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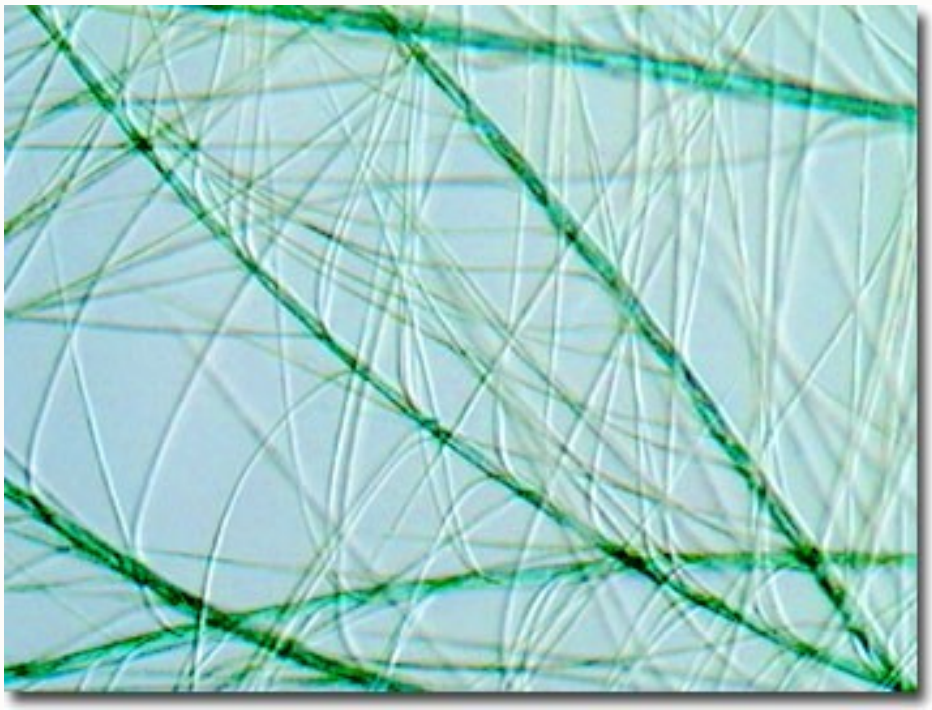
Chapter 8: Fibrous Proteins

Voet & Voet: Pages 231-240



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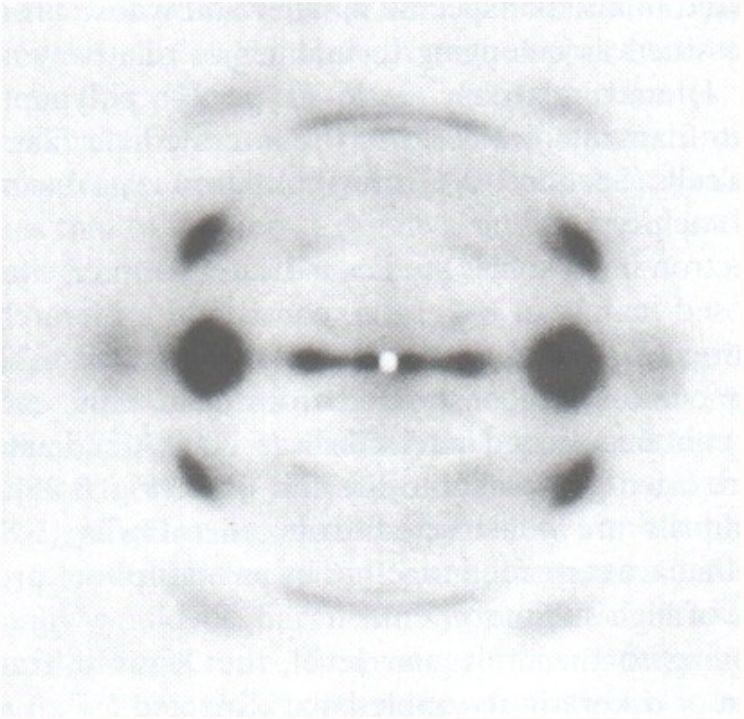
Fibrous Proteins

Fibrous proteins are highly elongated polypeptides composed of a single secondary structure element

Primary component of skin, tendon, bone, connective tissues, *etc*

- Function as **structural material** that have protective, connective or supportive roles
- Simplicity of structure makes relation between structure and function relatively obvious



Rarely crystallize (also difficult for NMR) so structural information a variety of indirect techniques

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Keratins

Mechanically durable and unreactive protein of vertebrates

- **up to 85% of protein in horns, hair, nails & feathers**
- **α-keratins occur in mammals; β-keratins occur in birds and reptiles**

> 30 keratin genes expressed in mammals

- **α-keratins are classified as relatively acidic (Type I) or basic (Type II)**

Keratins have complex quaternary structures

- **(a) keratins are dimers composed of a Type I and Type II subunit**
- **(b) many dimers associate to form protofilaments**
- **(c) protofilaments dimerize to form protofibrils**
- **(d) protofibrils form tetramers called microfibrils**
- **(e) microfibrils associate into macrofibrils**

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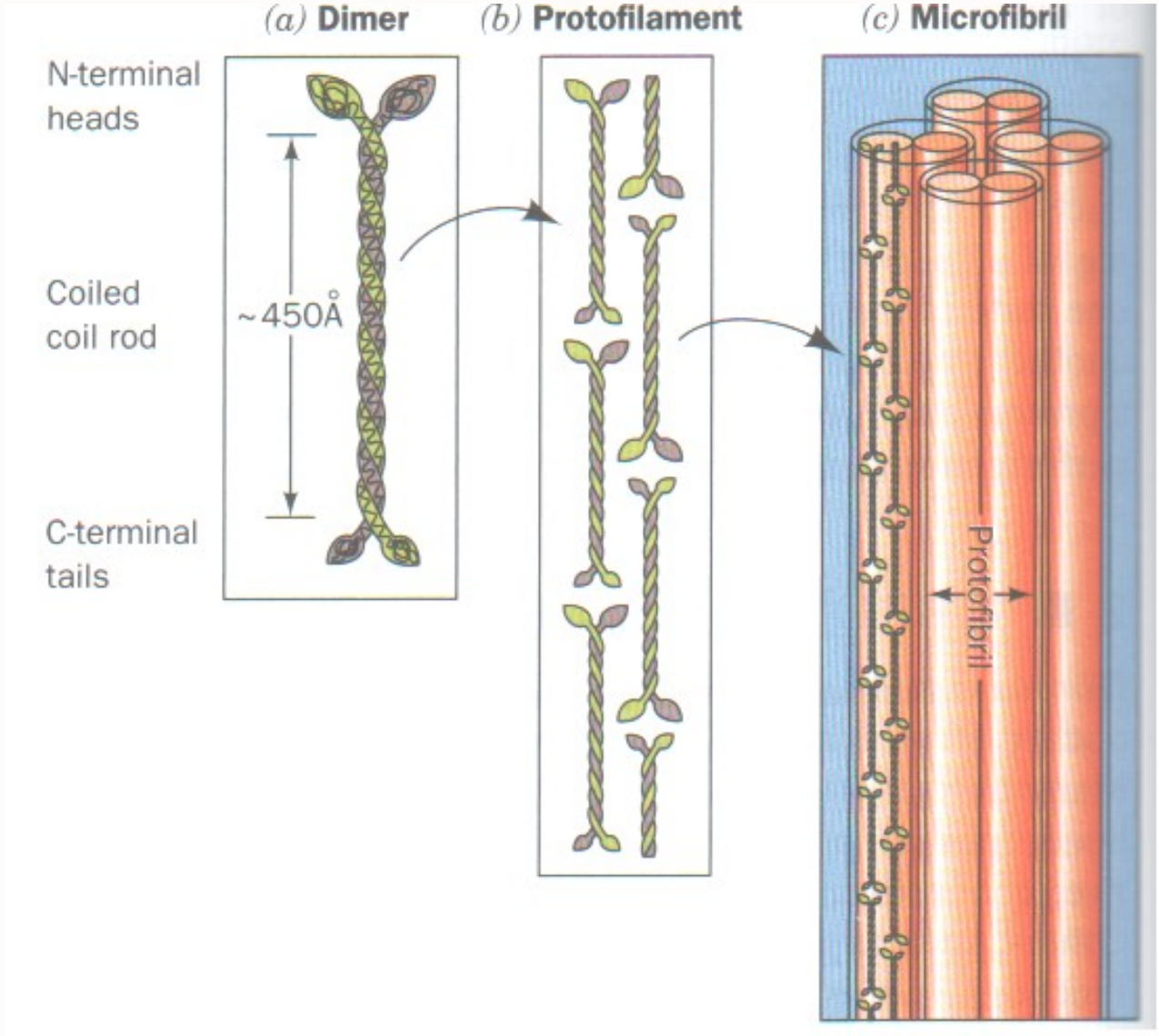
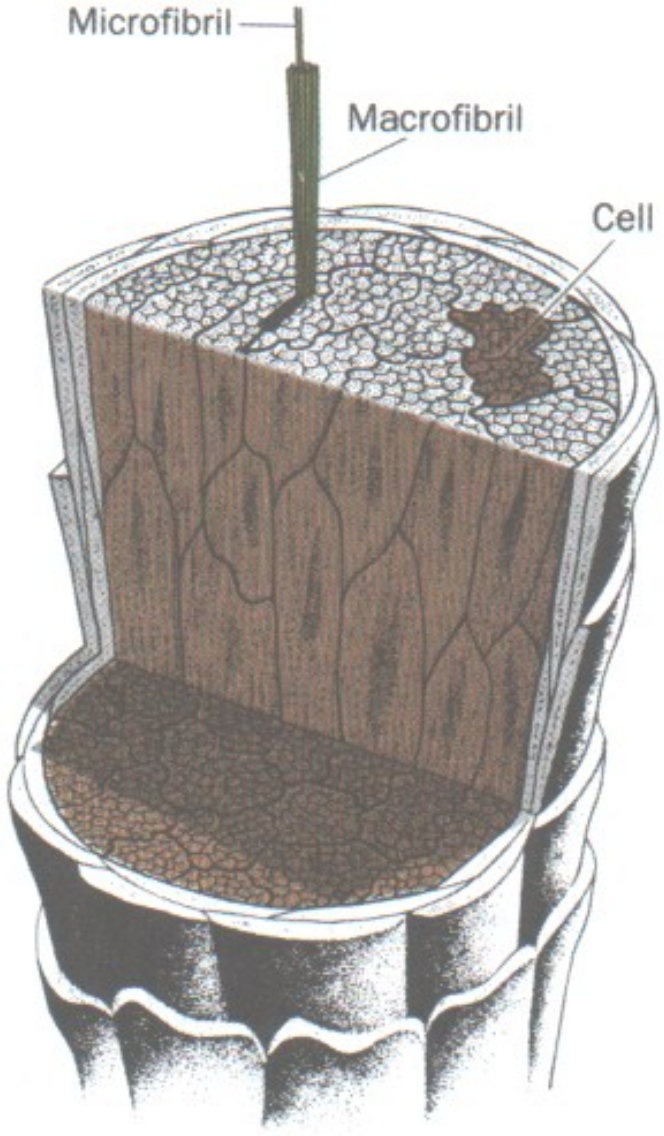
Keratin Quaternary Structure





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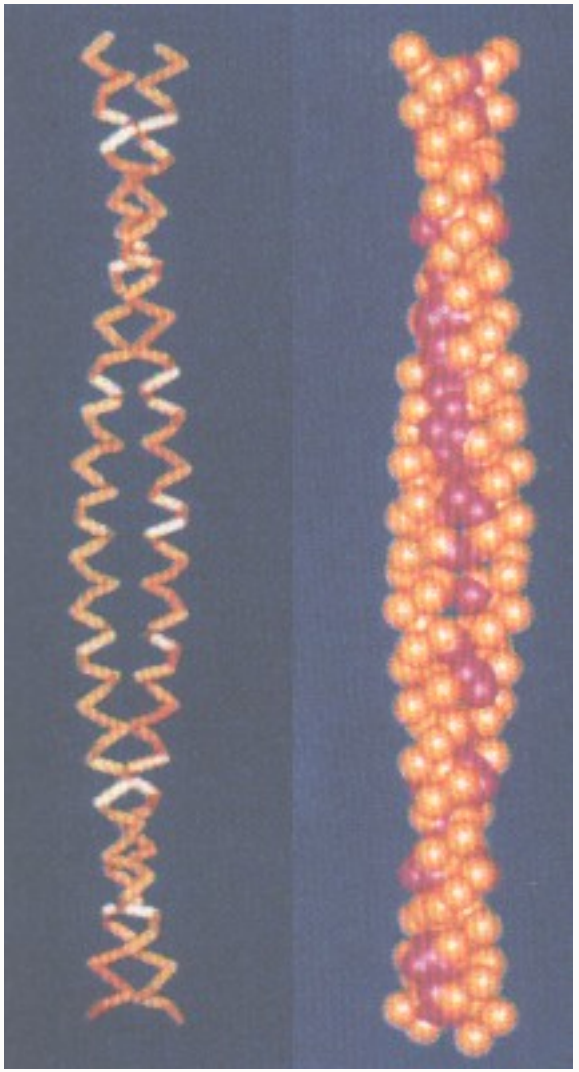
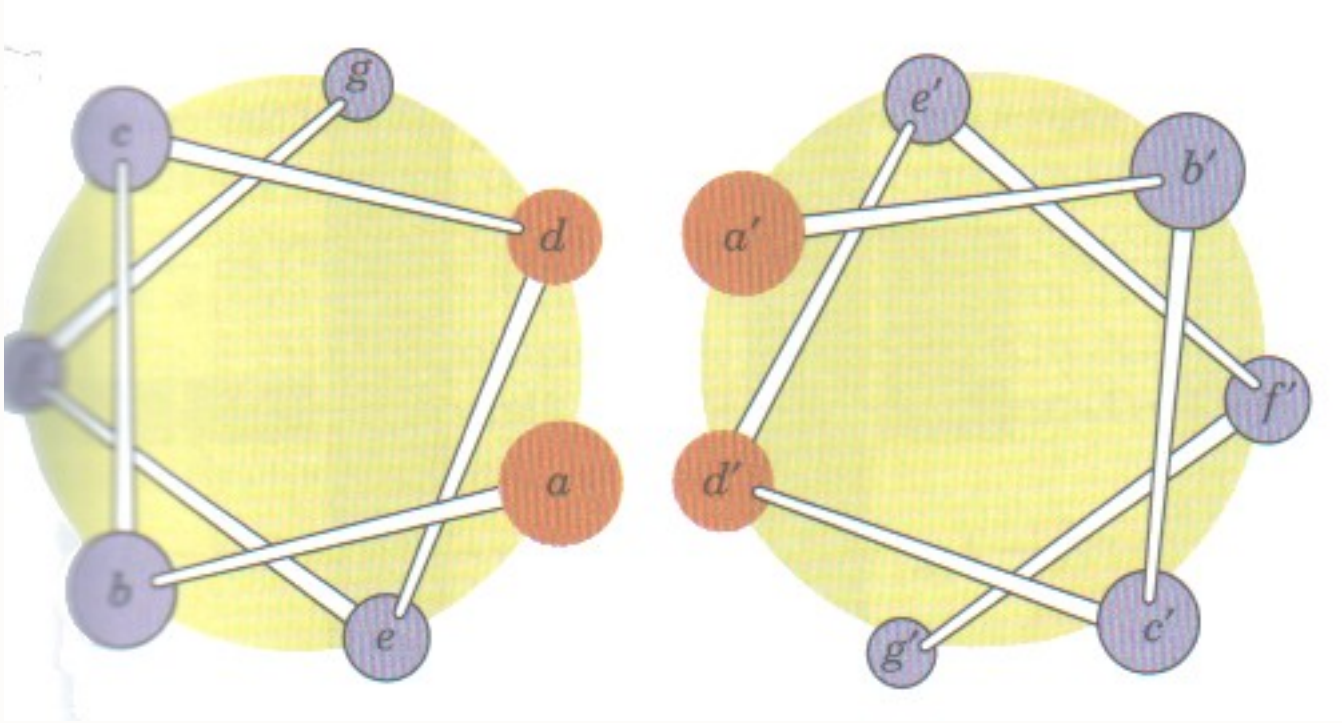
α-Keratin Structure

- X-ray scattering and diffraction studies indicate α-keratin has a helical structure with a 5.1 Å pitch (distance per turn of helix)
  - Variety of experimental evidence indicate α-keratin is a dimer composed of one Type I and one Type II subunit
  - Each subunit is a right-handed α-helices; the two helices wrap around one another to form a left-handed, parallel coiled-coil
- The twist of the individual α-helices in the coiled-coil is responsible for the short helical spacing (normally 5.4 Å)



Coiled Coil

- α-keratin contains a 7 residue pseudorepeat (*a-b-c-d-e-f-g*) within the central ~310 segment of the molecule
  - Residues at position a and d are hydrophobic and are responsible for the association of the two subunits
  - The 5.1 Å spacing of the α-helices optimize the association of the hydrophobic residues



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Cys content

α-keratin is rich in Cys residues and disulfide bridges

- One of the difference between the many α-keratin proteins is their Cys content
- The mechanical strength of horns, nails and skin depends upon the content of Cys residues and the number of disulfide bridges
  - The more disulfide bridges the more rigid the resulting structure

Disulfides occur between dimers and with other proteins that are part of microfibrils and macrofibrils

Extensive disulfide bridging accounts for the insolubility and resistance to stretching of α-keratins

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Real World Examples

Biochemistry of the "Hair Perm"

1) Mercaptans are reducing agents that break the naturally occurring disulfide bridges occuring within the α-keratin of hair
   (and also give rise to the "special" smell)

2) Moist heat and curlers are used to mechanically change the shape (stretch) of the hair

   actually causes a helix to coil transition along parts of the α-keratin

3) Oxidizing agents are used to reform the disulfide bridges while the hair is held in the changed shape

New hair growth will still produce the naturally occurring disulfide bridges and the "changed shape" will be lost as the hair grows

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Keratin Defects (mutations)

Several inherited diseases result from mutations in keratin genes

- **EBS (epidermolysis bullosa simplex) and EHS (epidermolytic hyperkeratosis) are characterized by skin blistering**
  - **Range of effects from barely noticeable to incapacitating (depending upon mutation)**
- **EBS results from mutation in either keratin 5 or 14**
- **EHS result from mutation in either keratin 1 or 10**

Mutation affects protofilament and/or protofibril formation

- **Aberrant protofilament or protofibril formation results in a loss of mechanical integrity of basal skin cells**

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Collagen

Present in all multicellular organisms and the most abundant protein in vertebrates

- **extracellular protein that forms insoluble fibers of great tensile strength**
- **major stress bearing component of connective tissue – bone, teeth, cartilage, tendon, skin, *etc.***

Mammals have at least 33 distinct collagen subunits that form >20 different quaternary structures

TABLE 8-2 The Most Abundant Types of Collagen

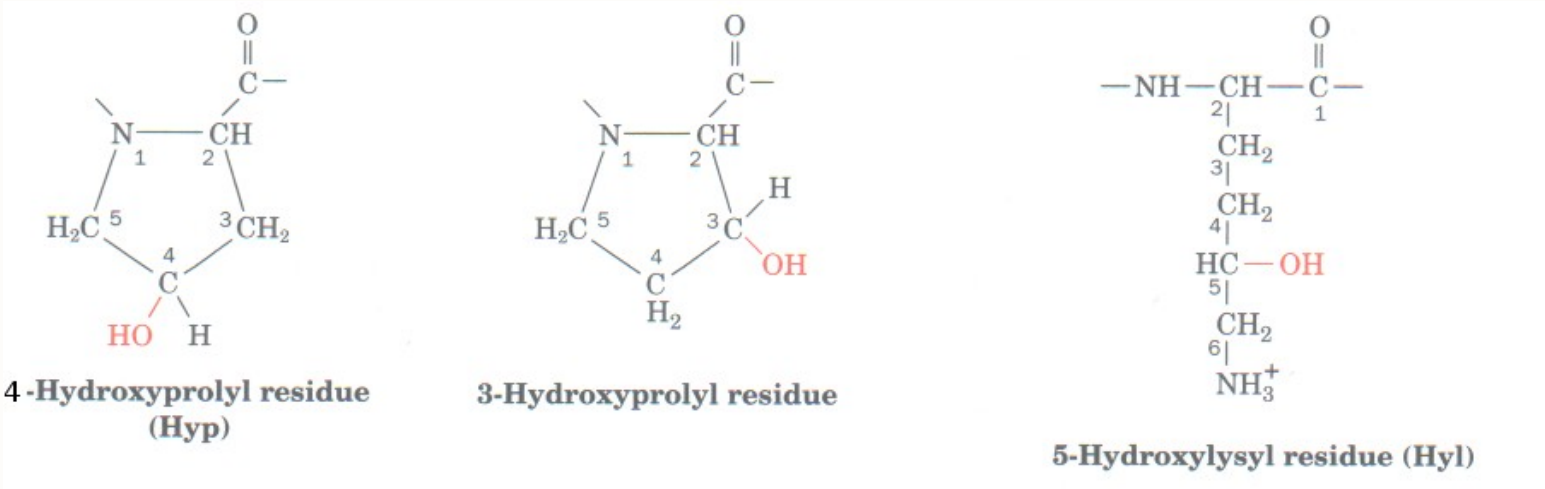
Type	Chain Composition	Distribution
I	$[\alpha 1(I)]_2\alpha 2(I)$	Skin, bone, tendon, blood vessels, cornea
II	$[\alpha 1(II)]_3$	Cartilage, intervertebral disk
III	$[\alpha 1(III)]_3$	Blood vessels, fetal skin

Source: Eyre, D.R., *Science* **207,** 1316 (1980).

Primary Structure

Collagens have a distinctive amino acid composition

- **nearly 1/3 of the residues are Gly**
- **another 15-30 % of the residues are either Pro or a post-translational modification of Pro (4-hydroxyproline)**
- **smaller amount of the modified amino acids, 3-hydroxyproline and 5-hydroxylysine also occur in collagen**



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4-hydroxyproline

Prolyl hydroxylase is the enzyme that converts proline to 4-hydroxyproline

- requires ascorbic acid (vitamin C) for activity; lack of dietary vitamin C leads to scurvy as 4-hydroxyproline cannot be synthesized
  - absence of 4-hydroxyproline prevent proper collagen fiber formation
- Scurvy is characterized by skin lesions, blood vessel fragility, poor wound healing and is ultimately fatal

4-hydroxyproline is responsible for stabilizing collagen structure

- collagen synthesized without 4-hydroxyproline denatures at 24° C, whereas normal collagen denatures at 39° C
- apparently, stabilizing is via hydrogen bonds to 'bridging' water molecules located between individual subunits of the collagen fiber

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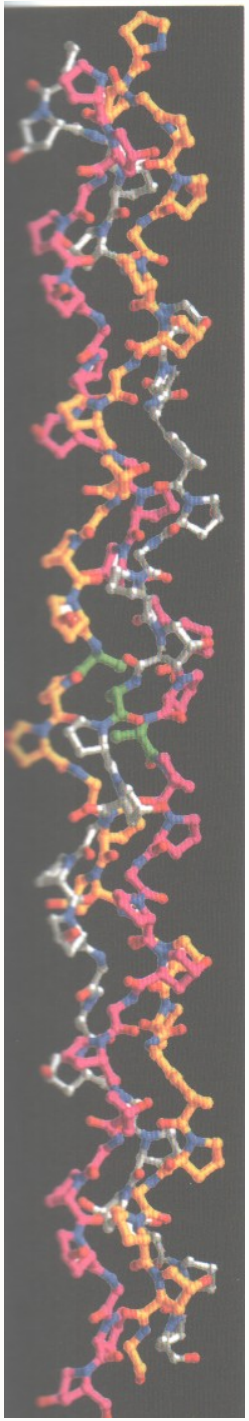
Quaternary Structure

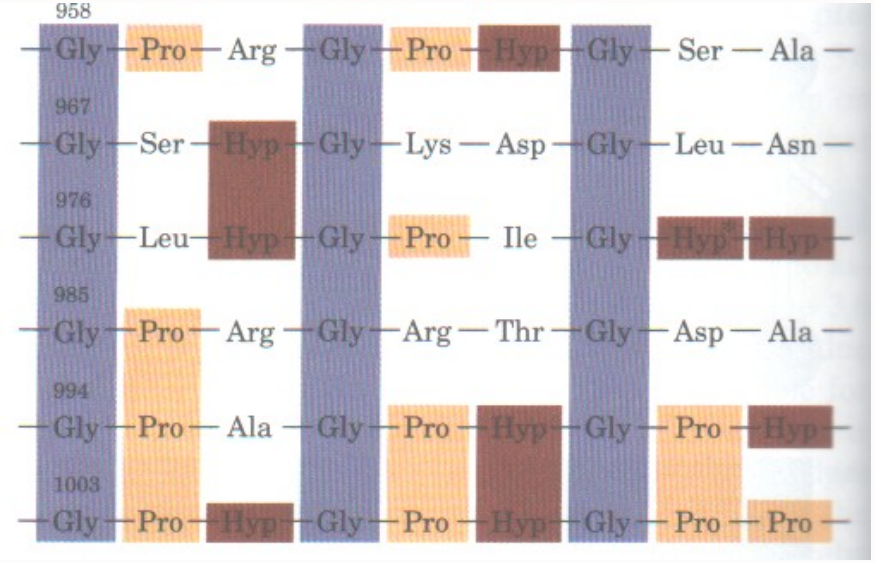


Collagen is composed of a repeating Gly – X – Y sequence

- the X position is typically Pro and the Y position is often hydroxyproline
- allows three collagen polypeptides to wrap around one another forming a right-handed triple helix
- each polypeptide is helical with n=3.3 and a pitch of 10.0 Å

Gly required at every 3rd position to allow close packing of subunits

- any other residue disrupts triple helix
- extreme tensile strength due to close packing



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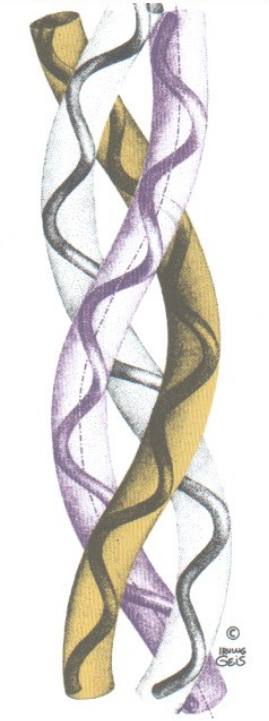
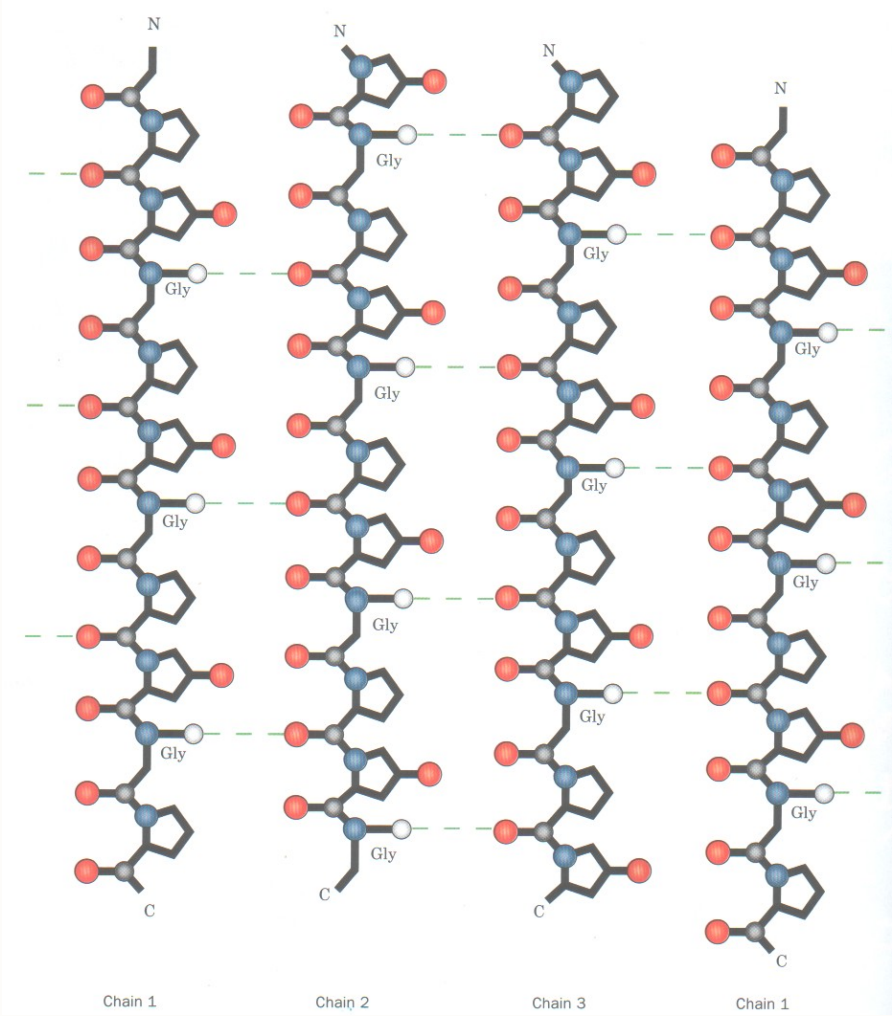
Triple Helix





- Three polypeptides of collagen helix are staggered relative to one another
  - places Gly at the center of the triple helix at every position along the collagen fiber
  - polypeptide helices are highly extended and have a left-handed twist while the triple helix is right-handed
- Gly at every third position contributes to tensile strength of helix

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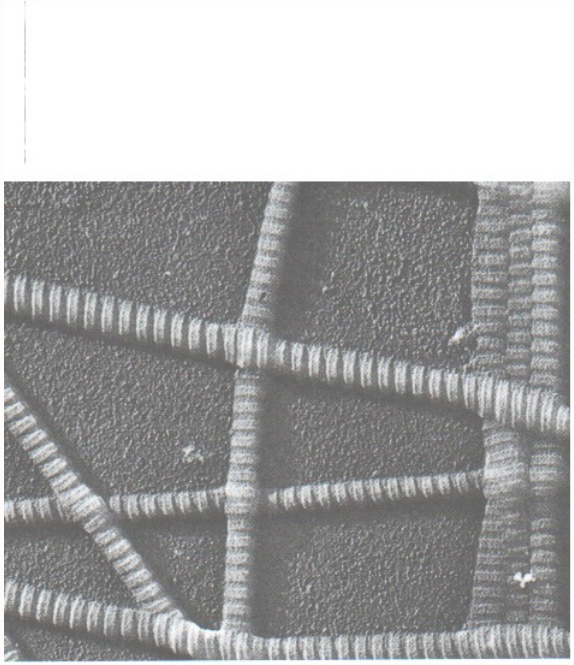
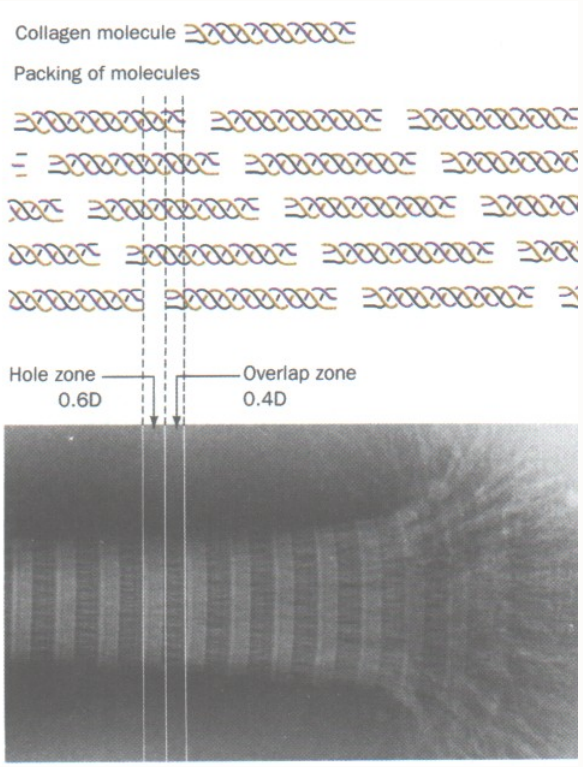
Collagen Fibrils

Several common collagens form distinctively banded fibrils

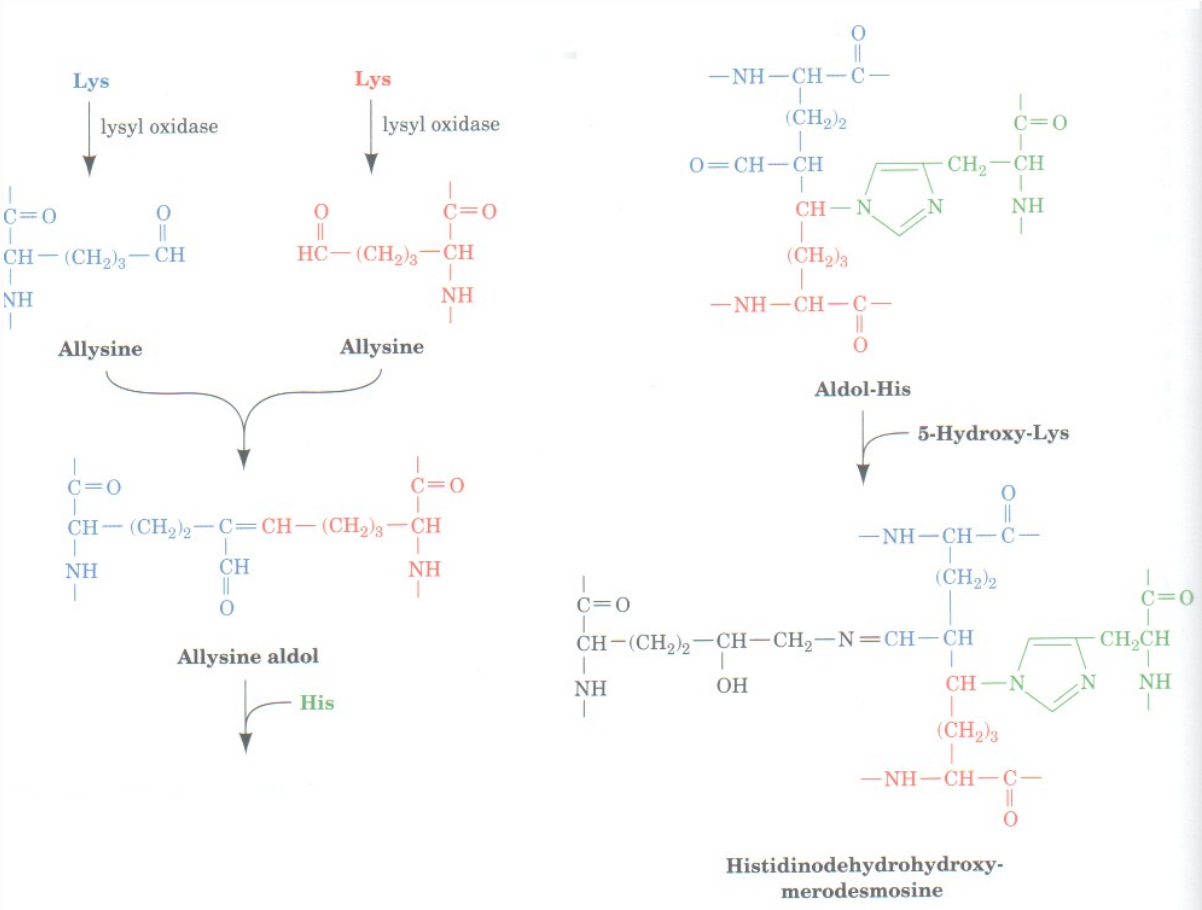
- Type I collagens have a diameter of 100-2000 Å and a periodicity of 680 Å
- Banding arises from packing of collagen molecules in the fibril
  - hydrophobic interactions are the driving force for association of collagen triple helices into a fibril





Covalent cross-links

- **Strength and insolubility of collagen fibrils is also due to intra- and intermolecular covalent cross-links**
  - **Not disulfide bonds as collagen is nearly devoid of Cys**
  - **Cross-links are between Lys (and His) residues**
    - **up to 4 residues can be involved in a single cross-linked**



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Lathyrism

Lathyrus odoratus is a sweet pea that contains significant amounts of β-aminopropionitrile

- β-aminopropionitrile is an inhibitor of lysyl oxidase and prevents the cross linking of collagen fibrils
- Increased fragility of collagen fibrils leads to abnormalities of bones, joints and large blood vessels

Aging increases the cross-linking of collagen

- not central cause of aging as lathyrism has no effect on lifespan
- explains why meat from older animals is tougher and why older individuals have less flexibility

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Collagen defects and disease

Several rare heritable disorders of collagen are known

- **brittle bone disease can arise from a single point mutation in collagen of bone**
- **hyperextensibility of joints arises from lessening of cross linking in collagen of ligaments**
- **osteoarthritis and atherosclerotic plaques arise from disruption of collagen in cartilage**

Each disease results in the disruption of the triple helical structure of a specific collagen