under the curlers, drier, *etc*.

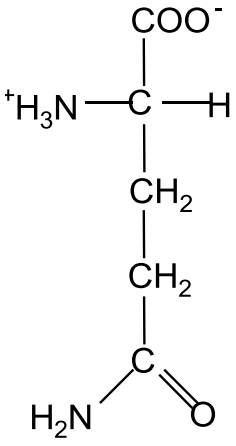


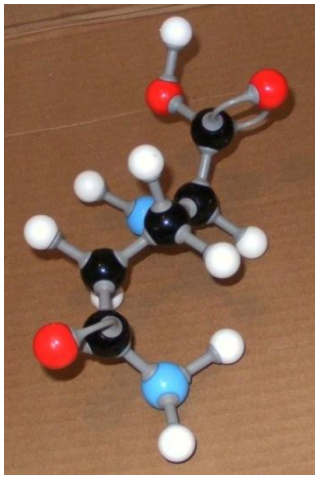


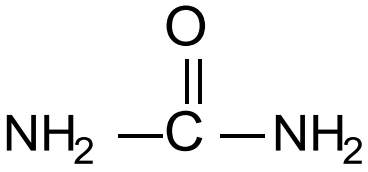




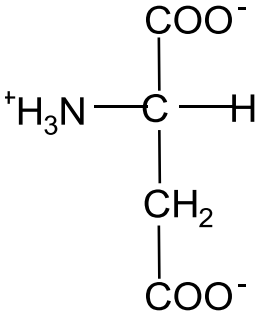
Asparagine (asn; top images, left and right) and glutamine (gln; images beneath the asn images, this page) are 4 and 5 carbons in length, respectively. They are derivatives of the dicarboxylic amino acids aspartate and glutamate (coming up below). Note that each has an extra NH_2 group on the carbon double bonded to an oxygen farthest from the α-carbon. These two molecules serve as ammonia transporters to the liver and kidney for urea synthesis. Urea (Image centered immediately below) is a small, non-toxic compound (compared to ammonia's effects on the cell) that is excreted via the urine.

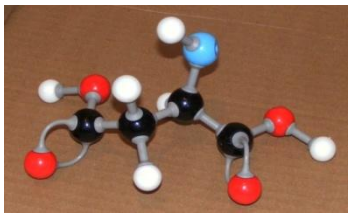


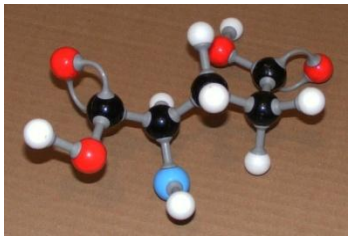


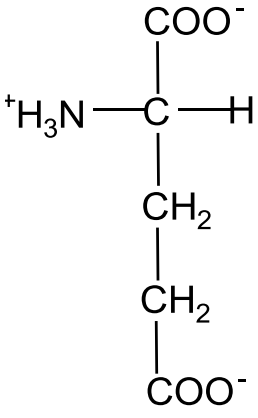


Amino Acids with Negatively Charged R Groups at Physiological pH





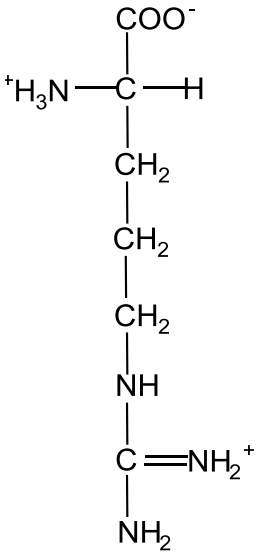


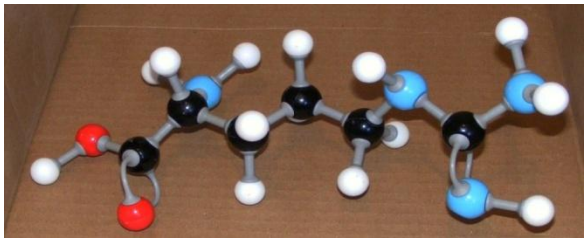


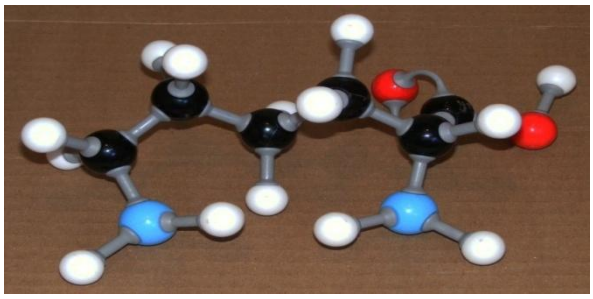
The next two amino acids under study are the acids aspartate (asp; above left and center top, above) and glutamate (glu; above right and center bottom, above) -- the precursor amino acids of asn and gln, respectively. Both are dicarboxylic amino acids, i.e., there is a COOH group on each end of the molecules.

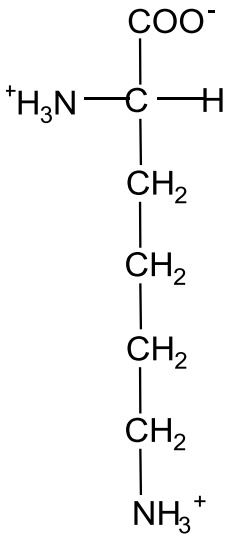
Amino Acids with Positively Charged R Groups at Physiological pH

Arginine (arg; below left and top center) and lysine (lys; below right and bottom center) have positively charged R groups at physiological pH.

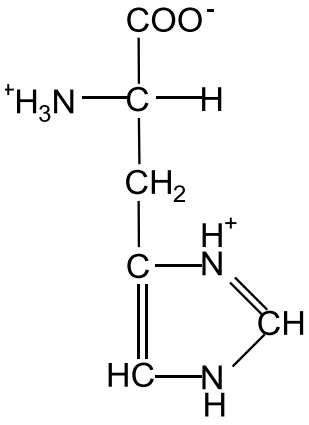






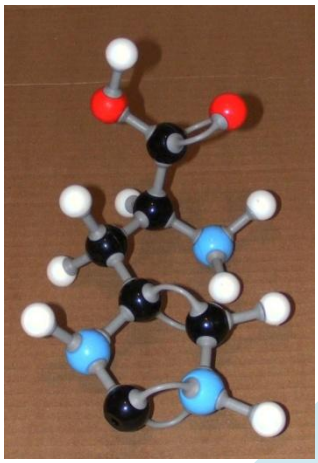


Lysine is heavily involved in connective tissue biosynthesis. Children with low levels of arginine tend to be mentally retarded (hypoargininemia).



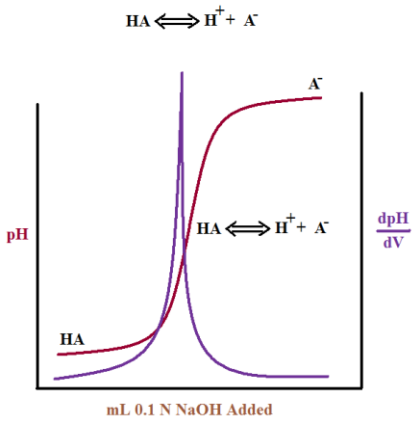
Arginine is the last product of the urea cycle from which urea is clipped for excretion.

Histidine (his; left and right images) is positively charged at a pH of approximately 6 or below. His is the precursor molecule to histamine, the compound that causes many allergic reactions and which may be blocked by the use of anti-histamines. Histamine synthesis may be stimulated by the



influence of norepinephrine or psychological stress. Because of this, many people who have itching-related health problems may be prescribed a drug like doxepin which has both histamine antagonistic properties and anxiolytic properties: both of which combat the health problem by reducing the anxiety felt by the patient which reduces the itching, which reduces the anxiety which reduces the itching, *ad nauseum*.

Of these 20 amino acids, 8 are essential (humans require them in their diets as humans lack the enzymes to synthesize them from scratch) and 2 are semi-essential (required for growth by the young human). The essential amino acids are phe, val, trp, thr, ile, met, lys, leu. The semi-essential amino acids are his and arg. A helpful mnemonic to remember these is: PVT TIM HALL, where the first letter of each amino acid makes up this mnemonic.

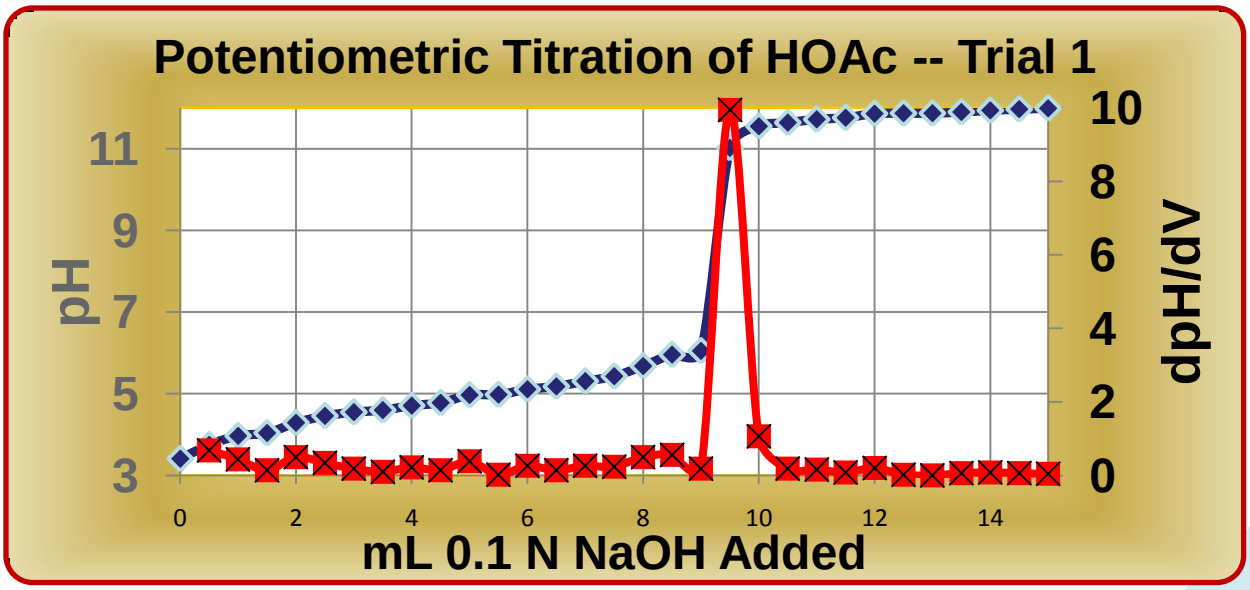


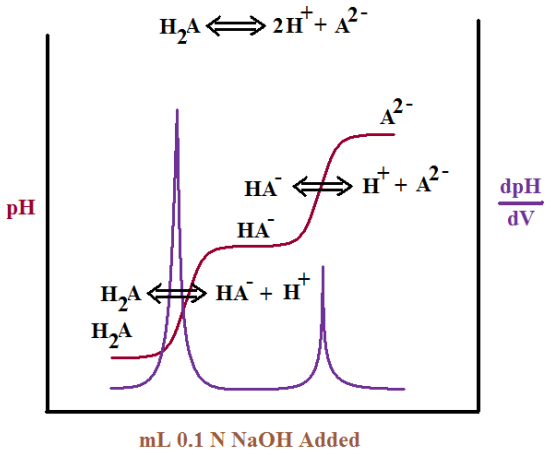
Acid-Base Titrations: Amino Acid Applications

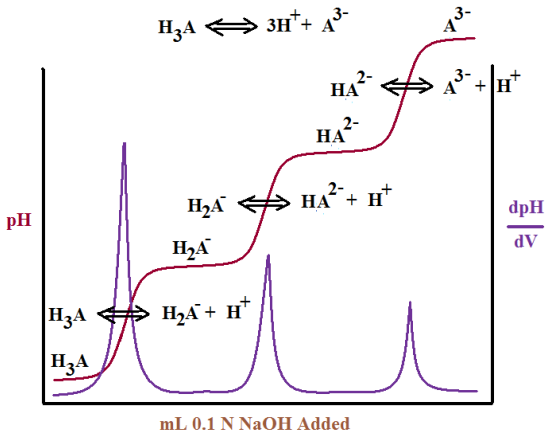
Mono-protic Acid Dissociation -- The dissociation of a monoprotic acid follows the general reactions below:

$$HA \leftrightarrow H^+ + A^- \text{ Or } HA + MOH \leftrightarrow HOH + MA$$

As you learned in your pre-requisite courses, the acid does not dissociate all at once, rather the deprotonation occurs slowly and sequentially … even with a mono-protic acid (See titration/first derivative curve in image at upper right to refresh your memory). The image below is actual data for the titration of HOAc performed by one of your predecessors at WNC in Spring 2008.







Di-protic Acid Dissociation

The dissociation of a di-protic acid follows the general reactions below:

$$H_2A \leftrightarrow 2H^+ + A^{-2} \text{ Or } H_2A + 2MOH \leftrightarrow 2HOH + M_2A$$

As you learned in your pre-requisite courses, the acid does not dissociate all at once, rather the deprotonation occurs slowly and sequentially (See titration/first derivative curve in image at upper left to refresh your memory).

Tri-protic Acid Dissociation

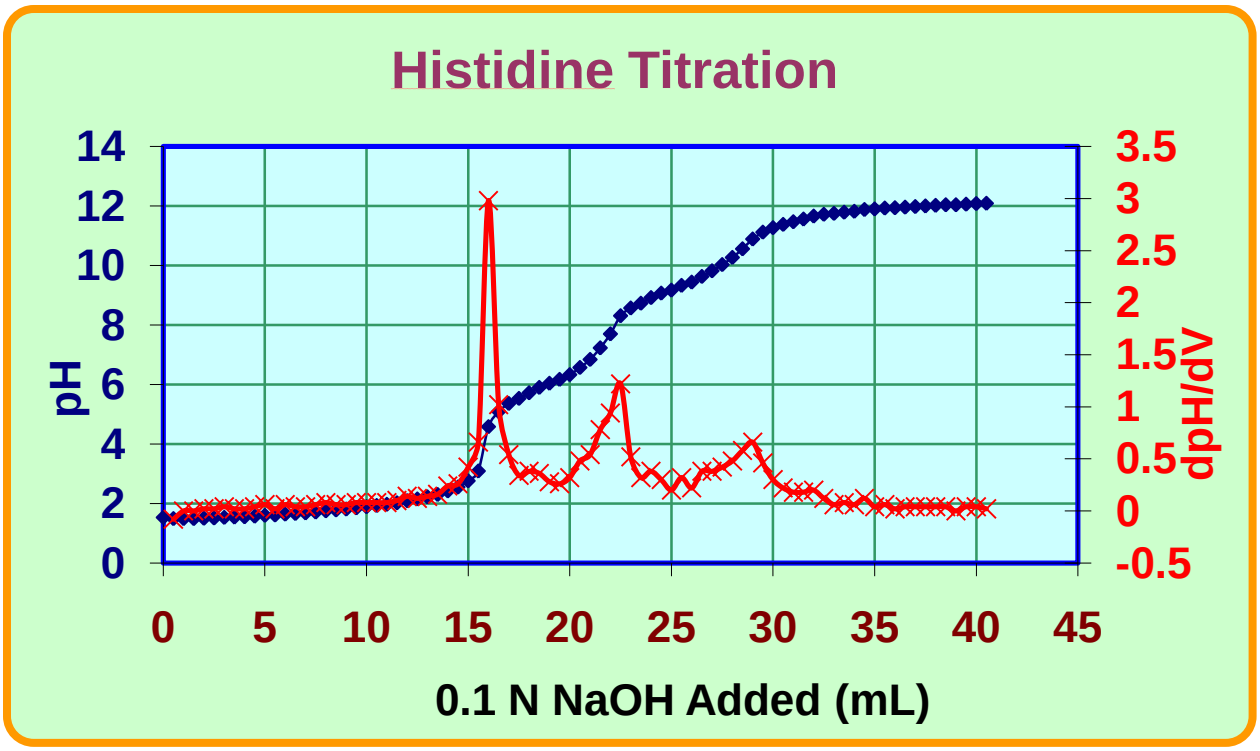
The dissociation of a di-protic acid follows the general reactions below:

$$H_3A \leftrightarrow 3H^+ + A^{-3} \text{ Or } H_3A + 3MOH \leftrightarrow 3HOH + M_3A$$

As you learned in your pre-requisite courses, the acid does not dissociate all at once, rather the deprotonation occurs slowly and sequentially (See titration/first derivative curve in image at upper right to refresh your memory).

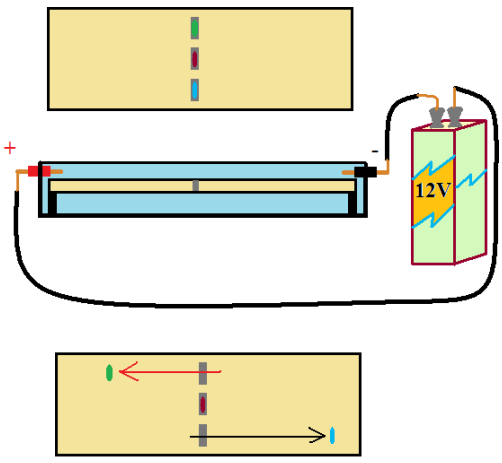
Amino acids are also titratable. For example, the titration of Alanine follows diprotic acid titration patterns. The carboxyl group is deprotonated first; eventually, the amine is protonated. This forms the zwitterionic form with the carboxylate group and the ammonium group.

The titration of Glutamate follows tri-protic acid titration. The carboxyl group bound to the α carbon is deprotonated first. The R group (another carboxyl group as you'll recall) is deprotonated, next. Lastly, the amine is protonated.



Another of your predecessors at WNC titrated Histidine. Histidine's carboxyl group is deprotonated first. The imidazole ring is deprotonated secondly. Lastly, the amine is protonated. Each endpoint is clearly identifiable not only on the first derivative curve in the graphic above, but on the pH curve, as well.

So, what is the value of being able to titrate amino acids? We all know that structure gives function. At specific pH's, each R group in a protein takes on specific charges. Not only do the charges give structure to the protein, we can also take advantage of that property of amino acids and proteins and separate them for identification by a process known as electrophoresis. Electrophoresis is defined as the separation of (in this case) amino acids or proteins in a gel by an electrical charge. The graphic at lower right illustrates Electrophoresis. The top portion of the graphic is a top view of a gel (usually polyacrylamide) that has three wells in it – each well has a specific sample in it (an amino acid OR a protein).



The gel is placed in a tank on a support and the samples are loaded into the wells. A buffer (determined by earlier research) is poured all around the gel and the electrophoresis tank is covered.

Don't let "tank" fool you – these are usually small. Some tanks can be as small as 4" by 4".

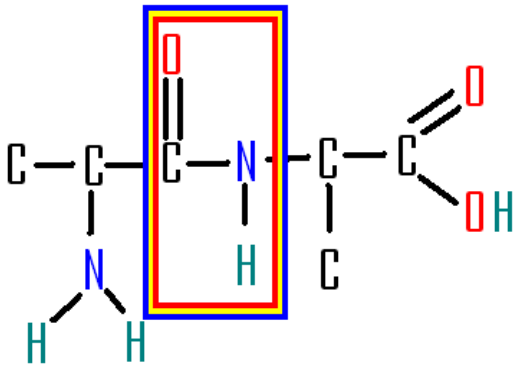
A power supply is attached to the electrophoresis tank and turned on. The current runs for a pre-determined amount of time, then it is turned off. Once the gel is removed from the tank it is stained and the bands, as they are called, are visualized.

The bottom part of the electrophoresis graphic shows that two of the samples migrated towards opposite ends of the gel. One remained at the origin. The top sample migrated to the positively charged electrode – this means that the amino acid or protein had an overall charge that was negative (which amino acids would give a negative charge?). The "blue sample" migrated to the negative electrode. This means that it had an overall charge that was positive (what amino acids will give overall positive charges?).

So, how are amino acids "put together" to make proteins?

Peptides and Peptide Bond

Amino acids are the building blocks of proteins. In order for the amino acids to link together to form the numerous proteins necessary to keep a human functioning, they form a special bond between each other: the peptide bond (highlighted in the image at right).

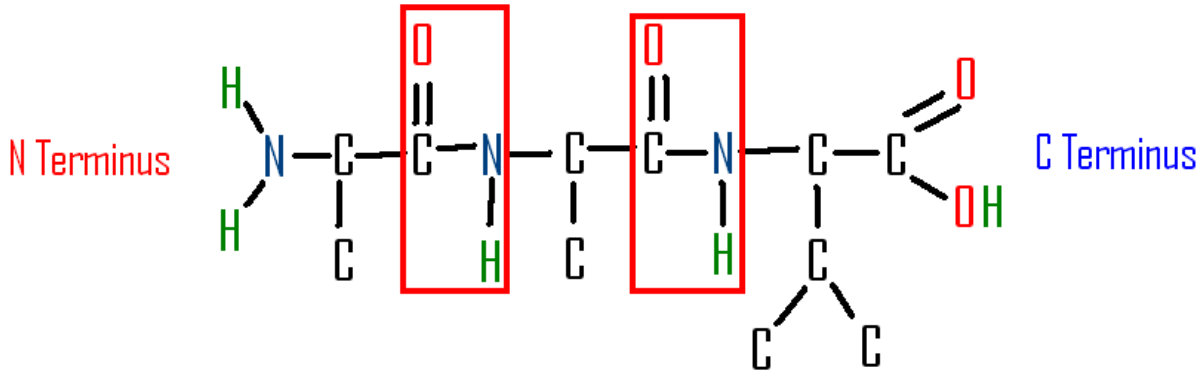


The peptide bond is formed between the carboxyl group of the first amino acid and the amino group of the second amino acid to form a dipeptide. The peptide bond is unique in that it appears to be a single bond, but has the characteristic of a double bond, i.e., it is a rigid bond. This kind of bond only occurs between amino acids.

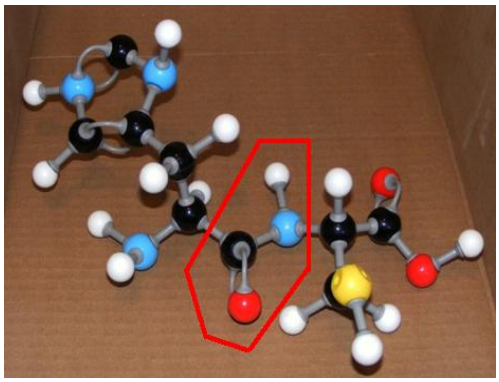
As the amino acid chain increases, the next amino acid adds onto the previous carboxyl group by its amino group.

Peptide Bond and Peptides

By convention, the left amino acid is always the #1 amino acid; is the free amino end or the N-terminus (see image top of following page). The farthest amino acid residue to the right is the amino acid in the protein that has the highest number and, as a general rule, is the free carboxyl end or the C-terminus. In some cases, the -OH may be replaced with an NH_2, making it an amide.



When dealing with peptides, there is always one LESS peptide bond than there are amino acid residues in the protein, i.e., a tripeptide has 2 peptide bonds and three amino acids; a hexapeptide has five peptide bonds and six amino acids, *ad nauseum*.

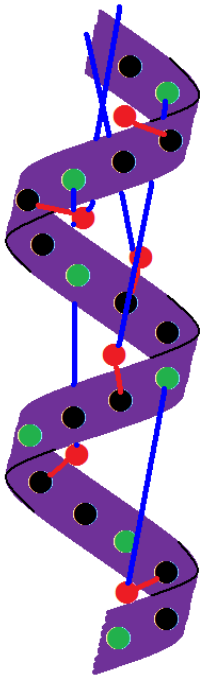


At right is an image of a stick-n-ball model of a dipeptide – the peptide bond is marked by the red box, as it is in the image above.

Primary Structure of Proteins

The sequence[s] of the amino acids held together by peptide bonds ONLY is called the primary structure of a protein.

Secondary Structure of Proteins

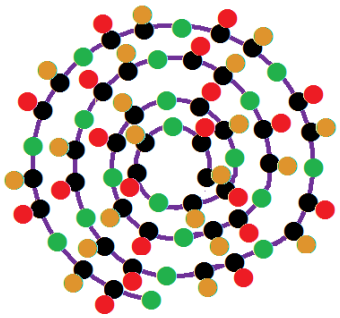


The secondary structure of proteins is determined by how the amino acid sequence (primary structure) folds upon itself and bonds with hydrogen bonds, i.e., non-covalent attractive forces. There are, for this course, 3 secondary structures: α-helix; β-pleated sheet; Thermodynamic random coil.

α-helix

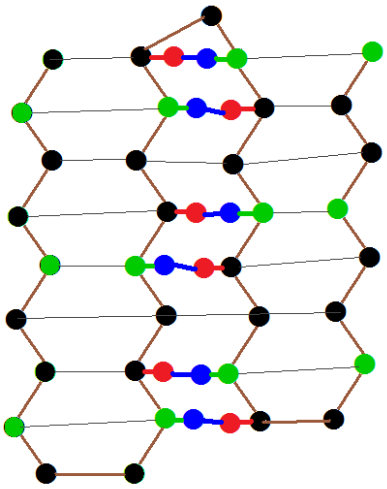
A protein that coils on itself in a right handed turn is called an α-helix. The α-helix permits tissues to stretch a bit, like hair (or a coiled spring). Note that the H bonds are between the carbonyl oxygen and the amino hydrogen. Only a PORTION of a protein is in alpha-helix, NOT the whole protein. This is illustrated at right. Green = N; black = C; red = O, blue = H bonds ca 3.6 amino acid residues apart. The H bonds are what give this structure its shape and stability. Helices are capable of stretching … much like coiled springs.

When one looks down an α-helix, it's much like looking down the bore of a piece of hose, image at right. Black = C; red = O; green = N; orange'ish = R groups; no H bonds are shown as it would be hideous to look at.



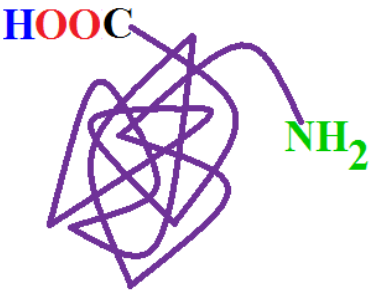
β-pleated sheet

The second of the secondary structures of proteins is called the pleated sheet or, some times, the β-pleated sheet (Image, center right of this page). The pleated sheet is in the anti-parallel organization, i.e., the peptide chains making up the sheet are running in opposite directions to each other. Pleated sheets tend to make proteins that do not "give", e.g., silk, it doesn't seem to be of great importance to other proteins. Colors are as before. When thinking of a pleated sheet do as Linus Pauling did: he figured it out after surgery in the hospital by folding the bed sheet into a "fan shape" … like we all did with construction paper in kindergarten. That was Nobel Prize #1 for Dr. Pauling.



Thermodynamic Random Coil

The last secondary structure about which we have interest is the thermodynamic random coil (Image at right). Although we call this a random coil, nature tells us that there is a reason for every structure. We call it random as we have not worked out the "code" of this structure. In addition, if we denature this structure, the protein loses its function.

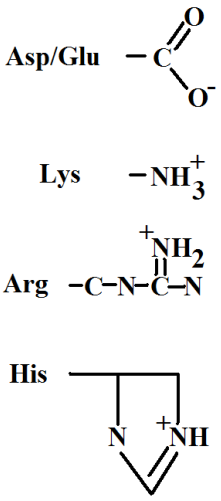


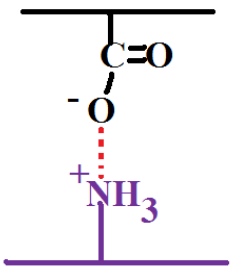
Tertiary Structure

The tertiary structure of a protein is, for all intents and purposes, the three dimensional shape of the protein brought about by interaction forces of ionic, hydrophobic and covalent disulfide links of the one protein chain. Tertiary structure, put another way, is the manner in which the R groups assist the protein in secondary structure formation to fold, twist, bend, kink, AGAIN, upon itself.

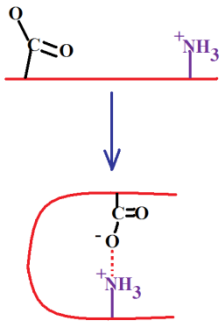
Water soluble proteins fold so that hydrophobic R groups are tucked inside the protein and hydrophilic R groups are on the outside of the protein. WHY? This way, the protein may interact with the solvent (water) and not precipitate or otherwise be inactivated.

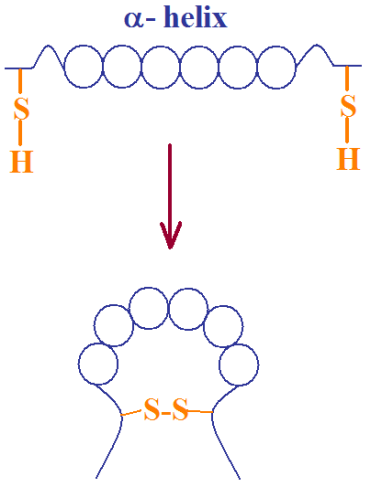
Water insoluble proteins fold so that hydrophilic R groups are tucked inside the protein and hydrophobic R groups are on the outside of the protein. WHY? This is so that a protein, e.g., an ion channel in a cell membrane, may insert itself in a non-polar environment so that polar particles may be transported into or out of regions compartmentalized from each other.





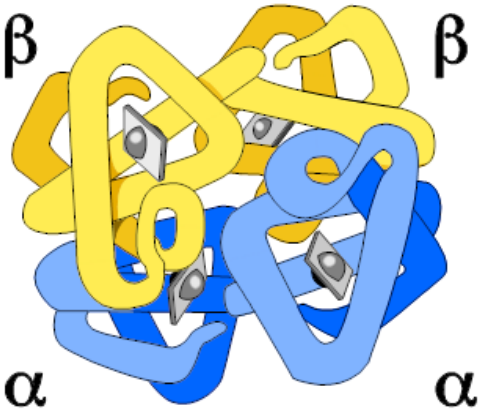
Ionic interactions also stabilize tertiary structures. Where, though, are the ionic groups? They are the R groups (Image at right)! The carboxyl groups on asp and glu; the ε-amino group on lys; the guanidino group on arg; the imidazole ring on his. R groups cross-link to form "salt links" (image at immediate left; black and purple lines represent remainder of protein chains – 2 chains linked by R groups; lower middle left image is an intra-chain linkage).





Disulfide bonds assist in tertiary structure by allowing the protein chain to interconnect itself and introduce a hair-pin into its structure (image at right) -- just like how straight hair is curled and curly hair is straightened out.

Two tertiary structure examples include the β-chain of hemoglobin and the myoglobin molecule, Image below of hemoglobin).



Quaternary Structure

The last structure of proteins in which we have interest is called the quaternary structure: the organization of two or more protein chains to bind together in such a manner as to give the group of proteins a single function, e.g., the tetramer of hemoglobin.

The 4 proteins are held together by salt links, hydrophobic and hydrophilic interactions. In Hemoglobin (also in image at lower left), disruption of

these forces (to form deoxy hemoglobin) cause the hemoglobin molecule to become smaller than oxy-hemoglobin.

Protein Denaturation

The denaturation of proteins includes anything that disrupts secondary, tertiary and/or quaternary structure of proteins: heat, alcohol, salts, heavy metals, freeze/thaw, acids/bases. All cause inactivation of proteins.

Groups of Proteins

Fibrous proteins include:

- Collagens: connective tissue; after it's boiled, the soluble part is called gelatin (Bill Cosby sells this as JELLO^{tm})
- Elastins: in stretching tissues
- Keratins: water-proofing proteins
- Myosins: in muscle
- Fibrin: blood clotting protein

Globular proteins include:

- Albumins: water soluble; transporters and increase blood osmotic pressure
- Globulins: saline soluble; transporters and antibodies
- Enzymes: biological reaction catalysts

Enzymes

Of significance, of course, is the fact that the shape of the enzyme gives it its function (the shape of a protein gives it its function). Enzymes speed up the reaction rate in biological systems 100,000 - 1,000,000 fold! Some are known to increase the reaction rate > 10^{20}-fold! Enzymes have specific substrates (chemical group upon which the enzyme works), but can work on limited kinds of substrates.

Enzymes Have Specific Functions

Enzymes are categorized into one of 6 biological activities according to the Enzyme Commission (E.C.):

- Oxidoreductases: catalyze redox reactions -- involve NAD and FAD (E.C. 1.X.X.X)
- Transferases: catalyze group transfers (E.C. 2.X.X.X)
- Hydrolases: use water to lyse bonds (E.C. 3.X.X.X)
- Lyase: nonhydrolytic and non-oxidative group removal (E.C. 4.X.X.X)
- Isomerases: catalyse isomerization reactions (E.C. 5.X.X.X)
- Ligase: catalyzes reactions requiring ATP hydrolysis (E.C. 6.X.X.X)

Enzyme "Add-On's" – Terminology

- Active site ≡ 3-dimensional cleft in the enzyme caused by/coded by the primary structure of the protein; complimentary to the shape (geometry of the substrate)
- Apoenzyme ≡ active enzyme minus the cofactor; catalytically inactive
- Coenzyme ≡ a carbon-based molecule required by an enzyme for complete catalytic capacity, e.g., NAD^+, FAD, vitamins – bound loosely to the apoenzyme
- Cofactors ≡ a molecule or ion of a non-protein nature that is required by an enzyme for complete catalytic capacity, e.g., Mn^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Mo^{2+}
- Constitutive enzymes ≡ always in the cell without regard to the availability of substrate
- E.C. nomenclature ≡ comes from Enzyme Commission. The Commission (a sub-committee of the International Union of Biochemists) mandated a standardized name and numbering system for enzymes to make it easier for everyone to know which of their favorite enzymes they discuss. The numerical system is a 4 number system – each of the 4 numbers is separated by a dot ("."). Only the first number is important for this course. "E.C. 1" = oxidoreductases; "E.C. 2" = transferases; "E.C. 3" = hydrolases; "E.C. 4" = lyases; "E.C. 5" = isomerases; "E.C. 6" = ligases.
- Holoenzyme ≡ apoenzyme plus prosthetic group
- Induced enzyme ≡ present in the cell ONLY when substrate activates gene mechanisms causing intracellular release of active enzyme.
- Prosthetic group ≡non-protein moiety tightly bound to apoenzyme
- Specificity characteristics ≡ due to the active site; crevice allows binding of 1) only one substrate or 2) 1 kind of R group

- Zymogens ≡ immature enzymes that need "clipping" for activation – more later in course

Enzymes are globular proteins. The exception to this rule is a class of RNA molecules that possess enzyme activity: ribozymes. Without enzymes, cellular reactions go too slowly to be conducive to life. All enzyme names end in "ase".

Efficiency of Enzymes

Enzymes Increase the rate of reaction without being consumed themselves. Enzymes lower the E_a; have no effect on K_{eq}; Enzymes permit reactions to reach equilibrium quicker; Enzymes have pH and temperature requirements; Enzymes cause reactions to go within seconds as opposed to lab reactions that may take years; Enzymes are an absolute necessity to/for life:

$$\text{E.g., } CO_2 + H_2O \leftrightarrow H_2CO_3$$

Catalyzed by carbonic anhydrase at a rate of $6*10^5$ molecules of CO_2 condensed per second!

Specificity of Enzymes

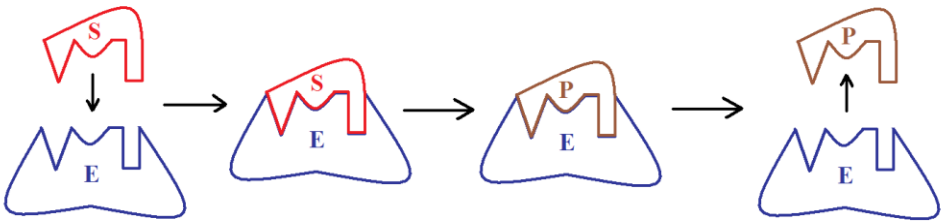
Enzyme specificity occurs in either the reaction types catalyzed or in the substance involved in the reaction (substrate; S):

- Absolute specificity ≡ catalyzes reaction with only one S
- Relative specificity ≡ catalyzes reaction of substrates with similar structures, e.g., functional groups
- Stereochemical specificity ≡ D vs L – this has to do with "handedness: right (D) or left (L) "handed"

Enzyme Regulation

The cell regulates which enzymes function and when, i.e., not ALL enzymes are working at the same time. Some enzymes catalyze uni-directionally; some catalyze bi-directionally.

Enzyme Activity is defined as the catalytic capacity of enzyme to increase reaction rate; the Turnover number is defined as the number of S molecules acted upon by ONE enzyme molecule per minute; Enzyme assays measure enzyme activity. Enzyme activity is measured in International Units ≡ IU. The amount of enzyme that catalyzes 1 μmol of substrate to be altered to product per minute at a given pH, T and [S]. It measures the amount of enzyme present, therefore, an enzyme level of 150 IU = an enzyme concentration 150 times greater than the standard – useful in diagnosing diseases.



Enzyme Models

There are two generally accepted models for the functioning of enzymes: the lock and key model and the induced fit model. We will address the lock and key model first.

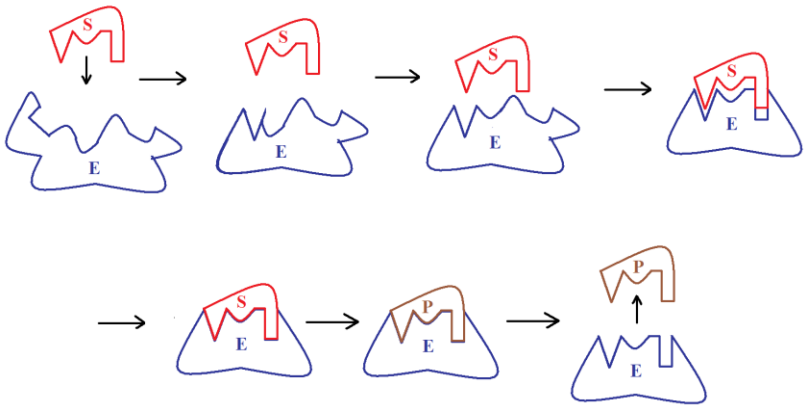
Model #1: Lock-n-Key

In this model, see graphic, above, top of page, the substrate (S) is complimentary to the binding/active site in the enzyme (E). This is likened to the lock and key, where the lock is complimentary to the key. As the E and S bind, they form the Enzyme-Substrate complex (ES). The enzyme acts as a sort of scaffold, holding the substrate so that one specific reaction may occur. ES is an intermediate in the reaction that will cause S to be changed into a product (P) to form the enzyme-product complex (EP). In this case, a bond (or bonds) is (are) broken as the enzyme changes its shape ever so slightly, causing the substrate to break exactly where it's supposed to, releasing the new products and the enzyme for use, again. Once EP is formed, it's a matter of time for P to be released and E to start the process all over. Remember that the active sites of the enzyme are complimentary to the SHAPE of the substrate.

Model #2: Induced Fit

The second model is called the induced fit model. This means that as the S gets closer to the E, the E actually undergoes a conformational change (shape change) to fit the S, i.e., its shape is INDUCED to change by the presence of the substrate (Image below).

Note that as S gets closer to E, the active sites change shape to match the complimentary site on S. As S continues to get even closer, the next site shifts its shape, as does the last site when S is all but bound to the enzyme. Once ES is formed, this model conforms



to the remainder of the lock and key theory of enzyme-substrate binding, i.e., it goes through ES to get to EP to form E plus P.

Enzyme Inhibition: Descriptive Introduction

The upper left graphic represents the normal ES complex for comparison with the three types of inhibition patterns. Note the green "pellet-shaped" region in the top center of the graphic – this is S for all future comparisons. The remainder of the graphic represents the enzyme (E).

The upper right graphic represents competitive inhibition of an enzyme, i.e., an inhibitor specific to this enzyme COMPETES with the substrate for the active site of this enzyme. It is reversible; will block S from binding. One example of this sort of inhibition is carbamoyl choline that competitively inhibits acetylcholinesterase. Note the green-outlined red region in the center with the white region above it. This is the inhibitor – it "looks" in part like the S – it's just different enough, however, to plug the crevice and inhibit the enzyme.

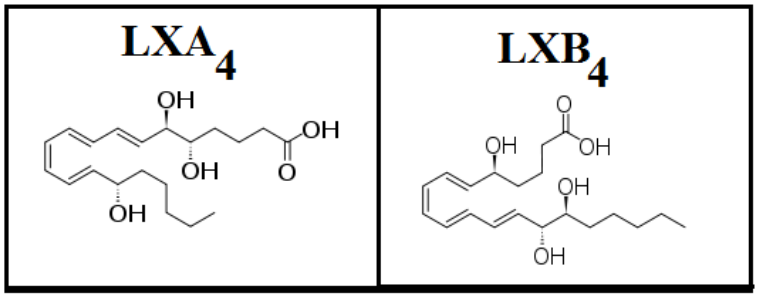
The bottom left graphic represents uncompetitive inhibition. This sort of inhibition involves covalently bound inhibitor and inactivates the enzyme irreversibly. The "pitchform" represents the inhibitor – it looks nothing like the S. Two examples of this sort of inhibitor are nerve gas and organophosphates that inhibit acetylcholinesterase. Organophosphate poisoning may be reversed by injecting a drug called 2-PAM. Valium and atropine are useful to treat muscle spasms and breathing difficulties, as well.

The bottom right graphic represents noncompetitive inhibition. Note that the inhibitor does NOT bind to the active site of the enzyme, rather it has its own unique binding site (the red bar). When a noncompetitive inhibitor binds to an enzyme, it causes the enzyme to change shape (see the white gap in the active site on the right side of the "pellet-shaped" S?) and shuts off its activity

reversibly by not allowing S to bind completely. This sort of inhibition is also referred to as allosteric inhibition and plays major roles in metabolic regulation.

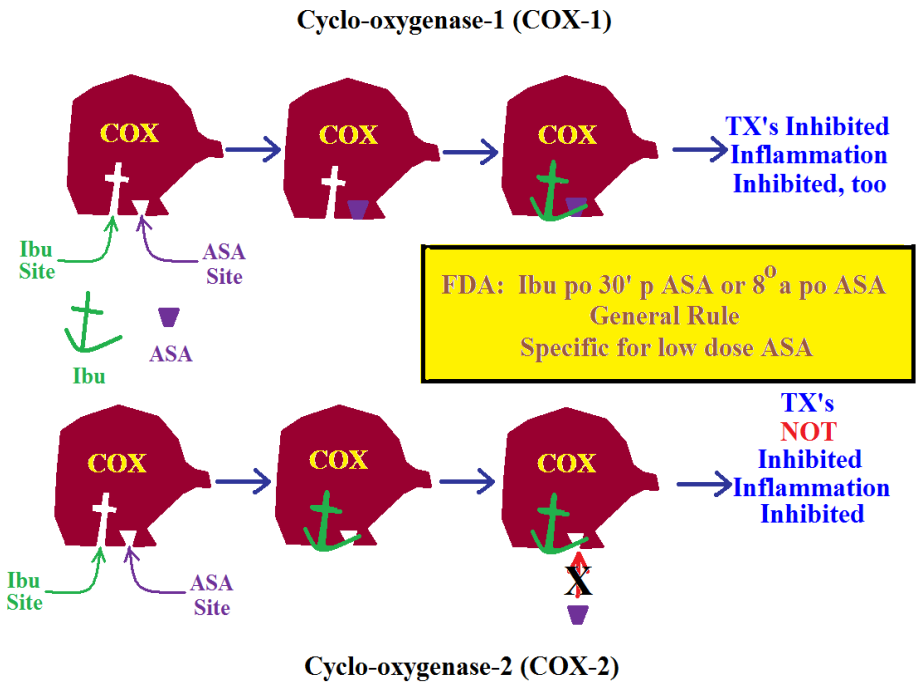
An example of mixed inhibitor types is aspirin (ASA) and Ibuprofen (IBU). ASA is an **UN**competitive inhibitor of COX-1 (CycloOXygenase type 1). ASA and IBU inhibit cyclo-oxygenase variants which is the main enzyme in prostaglandin biosynthesis. Prostaglandins mediate pain, Inflammation, blood pressure, gastric mucous secretion, blood clotting, labor and delivery, dysmenorrhea, to name a few. This inhibition is IR-reversible – unlike other NSAID's (Non-Steroidal Anti-Inflammatory Drug's). ASA acetylates COX-1 to inhibit it. The half life ($t_{½}$) of ASA varies by dosage: 250 mg dose $t_{½}$ = 2-4.5 hrs; 1 g dose $t_{½}$ = 5 hrs; 2 g dose $t_{½}$ = 9 hours; > 4 g $t_{½}$ = 15-30 hrs.

ASA CHANGES COX-2 activity to produce anti-inflammatory lipoxins ("LX's"; derived from ω3 fatty acids (EPA) as well as ω6 fatty acids such as 20:4$^{\Delta 5,8,11,14}$), see image at right.



Lipoxins
anti-inflammatory

IBU is a **NON**competitive inhibitor of COX-2. It is reversible in its inhibition. IBU works primarily through COX-2 (Like Vioxx and Celebrex by reducing PGI_2. This permits "normal" TX(A_2 or B_2) production which increases the incidence of blood clots [IBU has lowest incidence of GI/Hematological Sx of the NSAID's, by the way]). The half life for IBU is unique in that it's about 1.8-2 hrs while its duration of action is about 2-4X the $t_{½}$.



The problem with these two medications is that IBU binds to COX-2 inhibiting the production of PGI_2 –

the natural titrant of TX's. What does this mean? Blood clots, potentially. If one is a cardiac patient and is taking low dose po ASA to prevent blood clot formation, yet needs IBU for pain control, what is one to do?

Per http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm125222.htm, the patient needs to take their ASA first and wait 30 minutes to take their IBU (top of graphic at bottom of previous page) or take their IBU 8 hours before their ASA dose. If IBU is taken first or is not taken long enough before the ASA dose, IBU not only binds, it also blocks the binding of ASA, to COX-1 and COX-2 (bottom graphic at bottom of previous page). Should this occur, the potential for a fatal MI due to thrombosis of [a] coronary arter[y]ies is elevated.

Isoenzymes/Isozymes

Multiple forms of enzymes in different tissues with the same activity are called isoenzymes or isozymes. They possess identical cofactors but slightly different apoenzymes. Two best examples: LDH (or LD) and CPK (for those of us old enough to remember it this way; nowadays, it's CK).

LDH – Lactate Dehydrogenase

LDH is a cellular enzyme that generally is activated during anaerobic glycolysis. There are at least five (5) variants (isozymes). These variants are summarized in the table below.

LDH				
LD_1	LD_2	LD_3	LD_4	LD_5
H_4	H_3M	H_2M_2	HM_3	M_4
Heart	Heart (⇑); Brain = Kidney	Brain = Lung	Lung (⇑); Skeletal muscle	Liver = Skeletal Muscle
H = heart sub-units; M = muscle sub-units				

LD_1 was first discovered in the heart and has four (4) identical protein sub-units. Since they are identical and from the heart, each sub-unit is called an "H" sub-unit. There are four (4) H sub-units in LD_1. LD_5 was identified in muscle and it, too, had four (4) identical sub-units, called "M" for muscle. LD_{2-4} were identified afterwards and their sub-units were actually combinations of the H and M sub-units as indicated in the table. LD_2 is highest in the heart; brain and kidney

activities are about the same. LD_4 has the greatest activity in the lungs, although there is activity in skeletal muscle.

CPK or CK– Creatinephosphokinase or creatine kinase

CK was first isolated in skeletal muscle, followed by brain tissue. In the late 1970's, we were assaying total CPK levels and obtaining astronomical values … that didn't fit the MI damage. Turned out there were 3 variants (at least – some sources indicate there may be as many as 3 sub-variants of CK-MB, for example). CK-MB was specific for the heart. In the early 1980's, this assay was not STAT – it was "when you can get it". The assay, itself, took almost 3 hours to run. Nowadays, these are run at the bedside.

C[P]K		
BB	MM	MB
1° Brain	1° Skeletal muscle	1° Heart

Medical Uses of Enzymes and Enzyme Assays

When cells die or are injured, they dump some or all of their E's into the blood. Assays are used to make diagnoses, e.g.,

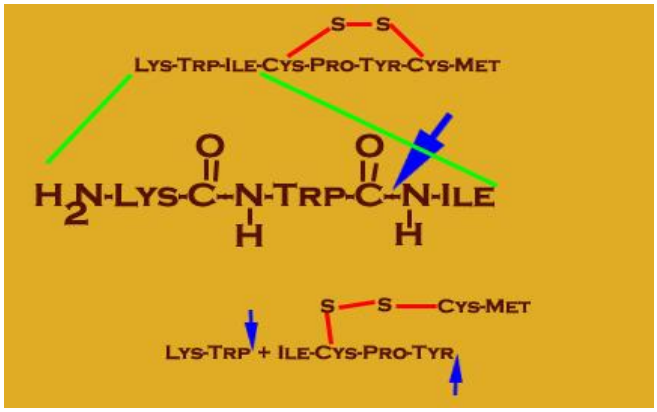
- C[P]K, LDH 2° MI (myoglobins and troponins are being used, as well)
- GPT (ALT) 2° liver problems
- GOT (AST) 2° MI or liver problems
- Ratios
    - GPT:GOT – normal = 0.75; viral hepatitis = 1.6
    - LD_1:LD_2 – normally < 1; 48° after MI, > 1 and is called the LD_1-LD_2 "flip"

Calcium ion channel blockers – block calcium ion influx via integral protein membrane channel which leads to reduced calcium ion being taken up inside the cell which leads to reduced muscle contraction. This reduction in contraction of the heart muscle makes it easier for the heart to beat to reduce the risk of MI or death after MI. More on calcium ion channel blockers in a later monograph (The Cell).

Pepsin

Pepsin hydrolyzes proteins at the C-terminus of			
Trp	The aromatic amino acids	Met	Sulfur-containing amino acid
Phe		Leu	BCAA
Tyr			

Pepsin Activity – Image at middle right illustrates some of pepsin's proteolytic activity. Pepsin is an enzyme from the stomach that initiates protein digestion in humans. Note the disulfide bond; Note the pepsin cleavage sites; Note the products.



Pepsin Activity – Image at lower right is an additional example of pepsin's proteolytic action. Again, note the disulfide bond (pepsin won't cleave this bond; it requires a desulfhydratase); Note the cleavage sites; Note the products. Pepsin can only do so much in the stomach. More enzymes are needed throughout the GI system to assist in protein digestion.

Proteases from Small Bowel

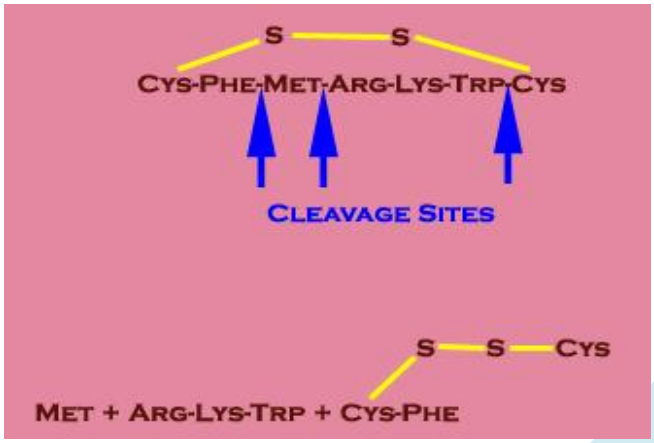
Aminopeptidase – removes N-terminal amino acid from peptide:

Asp-Gly-Pro-Lys-Arg-Cys-Phe + aminopeptidase

Yields

Asp + Gly-Pro-Lys-Arg-Cys-Phe

If repeated one AA at a time, this would disassemble peptide interminably slowly.



Dipeptidase: is the final protease that hydrolyzes dipeptides to free amino acids:

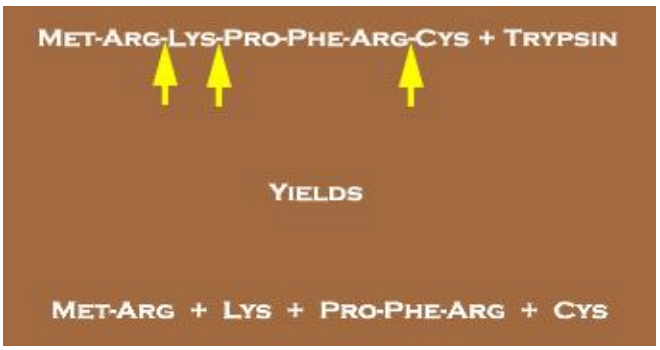
Pro-Met + dipeptidase

Yields

Pro + Met

Pancreatic Proteases

Trypsin	Chymotrypsin	Carboxypeptidase	Elastase
Cleaves at the C-terminus of Arg and Lys	Cleaves at the C-terminus of Phe, Trp, Tyr	Removes the C-terminal amino acid -- one at a time	Cleaves at the C-terminus of Ser, Thr, Tyr, Asn, Gln and Cys



Note that chymotrypsin has some of the same activity of pepsin. Pepsin, though, functions BEST at a pH of around 2. It STILL has SOME function at higher pH's, although it "prefers" the acidic conditions of the stomach for its "pH optimum". The pH optimum is the pH at which optimal activity is attained. Chymotrypsin functions best at an alkaline pH, as is found in the small bowel.

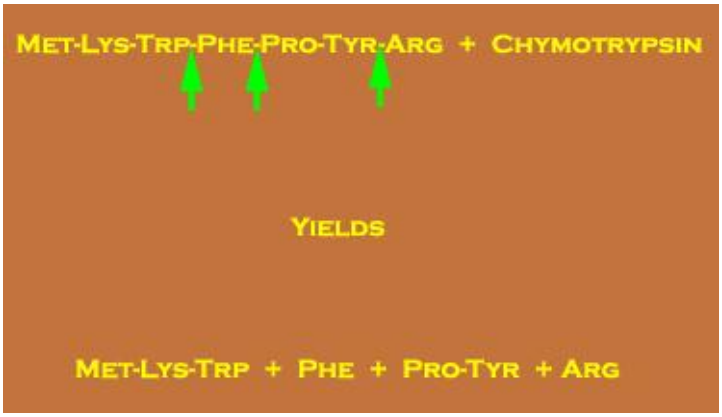
Trypsin

Trypsin is secreted as trypsinogen and activated by enterokinase Trypsin cleaves peptides/proteins at the C-terminus of Arg and Lys – the positively charged amino acids (image above right).

Cystic Fibrosis

A quick and dirty lab test to detect the potential for a newborn patient to develop cystic fibrosis (CF) tests for fecal trypsin activity. Although we usually think of CF as a pulmonary disease, it has multiple ramifications, including bowel disorders. This disorder comes about because the pancreas gets plugged by this disease in its process, rendering digestion difficult, to say the least. Since the pancreas gets plugged, it can not secrete digestive enzymes like trypsin.

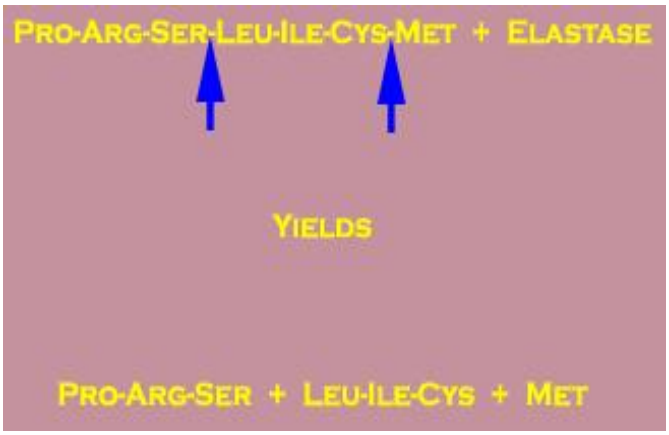
To perform this easy screening procedure, you need do the following. Place the bottom of a Petri dish flat on a lab surface, break an applicator stick in two and place them in the dish on top of a moistened paper towel. With another applicator stick, smear a little baby poop on the piece of x-ray film and mix it with normal saline.



Place the film on the applicator sticks and cover with the top of the Petri dish. Incubate at 37° C. After incubation, rinse off the film and examine it.

If the surface of the film doesn't look "chewed up" or "roughened", this means that there is no trypsin in poop. The pancreas is plugged and this infant needs to be tested further for CF.

If there's a rough surface or a sort of "hole" in film, this means that there is trypsin in poop. The pancreas is ok and no further tests for CF are necessary.



Chymotrypsin[ogen]

Chymotrypsinogen is activated by Trypsin. Chymotrypsin cleaves at the C-terminus of Trp, Phe, Tyr – the aromatic amino acids (image top right, this page).

Elastase

Elastase cleaves at the C-terminus of the neutral amino acids: Ser, Thr, Tyr, Cys, Asn, Gln (image middle right, this page).

Elastase Aside

Elastase is present in high quantities in lung tissue. Elastase activity is inhibited under normal conditions by α_1-PI – alpha one-protease inhibitor. This allows lungs to remain pliable and "stretchy-able". Smoking inhibits α_1-PI – alpha one-protease inhibitor. Elastase is activated and the lungs lose pliability and the patient is working on "getting" COPD.

Carboxypeptidase

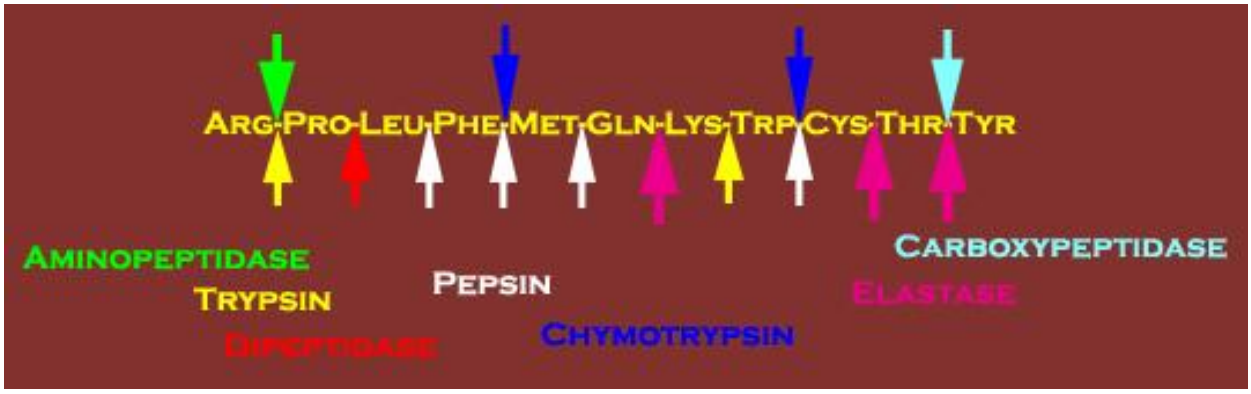
Carboxypeptidase removes the C-terminal amino acid from a peptide, e.g.,

Cys-Pro-Leu-Arg-Gly-**Lys** + Carboxypeptidase

Yields

Cys-Pro-Leu-Arg-Gly + **Lys**

Proteases



If each enzyme were to work one at a time, this process would take forever. Therefore, all of these enzymes (small bowel and pancreas) work at the same time to disassemble proteins. Pepsin, of course, only works, optimally, in the stomach. Note in the graphic, above, the back-up enzymes and specificity. This "assembly line" approach renders proteolysis incredibly effeicient and effective.

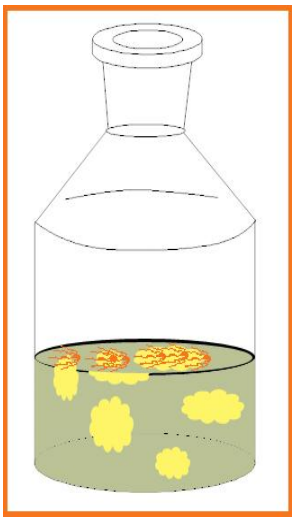
Experimental: Qualitative Amino Acid and Protein Methods

Char test: Place about a pea-sized bit of **casein** in an evaporating dish and ignite it with the Bunsen burner. What does the odor remind you of? What do you think it smells like?

Xanthoproteic reaction: Add 10 gtts **concentrated nitric acid** to a third of a pea-sized amount of **egg albumin** in 30 drops of water. Heat this tube to boiling in your hot water bath. Did it turn yellow?

Now add 12 gtts **6 M NaOH** to your mixture and examine the surface of the mixture in the tube. Did it turn orange?

If it turned orange (as in the graphic at right), this is a positive test. This test tests for the presence of aromatic (contains a benzene ring for our purposes) amino acids. Draw the 3 aromatic amino acids in the space below:



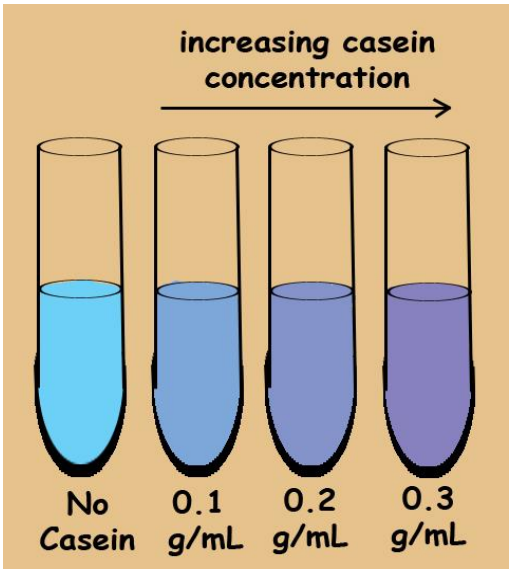
Biuret test: Obtain **5 test tubes**. Label them 1-5. Leave the #1 tube empty for now. Into each of the next 4 tubes add solid **egg albumin** in the following manner:

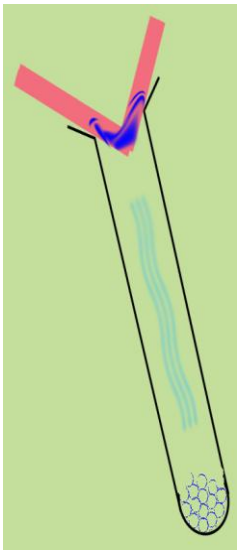
Approximate Sample Size of Egg Albumin			
Tube 2	Tube 3	Tube 4	Tube 5
About a quarter the size of a small pea	About a third the size of a small pea	About a half the size of a small pea	About two-thirds the size of a small pea

Now, into each of the 5 tubes, add 20 gtts water and vortex to mix. Add 20 gtts **6 M NaOH** to each of the five tubes and re-vortex. Add 3 gtts of the **$CuSO_4$ solution** to each of the 5 tubes and re-vortex. Record your observations (look at the color[s] and intensities):

Biuret Test Observations				
Tube 1	Tube 2	Tube 3	Tube 4	Tube 5

Note (and remember) that your first tube (tube #1) has NO albumin in it. As a general rule, the more protein present, the more peptide bonds that are present. Remember that the oxygen in the peptide bond has 2 unbonded pairs of electrons. The nitrogen has one pair of unbounded electrons. It is through coordinate covalent bonding that the Copper(II) ion reacts with the protein to deepen the color. Are your results consistent with your observations and the graphic at right? Why or why not?





Urea hydrolysis: Place about a half cm of **urea** in the bottom of an **ignition tube** (hold the tube with a **three fingered clamp to the ring stand** – "aim" it away from people) and place a piece of moistened **RED litmus paper** folded in a "V" shape in the neck of the tube (as in the image at right). Heat it gently with your **Bunsen burner** (the urea will bubble). CAREFULLY waft the odor towards you. What is the gas that is emitted?

What color did the litmus paper turn?

Define "thermolysis" in the space below.

Experimental: Quantitative Amino Acid and Protein Methods

Potentiometric Titration of an Amino Acid

In previous course-work, you explored the potentiometric titration of a weak acid (HOAc). In this experiment, you will explore the titration of an amino acid. The information you will obtain from this experiment will demonstrate the acidity of the carboxyl group (COOH), the alkalinity of the amino group ($-NH_2$) and the acid-base characteristics of the R-group -- if any on the amino acid, at all.

Remember from lecture that amino acids under physiological conditions undergo double ionization and are called "zwitterions" -- twin ions. This term comes about from the deprotonation of the carboxyl group to the carboxylate ion (COO^-) and the protonation of the amino group to the ammonium ion ($-NH_3^+$). R-groups, such as the imidazole ring, carboxyl group, amino group, guanidino group, will protonate or deprotonate depending upon their chemical characteristics and the pH of the solution in which they are solvated. The table, below, lists the pK values for some of the amino acids and the isoelectric point (pI) for these amino acids, as well:

Name of Amino Acid	pK_{COOH}	pK_{NH2}	pK_R	pI
Alanine (ala)	2.34	9.69		6.02
Arginine (arg)	2.17	9.04	12.48	10.76
Aspartic acid (asp)	2.09	9.82	3.86	2.98
Cysteine (cys)	1.71	10.78	8.33	9.56
Glutamic acid (glu)	2.19	9.67	4.25	3.22
Glycine (gly)	2.34	9.6		5.97
Histidine (his)	1.82	9.17	6.0	7.59
Isoleucine (ile)	2.32	9.75		6.04
Leucine (leu)	2.36	9.60		5.98
Lysine (lys)	2.18	8.95	10.53	9.74
Methionine (met)	2.20	9.05		5.63
Proline (**pro**)	2.00	10.6 (Imine)		6.3
Serine (ser)	2.21	9.15		5.68
Threonine (thr)	2.63	10.43		6.53
Tryptophan (trp)	2.35	9.33		5.84
Tyrosine (tyr)	2.20	9.11	10.07	9.59
Valine (val)	2.29	9.72		6.01

Remember that the calculation of the pI is to take the sum of the 2 closest pK values and divide them by 2. This is the point at which the amino acid is electrically neutral.

There are some caveats to remember when titrating an amino acid: while this is an accepted technique among some analytical chemists, among biochemists it's not believed to be as accurate as something like HPLC or 2-D paper chromatography for amino acid identification. It is also important to remember that pK's within about 2 pK units will probably NOT be detected by this method, e.g., the 2 carboxyl groups on asp or glu or the 2 amines on lys may not be picked up.

The purpose of this portion of the experiment is for the student to observe the amphiprotic characteristics of amino acids and use a "pre-fabb'd" Excel spreadsheet to demonstrate the zwitterionic characteristics of an amino acid. The techniques for determining the titration and first derivative curves are as per the potentiometric titration of a weak acid experiment that you performed in previous courses, i.e., carry that knowledge into this experiment.

Materials and Methods

Chemicals	Equipment
1 M HCl	Weighing boats
0.1 N NaOH	Spatula
Amino acid (assigned to each student)	Electronic pan balance
pH 4 and 7 buffers	Buret and clamp
1-calibrated pH Checker	Ring stand
2-125 mL Erlenmeyer flasks	

Method

Mass out 2 samples of your amino acid such that they are not more than 0.1000 g, apiece. **Record the amino acid and the masses in the table, below**:

Name of Amino Acid:	
Sample #1 mass (g)	Sample #2 mass (g)

Pour each sample into each of the pre-labeled Erlenmeyer flasks and dissolve the amino acid in about 25 mL of water.

Obtain your pH checker and turn it on. Calibrate it according to instructions. Once the pH checker is satisfactorily calibrated, insert it into the amino acid solution and allow it to stabilize. Once it has stabilized, add enough 1 M HCl to it to adjust the pH of the solution to a pH of about 1 to 1.5. **Record that pH and the volume of HCl added to get to that pH in the box below**:

Sample 1 pH after _____ mL HCl added	Sample 2 pH after _____ mL HCl added
pH =	pH =

Obtain your buret and titrating supplies. Clean and prepare the buret as in the past, **remembering to put the 0.1 N NaOH in the buret**. Remember, too, to read your buret from top down. Once you have this assembled, you are ready to begin titrating your sample. Read the following table carefully and record your pH readings at the volumes indicated. Note that this is very painstaking and that readings are characteristically taken after the addition of every 0.5 mL 0.1 N NaOH. This is necessary for good first (and sometimes, second) derivative curves. You will perform this titration in duplicate.

TRIAL 1

Vol 0.1 N NaOH (mL)	pH	Vol 0.1 N NaOH (mL)	pH	Vol 0.1 N NaOH (mL)	pH
0.0		15.0		30.0	
0.5		15.5		30.5	
1.0		16.0		31.0	
1.5		16.5		31.5	
2.0		17.0		32.0	
2.5		17.5		32.5	
3.0		18.0		33.0	
3.5		18.5		33.5	
4.0		19.0		34.0	
4.5		19.5		34.5	
5.0		20.0		35.0	
5.5		20.5		35.5	
6.0		21.0		36.0	
6.5		21.5		36.5	
7.0		22.0		37.0	
7.5		22.5		37.5	
8.0		23.0		38.0	
8.5		23.5		38.5	
9.0		24.0		39.0	
9.5		24.5		39.5	
10.0		25.0		40.0	
10.5		25.5		40.5	
11.0		26.0		41.0	
11.5		26.5		41.5	
12.0		27.0		42.0	
12.5		27.5		42.5	
13.0		28.0		43.0	
13.5		28.5		43.5	
14.0		29.0		44.0	
14.5		29.5		44.5	

TRIAL 2

Vol 0.1 N NaOH (mL)	pH	Vol 0.1 N NaOH (mL)	pH	Vol 0.1 N NaOH (mL)	pH
0.0		15.0		30.0	
0.5		15.5		30.5	
1.0		16.0		31.0	
1.5		16.5		31.5	
2.0		17.0		32.0	
2.5		17.5		32.5	
3.0		18.0		33.0	
3.5		18.5		33.5	
4.0		19.0		34.0	
4.5		19.5		34.5	
5.0		20.0		35.0	
5.5		20.5		35.5	
6.0		21.0		36.0	
6.5		21.5		36.5	
7.0		22.0		37.0	
7.5		22.5		37.5	
8.0		23.0		38.0	
8.5		23.5		38.5	
9.0		24.0		39.0	
9.5		24.5		39.5	
10.0		25.0		40.0	
10.5		25.5		40.5	
11.0		26.0		41.0	
11.5		26.5		41.5	
12.0		27.0		42.0	
12.5		27.5		42.5	
13.0		28.0		43.0	
13.5		28.5		43.5	
14.0		29.0		44.0	
14.5		29.5		44.5	

Calculations

Once you have completed your work and disposed of your waste as instructed, get your computer, download the Excel file (http://www.drcarman.info/kem121lb/hoac.xls) onto your desktop and set up the titration curves and first-derivative curves as in the previous lab.

For pK_{COOH}: find the first endpoint volume (from your first derivative curve) and divide that in half. Back determine the pH at that new (half) volume from your titration curve. Record both experimental $pK_{COOH's}$ here:

Sample 1 pK_{COOH}	Sample 2 pK_{COOH}

For pK_{NH2}: find the first and second endpoint volumes from your first derivative curve. Find the halfway volume between the two. Back determine the pK_{NH2} at that volume from your titration curve. Record both experimental $pK_{NH2's}$ here:

Sample 1 pK_{NH2}	Sample 2 pK_{NH2}

For those of you with three titratable groups -- pK_R: find the 2d and 3d endpoint volumes from your first derivative curve. Find the halfway volume between the two. Back determine the pK_R at that volume from your titration curve. Record both experimental $pK_{R's}$ here:

Sample 1 pK_R	Sample 2 pK_R

Summarize the average pK values in the table below:

	pK_{COOH}	pK_{NH2}	pK_R
Text pK Values			
Experimental pK Values			

How do the values compare?

Questions

You have plenty to do without the added aggravation of questions. Attach your graphs to the lab for turn-in.

Sources

Harris: Exploring Chemical Analysis, Second Edition. (W.H. Freeman and Co.: NY)© 2001.

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