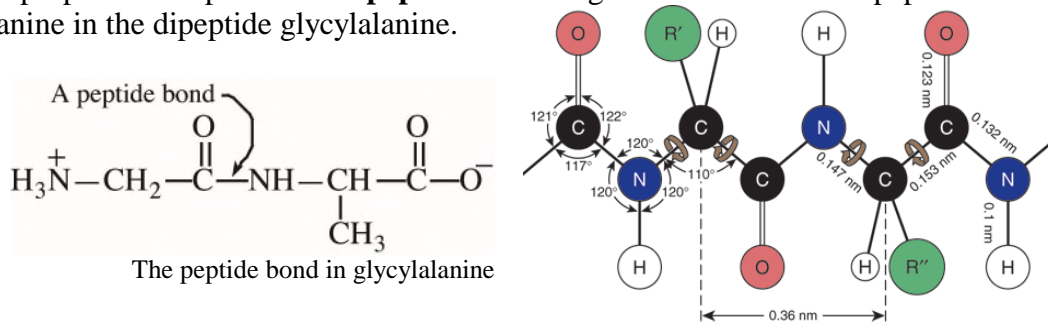


PROTEINS

I.3 Polypeptides and proteins

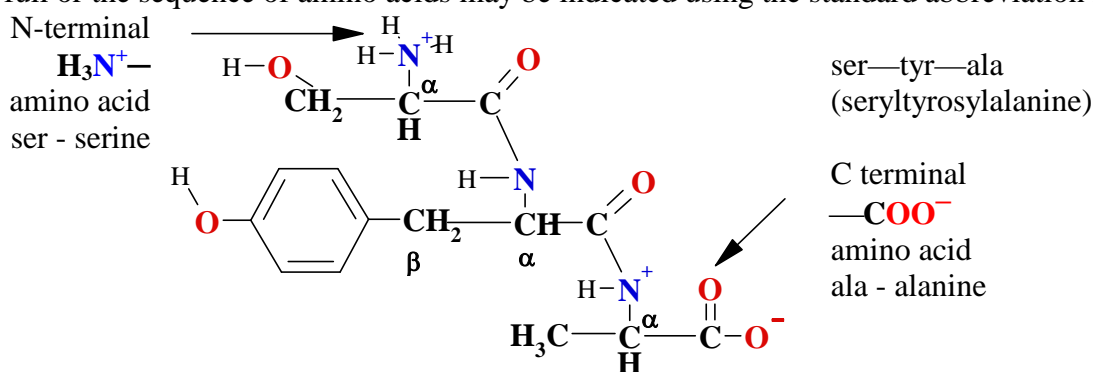
<http://aris.gusc.lv/NutritionBioChem/32ProteinsC.doc>

In 1902, Emil Fischer proposed that proteins are long chains of amino acids joined together by amide bonds between the α -carboxyl group of one amino acid and the α -amino group of another. For these amide bonds, Fischer proposed the special name **peptide bond**. Figure below shows the peptide bond formed between glycine and alanine in the dipeptide glycylalanine.



A molecule containing two amino acids joined by an amide bond is called a **dipeptide**. Those containing larger numbers of amino acids are called **tripeptides**, **tetrapeptides**, and **pentapeptides** and so on. Molecules containing 10 or more amino acids are generally called **polypeptides**. Proteins are biological macromolecules of molecular weight 5000 or greater, consisting of one or more polypeptide chains.

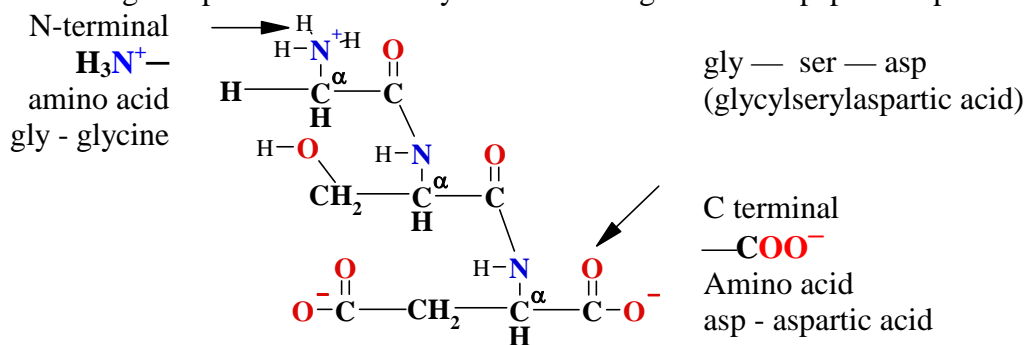
By convention, polypeptides are written from the left, beginning with the amino acids having the free H_3N^+ group and proceeding to the right toward H_3N^+ group is called the **N-terminal amino acid** and that with the free $-\text{COO}^-$ group is called the **C-terminal amino acid**. The structural formula for a polypeptide sequence may be written out in full or the sequence of amino acids may be indicated using the standard abbreviation for each.



Polypeptides are named by listing each amino acid in order, from the N-terminal end of the chain to the C-terminal end. The name of the C-terminal amino acid is given in full. The name of each other amino acid in the chain is derived by dropping the suffix *-ine* or *-ic* and adding *-yl*. For example, if the order of amino acids from the N-terminal end is serine-tyrosine-alanine (ser-tyr-ala), the name of the tripeptide is seryltyrosylalanine.

Example I.4 Name and draw a structural formula for the tripeptide gly-ser-asp. Label the N-terminal amino acid and the C-terminal amino acid. What is the net charge on this tripeptide at $\text{pH}=6.0$?

Solution In writing the formula for this tripeptide, begin with glycine to the α -amino group of serine by a peptide bond. Finally, connect the α -carbonyl of serine to the α -amino group of aspartic acid by another peptide bond. If you have done this correctly, the backbone of the peptide chain be a repeating sequence of nitrogen-alpha carbon-carbonyl. The net charge on this tripeptide at $\text{pH}=6.0$ is -1 .



Problem I.4 Name and draw a structural formula for lys-phe-ala. Label the N-terminal amino acid and the C-terminal amino acid. What is the net charge on this tripeptide at $\text{pH}=6.0$?

I.4 Amino Acid Analysis

The first step in analyzing a polypeptide is hydrolysis and quantitative determination of its amino acid composition. Recall that amide bonds are very resistant to hydrolysis. Hydrolysis of polypeptides requires heating in 6M HCl at 110°C for 24-70 hr or heating 2-4M NaOH at comparable temperature and times. Once the polypeptide is hydrolyzed, the resulting mixture of amino acids is analyzed by ion-exchange chromatography. Amino acids are detected as they emerge from the column by reaction with ninhydrin (See previous). Current procedures for hydrolysis of polypeptides and analysis of amino acid mixtures have been refined to the point where it is possible to obtain amino acid composition from as little as 50 nanomoles ($50 \cdot 10^{-9}$ mol) of polypeptide. Figure I.5 shows the analysis of a polypeptide hydrolysate by ion exchange chromatography. Note that during hydrolysis, the side-chain amides of asparagine and glutamine are hydrolyzed and these amino acids are detected as glutamic acid and aspartic acid. For each glutamine or asparagine hydrolyzed, there is an equivalent amount of ammonia formed.

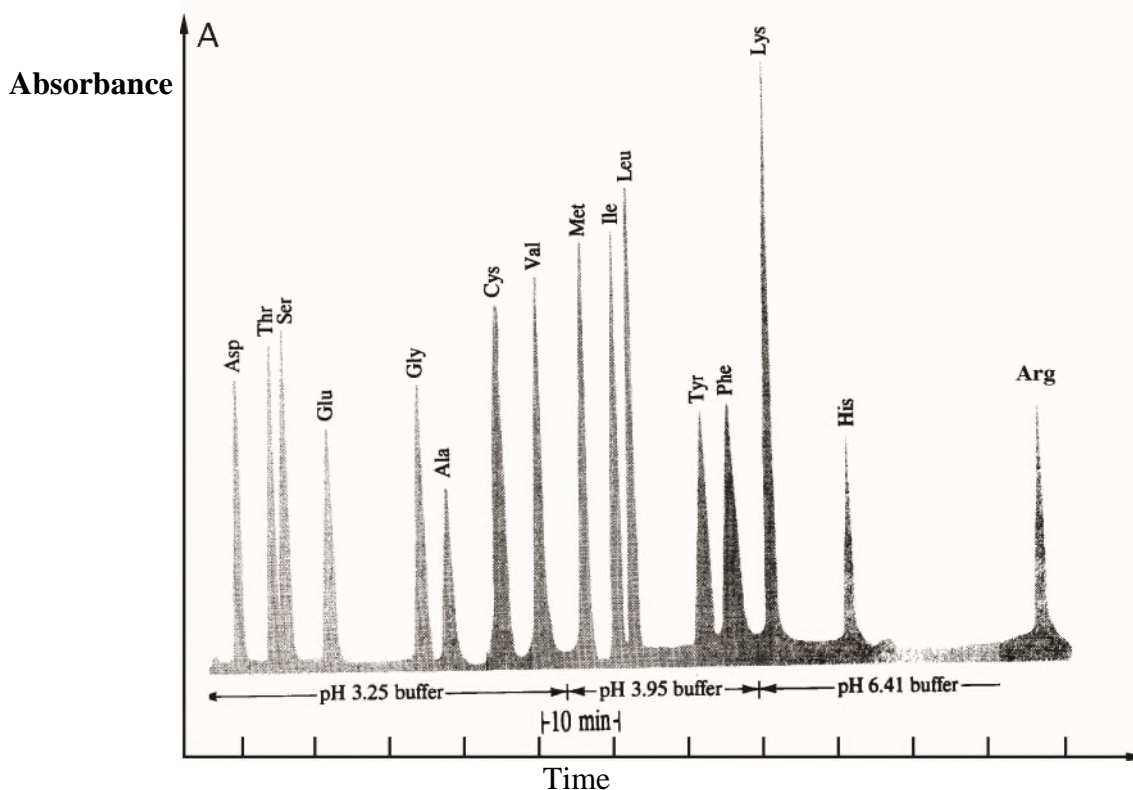


Figure I.5 Analysis of mixture of amino acids by ion-exchange chromatography.

I.5 Primary Structure of Polypeptides and Proteins

Primary (1°) structure of polypeptides and proteins refers to the sequence of amino acids in a polypeptide chain. In this sense, primary structure is a complete description of all covalent bonding in a polypeptide or protein.

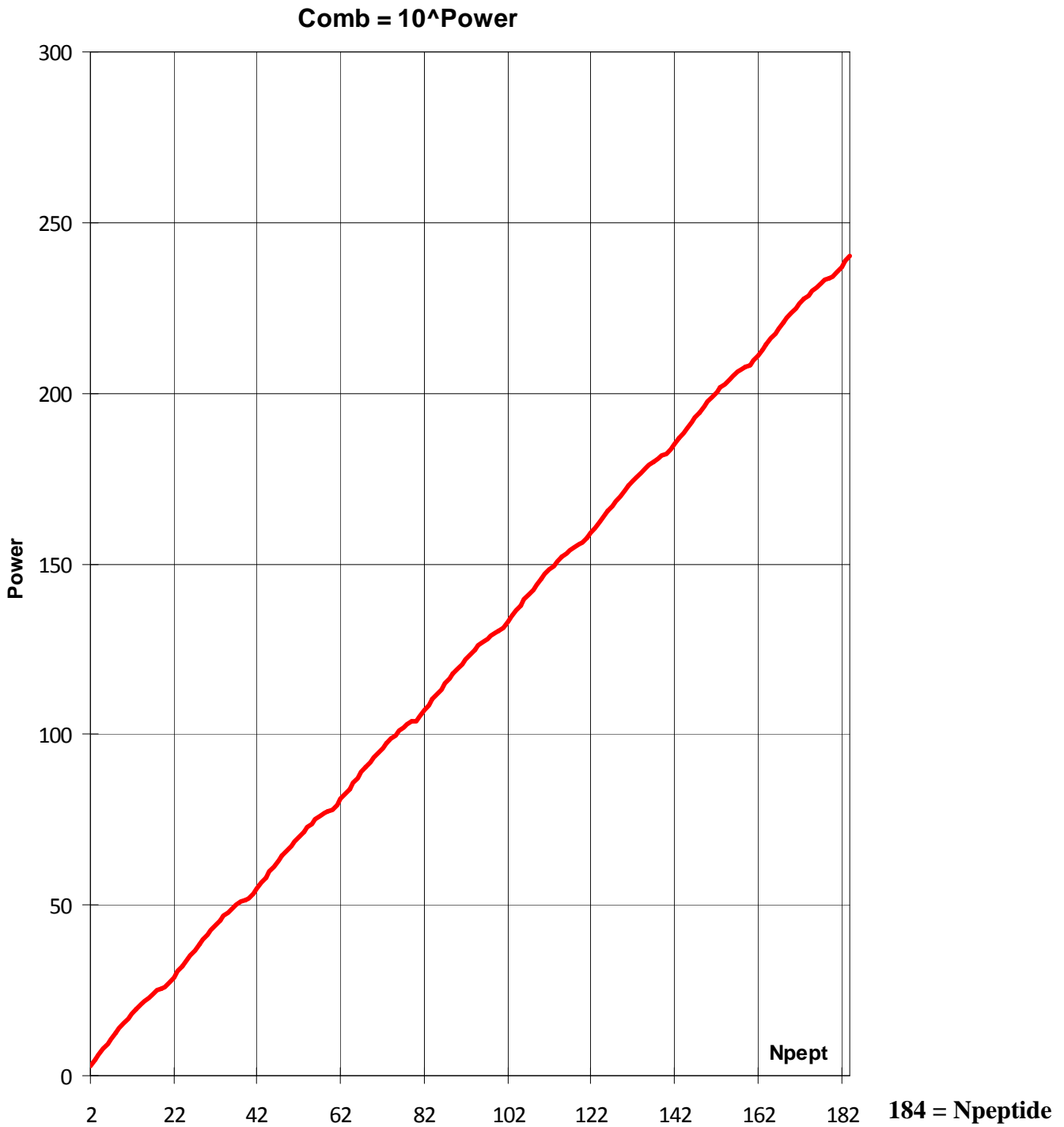
It is difficult to appreciate the incredibly large number of different polypeptides that can be constructed from 20 amino acids, where the number of amino acids in a polypeptide can range from under ten to well over a hundred. With only three amino acids, there are **27** different tripeptides possible. For **glycine, alanine** and **serine**, the **27** tripeptides are:

gly—gly—gly	ser—ser—ser	ala—ala—ala
gly—gly—ser	ser—ser—gly	ala—ala—gly
gly—gly—ala	ser—ser—ala	ala—ala—ser
gly—ser—gly	ser—gly—ser	ala—gly—ala
gly—ala—gly	ser—ala—ser	ala—ser—ala
gly—ser—ala	ser—gly—ala	ala—gly—ser
gly—ala—ser	ser—ala—gly	ala—ser—gly
gly—ser—ser	ser—gly—gly	ala—gly—gly
gly—ala—ala	ser—ala—ala	ala—ser—ser

Statistical analyze of human protein average polypeptide chain length shows number **Npeptide = 184** of the 20 = **nAA** different amino acids (Combinations-Variations) **Comb**, the number of possible polypeptides is

$$i = \text{INT}\left(\frac{\text{Npeptide}}{\text{nAA}}\right) = 9 ; m = \text{Npeptide} - i \cdot \text{nAA} = 4$$

Comb = $(\text{nAA}^{\text{nAA}})^i \cdot m^m \cdot \frac{\text{nAA}!}{(\text{nAA} - m)! \cdot m!} = 1.9 \cdot 10^{240}$ truly countless variation-combination of 20 amino acids arrangement on the polypeptide chain starting from N-terminal and finishing on C-terminal.



I.6 Three-Dimensional Shapes of polypeptides and Proteins A. Geometry of a Peptide Bond

In the late 1930s, Linus Pauling began a series of studies designed to learn more about the three-dimensional shapes of proteins. One of his first discoveries was that a peptide bond itself is planar. As shown in Figure I.6, the four atoms of a peptide bond —**C**—**O**—**H****N**— and the two alpha-carbons joined to it all lie in the same plane

Had you been asked earlier to predict the **geometry of a peptide bond**, you probably would have reasoned in the following way. There are three bundles of electron density around the carbonyl carbon; therefore predict bond angles of 120° about the carbonyl carbon. There are four bundles of electron density around the amide nitrogen; therefore predict bond angles of 109.5° about this atom. These predictions agree with the observed bond angles of approximately 120° about the carbonyl carbon. However, a bond angle of 120° about the amide nitrogen is unexpected. To account for this observed geometry, Pauling proposed that a peptide bond is more accurately represented as a resonance hybrid of two important contributing structures:

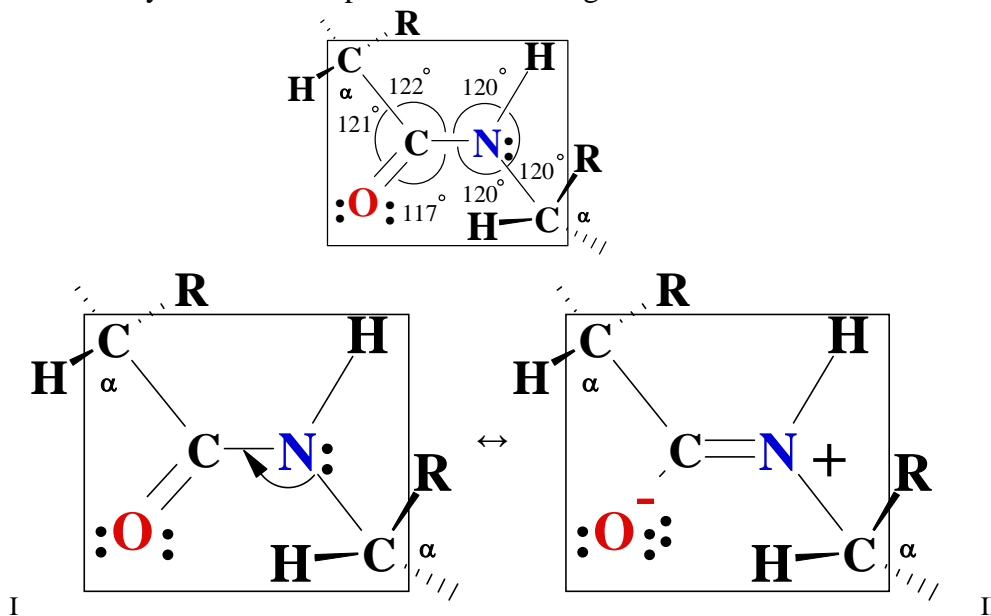
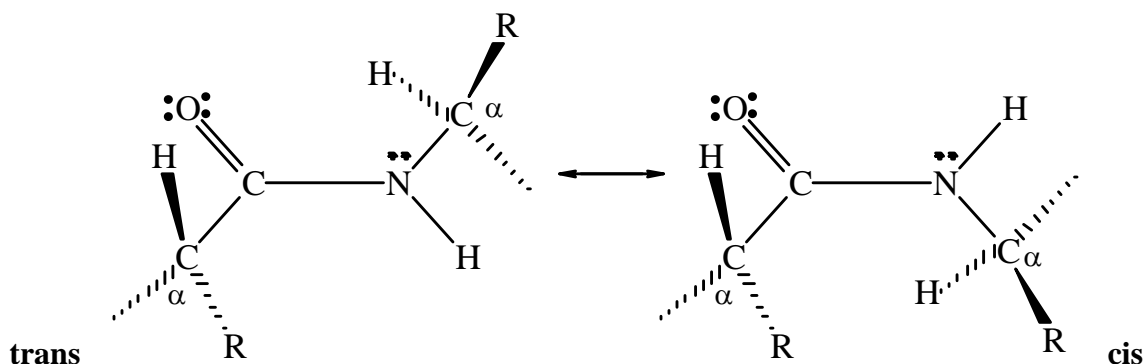


Figure I.6 Planarity of a peptide bond. Bond angles about the carbonyl carbon and the amide nitrogen are approximately 120° .

Contributing structure I show C=O double bond and a C—N single bond. Structure II shows a C—O single bond and a C=N double bond. If structure I is the major contributor to the hybrid, the C—N—C bond angle would be nearer 109.5° . If, on the other hand, structure II is the major contributor, the C—N—C bond angle would be nearer 120° . The fact, first observed by Pauling, is that the C—N—C bond angle is very near 120° , which means that the peptide bond is planar and structure II is the major contributor to the resonance hybrid.

Two configurations are possible for the atoms of a planar peptide bond. In one configuration, the two α -carbons are **cis** to each other; in the other, they are **trans** to each other:



The **trans** configuration is more favorable because the bulky α -carbons are farther from each other than they are in the **cis** configuration. Virtually all peptide bonds in natural proteins have the **trans** configuration.

B. Secondary Structure

Secondary (2°) structure refers to ordered arrangements (conformation) of amino acids in localized regions of a polypeptide or protein, molecule. The first studies of polypeptide conformations were also carried out by Linus Pauling and Robert Corey, beginning in 1939. They assumed that in conformations of greatest stability, (1) all atoms in a peptide bond lie in the same plane and (2) each amide group is hydrogen-bonded between the **N—H** of one peptide bond and the **C=O** of another, as shown in Figure I.7. On this basis of model-building, Pauling and Corey proposed that two folding patterns should be particularly stable: the **α -helix** and the antiparallel **β -pleated sheet**. In the **α -helix** pattern shown in Figure I.7, a polypeptide chain is coiled in a spiral.

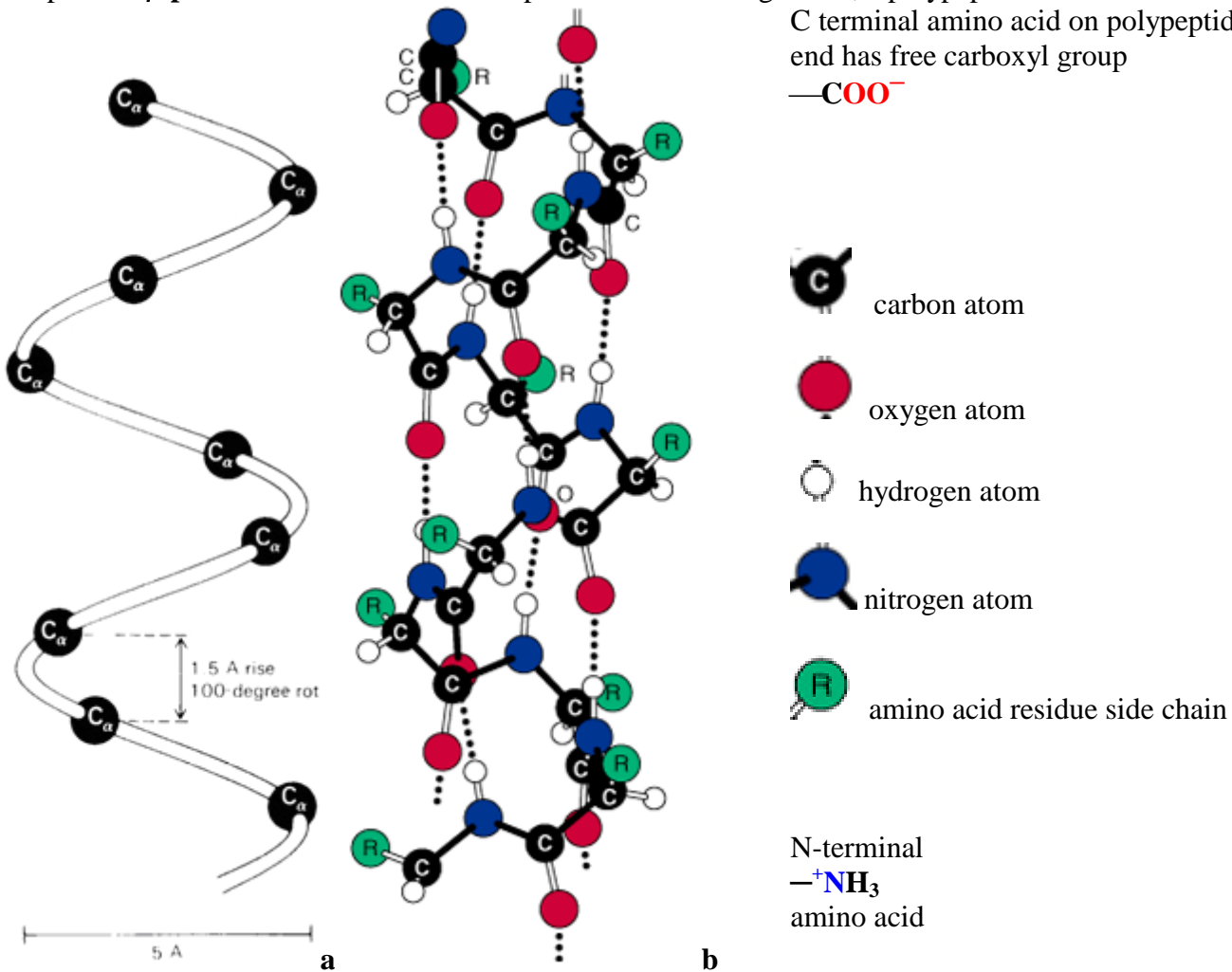


Figure I.7 (a) A right-handed **α -helix** space-filling **α -C** model on the carbon-nitrogen backbone trace of an **α -helix**. (b) Ball-and-stick model of an **α -helix** showing intra chain hydrogen bonds **•••**. **α -helix** in one turn has 3.6 amino acid residues and one step amino acid turn stage have 1.5 Å and 100° angle.

As you study the **α -helix** in Figure I.7, note the following:

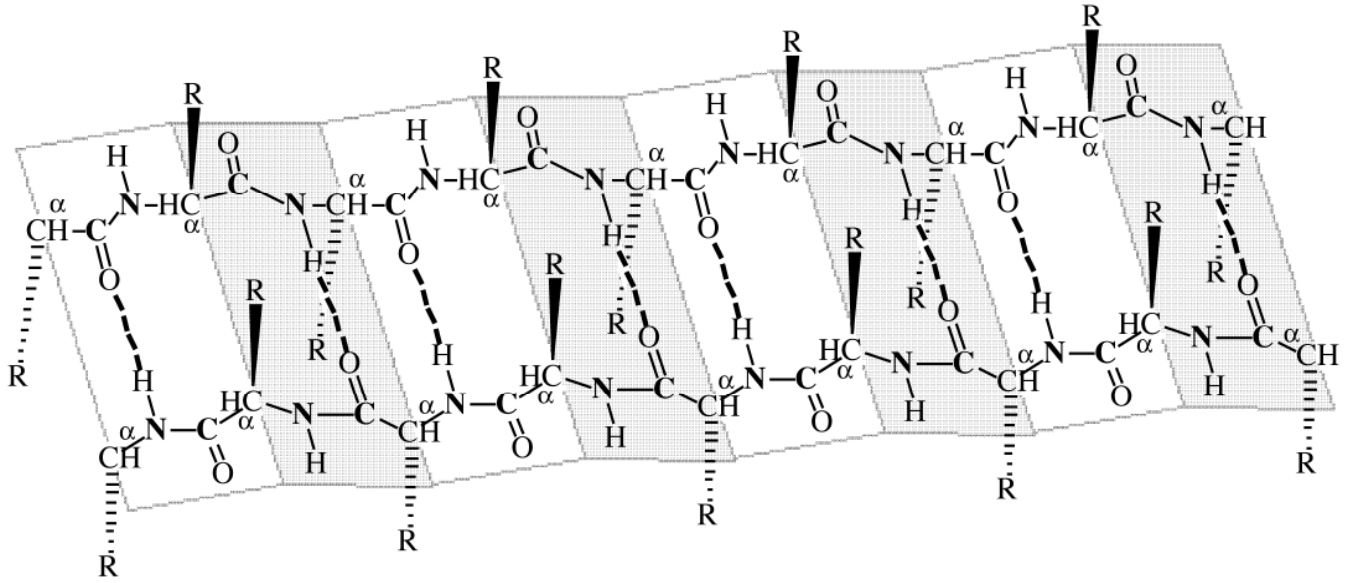
1. The helix is coiled in a clockwise or right-handed manner. Right-handed means that if you turn the helix clockwise, it twists away from you. In this sense, a right-handed **helix** is analogous to the right-hand thread of a common wood or machine screw.
2. There are 3.6 amino acids per turn of the **helix**.
3. Each peptide bond is **trans** and **planar**.
4. The **N—H** group of each peptide bond points roughly upward, parallel to the axis of the **helix**; and the **C=O** of each peptide bond points roughly downward, also parallel to the axis of **helix**.
5. The carbonyl group of each peptide bond is hydrogen-bonded to the **N—H** group of the peptide bond four amino acid units away from it. Hydrogen bonds are shown as dotted lines.
6. All R— groups point outward from the **helix**.

Almost immediately after Pauling proposed the **α -helix** structure, other researchers proved the presence of **α -helix** in keratin, the protein of hair and wool. It soon became obvious that the **α -helix** is one of the fundamental folding patterns for **secondary structure 2°** of polypeptide chains.

β -pleated sheets consist of extended polypeptide chains with neighboring chains running in opposite (antiparallel) directions. Unlike the **α -helix** arrangement, **N—H** and **C=O** groups lie in the sheet and are roughly

perpendicular to the long axis of the sheet. The $\text{C}=\text{O}$ group of each peptide bond is hydrogen-bonded to the $\text{N}-\text{H}$ group of a peptide bond of a neighboring chain $>\text{C}=\text{O} \cdots \text{H}-\text{N}<$.

$\text{H}_3^+\text{N}-$ Amino terminus \longrightarrow Polypeptide chain \longrightarrow Carboxyl terminus $-\text{COO}^-$ chain



$-\text{COO}^-$ Carboxyl terminus \longleftarrow Polypeptide chain \longleftarrow Amino terminus $-\text{NH}_3^+$

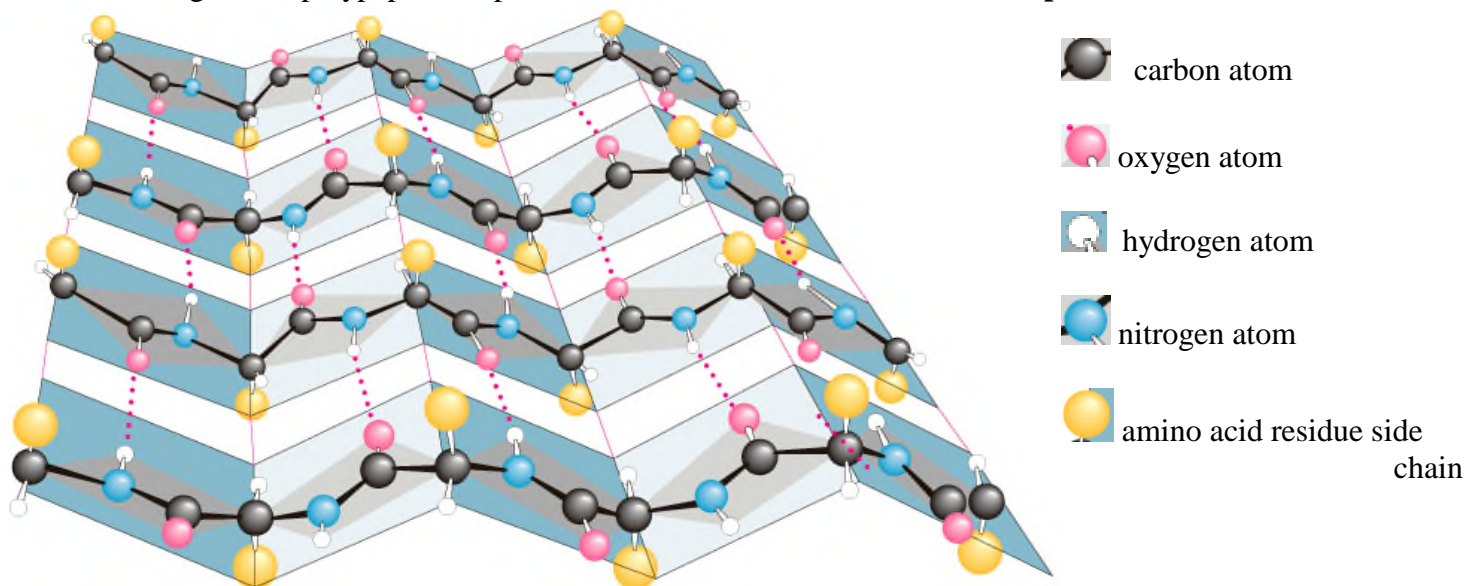
Figure I.8 β -pleated sheet conformation with two polypeptide chains running in opposite (antiparallel) directions. Hydrogen bonding between chains is indicated by dotted lines $\text{C}=\text{O} \cdots \text{H}-\text{N}$.

As you study the secondary 2° structure of **β -pleated sheet** shown in figure I.8, note the following:

1. The two polypeptide chains lie adjacent to each other and run in opposite (antiparallel) directions.
2. Each peptide bond is **trans** and **planar**.
3. The polypeptide is a chain of flat or planar sections connected at amino acid **α -carbons**.
4. The $\text{C}=\text{O}$ and $\text{N}-\text{H}$ groups of peptide bonds from adjacent chains point at each other and are in the same plane, so hydrogen bonding is possible between adjacent polypeptide chains.
5. The $\text{R}-$ groups on any one chain alternate, first above the plane of the sheet and below the plane of the sheet.

The **pleated sheet** conformation is stabilized by hydrogen bonding between $\text{N}-\text{H}$ groups of one chain and $\text{C}=\text{O}$ groups of an adjacent chain $\text{C}=\text{O} \cdots \text{H}-\text{N}$. By comparison, the **α -helix** is stabilized by hydrogen bonding between $\text{N}-\text{H}$ and $\text{C}=\text{O}$ groups within the same polypeptide chain.

Secondary 2° structure is used to describe **α -helix**, **β -pleated sheet** and other types of periodic conformations in localized regions of polypeptide or protein molecules like as **beta turns** or **loops** as well **bends**.



Loops, Turns & Bends

Roughly half of the residues in a "typical" globular protein reside in **α -helices** and **β -sheets** but second half in **loops**, **turns**, **bends** and other extended conformational features. **Loops**, **turns** and **bends** refer to short

segments of amino acids that join two units of secondary structure, such as two adjacent strands of an antiparallel β -sheet. A β -turn involves four aminoacyl residues, in which the first residue is hydrogen-bonded to the fourth, resulting in a tight 180-degree turn (Figure 5–7). Proline and glycine often are present in β -turns.

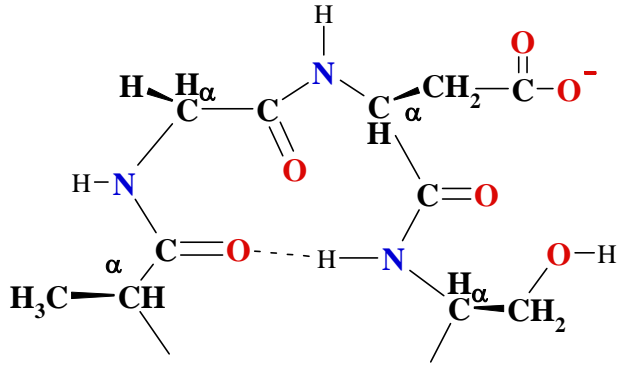


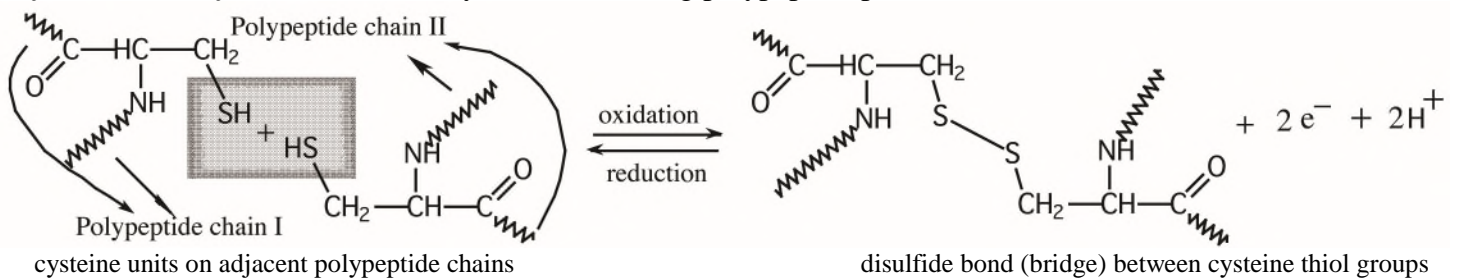
Figure I.8A A β -turn that links two segments of anti-parallel β -sheet. The dotted line indicates the hydrogen bond between the first and fourth amino acids of the four-residue segment Ala-Gly-Asp-Ser.

The term secondary structure is used to describe α -helix, β -pleated sheet and β -turns of periodic conformations in localized regions of polypeptide or protein molecules.

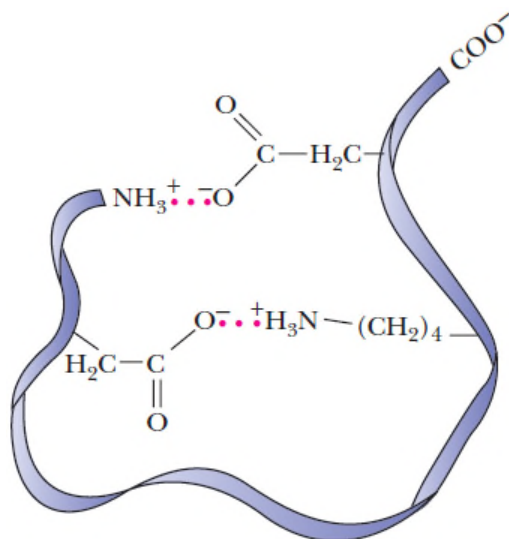
C. Tertiary Structure

Tertiary (3^o) structure refers to the overall folding pattern and arrangement in space of all atoms in a single polypeptide chain. Actually, there is no sharp dividing line between secondary and tertiary structure. Secondary structure refers to the spatial arrangement of amino acids close to one another on a polypeptide chain. Tertiary structure refers to the secondary structure folding patterns in three-dimensional arrangement.

Disulfide bonds (Section of Thiols) are important in maintaining tertiary structure. **Disulfide bonds** are formed between side chains of cysteine by oxidation of two thiol groups (Cys—SH) to form the **disulfide bond** (Cys—S—S—Cys), as shown for cysteine containing polypeptide parts:



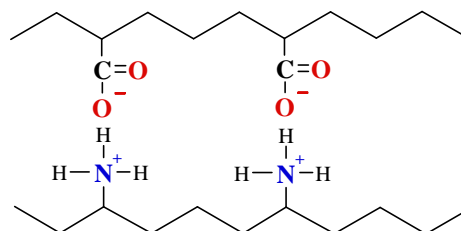
Treatment of a **disulfide bond** with a reducing agent regenerates the thiol group that is reduction.



Intra molecular ionic bonds between oppositely charged groups on amino acid residues in a protein like as **salt bridge** indeed, for example, of aspartic acid salt and lysine positive charged basic amino group Asp—COO⁻...H₃⁺N—Lys

Salt bridge forms between negative charged carbonic acid salt and positive charged neighbor on protein chains ammonium

functional groups —COO⁻...H₃⁺N— and is one of five intermolecular forces :

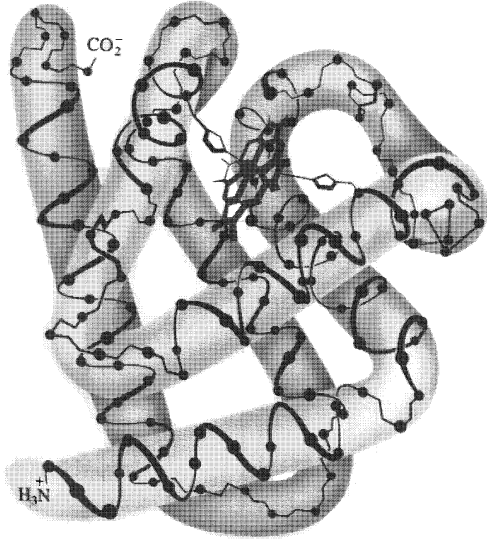


1. Hydrogen bonds,
2. **Salt bridge**,
3. **Disulfide bonds**,
4. Hydrophobic bonds and
5. Coordinative bonds.

<http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/1MBDeOxyLopez.kin>

Myoglobin protein supply stored molecular oxygen in cytosols by oxy \leftrightarrow deoxy sensitive equilibrium at decreasing concentration below [O_{2(aqua)}]=1.5·10⁻⁵ M value. **Myoglobin** consists of a single polypeptide chain of 153 amino acids starting from Val1 to Gly153 coordinated around the single iron(II)atom heme complex center.

J.C. Kendrew awarded by Nobel Prize in chemistry in 1962 for **myoglobin**. The secondary and tertiary structure of **myoglobin** shown in **Figure** of the three-dimensional structure. The **heme** group is shown as on one plane disposed symmetrical joined adjacent



polygons. The **N**-terminal amino acid Val1 indicated by —**NH₃⁺** is at the lower left. **C**-terminal amino acid Gly153 indicated by —**COO⁻** is at the upper left. Alpha carbon atoms between the peptide bonds are located on **backbone trace**.

1. The **backbone** consists of eight sections of **α-helices A, B, C, D, E, F, G, H**, each separated by a **β-bend** with **hydrogen bonds** between peptide bonds groups **>N—H...O=C<**.

2. Non polar side chains of 29 amino acids: Phe, ala, Val, Leu, ile, Gly and Met are clustered around heme pocket, which shield oxygen **O₂** from water and hydronium ions **H₃O⁺** contacts. **Hydrophobic interactions** between

nonpolar side chains fold eight **α-helices** into tertiary 3° structure of **myoglobin protein**.

3. The **myoglobin** surface is coated with hydrophilic amino acids Lys, Arg, Ser, Glu, His and Gln, which interact with the aqueous environment create water soluble **hydrate coat**.

4. Tertiary 3° structure support electrostatic attractions called **salt bridges**. Positive Lys—**NH₃⁺** attract with negative charged Glu carboxylic —**COO⁻** group.

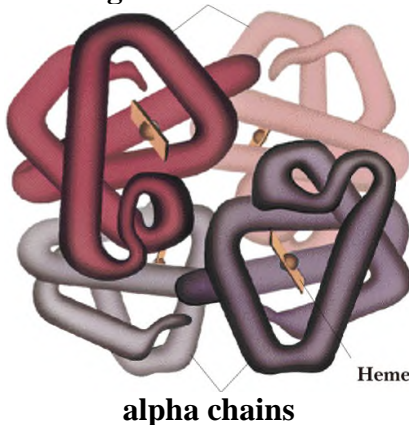
The tertiary 3° structures of globular proteins fold **α-helix** and **β-pleated sheet** secondary 2° structures in **Cytochrome, Hemoglobin, Catalase, Peroxidase** etc. .

Quaternary 4° Structure: <http://aris.gusc.lv/ChemFiles/ChromoHem/HbOxDeoxCO/2HCOProTour8.kin>

Max Perutz awarded by Nobel Prize in chemistry in 1962 for **hemoglobin**.

Quaternary 4° Structure. Hemoglobin consists of 4 synthesised protein monomers

Figure. beta chains



- subunits: **α1, α2** 141 amino acids on **chains** and **β1, β2** 146 amino acids on **chains** with

five **5 intermolecular forces** bounded together. The flat disks represent four **hemes** in its pockets.

The chief factors stabilizing the binding of protein subunits is **hydrophobic interaction** and ten **10 salt bridges** support oxy-deoxy venous oxygen concentration [**O₂_{aq}**]=1.85·10⁻³M sensitive equilibrium as conformation changes to deoxy state.

- 1 - a1Arg141—**COO⁻**...**H₃⁺N**—a2Val1,
- 2 -a1Arg141—**COO⁻**...**H₃⁺N**—a2Lys127,
- 3-a2Arg141—**N H₃⁺**...**OOC**—a1Asp126,
- 4 -b2Asp94—**COO⁻**...**H₃⁺N**—b2His146,

5-b2His146—**COO⁻**...**H₃⁺N**—a1Lys40,

4th page on **Proteins** <http://aris.gusc.lv/NutritionBioChem/38Olbalt10311Eng.doc>

Sickle cell hemoglobin SC **hydrophobic** spots stick excluding water causing aggregation. Adjacent β chain Val6 bound to neighbor molecule Ala70 and Leu88.

<http://aris.gusc.lv/ChemFiles/hemoglobEricMarzUMas/INDEX.htm>

Table I.7

Table I.7	Protein	Mol.Wt.	Number of Subunits	Subunit Mol.Wt.	Biological Function
Quaternary					

structure of selected proteins.	insulin	11 466	2	5 733	a hormone regulating glucose metabolism
	hemoglobin	64 500	4	16 100	oxygen transport in blood plasma
	alcohol dehydrogenase	80 000	4	20 000	an enzyme of alcoholic fermentation
	lactate dehydrogenase	134 000	4	33 500	an enzyme of anaerobic glycolysis
	aldolase	150 000	4	37 500	an enzyme of anaerobic glycolysis
	fumarase	194 000	4	48 500	an enzyme of the tricarboxylic acid <u>Krebs cycle</u>
	tobacco mosaic virus	40 000 000	2280	17 500	plant virus coat

Tobacco mosaic virus single stranded **RNA** helix containing 6390 nucleotides

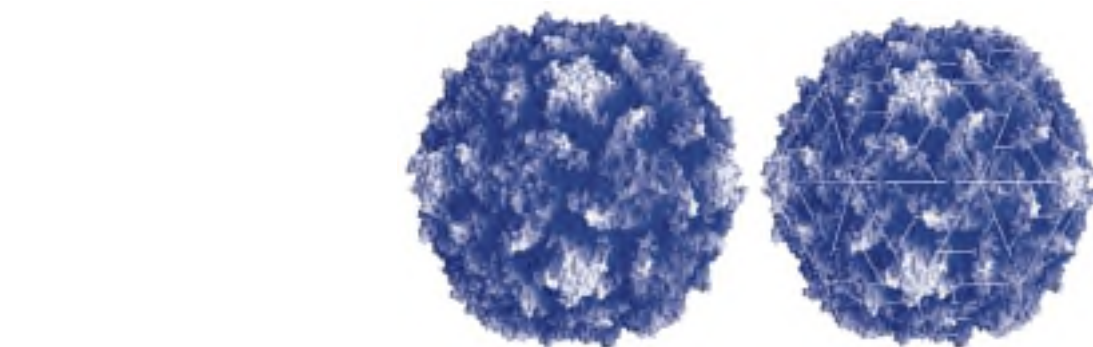
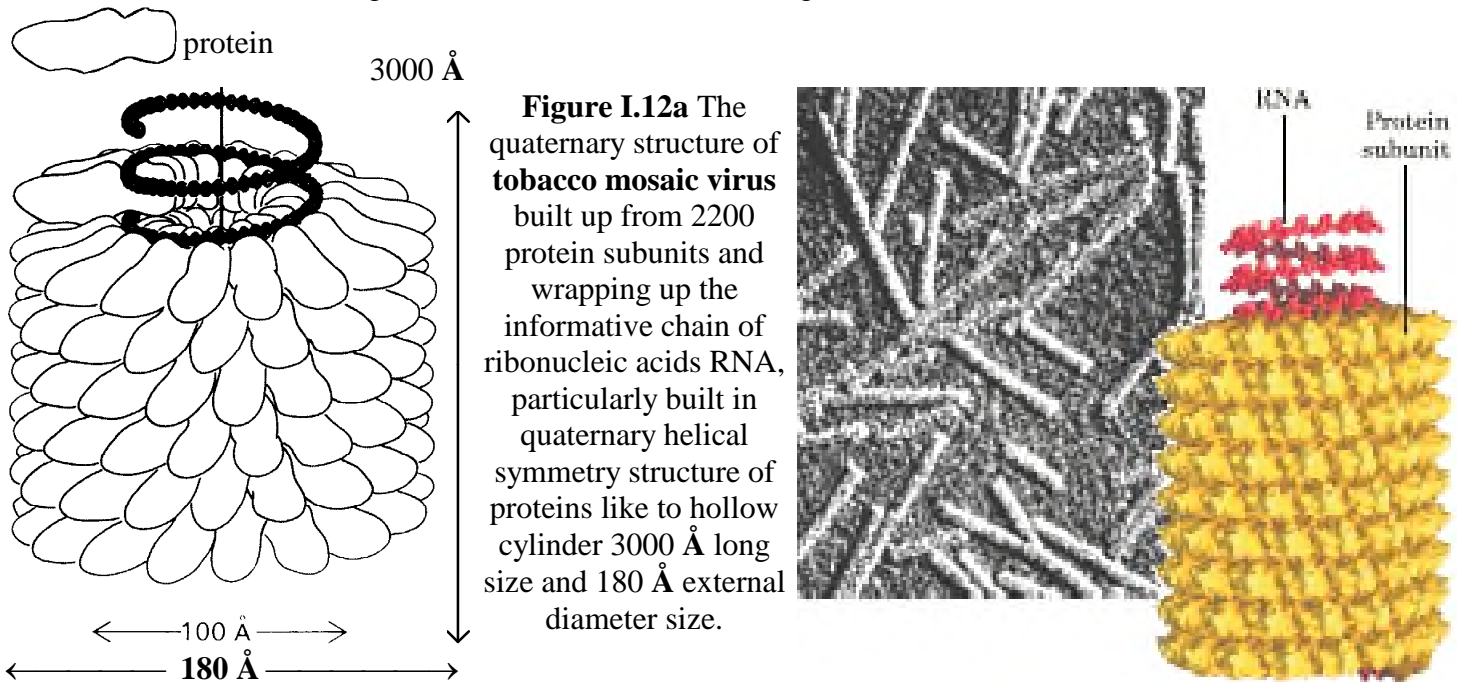


Figure I.12b The quaternary structure of **polio virus capsids** built up from protein subunits stuck in icosahedral rotational symmetry by 300 Å size and wrapping up the informative chain of ribonucleic acids RNA, particularly built in quaternary structure of proteins.

E. 1° Structure Determines 2°, 3° and 4° Structure

The primary structure of a protein is determined by information coded within genes. Once the primary structure of a polypeptide is established, the structure itself directs the folding of the polypeptide chain into a three-dimensional structure. In the other words, information inherent in the primary structure of a protein determines its secondary, tertiary and quaternary structures.

If three-dimensional shape of a polypeptide or protein is determined by its primary structure, how can we account for the observation that **denaturation** is irreversible for some proteins and not others?

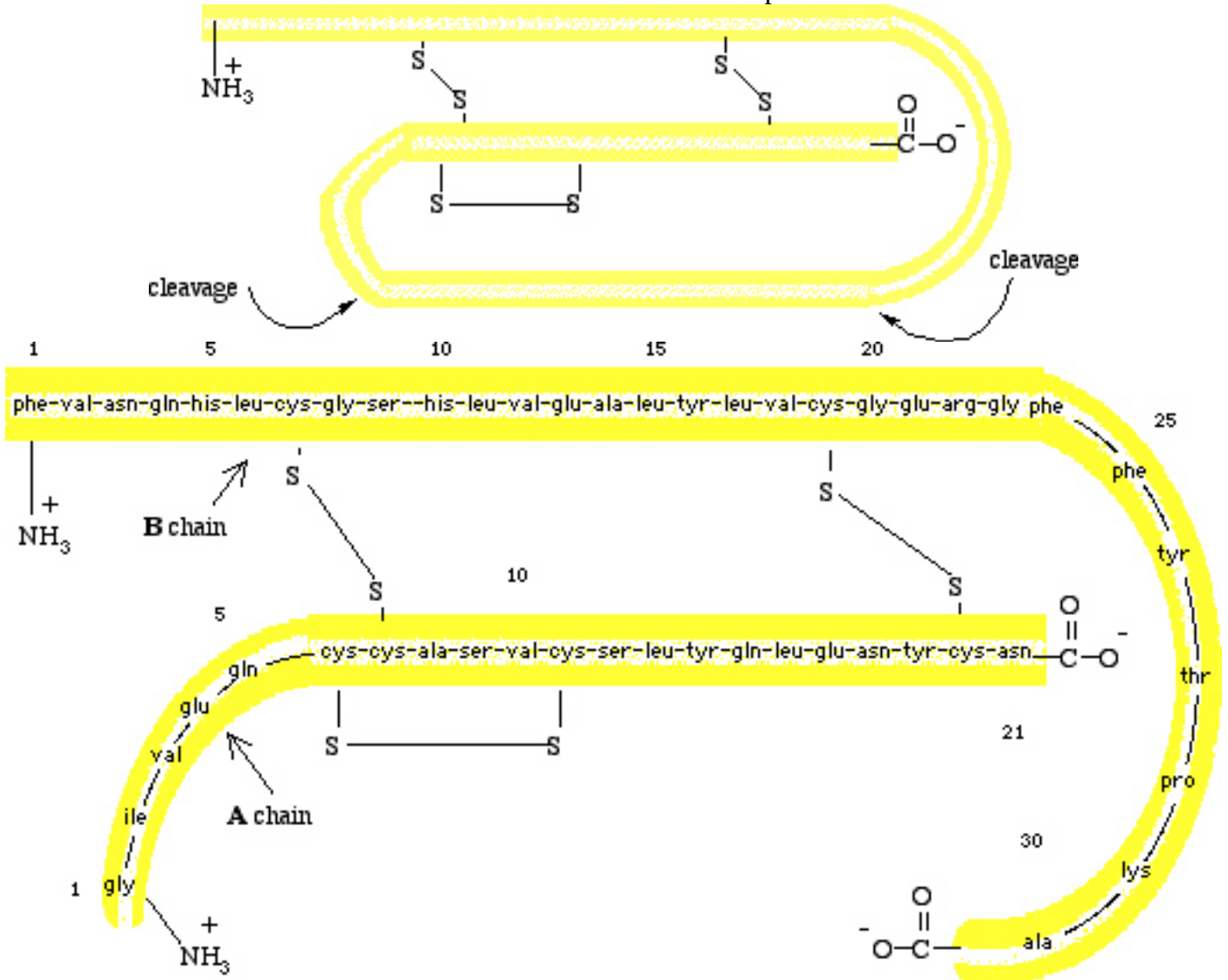


Figure I.13 (Top) A schematic diagram of pro insulin, a single polypeptide chain of 84 amino acids. (Bottom) The amino acid sequence of bovine insulin.

The reason for this difference in behavior from one protein to another is that some proteins, like ribonuclease, are synthesized as single polypeptide chains, which then fold into unique three-dimensional structures with full biological activity. Others, like insulin, are synthesized as larger molecules that are not biologically active at first but "activated" later by specific enzyme-catalyzed peptide-bond hydrolysis. Insulin is synthesized in the beta cells of the pancreas as a single polypeptide chain of 84 amino acids. This molecule, called pro insulin, has no biological activity. When insulin is needed, a section of 33 amino acids is hydrolyzed from pro insulin in an enzyme-catalyzed reaction to produce the active hormone (Figure I.13). Bovine insulin contains 51 amino acids in two polypeptide chains. The **A chain** contains 21 amino acids and has glycine (**Gly[G]**) at the —NH_3^+ terminus and asparagine (**Asn[N]**) at the —COO^- terminus. The **B chain** contains 30 amino acids with phenylalanine (**Phe[F]**) at the —NH_3^+ terminus and alanine (**Ala[A]**) at the —COO^- terminus.

Because the information directing the original folding of the single polypeptide chain of pro insulin is not present in the **A** and **B chains** of the active hormone, refolding of the denatured protein is irregular and denaturation is irreversible.

Zymogens are enzyme produced as inactive proteins, which are then activated by hydrolyze of one or more of the polypeptide bonds. The process of producing a protein in an inactive, storage form is common. For example, the digestive enzymes trypsin and chymotrypsin are produced in the pancreas as inactive proteins, named trypsinogen and chymotrypsinogen. There is a logical and simple reason for the synthesis of zymogens. In the case of trypsin and chymotrypsin, their function is to catalyze the hydrolysis of dietary proteins reaching the intestinal track. Proteins there are hydrolyzed to their component amino acids and then absorbed through the wall of the intestine into bloodstream. If trypsin and chymotrypsin were produced as active enzyme, they might well catalyze their own hydrolysis as well as that of other proteins in the pancreas-in effect, a "self-destruct" system. But nature has protected against this happening by synthesizing and storing zymogens instead.

F. Denaturation

Globular proteins found in living organisms are remarkably sensitive to changes in environment (pH, T, SAC, solvent composition, mechanical treatment, urea). Relatively small changes in pH, temperature or solvent composition, even for only a short period, may causes them to become **denatured**. **Denaturation** causes mechanical-physical activity. Except for cleavage of **disulfide** and **coordinative** bonds, **denaturation** stems from changes in **secondary**, **tertiary** or **quaternary** structure through disruption of non covalent intermolecular interaction forces, such as **hydrogen** bonds, **salt linkages** and **hydrophobic** bonds. Common **denaturing agents** include the following:

1. **Heat**. Most globular proteins become **denatured** when heated above $T > 50-60^{\circ} \text{C}$. For example, boiling or frying an egg causes egg-white and yolk proteins to become **denatured**, forming an insoluble mass.

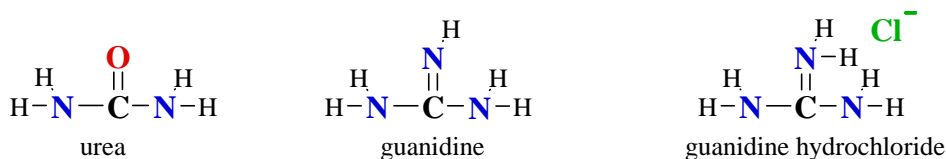
2. **Large change in pH**. Adding concentrated acid or alkali to a protein in aqueous solution causes changes in the charged character of ionizable side chains and interferes with **salt linkages**. For example, in certain clinical chemistry tests where it is necessary first to remove any protein material, trichloroacetic acid (a strong organic acid) is added to **denature** and **precipitate** any protein present.

3. **Detergents**. Treating a protein with surface active compounds (**SAC**) like as sodium dodecylsulfate (SDS), (See next lecture), a detergent, causes the native conformation to unfold and exposes the nonpolar protein side chains to the aqueous environment. These side chains are then stabilized by **hydrophobic** interaction with hydrocarbon chains of the detergent.

4. **Organic solvents** such as alcohols, acetone or ether.

5. **Mechanical treatment**. Most globular proteins are **denatured** in aqueous solution if they are stirred or shaken vigorously. An example is whipped egg whites to cook beze biscuits or pudding.

6. **Urea and guanidine hydrochloride** cause disruption of protein **hydrogen** bonding and **hydrophobic** interactions. Because urea is a small molecule with a high degree of polarity, it is very soluble in water. A solution of 8M urea (480 g urea/L of water) is commonly used to **denature** proteins. Guanidine is a derivative of urea in which $>\text{C}=\text{O}$ is replaced by $=\text{NH}$. Guanidine is a strong base and reacts with **HCl** and to form the salt guanidine hydrochloride.



Denaturation can be partial or complete. It can also be reversible or irreversible. For example, the hormone insulin can be **denatured** with 8M urea and then the three **disulfide bonds** $\text{Cys}-\text{S}-\text{S}-\text{Cys}$ reduced to six free $\text{Cys}-\text{SH}$ groups. If urea is then removed and the **disulfide bonds** re-formed by oxidation, the resulting molecule has less than 1% of its former biological activity. In this case, **denaturation** is both complete and irreversible. Consider another example, ribonuclease, an enzyme that consist of a single polypeptide chain of 124 amino acids folded into a compact, three-dimensional structure partly stabilized by four **disulfide bonds** $\text{Cys}-\text{S}-\text{S}-\text{Cys}$. Treatment of ribonuclease with urea causes the molecule to unfold, and the **disulfide bonds** can then be reduced to thiol groups $\text{Cys}-\text{SH}$. At this point, the protein is completely **denatured**-it has no biological activity. If urea is removed from solution and the thiol groups $\text{Cys}-\text{SH}$ reoxidized to **disulfide bonds** $\text{Cys}-\text{S}-\text{S}-\text{Cys}$, the protein regains its full biological activity. In this instance, **denaturation** has been complete but reversible.

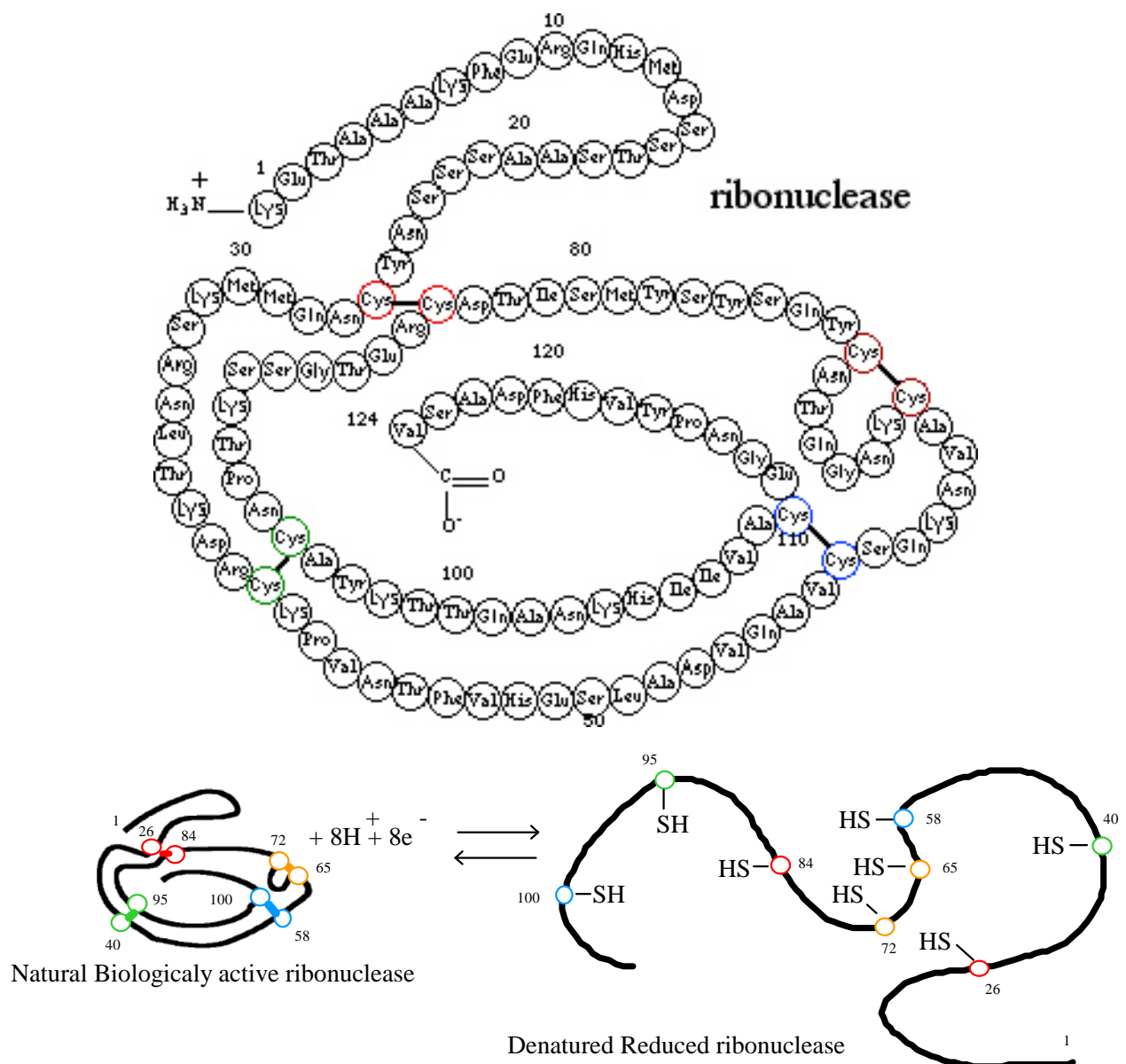


Figure I.13 Structure of biologically active enzyme: the ribonuclease.

Denaturation of ribonuclease by reducing agent and oxidation by oxidizing agent.

I.7 Fibrous Proteins

Fibrous proteins are stringy, physically tough macromolecules composed of rod like polypeptide chains joined together by several types of cross-linkages to form stable, insoluble structures. The two main classes of **fibrous proteins** are the keratins of skin, wool, claws, horn and feathers and the collagens of the tendon and hides.

A. The Alpha-Keratins

Hair and wool are very flexible and also elastic, so when tension is released, the **fibers** revert to their original length. At the molecular level, the fundamental structural unit of hair is a polypeptide wound into an **α -helix** conformation (Figure I.14). Several levels of structural organization are built from simple **α -helix**. First, three strands of **α -helix** are twisted together to form a larger cable called a **protofibril**. **Protofibrils** are then wound into bundles to form an 11-strand cable called a **micro fibril**. These, in turn, are embedded in a larger matrix that ultimately forms a hair **fiber** (Figure I.14). When hair is stretched, **hydrogen bonds** along turns of each **α -helix** are elongated. The main force causing stretched hair **fibers** to return to their original length is re-formation of **hydrogen bonds** in the **α -helices**.

The **α -keratins** of horns and claws have essentially the same structure as hair but with a much higher concentration of cysteine (**Cys**) and greater degree of **disulfide cross-linking** **Cys—S—S—Cys** between individual **helices**. These additional **disulfide bonds** **Cys—S—S—Cys** greatly increase resistance to stretching and produce the hard **keratins** of horn and claw.



Figure I.14 Detailed structure of a hair fiber.

B. Collagen Triple Helix

Collagens are constituents of skin, bone, teeth, blood vessels, tendons, cartilage and connective tissue. They are the most abundant protein in higher vertebrates and make up almost 30% of total body mass in humans. Table I.8 lists the **collagen** content of several tissues. Note that bone, the Achilles tendon, skin, and the cornea of the eye are largely **collagen**.

Table I.8		Collagen	
Collagen content	Tissue	(% Dry Weight)	Tissue
of some body tissues	bone, mineral-free	88	cartilage
	Achilles tendon	86	ligament
	skin	72	aorta
	cornea	68	46-63
			17
			12-24

Because **collagen** is abundant and widely distributed in vertebrates and because it is associated with a variety of diseases and problems of aging, more is known about this **fibrous** protein than about any other. **Collagen** molecules are very large and have a distinctive amino acid composition. One-third of all amino acids in **collagen** are glycine and another 21% are either hydroxylysine or hydroxyproline (See former amino acids). Both hydroxylated amino acids are formed after their parent amino acids (L-proline and L-lysine) and incorporated in **collagen** molecules. Because cysteine is almost entirely absent, there are no **disulfide cross-links** in **collagen**. When **collagen fibers** are boiled in water, they are converted to insoluble gelatins.

The polypeptide chains of **collagen** fold into a conformation that is particularly stable and unique to **collagen**. In this conformation, three protein strands wrap around each other to form a left-handed **super helix** called the **collagen triple helix**. This unit, called **tropocollagen**, looks much like a **three-stranded rope** (Figure I.15).

The hydroxyl groups of hydroxyproline and hydroxylysine residues help to maintain the **triple helix** structure by forming **hydrogen bonds** $>C=O \cdots H-N<$ between adjacent chains. **Fibers** in which proline and lysine groups have not been hydroxylated are far less stable than **fibers** in which these groups have been hydroxylated. one of the important functions of vitamin C is in hydroxylation of **collagen**. Without adequate supplies of vitamin C, **collagen** metabolism is impaired, giving rise to scurvy, a condition in which **tropocollagen fibers** do not form stable physically tough **fibers**. Scurvy produces skin lesions, fragile blood vessels and bleeding gums.

Collagen fibers are formed when many **tropocollagen** molecules line up side by side in regular pattern and are then **cross-linked** by newly formed covalent **cross-bonds**. Most covalent **cross-linking** involves the side chains of lysines. The extent and type of **cross-linking** vary with age and physiological condition. For example, the **collagen** of rat Achilles tendon is highly **cross-linked** and **collagen** of the more flexible tendon of rat tail is less highly **cross-linked**. Further, it is not clear when, if ever, the process of **cross-linking** is completed. Some believe it continues throughout life, producing increasingly stiffer skin, blood vessels and other tissues, which then contribute to the medical problems of aging and the aged, making more fragile bones and tissues.

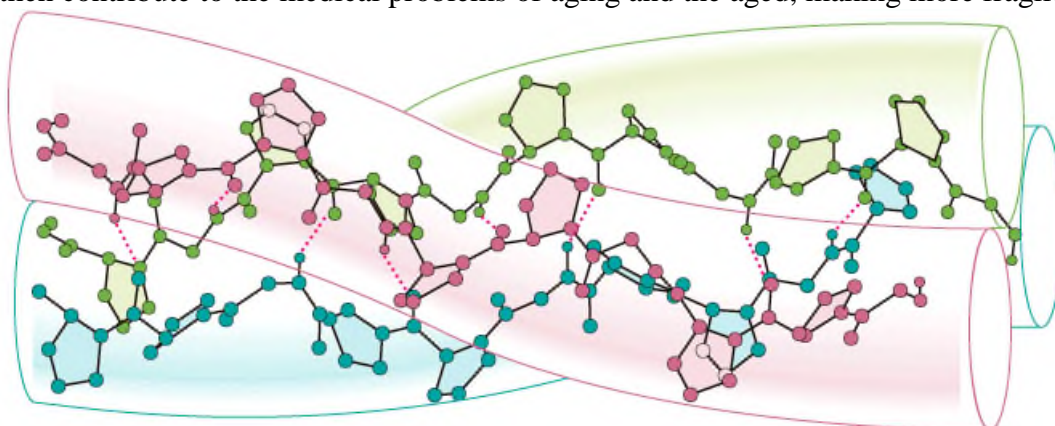


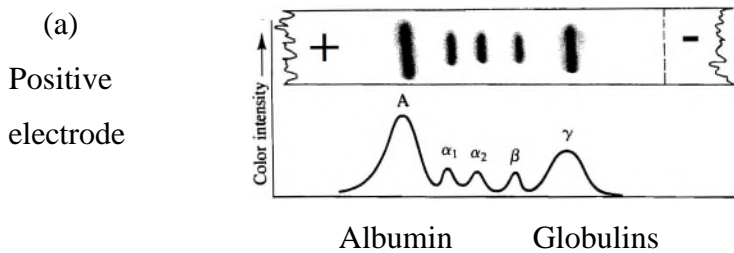
Figure I.15 Collagen triple helix.

I.8 Plasma Proteins: Examples of Globular Proteins

Human blood consists of a fluid water (90-92%) solution portion (plasma- various inorganic ions and a heterogeneous mixture of organic molecules) and cellular components. The cellular components, which make up 40-50% of volume of whole blood, consist of red blood cells (erythrocytes), white blood cells (leukocytes) and blood platelets (thrombocytes). **Electrophoresis** is the method of separating **proteins** of biological fluids into fractions in human plasma, urine and cerebrospinal fluid.

A sample of plasma is applied as a narrow line to a cellulose acetate strip. The ends of the strip are then immersed in a buffer of pH 8.8 and a voltage is applied to the strip. At pH 8.8, **plasma proteins** have net negative charges and migrate toward the positive electrode. The **protein** in spot is determined as peak.

Proteins move in this direction ← Origin



Negative electrode

Figure Separation of serum **proteins** by **electrophoresis**.

(a) A sample is applied as a narrow line at the origin. After **electrophoresis** at pH 8.8,

(b)

the paper is dried and stained. (b) A plot of peaks of each spot.

Table	Fraction	(g/L)	%	
	albumin	35-50	52-67	One of the albumins functions is to regulate the osmotic pressure in blood. Albumin is seven 7 fatty acids and certain drugs such as aspirin and warfarin transporter through blood circulation in human organism.
globulins	α_1	1-4	2.5-4.5	
human serum	α_2	5-11	6.6-13.6	
proteins	β	6-12	9.1-14.7	
electrophoresis.	γ	05-15	9.0-21.6	

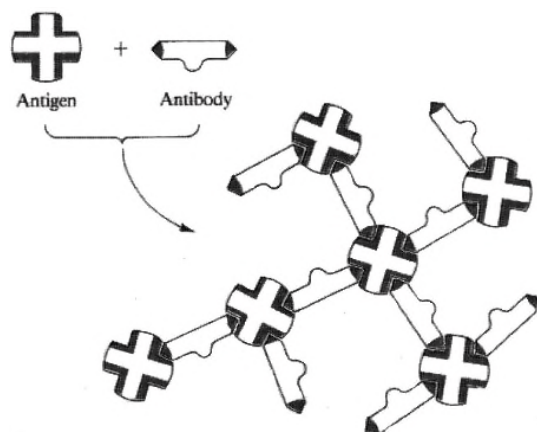
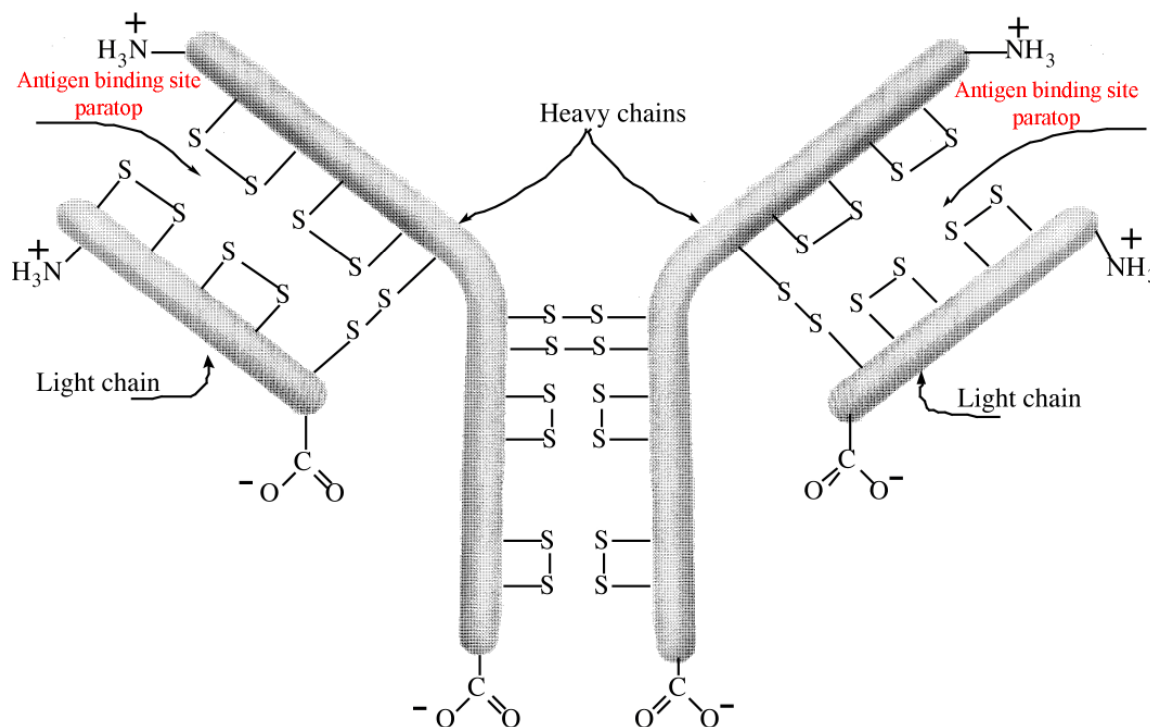
The α_1 and α_2 fractions (lipoprotein vesicles and lipocalins) transport fats, cholesterol, phospholipids and lipocalins load and unload steroids as well vitamins K, E, D, A along movement.

The four **globulin** fractions are arbitrarily designated α_1 , α_2 , β and γ according to their **electrophoretic** mobility. Serum **albumin** has an isoelectric point **pH 5** and migrates farthest toward the positive electrode. **Gamma-globulin (immunoglobulin)** has an isoelectric point **pH 7.9** and migrates the shortest distance.

The α_1 fraction also contains antitrypsin, a protein that inhibits the protein-digesting enzyme trypsin. The α_2 fraction contains **haptoglobulin**, which binds any **hemoglobin** released from destroyed red blood cells and **ceruloplasmin**, the principal

copper-containing **protein** of the body. The α_2 fraction also contains **prothrombin**, an inactive form of the blood-clotting enzyme thrombin. The β fraction contains a variety of specific transport **proteins**, as well as substances involved in blood clotting.

The γ -globulin fraction **antibodies (immunoglobulin)**, whose function is to combat **antigens** (non host **proteins**) introduced into the host body. The response is the basis for immunization against such infectious diseases (poliomyelitis, tetanus and diphtheria etc.). An **antibody** is quaternary structure of two **heavy** (high-molecular-weight) and two **light** (low-molecular-weight) polypeptide chains held together by four **disulfide bonds** Cys—S—S—Cys. Each **antibody** has two identical binding sites **paratops** that react with specific **antigen** to form an insoluble complex called **precipitin** and binding it remove following breakdown by white blood cells (leucocytes-macrophages).



Four protein subunit chains projection of the quaternary structure **antibody**. The interaction between **antibody** and its specific **antigen** to form an inactive **precipitin** complex. The precipitated **antigen-antibody** complex is then ingested and broken down with blood cells. **Precipitin** complex (insoluble) building of antibody immunoglobulin IgG1 for antigenic bodies binding in plasma.

<http://aris.gusc.lv/ChemFiles/ChimAntibodyMarz/2frmcont.htm>