

BCMB 3100: Partial notes

Chapter 4 (Part 1)

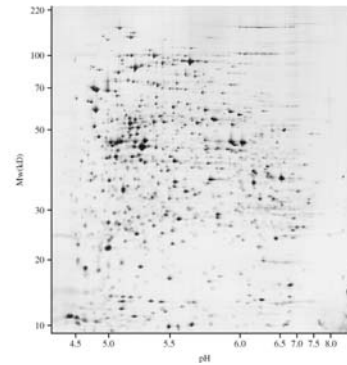
- [Diversity of proteins](#)
- [3D structure of proteins](#)
- [Fibrous vs globular proteins](#)
- **Conformation vs configuration**
- 1°, 2°, 3° and 4° structure
- **Peptide groups in polypeptide**
- ϕ vs Ψ angles, Ramachandran plot
- **Xray crystallography & NMR**
- α helix vs β -sheet

1

E. coli proteins on 2D gel electrophoresis

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E. coli
expresses
~4000
proteins



3

Diversity of proteins

- _____ - study of large sets of proteins, such as the entire complement of proteins produced by a cell
- *E. coli* has about _____ different polypeptides (average size 300 amino acids, M_r 33,000)
- Fruit fly (*Drosophila melanogaster*) about 16,000, humans, other mammals about 40,000 different polypeptides

2

3D STRUCTURE OF PROTEINS

Two classes of proteins:

_____ : water insoluble, static, "tough", extended, provide mechanical support (α -keratin, collagen)

_____ : compact, "spherical", usually: hydrophobic interior & hydrophilic exterior enzymes

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Levels of Protein Structure

_____ : the covalent backbone of a polymer

_____ : the residue-by-residue conformation of the backbone of a polymer

_____ : the 3D conformation of a polymer in its native folded state

_____ : the 3D structure of a multisubunit, particularly the manner in which the subunits fit together

Supersecondary structure: clusters of secondary structure (e.g. $\beta\alpha\beta$)

Domain: a distinct structural unit of a polypeptide; domains have separate functions and may fold as independent, compact units

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The biological activity of a protein depends on its conformation

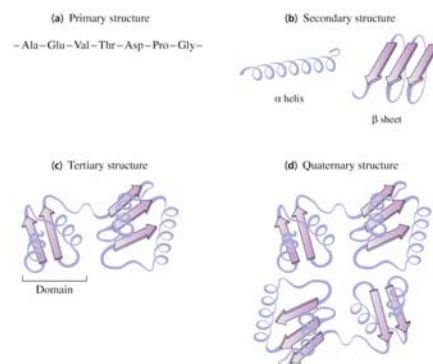
_____ : spatial arrangement of substituent groups that are free to assume different positions in space, without breaking any bonds, because of the freedom of bond rotation

The number of potential conformations of a protein is _____. Under physiological conditions the protein assumes a single stable shape: native conformation

_____ : a spatial arrangement of atoms that can not be changed without breaking covalent bonds

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Fig. 4.1 Levels of protein structure



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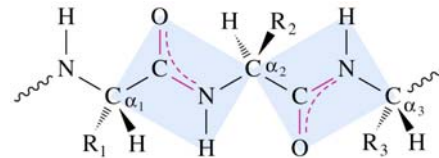
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Fig. 4.6 Planar peptide groups in a polypeptide chain

- Rotation around C-N bond is restricted due to the double-bond nature of the resonance hybrid form
- Peptide groups (blue planes) are therefore planar



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Conformation of the Peptide Group: The peptide group consists of 6 atoms

Fig. 4.5 Resonance structure of the peptide bond

(a) Peptide bond shown as a C-N single bond

(b) Peptide bond shown as a double bond

(c) Actual structure is a hybrid of the two resonance forms. Electrons are delocalized over three atoms: O, C, N

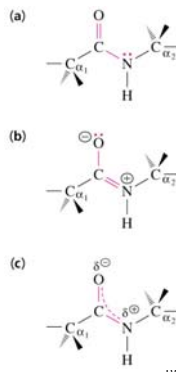
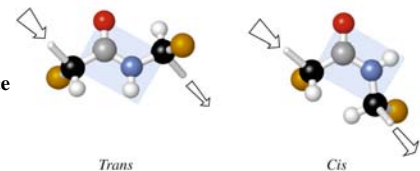


Fig. 4.7 *Trans* and *cis* conformations of a peptide group

Nearly all peptide groups in proteins are in the *trans* conformation

- _____ conformation is less favorable than *trans* due to steric interference of α -carbon side chains



- _____ conformation is established protein during synthesis

● α -carbon ● Hydrogen ● Oxygen
 ● Carbonyl carbon ● Nitrogen ● Side chain

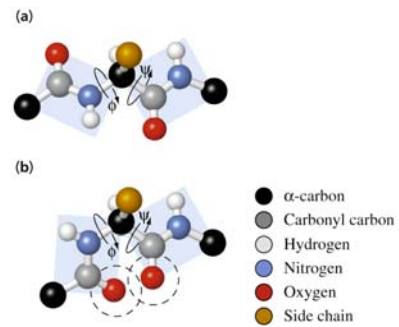
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Fig. 4.8 Rotation around the N-C $_{\alpha}$ and C $_{\alpha}$ -C bonds that link peptide groups



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Rotation of atoms in a peptide group

- Peptide group has a repeating N-C $_{\alpha}$ -C backbone
- Rotation about both the N-C $_{\alpha}$ (ϕ) and C $_{\alpha}$ -C (Ψ) bonds is possible
- Rotation of the N-C $_{\alpha}$ bond in proline is restricted because of the pyrrolidine ring structure

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_____:

steric contour diagram of allowed values of rotation permitted about the N-C $_{\alpha}$ (ϕ) and C $_{\alpha}$ -C (Ψ) bonds.

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Electrons in protein scatter X-rays (amplitude of wave is proportional to number of electrons)

Scattered waves recombine:
in phase waves → complement
out-of-phase waves → cancel

Pattern of recombinant waves (angles and intensities) depend upon atomic structure. Pattern converted to atomic image by computer analysis (Fourier transformation).

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NMR determination of protein structure

• _____ a method used to determine structure of small proteins in solution

- **Fig 4.4 Ribonuclease A** determined by NMR (polypeptide chain backbone)

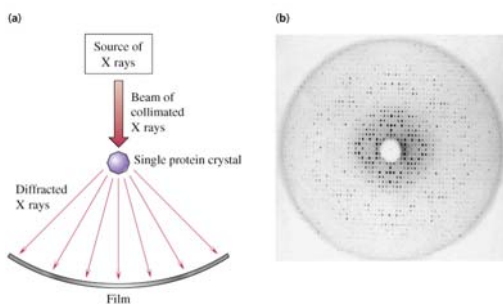
Protein structures are deposited in the databases:
e.g., **Protein Data Bank (PDB)**



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4.2 Methods for Determining Protein Structure

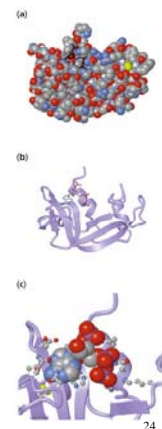
- X-ray crystallography is used to determine the three-dimensional conformation of proteins



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Fig 4.3 Ribonuclease A

- (a) Space-filling model (bound substrate analog black)
- (b) Cartoon ribbon model (shows secondary structure)
- (c) Substrate-binding site view



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[right-handed](#)

carbonyl O of aa residue n is H-bonded to α -amino N of residue n+4

[rise](#) = 0.15nm, [pitch](#) = 0.54, [3.6 residue per turn](#)

Fibrous proteins (e.g. keratin) may be largely α -helix

Globular proteins vary greatly in α -helix content: average α helix content of 26%

All side chains point outward from cylinder of helix

Ala often found in α helix; **Pro** & **Gly** usually not present in helix but may be at ends

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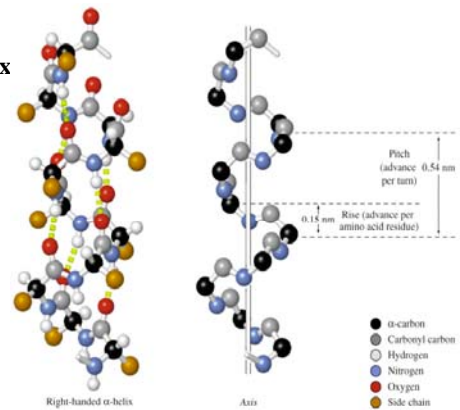
Secondary structure in proteins

Linus Pauling & Robert Corey (1950s)

proposed _____ and _____ as types of structures in proteins

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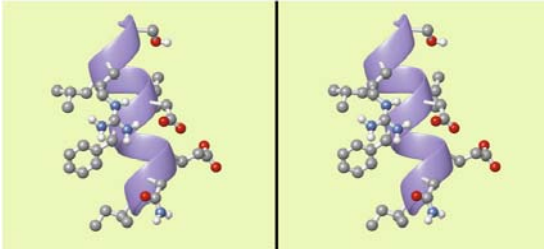
Fig. 4.10
The α -helix



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Fig. 4.11 Stereo view of right-handed α helix

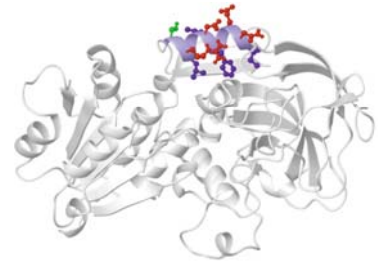
- All side chains project outward from helix axis



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Fig. 4.13 Horse liver alcohol dehydrogenase

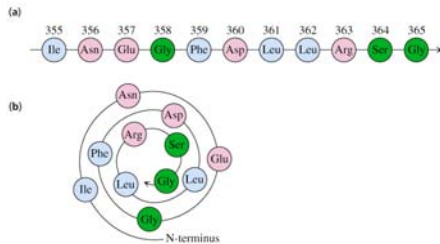
- Amphipathic α helix (blue ribbon)
- Hydrophobic residues (blue) directed inward, hydrophilic (red) outward



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Fig. 4.12 Helix in horse liver alcohol dehydrogenase

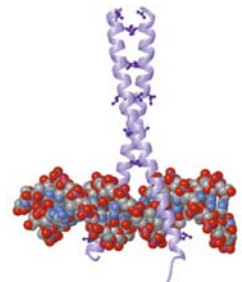
(a) Amino acid sequence, (b) Helical wheel diagram



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Fig. 4.14 Leucine zipper of yeast

- DNA binding region consists of two amphipathic α helices, one from each of two protein subunits.



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β sheet: extended polypeptide strands (β strands) stabilized by H-bonds between carbonyl O and amide H.

β strands may be parallel or antiparallel

side chains point alternatively above and below plane of strand

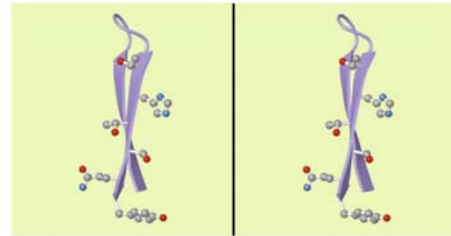
β sheets contain 2-15 strands with average of 6 aa residues per strand

β strand content of globular proteins is variable: Average of 19% β structure

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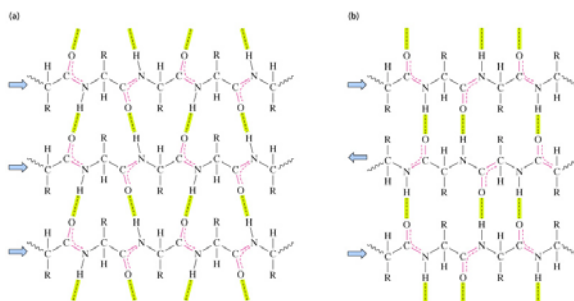
Fig 4.16 Stereo view of antiparallel β sheet

- Side chains (front β strand) alternate sides
- β -Strands twist in a right-handed direction



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Fig 4.15 β -Sheets (a) parallel, (b) antiparallel



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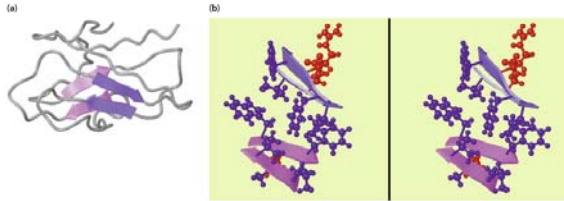
Interactions of β sheets

- β Sheet side chains project alternately above and below the plane of the β strands
- One surface of a β sheet may consist of hydrophobic side chains that can interact with other hydrophobic residues in protein interior
- _____ helices have hydrophobic side chains projecting outward that can interact with hydrophobic faces of β sheets or other helices

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Fig 4.17 Structure of PHL P2 protein

- (a) Blue/purple antiparallel β -sheets within a protein
- (b) Stereo view of the β sandwich. Polar residues (red), hydrophobic residues (blue)



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Loops and Turns (2)

 : 4 aa, stabilized by H-bonds between α -carbonyl C of residue n and α -NH of residue (n+3); Pro is often 2nd residue

 : (also called glycine turn, β bend) like Type I Turn but 3rd residue is Gly

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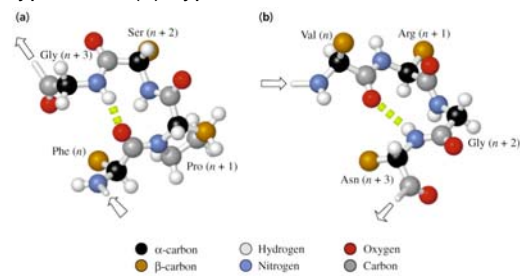
4.6 Loops and Turns (1)

- Loops and turns connect α helices and β strands and allow a peptide chain to fold back on itself to make a compact structure
- - often contain hydrophilic residues and are found on protein surfaces
- - loops containing 5 residues or less
- - connect different antiparallel β strands (also called hairpin loop)

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Fig. 4.18 Reverse turns

- (a) Type I, and (b) Type II



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BCMB 3100 Partial Lecture Notes
for Chapter 4 (Part 2)

- Supersecondary Structures (Motifs), Domains, Folds, Quaternary Structure
- Anfinsen's Experiment
denaturation, reduction & refolding
- Protein folding, Chaperones
- Collagen, Myoglobin & Hemoglobin

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- independently folded, compact, distinct structural unit in proteins
- ~25 to ~300 amino acid residues
- connected to each other by loops, bound by weak interactions between side chains
- may have separate functions
- illustrate evolutionary conservation of protein structure

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Fig. 4.19
Common motifs

Motifs -
recurring
protein
structures

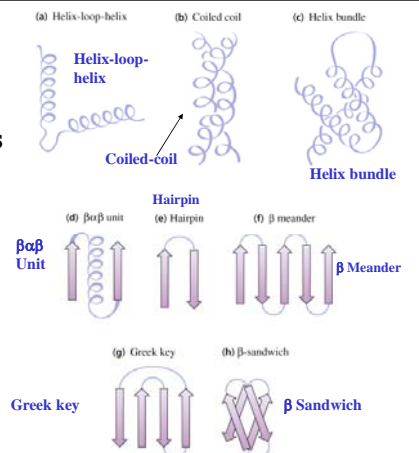
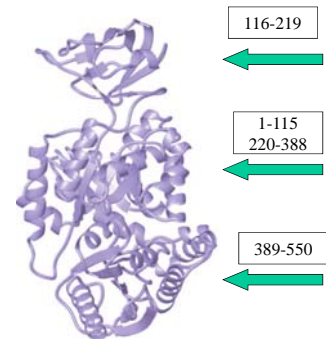


Fig 4.20 Pyruvate Kinase

- Main polypeptide chain folds into three distinct domains



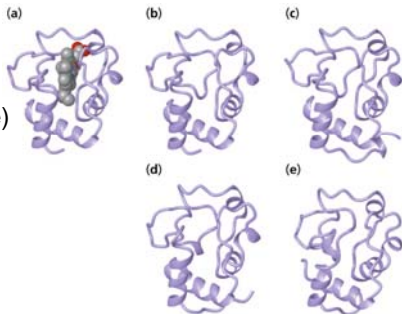
44

Fig. 4.21 Cytochrome c

- Conservation of cyt c structure

- (a) Tuna (+heme)
- (b) Tuna
- (c) Rice
- (d) Yeast
- (e) Bacteria

See Fig 3.22 for
Phylogenetic Tree



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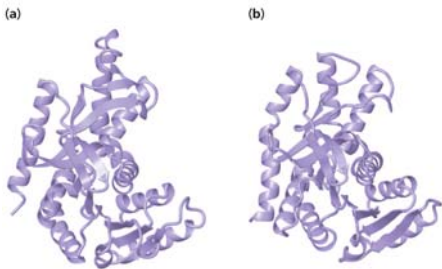
Four categories of protein domains

- (1) **All α** - almost entirely α helices and loops
- (2) **All β** - only β sheets and non-repetitive structures that link the β strands
- (3) **Mixed α/β** - alternate regions of α helix and β strand (e.g. $\alpha\beta\alpha$ motif)
- (4) **$\alpha + \beta$** - local clusters of α helices and β sheet in separate, contiguous regions of the polypeptide chain

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Fig. 4.22 Structural similarity of (a) lactate dehydrogenase, (b) malate dehydrogenase
(only 23% identical amino acids!!)

- (a) *B. stereothermophilus*, (b) *E. coli*



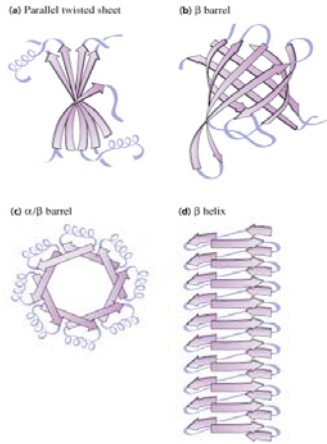
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Folds

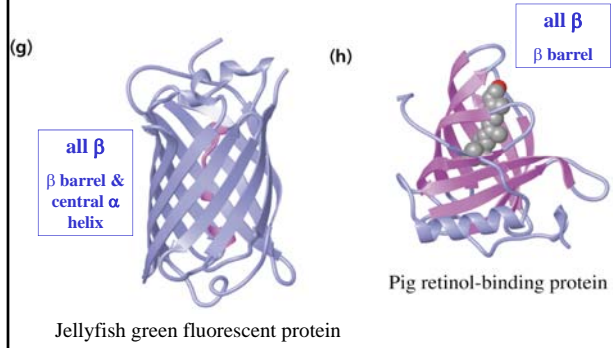
- A “fold” is a combination of secondary structures that form the core of a domain
- Some domains have simple folds, others have more complex folds

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Fig. 4.24 Common domain folds

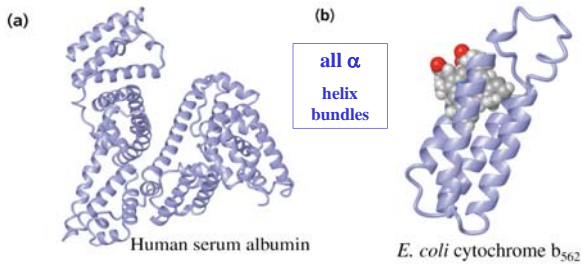


Tertiary Protein Structures (cont)



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Fig 4.23 Examples of tertiary structure



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Domain Structure and Function

- A _____ may have a particular function
- _____ between 2 domains provide crevices, grooves, and pockets on the surface of a protein for binding or catalytic sites
- In multifunctional enzymes, each catalytic activity can be on one of several domains

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Quaternary Structure

- organization of subunits in a protein with multiple subunits (an “oligomer”)
- Subunits (may be identical ($\alpha\alpha$) or different ($\alpha\beta$)) have a defined stoichiometry and arrangement
- Subunits are held together by many weak, noncovalent interactions (hydrophobic, electrostatic)

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Fig. 4.25 (continued)

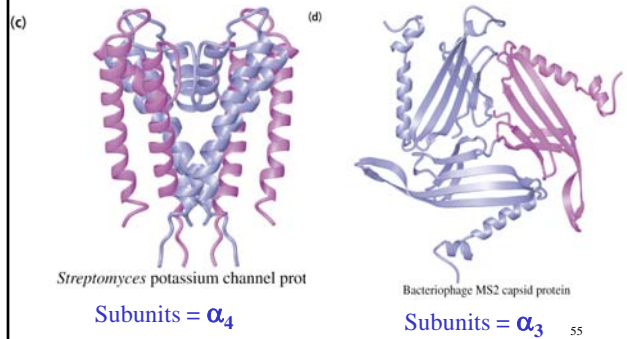


Fig 4.25 Quaternary structure of multidomain proteins

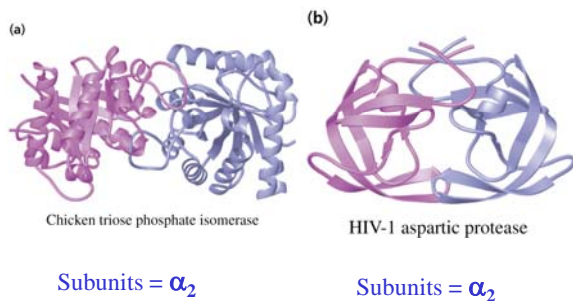
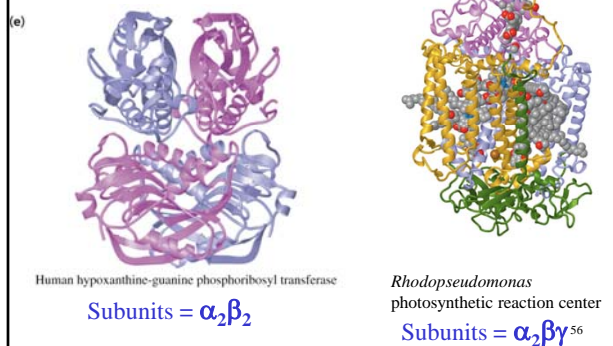


Fig. 4.25 (cont.)



Protein Denaturation and Renaturation

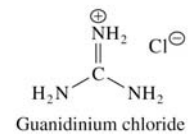
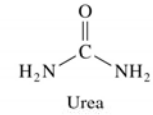
_____:

- partial or complete unfolding of native conformation
- causes loss of biological activity
- caused by heat, extreme pH, detergents, chaotropic agents; due to disruption of non-covalent interactions
- some proteins can be refolded or renatured

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Fig 4.27 Urea and guanidinium chloride Two chaotropic agents

Disrupt hydrophobic interactions in interior of protein



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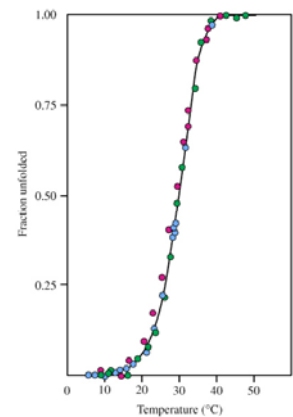
_____ ("chaos-promoting"):
chemicals that denature proteins (e.g. **urea**,
guanidinium chloride)

chaotropic agents do NOT cleave covalent bonds
but disrupt 2°, 3° and 4° structure

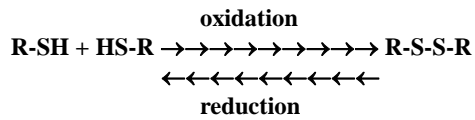
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Fig 4.26

- Heat denaturation of ribonuclease A
- Unfolding monitored by changes in ultraviolet (blue), viscosity (red), optical rotation (green)



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_____ : the loss of electrons from a compound

_____ : the gain of electrons by a compound or ion

Common reducing agents: β mercaptoethanol; dithiothreitol

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_____ showed that the information necessary to specify **3D structure** of ribonuclease came from the amino acid sequence

The amino acid sequence specifies 3D structure!!

The native form of a protein (e.g. ribonuclease) appears to be the thermodynamically most stable structure

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Fig. 4.28 Disulfide bridges in bovine ribonuclease A

Ribonuclease:

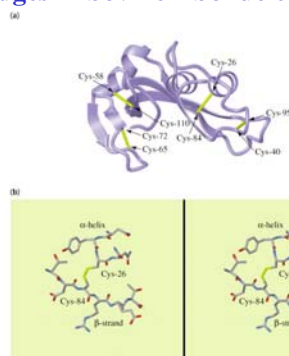
124 aa,

mainly β sheet,

4 disulfide bonds

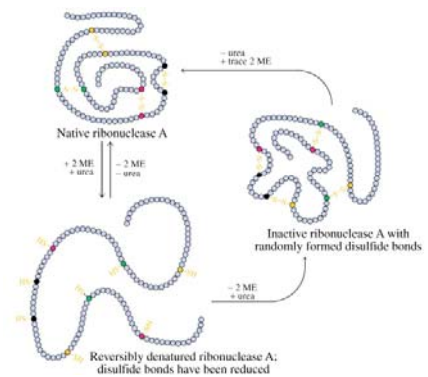
(a) Location of disulfide bridges

(b) Stereo view of Cys-26 and Cys-84



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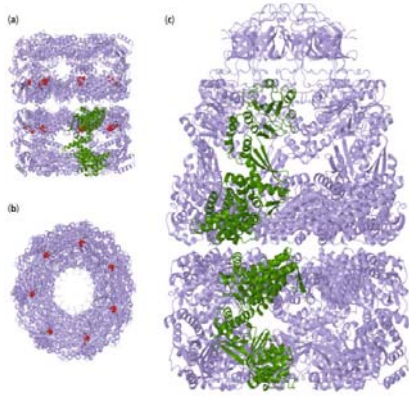
Fig. 4.29 Denaturation and renaturation of ribonuclease A



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Fig 4.32
E. coli
chaperonin

(a) (b) Core consists of 2 identical rings (7 GroE subunits in each ring)
(c) Protein folding takes place inside the central cavity



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COLLAGEN TRIPLE HELIX (1)

_____ : most abundant vertebrate protein (25-35% total protein); fibrous; found in bone, tendons, skin, blood vessels, cartilage; gelatin, glue

collagen is an aggregate of 3 left-handed helices coiled into a right-handed super helix!

has high percentage of Pro, Gly, Hyp (4-hydroxyproline),

also contains Hyl (hydroxylysine)

Structure elucidated by G. N. Ramachandran

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Fig 4.33 Chaperonin-assisted protein folding

- Hydrolysis of several ATP molecules is required

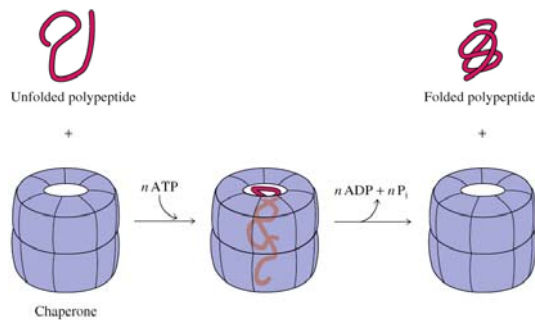
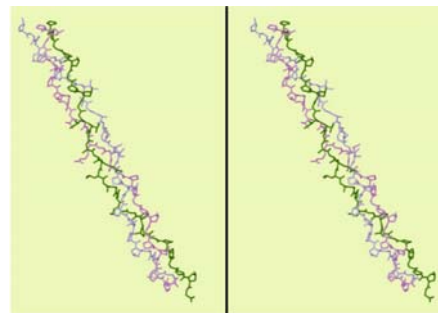


Fig 4.36 Stereo view of human Type III collagen triple helix



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COLLAGEN TRIPLE HELIX (2)

Hyp & Hyl are formed posttranslationally

Hyp allows more _____ H-bonding & stabilization of collagen triple helix

Pro & Hyp prevent formation of α helices & make collagen fibers rigid

Strengthened by inter- and intra- covalent crosslinks between allysine-Lys (allysine)

Vitamin c (ascorbate) deficiency \rightarrow decrease in Hyp & Hyl formation \rightarrow _____ (weakness in blood vessels & skin)

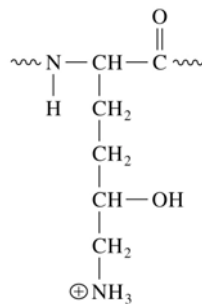
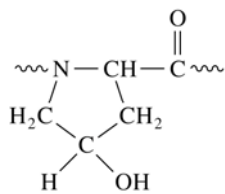
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Collagen triple helix (3)

- Multiple repeats of -Gly-X-Y- ; X often proline, Y often 4-hydroxyproline
- Glycine residues located along central axis of a triple helix (other residues cannot fit)
- For each -Gly-X-Y- triplet, one interchain H bond forms between amide H of Gly in one chain and -C=O of residue X in an adjacent chain
- No intrachain H bonds exist in the collagen helix

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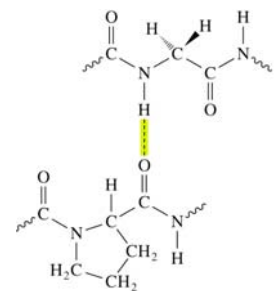
Fig 4.35 4-Hydroxyproline Fig 4.37 5-Hydroxylysine



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Fig. 4.35 Interchain H bonding in collagen

- Amide H of Gly in one chain is H-bonded to C=O in another chain



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Some proteins require **cofactors** or **prosthetic groups** at their active sites for activity

_____ : small, usually nonprotein molecule required for enzyme activity

_____ : a metal ion or other non-amino acid molecule tightly bound to a protein and essential for its activity

_____ : active protein with all its cofactors

_____ : protein without its cofactors

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Hemoglobin (Hb)

Binds O_2

Transports O_2 in vertebrate blood

Prosthetic group: heme = Fe-protoporphyrin IX

Tetramer: $\alpha_2\beta_2$; α has 141 aa; β has 146 aa; acts as dimer of $\alpha\beta$

3° structure of each subunit similar to myoglobin

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Myoglobin (Mb)

Binds O_2

Stores & transports O_2 in muscle

Prosthetic group: heme = Fe-protoporphyrin IX

Single polypeptide (153 aa) + heme

Globular protein with 8 α -helices

Oxygenated myoglobin = oxymyoglobin

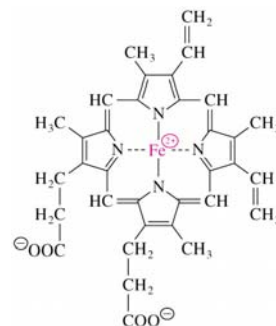
All polar residues (except 2 His's) are located on protein surface

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Fig. 4.39 Heme Fe(II)-protoporphyrin IX

- Porphyrin ring provides four of the six ligands surrounding iron atom

The ferrous iron is held in place by binding of nitrogens of the 4 pyrrole rings



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The structure of globins has been conserved in many species

amino acid changes (substitutions) may or may not effect protein conformation

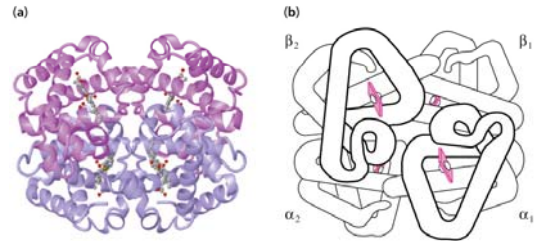
_____ do NOT significantly effect conformation (i.e. Val → Ile)

_____ DO effect conformation (Glu → Val)

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Fig. 4.42 Hemoglobin tetramer

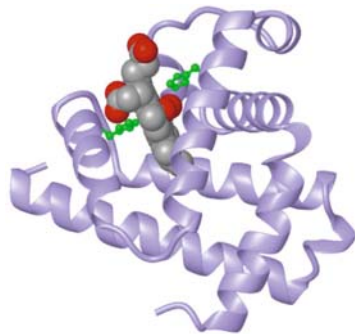
(a) Human oxyhemoglobin (b) Tetramer schematic



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Fig 4.40 Sperm whale oxymyoglobin

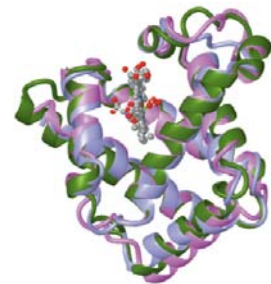
- Oxygen (red)
- His-93 and His-64 (green)



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Fig 4.43 Tertiary structure of myoglobin, alpha-globin and beta-globin

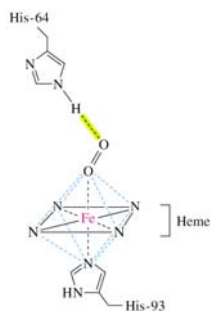
- alpha-Globin (blue)
- beta-Globin (purple)
- Myoglobin (green)



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Fig 4.44 Oxygen-binding site of whale oxymyoglobin

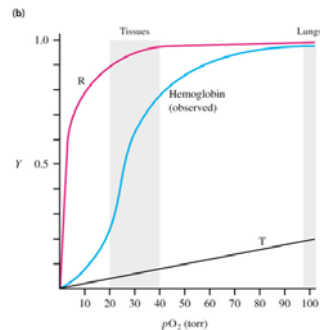
- Octahedral geometry of coordination complex (six ligands around iron)
- His-93 (proximal histidine) liganded to Fe
- His-64 (distal histidine)



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Fig. 4.46 (b) Oxygen-binding curves

- (b) Binding of the R (high-affinity) and T (low affinity) forms of Hb



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Fig 4.46 (a) Oxygen-binding curves

- (a) Comparison of O₂ binding to Mb and Hb

Hyperbolic curve
(single equilibrium constant)

$$\text{Mb} + \text{O}_2 \rightleftharpoons \text{MbO}_2$$

Fractional Saturation = Y

$$Y = \frac{[\text{MbO}_2]}{[\text{MbO}_2] + [\text{Mb}]}$$

p50 = partial pressure at half saturation

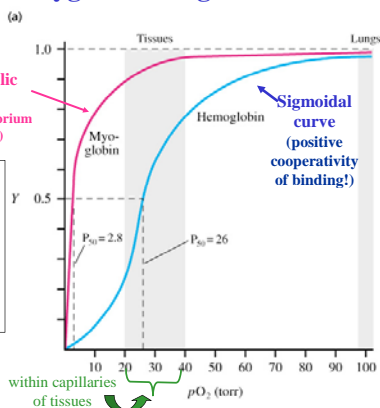
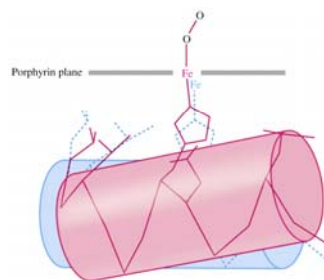


Fig 4.47 Conformational changes in a hemoglobin chain induced by oxygenation

- Oxygen binding to Fe pulls the His toward ring plane
- Helix with His shifts position, disrupting some ion pairs between subunits (blue to red position)



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Hemoglobin is an **allosteric protein**

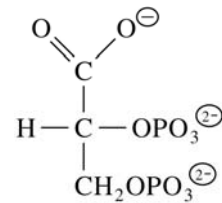
_____ : regulatory protein whose activity is modulated by noncovalent binding of a specific metabolite at a site other than the active site

_____ : small molecules that bind to allosteric proteins and regulate their activity

allosteric regulation is caused by small changes in native conformation of a protein

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Fig 4.48 2,3-Bisphospho-D-glycerate (2,3BPG)



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active shape = **R** (relaxed)
inactive shape = **T** (taut)

allosteric inhibitor
R →→→→→ **T**
←←←←←
allosteric activator

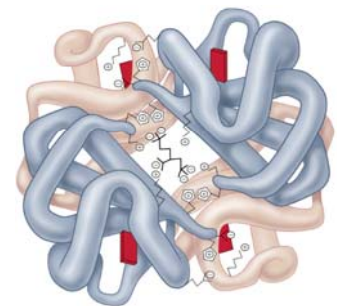
2,3-bisphospho-D-glycerate (2,3BPG) is an allosteric effector of hemoglobin. It lowers the affinity of deoxyhemoglobin for O₂ (raises P50). [Know physiological significance for Exam]

Bohr effect: the increase in P50 of hemoglobin caused by a lowered pH due to an increase in CO₂

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Fig 4.49 Binding of 2,3BPG to deoxyhemoglobin

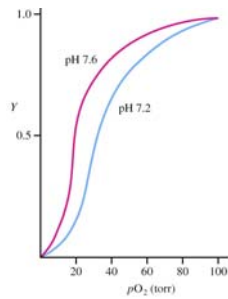
- (-) Charges on 2,3BPG pair with (+) charges lining the central cavity, stabilizing the DeoxyHb form
- α-Subunits pink, β-subunits blue, heme groups red



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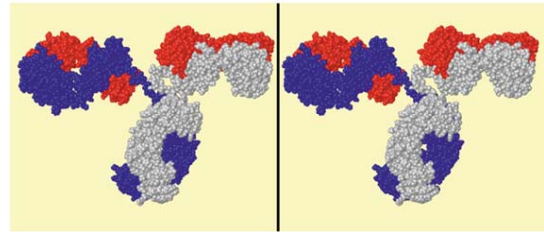
Fig 4.50 Bohr effect

- Lowering the pH decreases the affinity of Hb for oxygen



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Fig 4.52 (a) Human antibody structure



Light Chain contains 2 domains; Heavy Chain contains 4 domains
Each domain: 110 aa in common motif known as immunoglobulin fold

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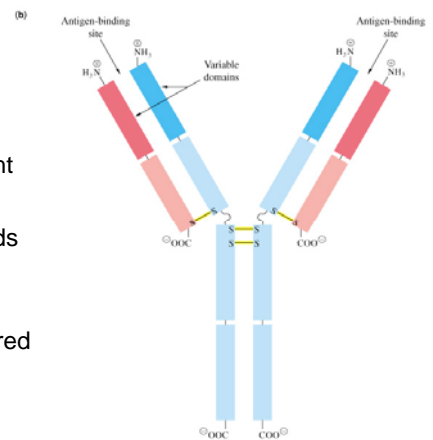
Antibodies Bind Specific Antigens

- Vertebrate immune systems synthesize protein **antibodies** (immunoglobulins) to eliminate bacteria, viruses, other foreign substances
- Antibodies specifically recognize and bind **antigens**
- Antibodies are synthesized by lymphocytes (white blood cells)

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Fig. 4.52 (b)

- Heavy chains (blue) and light chains (red)
- Disulfide bonds (yellow)
- Variable domains colored darker



The following are additional notes that will help you in your studying

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Four categories of protein domains

- (1) **All α** - domains almost entirely α helices and loops
- (2) **All β** - domains contain only β sheets and non-repetitive structures that link the β strands
- (3) **Mixed α/β** - supersecondary structures where regions of α helix and β strand alternate (e.g. $\alpha\beta\alpha$ motif)
- (4) **$\alpha + \beta$** - local clusters of α helices and β sheet in separate, contiguous regions of the polypeptide chain

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Review of Globular Protein 3D Structure

Most globular proteins have compact globular shape due to many reversible turns in direction combined with the α helix and/or β structure. Usually, hydrophobic aa residues are in the interior and hydrophilic aa residues on the exterior of the protein.

The loops & turns contain nonrepetitive regions of 2° structure.

loops: range from ~ 2-16 residues, many hydrophilic residues found at surface of protein (can H-bond with water)

turn: loops having only a few residues (<6)

Average structure of Globular Protein

α helix: 26%; β structure: 19%; turns: 15%;
simple loops 21%; complex loops: 10%

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Protein Denaturation and Renaturation

- **Denaturation** - disruption of native conformation of a protein, with loss of biological activity
- Most denatured proteins adopt a random-coil conformation
- Proteins denatured by heating or chemicals
- Some proteins can be refolded or renatured

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Protein Folding and stabilization (1)

Cooperativity of folding: formation of one part of structure (e.g. initial aa interactions) leads to formation of remaining structure.

Hydrophobic effect is MAJOR driving force in protein folding

Folded proteins occupy a low-energy well that makes the native structure most stable

Many proteins can fold spontaneously to this low-energy conformation

Proteins are thought to fold “cooperatively” ... the first few interactions assist subsequent alignment and folding

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- **Chaperones:** proteins that bind newly synthesized polypeptides & assist in proper folding
- **Chaperones** increase rate of correct folding and prevent the formation of incorrectly folded intermediates
- Chaperones bind to unassembled protein subunits to prevent incorrect aggregation before they are assembled into a multisubunit protein
- Most chaperones are **heat shock proteins** (synthesized as temperature increases)

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Protein folding (more detail)

- extremely rapid, native conformation is generally reached < 1 second
- During folding the polypeptide collapses in upon itself due to the hydrophobic effect
- An intermediate “**molten globule**” forms with elements of secondary structure
- The backbone is rearranged to achieve a stable native conformation

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Two conformations of hemoglobin: T and R

- **Active (R state)** and **inactive (T state)** forms are in rapid equilibrium in allosteric proteins
- Binding of substrates and allosteric activators stabilize the R state and shift the equilibrium in the R direction
- Allosteric inhibitors stabilize the T state and shift the equilibrium in the T direction

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