Properties of Amino Acids

Specific learning objectives

- Properties of amino acids.
- Structure organization of the Proteins:

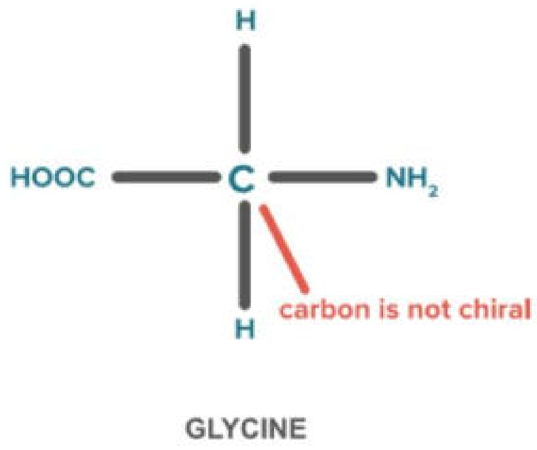
1) Primary structure
2) Secondary Structure
3) Tertiary Structure
4) Quaternary Structure

Properties of Amino Acids

Amino Acids have an Asymmetric Center

- Optically active molecules have an asymmetry such that they are not superimposable on their mirror image.

- Cα atoms of all aa are asymmetric centers and optically active except glycine, in which R=H two of the four substituents on the α-carbon atoms are hydrogen.



- ~~Cα is a chiral center, this carbon atom is attached to four different groups.~~

Absolute Configuration of Amino Acids

- All α-amino acids (aa) derived from proteins have the L-stereochemical configuration.
- They all have the same relative configuration about their Cα atoms.
- If the NH_3^+ projected to the left, the aa has L (Levo) absolute configuration.
- NH_3^+ projected to the right, the aa has D (Dextro) absolute configuration.

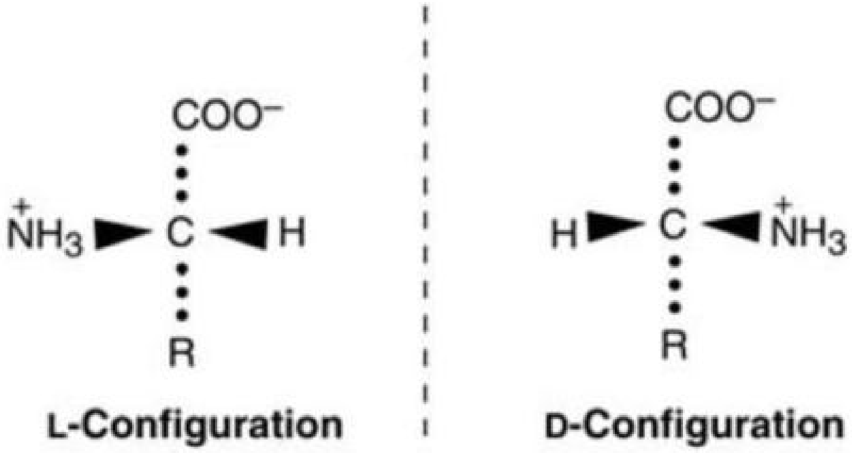


Fig. 2.7. Textbook of Biochemistry with Clinical Correlations, 4th edition by Thomas M Devlin

Acid-Base Properties of Amino Acids

- Amino acids in aqueous solution, contain weakly acidic α-carboxyl groups and weakly basic α-amino groups.

- Charged and uncharged form of the ionizable weak acid groups –COOH and $-NH_3^+$ exist in protonic equilibrium:

$$R\text{-}COOH \rightleftharpoons R\text{-}COO^- + H^+$$

$$R\text{-}NH_3^+ \rightleftharpoons R\text{-}NH_2^+ + H^+$$

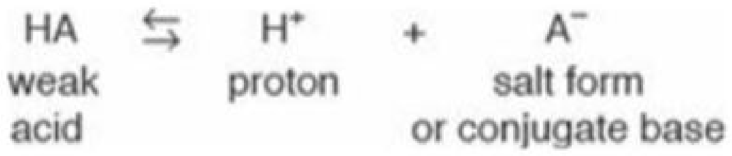
- Both R-COOH and $R\text{-}NH_3^+$ are weak acids. R-COOH are stronger acid than $R\text{-}NH_3^+$

- At physiological pH, carboxyl group exists as $R\text{-}COO^-$ and amino group as $R\text{-}NH_3^+$

Henderson-Hasselbalch equation: Quantitative relationship between pH and concentration of a weak acid (HA) and its conjugate base (A-).

Derivation of Henderson-Hasselbalch equation: Consider the release of a proton by a weak acid represented by HA:

The "salt" or conjugate base (A–) is the ionized form of a weak acid.



$$\underset{\text{weak acid}}{HA} \leftrightarrows \underset{\text{proton}}{H^+} + \underset{\text{salt form or conjugate base}}{A^-}$$

- Dissociation constant of the acid, Ka, is: $K_a = \frac{[H^+][A^-]}{[HA]}$ OR $[H^+] = \frac{K_a[HA]}{[A^-]}$

- By taking the negative logarithm of both sides: $-\log[H^+] = -\log K_a + \log\frac{[A^-]}{[HA]}$

- Substituting pH = -log[H+] and pK_a = -log K_a obtain Henderson-Hasselbalch equation:

$$pH = pK_a + \log\frac{[A^-]}{[HA]} = pK_a + \log\frac{[BASE]}{[ACID]}$$

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- Larger the K_a, the stronger the acid, because most of the HA has dissociated into H+ and A–.

- Conversely, the smaller the K_a, the less acid has dissociated and, therefore, the weaker the acid.

- pKa values for a particular molecule are determined by titration.

Titration of an Amino Acids

Dissociation of the carboxyl group of Alanine:

- At a low (acidic) pH, both of ionizable α-carboxyl and α-amino group are protonated.
- As pH of solution raised, – COOH group of **form I** can dissociate by donating a proton to the medium.
- Release of a proton results in the formation of carboxylate group, – COO– of **form II**, which is the dipolar form of the Alanine.

Ionic forms of alanine in acidic, neutral, and basic solutions

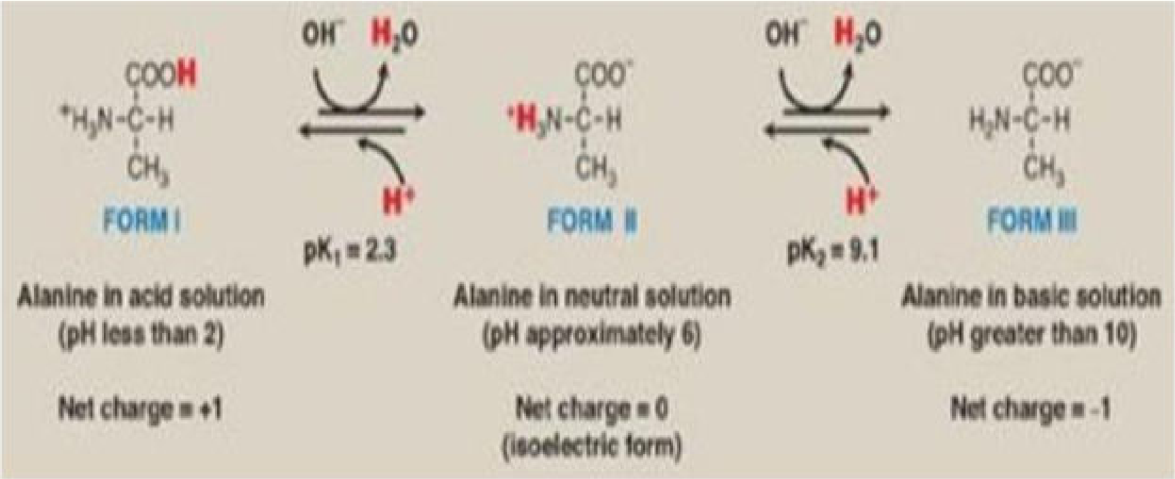

Fig. 1.10: Lippincott's illustrated reviews- Biochemistry 6th edition

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Dissociation of the amino group:

- Second titratable group of alanine is amino (– NH3+) group.
- This is a much weaker acid than the – COOH group and, i.e, has a much smaller dissociation constant, K_2.
- Release of a proton from protonated amino group of form II results in fully deprotonated form of alanine, form III.

Titration Curve of Alanine

- pKa for acidic group (–COOH) is pK_1 is 2.35, whereas pKa for next acidic group (– NH3+) is pK_2 is 9.69.

- Isoelectric pH (pI) of Alanine is:

$$\mathrm{pI} = \frac{\mathrm{p}K_1 + \mathrm{p}K_2}{2} = \frac{2.35 + 9.69}{2} = 6.02$$

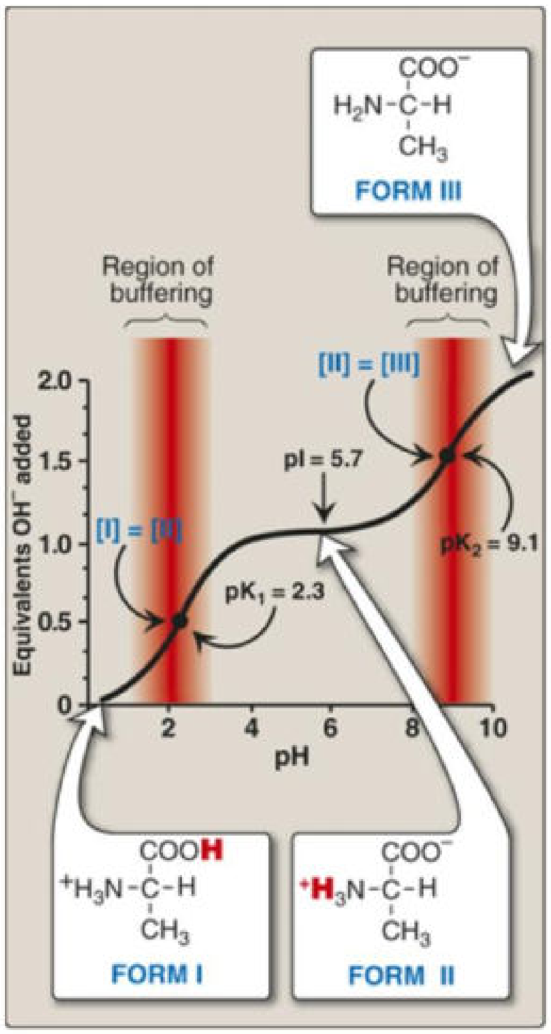

Fig. 1.11: Lippincott's illustrated reviews- Biochemistry 6th edition

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- In clinical laboratory, knowledge of the isoelectric pH guides, selection of conditions for electrophoretic separations.

- For example, two simple aa (with one COOH and one NH3+ group) separated by electrophoresis either at an acidic or basic pH that exploits subtle differences in net charge based on subtle differences in pK_1 or pK_2 values.

Ultraviolet Spectra of Tyr, Phe andTrp

- Amino acids do not absorb visible light.
- Tyr, Phe, and Trp absorb high-wavelength (250-290nm) UV light.
- Absorption of light at 280nm used to estimate the concentration of a protein in solution, if the number of Trp and Tyr residues in the protein is known.

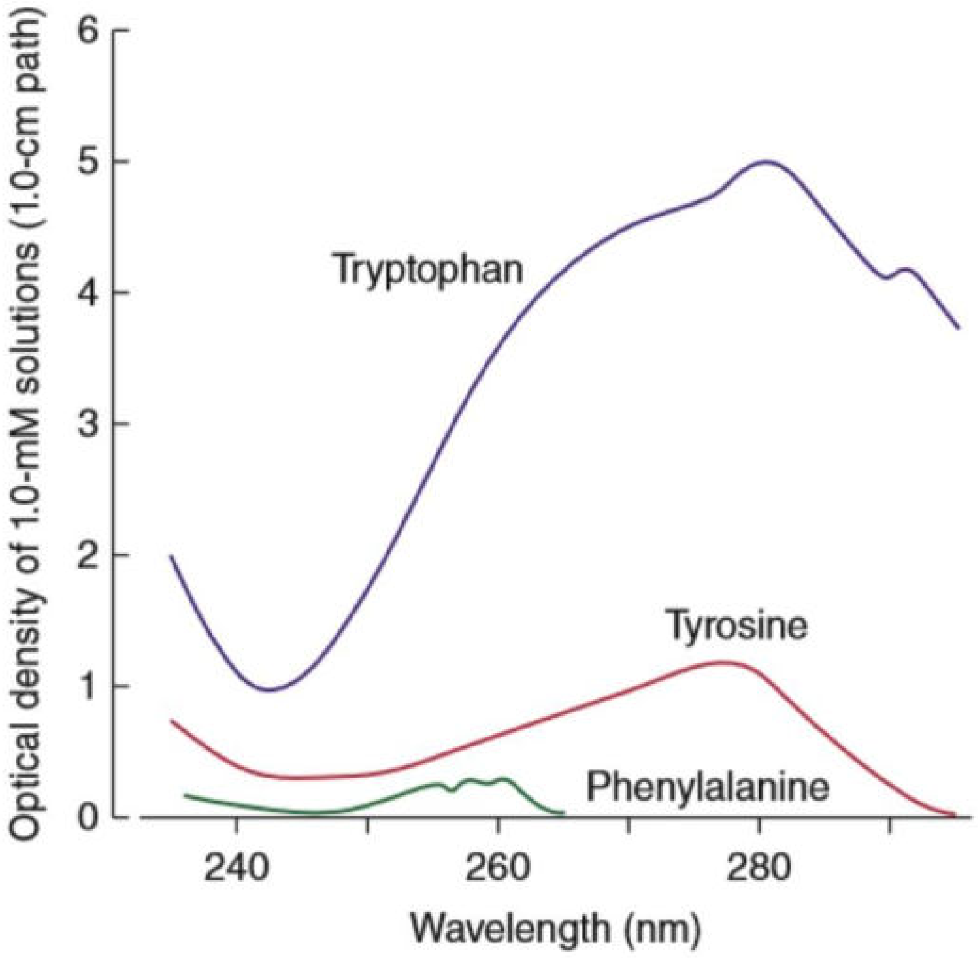

Fig.3.7. Harper's Illustrated Biochemistry 30th edition

Structural organization of Proteins

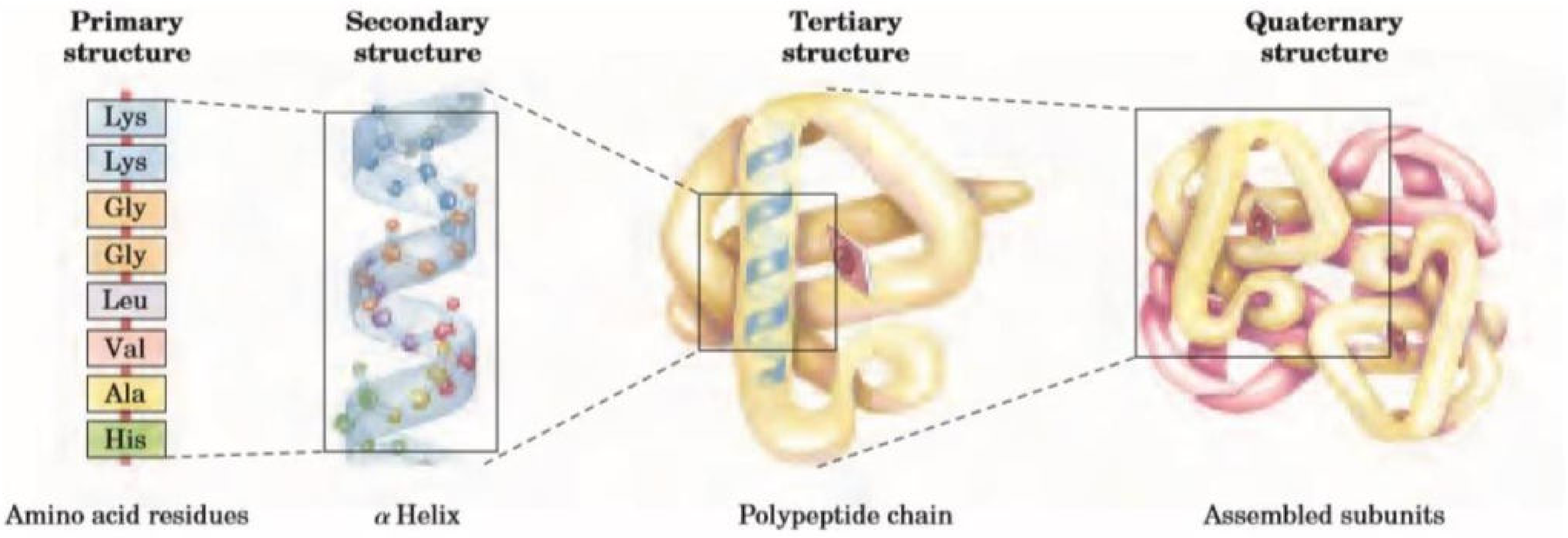

FIGURE 3-16 Levels of structure in proteins. The *primary structure* consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. The resulting polypeptide can be coiled into units of *secondary structure,* such as an α helix. The helix is a part of the *tertiary structure* of the folded polypeptide, which is itself one of the subunits that make up the *quaternary structure* of the multisubunit protein, in this case hemoglobin.

Fig.3.16. Lehninger Principles of Biochemistry

Primary Structure of Proteins

Primary Structure

- Amino acids are linked by peptide bonds to form polypeptide chains.

- Ordered sequence of aa in a polypeptide chains is the primary structure of the protein.

- It is the unique primary structure that enables a polypeptide chain to fold into a specific 3-D structure that gives the protein its chemical and physiological properties.

Amino Acids are Polymerized into Peptides and Proteins

- α-carboxyl group of an aa with side chain R1 forms a covalent peptide bond with α-amino group of the aa with side chain R2 by elimination of a molecule of H_2O.

- Dipeptide (contain two aa bonded to each other via a single peptide bond).

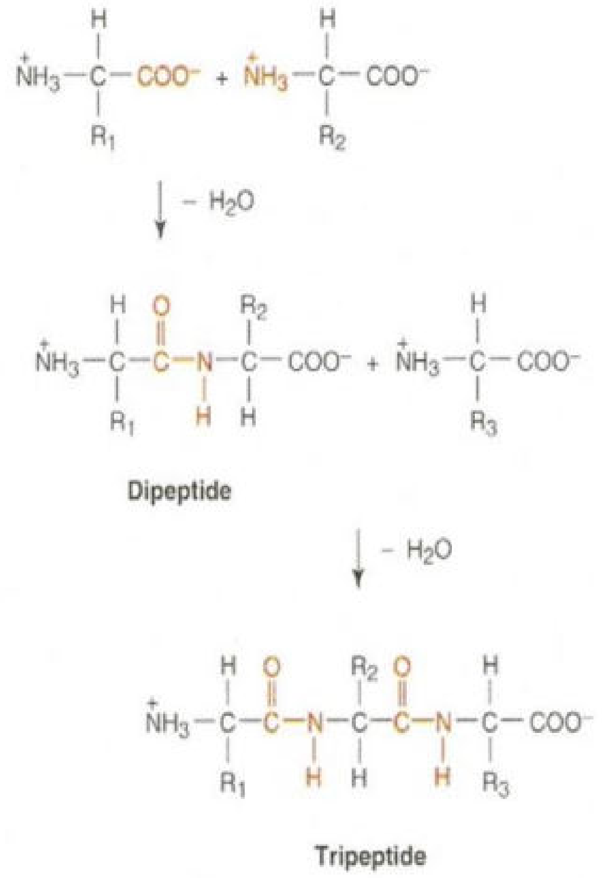

Fig. 2.8. Peptide bond formation:
Textbook of Biochemistry with Clinical Correlations
4th edition by Thomas M Devlin

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- Tripeptides (contains three aa) form a second peptide bond through its terminal carboxylic acid group and the a amino of a third amino acid (R3).

- Repetition of this process form a polypeptide or protein of specific aa sequence (R1R,2R,3R4·· ·Rn).

Components of a Polypeptide Chain

- It consists of a repeating part, backbone, and a variable part, comprising the distinctive side chains.
- Polypeptide backbone is rich in hydrogen-bonding potential. Each residue contains a carbonyl group, and, with the exception of proline, an NH group.
- Mass of a protein expressed in units of daltons; one Dalton (Da) is equal to one atomic mass unit.

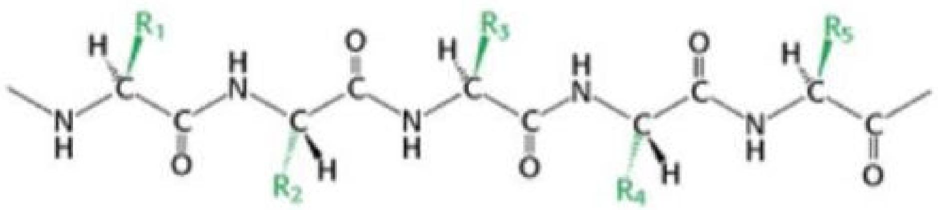
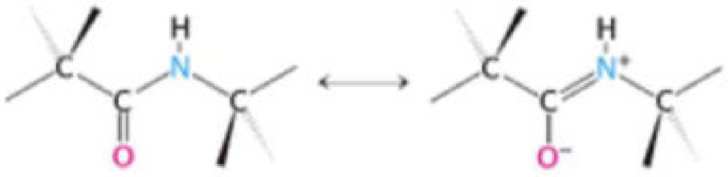


Fig.2.15: Components of a polypeptide chain: Biochemistry 7th edition by Berg, Tymoczko and Stryer

Peptide Bond has Partial Double-Bond Character

- Single bond linked the α-carboxyl and α-nitrogen atoms, this **peptide bond exhibits partial double-bond character**.
- Bond that connects a carbonyl carbon to an α-nitrogen cannot rotate and thus conformation of the peptide backbone is constrained.
- Almost all **peptide bonds in proteins are trans**.
- The O, C, N, and H atoms of a **peptide bond are coplanar.**



Peptide bond resonance structures

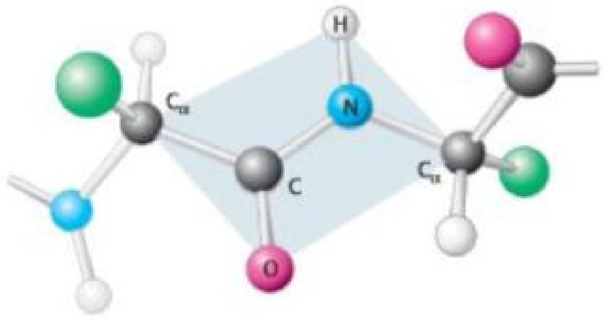


Fig.2.18: Peptide bonds are planar: Biochemistry 7th edition by Berg, Tymoczko and Stryer

Rotation about Bonds in a Polypeptide

- Structure of each aa in a polypeptide adjusted by rotation about two single bonds.
- Phi (ϕ) is angle of rotation about bond between N-C_{α} bond.
- psi (ψ) is angle of rotation about bond between C_{α}-C bond.
- Freedom of rotation about two bonds of each aa allows proteins to fold in many ways.

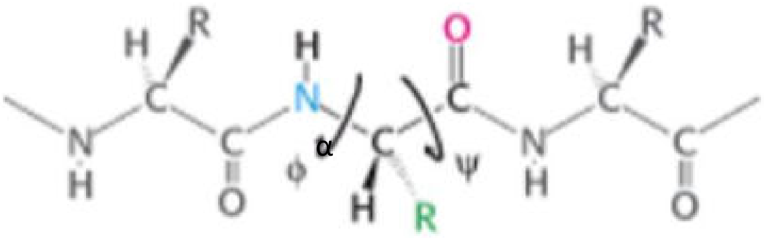


Fig.2.22: Biochemistry 7th edition by Berg, Tymoczko and Stryer

Ramachandran Plot

- In principle, values of ϕ and ψ bet. -180° and +180°, but many values are prohibited by steric interference between atoms in the polypeptide backbone and aa side chains
- Allowed/favorable regions with no steric overlap are shown in dark green; borderline regions are shown in light green.
- In peptides, for aa other than glycine, most combinations of ϕ and ψ angles are disallowed because of steric hindrance.

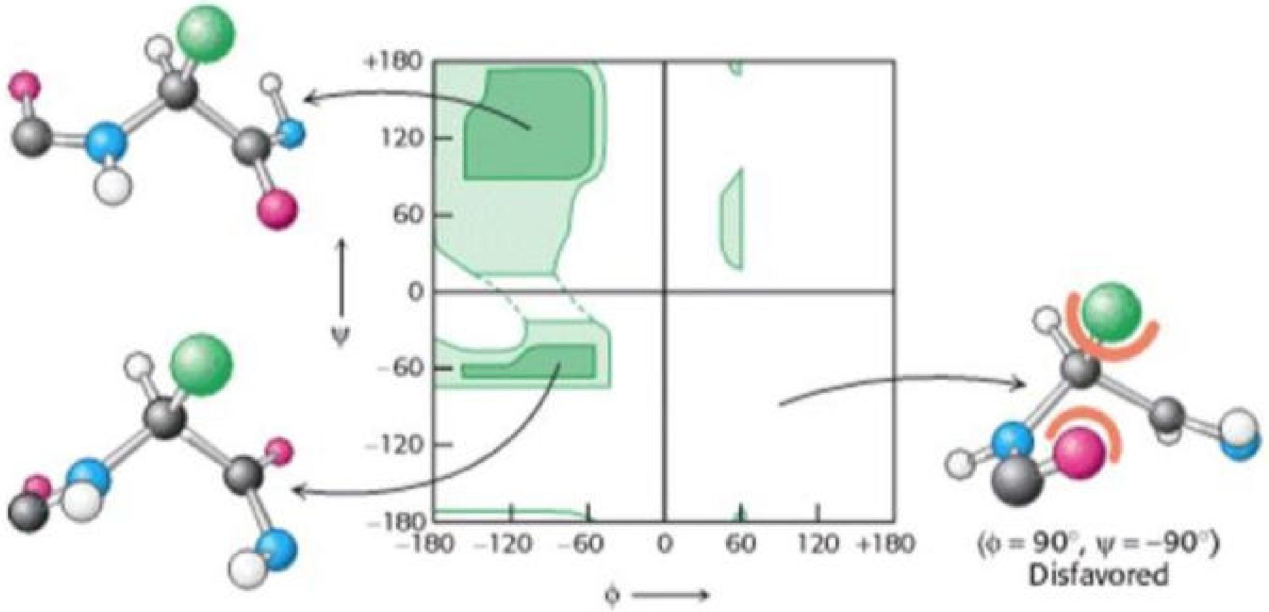


Fig.2.23: Biochemistry 7th edition by Berg, Tymoczko and Stryer

Amino Acid Sequences have Direction

- A polypeptide chain has polarity because its ends are different: an α-amino group is present at one end and an α-carboxyl group at the other.

- Amino end is taken to be the beginning of a polypeptide chain, and so the sequence of aa in a polypeptide chain is written starting with the amino-terminal residue.

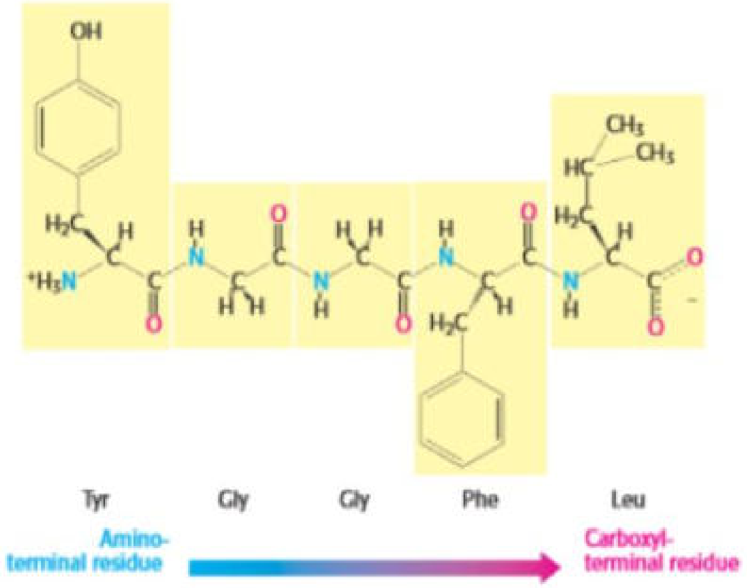


Fig.2.14: Amino acid sequences have direction: Biochemistry 7th edition by Berg, Tymoczko and Stryer

Cross-Links: Formation of a Disulfide Bond

- In some proteins, the linear polypeptide chain is cross-linked.

- The most common cross-links are disulfide bonds, formed by the oxidation of a pair of cysteine residues.

- The resulting unit of two linked cysteines is called cystine.

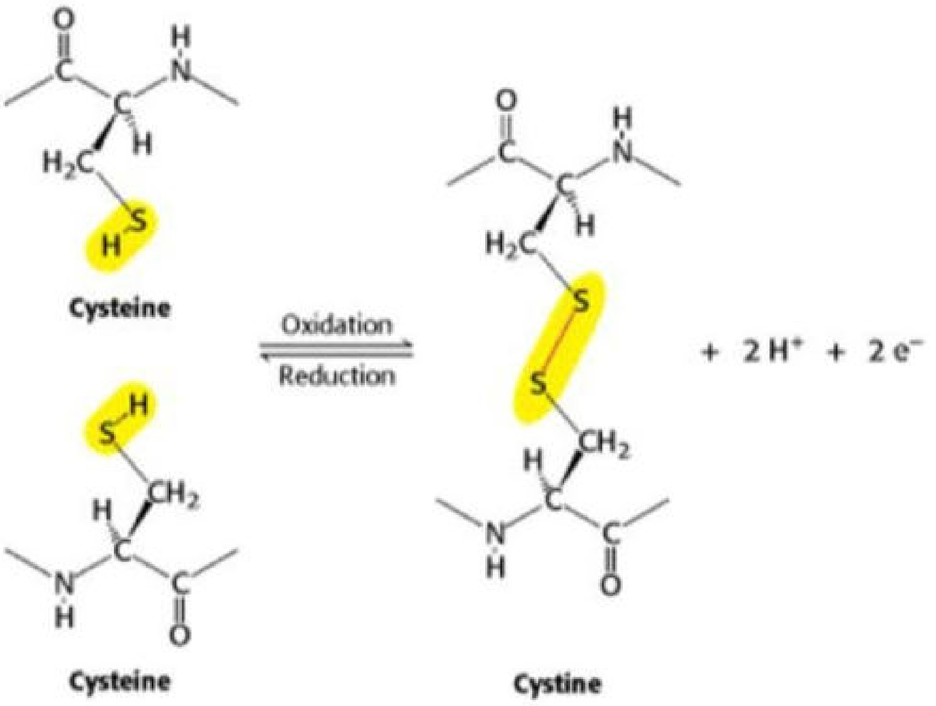


Fig.2.16: Cross-links: Biochemistry 7th edition by Berg, Tymoczko and Stryer

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- Extracellular proteins often have several disulfide bonds, whereas intracellular proteins lack them.
- For ex., collagen fibers in connective tissue and fibrin blood clots.

Summary

- All aa possess at least two weakly acidic functional groups, $R\text{-}NH_{3^+}$ and R-COOH.
- Many also possess additional weakly acidic functional groups such as phenolic -OH, -SH, guanidino, or imidazole moieties.
- The pKa values of all functional groups of an aa or of a peptide dictate its net charge at a given pH.
- pI, the isoelectric pH, is the pH at which an aa bears no net charge, and does not move in a direct current electrical field.

Cont--

- The most important element of primary structure is the sequence of amino acid residues.
- Nature of the covalent bonds in the polypeptide backbone places constraints on structure.
- Peptide bond has a partial double bond character that keeps the entire peptide group in a rigid planar configuration.
- The N-C_α and C_α-C bonds can rotate to assume bond angles of ϕ and ψ, respectively.

Group Discussion

- Subtopics of previous class discussed in groups.

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Reference Books

1) Harper's Illustrated Biochemistry-30th edition.
2) Textbook of Biochemistry with Clinical Correlations. 4th edition. Thomas M. Devlin.
3) Biochemistry. 4th edition. Donald Voet and Judith G. Voet.
4) Biochemistry 7th edition by Jeremy M. Berg, John L. Tymoczko and Lubert Stryer.
5) Lehninger Principles of Biochemistry.

Thank you

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