

Biochemistry

Amino acids

Orders of protein structure: -

There are four levels of protein structure: -

A.Primary structure: -

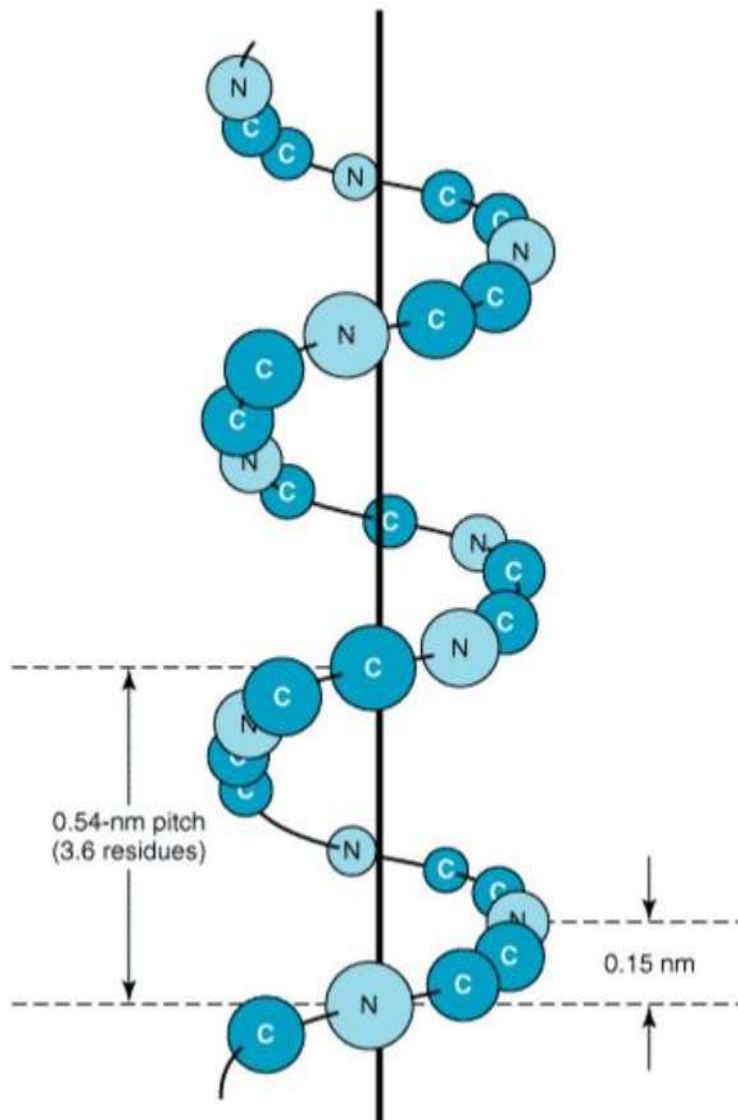
Primary structure refers to the order of the amino acids in the polypeptide and to the location of disulfide bonds if these are present, a description of all covalent bonds (mainly peptide bonds and disulfide bonds).

B.Secondary structure: -

Secondary structure refers to stable arrangements of amino acids residues in linear polypeptide chain depending on the nature of the R – group present, one of the most common structures is called α – helix.

α - helix

1. α – helix is a right – handed helix or rod with 3.6 amino acids residues per turn.
2. α – helix has a pitch of $5.4^\circ\text{\AA}$; that is for each turn, the helix rises $5.4^\circ\text{\AA}$ along the axis.
3. α – helix is stabilized by intrachain hydrogen bonds between $\text{C}=\text{O}$ of each peptide bond and the NH of the peptide bond four residues away



From this we can calculate the length of α – helix, for example: for 78 amino acid polypeptide:

$$\frac{5.4}{3.6} = \frac{1.5^\circ A}{1 \text{ residue}} = \frac{\text{lenght}}{78}$$

$$1.5^\circ A \times 78 = 117^\circ A$$

Note: if the H is called atom number 1 the hydrogen bonded oxygen is the 13th atom along the chain, thus the coil is designated a **3.6₁₃ helix**.

The α – helix is prevented from forming by: -

1. Two or more consecutive residue with like charges (e.g. lysine, glutamic acid).
2. Two or more consecutive residue with bulky R – group like Ile, Thr, Val.
 - In these cases the polypeptides chain may assume a random coil structure.
3. Proline also prevent forming α – helix because the nitrogen atom is a rigid ring. Thus, **no rotation** about α – carbon is possible. Also there is no hydrogen atom on the nitrogen of a Proline residue, so no intrachain hydrogen bonds can form.
4. Successive serine residues disrupt the α – helix because of the tendency of the OH group of serine to make hydrogen bond strongly with water.

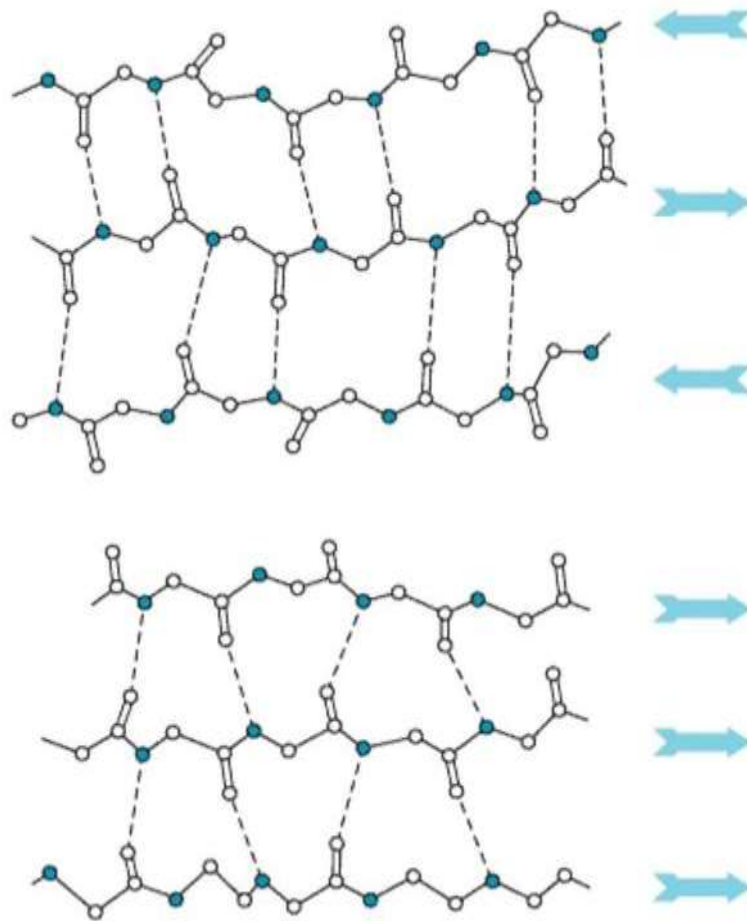
Note: stretches of Pro & Ser coil the polypeptide chain into random coil arrangements other than an α – helix.

β - sheet

β - sheet repeating sequence of amino acid with small compact R - group (like Gly, Ala) tend to form β or pleated sheet.

1. β - sheet consist of parallel or anti - parallel polypeptide chain
2. β - sheet is stabilized by interchain hydrogen bonds
3. β - sheet has a pitch $6.95^\circ\text{\AA}$ with two residue per turn

An example of antiparallel sheet is ***silk protein***.

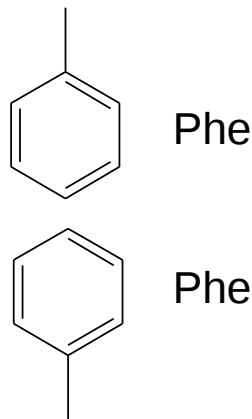


Copyright ©2006 by The McGraw-Hill Companies, Inc.
All rights reserved.

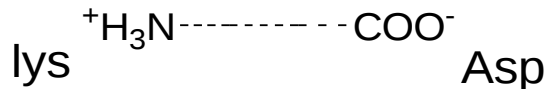
C. Tertiary structure: most non fibrous protein have a very precise and compact three dimensional formed when the α – helix & random coil of the polypeptide chain bends, twists & fold over & backup on itself.

Tertiary structure is stabilized by interactions of amino acids R – groups: -

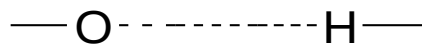
1. Covalent disulfide bond $[-S-S-]$.
2. Hydrophobic interaction



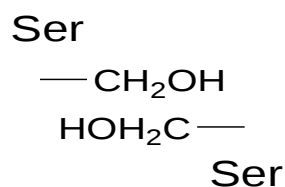
3. Ionic interaction (salt bridges or salt linkages)



4. Hydrogen bond



5. Dipole – dipole interactions



The biochemical function of protein is tied to its tertiary structure, in other words that is the protein to function in a certain way, must have a correct tertiary structure.

D.Quaternary structure: many proteins still have another order of structural complexity which is quaternary structure. This structure is formed by the ***non covalent association*** of tertiary structured units, in other words:

When a protein has two or more polypeptide subunits, their arrangement in space is referred to as quaternary structure which shows full activity for protein.

Example: lactate dehydrogenase (LDH) is a tetramer enzyme composed of two kinds of subunits called ***M & H***.

There are five possible isoenzymes of LDH:

HHHH, HHHM, HHMM, HMMM, MMMM

HHHH → found in heart

$\left\{ \begin{array}{l} \text{HHHM} \\ \text{HHMM} \\ \text{HMMM} \end{array} \right\}$	$\longrightarrow$	Other tissue, contain hybrid isoenzyme containing both M & H subunits
---	-------------------	--

MMMM → found in skeletal muscle

Q₁: Compare between α – helix & β – sheet.

Example: phosphorylase enzyme which contains two identical subunits that alone inactive but when joined as a dimer form the active enzyme.

The forces that stabilize the aggregation in structure are hydrogen bonds & electrostatic interaction formed between residues on the surfaces of the polypeptide chains.

During denaturation of protein by reagent like urea (detergent) or sodium dodecyl sulphate (SDS), the hydrogen bond, hydrophobic, electrostatic bonds are broken but not peptide or disulfide bonds.

- Reversible → return to its active form
- Irreversible → cannot return and coagulate.

Q₂: Why only α – amino group and α – carboxyl group involved in peptide bond?

Answer: Because only these groups are responsible for rotation.

Example: Insulin

This hormone consists of two polypeptide chains linked covalently by disulfide bonds {the figure in the next page}.

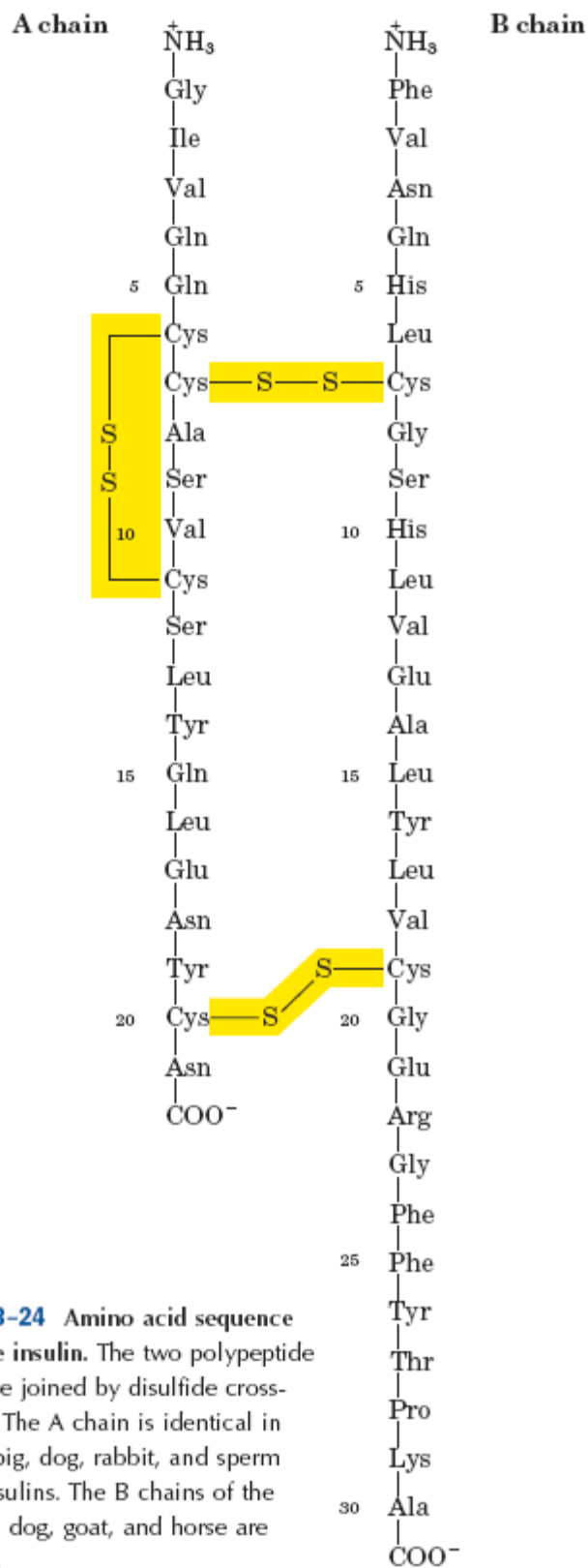


FIGURE 3-24 Amino acid sequence of bovine insulin. The two polypeptide chains are joined by disulfide cross-linkages. The A chain is identical in human, pig, dog, rabbit, and sperm whale insulins. The B chains of the cow, pig, dog, goat, and horse are identical.

Biosynthesis of insulin: -

Proinsulin formed first which is single polypeptide chain, and then this proinsulin undergoes proteolytic process to form active insulin.

The sequence of amino acid in the polypeptide chain can be established by selective chemical and enzymatic cleavage of the protein followed by separation and amino acid analysis and sequence determination of all peptide fragments.

The entire amino acid sequence is established by overlapping identical regions of the individual fragments.

Problems:

1. Partial hydrolysis of protein yield a number of polypeptides, one of them was purified. Declare the sequence of amino acid in this polypeptide from the following information: -
 - a. Complete acid hydrolysis yield:
$$\text{Ala} + \text{Arg} + 2\text{Ser} + \text{Lys} + \text{Phe} + \text{Met} + \text{Trp} + \text{Pro}$$
 - b. Treatment with fluorodinitrobenzene (FDNB), [Sanger reagent] yield dinitrophenylalanine (DNP – ala) and (E – DNP – Lys).
 - c. Neither carboxypeptidase A nor carboxypeptidase B released C – terminal amino acid.
 - d. Treatment with cyanogen bromide (CNBr) yields two peptide one contained the {Ser + Trp + Pro} and the other one contained the remaining amino acid (including the second Ser).

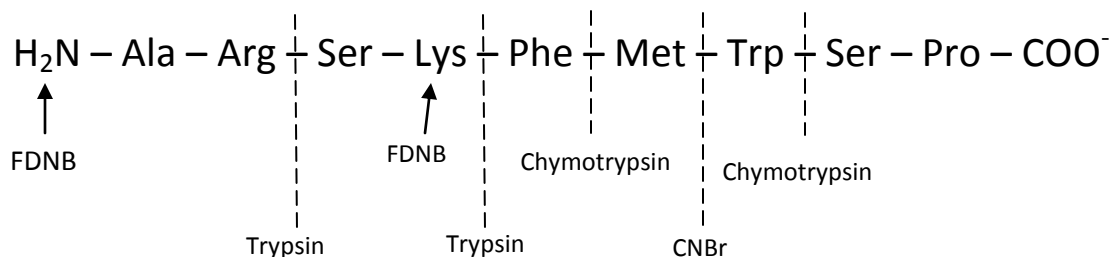
- e. Treatment with chymotrypsin yield three peptides one of them contain only → Ser + Pro, another contained only → Met + Trp, the third contained → Phe + Ser + Lys + Arg + Ala.
- f. Treatment with trypsin yielded three peptides, one contained only → Ala, Arg, and another contained only → Lys + Ser, the third contained → Phe + Trp + Met + Ser + Pro.

Solution:

- a. FDNB react with free amino group yielded the DNP amino acid derivative up on hydrolysis, the N – terminal amino acid is Ala. Lys is in the interior of the chain and has it E – amino group free, thus the peptide is linear to circular.
- b. Carboxy peptidase A will cleave all C – terminal amino acid except Arg, Lys, Pro carboxypeptidase B will cleave only C – terminal Arg or Lys. Neither will act on any C –terminal amino acid if the next to last amino acid is Proline. The lack of product with both enzymes suggests that Proline is last or penutimateresidue.
- c. Cyanogen bromide cleaves specifically on carboxyl side of Methionine residues. The data so far suggest that tripeptide released by CNBr is C – terminal. Thus, the fast four residues include: Met, Trp, Ser & Pro. But the sequence of the last three is still unknown.
- d. Chymotrypsin cleaves on the carboxyl side of Phe, Tyr, Trp provided the next amino acid (is not Pro). The composition of the original is Met – Trp – Ser – Pro. The amino acid preceding the Met must be Phe (the only remaining residue susceptible to chymotrypsin). Thus the terminal sequence is Phe – Met – Trp – Ser – Pro.

- e. Trypsin cleaves on the carboxyl side of Lys & Arg provided the next amino acid is not Pro. Since Ala is N – terminal, the beginning sequence must be Ala – Arg – Ser – Lys.

The overall sequence is shown bellow: -



2. Upon complete acid hydrolysis, a peptide yielded Gly + Ala + Arg + 2Cys + Glu + Ile + Thr + Phe + Val + NH_4^+ . Reduction of original peptide with mercaptoethanol followed by alkylation of the Cys residue with iodoacetate yielded two smaller peptides (A & B). Suggest a likely structure of the original peptide from the following data; -

Peptide A:

- Contained: Ala + Gly + Cys + Glu + Arg + Ile + NH_4^+
- Carboxy peptide A liberated Ile
- Treatment with phenylisothiocyanate (PITC, Edman reagent) yields the phenylthiohydantion derivative of Gly (OTH – Gly).
- Treatment with trypsin yield two peptides one contained Glu + Ile + NH_4^+ the other contained Gly + Ala + Cys + Arg.

Peptide B:

- Contained Thr + Val + Cys + Phe
- Carboxy peptidase A liberated Val

- g. Chymotrypsin liberated Val & tripeptide containing Cys + Thr + Phe.
- h. The Edman degradation yielded PTH – Threonine.