"Fibrous Proteins"

I. Overview

- Fibrous proteins are usually folded into either extended filaments or sheet-like structures, with repeated amino acid sequences.

- They are relatively insoluble.

- They provide structural or protective function in our tissues, such as in:

    - Connective tissues
    - Tendons
    - Bone
    - Muscle fibers

- Collagen and elastin are examples of commonly occurring, well-characterized fibrous proteins of the extracellular matrix (ECM).

- Collagen and elastin serve structural functions in the body.

- They are components of the:
    - Skin
    - Connective tissue
    - Blood vessel walls
    - Sclera of the eye
    - Cornea of the eye

- Each fibrous protein exhibits special mechanical properties, resulting from its unique structure.

- This unique structure is obtained by combining specific amino acids into repeated, secondary structural elements.

- This structural organization is in contrast to globular proteins, whose shapes result from:
    - Complex interactions between:
        - Secondary
        - Tertiary
        - Sometimes quaternary structural elements.

II. Collagen

A. General Description

- Collagen is the most abundant protein in the human body.
- A typical collagen molecule is a long, rigid structure in which:
    - Three polypeptides (referred to as α chains) are wound around one another in a rope-like triple helix.
- Although this triple helix is found in all collagen molecules throughout the body, the many subtypes of collagen are further organized and dictated by the structural role collagen plays in a particular organ.

B. Tissue-specific Organization

- In some tissues, collagen may be dispersed as a gel that gives support to the structure, such as in:
    - Extracellular matrix (ECM)
    - Vitreous humor of the eye

- In other tissues, collagen may be bundled in tight, parallel fibers that provide great strength, as in:
    - Tendons
- In the cornea of the eye, collagen is stacked so as to transmit light with a minimum of scattering.
- Collagen of bone occurs as fibers arranged at an angle to each other so as to resist mechanical shear from any direction.

C. Types

- The collagen superfamily of proteins includes:
    - More than 25 collagen types
    - Additional proteins that have collagen-like domains
- The three polypeptide α chains are held together by:
    - Interchain hydrogen bonds

- Variations in the amino acid sequence of the α chains result in:
    - Structural components that are about the same size (~1,000 amino acids long) but with slightly different properties
- These α chains are combined to form the various types of collagen found in tissues.

D. Examples of Collagen Types

- The most common collagen, type I, contains:
    - Two chains called α1
    - One chain called α2
    - Structure: $\alpha 1_2 \alpha 2$
- Type II collagen contains:
    - Three α1 chains
    - Structure: $\alpha 1_3$

E. Classification of Collagens by Function and Location

- The collagens can be organized into three groups, based on their location and functions in the body.

I. Fibril-forming Collagens

- Types: I, II, and III
- These are the fibrillar collagens, with a rope-like structure as described for a typical collagen molecule.
- Under the electron microscope, these linear polymers of fibrils show:
    - Characteristic banding patterns, reflecting the regular staggered packing of individual collagen molecules in the fibril.
- Type I collagen fibers (composed of collagen fibrils):
    - Found in supporting elements of high tensile strength
    - Examples: Tendons and Corneas

- Type II collagen fibers:
    - Restricted to cartilaginous structures
- Type III collagen fibers:
    - Prevalent in more distensible tissues
    - Example: Blood vessels

2. Network-forming Collagens

- Types: IV and VIII
- These form a three-dimensional mesh, rather than distinct fibrils.
- Example: Type IV molecules assemble into a sheet or meshwork that:
    - Constitutes a major part of basement membranes

Basement Membranes

- Are thin, sheet-like structures
- Provide:
    - Mechanical support for adjacent cells
    - Function as a semipermeable filtration barrier to macromolecules in organs such as the:
        - Kidney
        - Lung

Fibril-Associated Collagens and Structure of Collagen

A. 3. Fibril-Associated Collagens

- Types IX and XII bind to the surface of collagen fibrils.
- These collagens link the fibrils to one another.

B. Structure

- Unlike most globular proteins, which are folded into compact structures, collagen (a fibrous protein) has:
    - An elongated, triple-helix structure
    - The structure is stabilized by interchain hydrogen bonds.

1. Amino Acid Sequence

- Collagen is rich in proline and glycine, both of which are important in the formation of the triple-stranded helix.

- Proline:

    - Facilitates the formation of the helical conformation of each α chain.
    - Its ring structure causes "kinks" in the peptide chain.
    - Note: The presence of proline dictates that the helical conformation of the α chain cannot be an α helix.

- Glycine:

    - Is the smallest amino acid.
    - Is found in every third position of each polypeptide chain.
    - Fits into the restricted spaces where the three chains of the helix come together.

- The glycine residues are part of a repeating sequence:
    - –Gly–X–Y–, where:
        - X is frequently proline
        - Y is often hydroxyproline, but can be hydroxylysine
- Thus, most of the α chain can be regarded as a polytripeptide whose sequence can be represented as:
    - $(–Gly–Pro–Hyp–)_{333}$

Collagen: Posttranslational Modifications and Biosynthesis

2. Hydroxyproline and Hydroxylysine

- Collagen contains hydroxyproline and hydroxylysine, which are nonstandard amino acids not present in most other proteins.
- These unique amino acids result from the hydroxylation of some of the proline and lysine residues after their incorporation into polypeptide chains.

- Therefore, hydroxylation is a posttranslational modification.

- Note: The presence of hydroxyproline maximizes formation of interchain hydrogen bonds that stabilize the triple-helical structure.

3. Glycosylation

- The hydroxyl group of the hydroxylysine residues of collagen may be enzymatically glycosylated.

- Most commonly, glucose and galactose are sequentially attached to the polypeptide chain prior to triple-helix formation.

C. Biosynthesis

- The polypeptide precursors of the collagen molecule are synthesized in:
    - Fibroblasts
    - Or in the related:
        - Osteoblasts of bone
        - Chondroblasts of cartilage

- These precursors are enzymically modified and form the triple helix, which is secreted into the ECM.

- After additional enzymic modification, the mature extracellular collagen fibrils:

    - Aggregate
    - Become cross-linked to form collagen fibers.

1. Pro-α Chain Formation

- Collagen is one of many proteins that normally function outside of cells.

- Like most proteins produced for export, the newly synthesized polypeptide precursors of α chains (prepro-α chains) contain a special amino acid sequence at their amino (N)-terminal ends.

- This sequence:

    - Acts as a signal
    - In the absence of additional signals, targets the polypeptide being synthesized for secretion from the cell.

- The signal sequence:
    - Facilitates the binding of ribosomes to the rough endoplasmic reticulum (RER)
    - Directs the passage of the prepro-α chain into the lumen of the RER.

- The signal sequence is rapidly cleaved in the lumen to yield a precursor of collagen called a pro-α chain.

2. Hydroxylation

- The pro-α chains are processed by multiple enzymes within the lumen of the rough endoplasmic reticulum (RER) while the polypeptides are still being synthesized.

- Proline and lysine residues in the Y-position of the -Gly-X-Y- sequence can be hydroxylated to form:
    - Hydroxyproline
    - Hydroxylysine

- These hydroxylation reactions require:
    - Molecular oxygen (O_2)
    - Ferrous iron (Fe^{2+})
    - The reducing agent vitamin C (ascorbic acid)

- Without vitamin C, the hydroxylating enzymes:
    - Prolyl hydroxylase
    - Lysyl hydroxylase
    - Cannot function
- Deficiency of ascorbic acid leads to:
    - Impaired formation of interchain hydrogen bonds
    - Impaired formation of a stable triple helix
    - Inability to cross-link collagen fibrils
    - ↓ Tensile strength of collagen fibers
- The resulting disease is known as scurvy:
    - Characterized by ecchymoses (bruise-like discolorations)
    - Petechiae on the limbs due to subcutaneous extravasation of blood (from capillary fragility)
    - Other symptoms:
        - Gum disease
        - Loosening of teeth
        - Poor wound healing

3. Glycosylation

- Some hydroxylysine residues are glycosylated with:
    - Glucose
    - Or glucosyl-galactose

4. Assembly and Secretion

- After hydroxylation and glycosylation, three pro-α chains assemble to form procollagen.
- Procollagen:
    - Is a precursor of collagen
    - Has a central triple-helical region
    - Flanked by nonhelical N- and C-terminal extensions called propeptides
- Formation of procollagen begins with:
    - Interchain disulfide bonds between the C-terminal extensions of the pro-α chains
    - This aligns the three α chains to allow triple helix formation

- The procollagen molecules pass through the Golgi apparatus, where they are:
    - Packaged into secretory vesicles
- These vesicles fuse with the cell membrane, releasing procollagen into the extracellular space

5. Extracellular Cleavage of Procollagen Molecules

- Once procollagen is secreted into the extracellular space, it undergoes enzymatic cleavage.
- Enzymes involved:
    - N-procollagen peptidase
    - C-procollagen peptidase
- These enzymes remove the terminal propeptides, converting procollagen into:
    - Tropocollagen molecules (fully triple-helical)

6. Collagen Fibril Formation

- Tropocollagen molecules:
  - Spontaneously associate to form collagen fibrils
- Fibrils align in an ordered, parallel array:
  - Molecules are arranged in a staggered pattern
  - Each collagen molecule overlaps its neighbor by about three-quarters of its length
- This staggered array contributes to the banding pattern seen under electron microscopy

7. Cross-Link Formation

- The collagen fibrils serve as a substrate for the enzyme lysyl oxidase
- Lysyl oxidase:
  - A copper-containing extracellular enzyme
  - Performs oxidative deamination of:
    - Some lysine
    - Some hydroxylysine residues

- This reaction produces reactive aldehydes:
    - Allysine
    - Hydroxyallysine
- These aldehydes:
    - Can spontaneously condense with lysine or hydroxylysine residues of adjacent tropocollagen molecules
    - Form covalent cross-links
    - Result in mature collagen fibers with increased tensile strength
- Note: Cross-links can also form between two allysine residues

Copper-containing Enzymes (Lysyl Oxidase and Others)

- Lysyl oxidase is one of several enzymes that require copper as a cofactor.
- Other copper-containing enzymes include:
    -> Ceruloplasmin -> Cytochrome c oxidase
    -> Dopamine hydroxylase -> Superoxide dismutase
    -> Tyrosinase

- Disruption of copper homeostasis leads to:
    - Copper deficiency → X-linked Menkes syndrome
    - Copper overload → Wilson disease

D. Degradation of Collagen

Collagen Fiber Stability

- Normal collagen fibers are highly stable
- Half-life: Can be several years
- Despite stability, connective tissue is dynamic and continuously remodeled
    - Remodeling occurs in response to:
        - Tissue growth
        - Injury

Mechanism of Collagen Degradation

- Primary enzymes: Collagenases
    - Belong to the matrix metalloproteinase (MMP) family

- For Type I collagen:
    - Collagenase cleaves at a specific site
    - Produces:
        - A three-quarter length fragment
        - A one-quarter length fragment
- These fragments are further degraded by:
    - Other matrix proteinases

E. Collagenopathies

Definition and Cause

- Collagenopathies: Genetic diseases due to defective collagen synthesis
- Caused by mutations affecting any step in collagen fiber formation
- Result in:
    - Improper collagen fiber assembly
    - Reduced tensile strength of tissues

Genetic Basis

- Over 1,000 mutations have been identified
- Affect 23 genes
- These genes code for:
    - 13 different types of collagen

Consequences

- Defective collagen leads to:
    - Weak connective tissue
    - Clinical manifestations depending on the type of collagen affected
- Examples of collagenopathies are given below

1. Ehlers-Danlos Syndrome (EDS)

Definition

- A heterogeneous group of heritable connective tissue disorders
- Caused by defects in fibrillar collagen metabolism

Causes

- Deficiency of collagen-processing enzymes, such as:
    - Lysyl hydroxylase
    - N-procollagen peptidase
- Mutations in collagen genes:
    - Type I, III, and V

Forms of EDS

- Classic Form:
    - Caused by defects in type V collagen
    - Characterized by:
        - Skin extensibility
        - Skin fragility
        - Joint hypermobility
- Vascular Form:
    - Caused by defects in type III collagen
    - Most serious and potentially lethal
    - Associated with arterial rupture

Inheritance

- Both classic and vascular forms are autosomal dominant

Pathophysiology

- Mutant collagen chains may:
    - Have altered structure, secretion, or distribution
    - Be degraded more easily

- Dominant-negative effect:
    - Incorporation of just one mutant chain can:
        - Disrupt the entire triple helix
        - Lead to degradation of the molecule

2. Osteogenesis Imperfecta (OI)

Definition

- Also called "brittle bone disease"
- A genetic disorder of bone fragility

Clinical Features

- Bones fracture easily
- Fractures may occur with minor or no trauma

Cause

- Caused by dominant mutations in genes coding for:
    - α1 or α2 chains of Type I collagen
- Accounts for over 80% of OI cases

Molecular Pathology

- Most common mutations involve replacement of glycine (in -Gly-X-Y- sequence) with bulky amino acids
- This disrupts triple-helix formation in α-chains
- Leads to abnormal collagen structure

Phenotypic Severity

- Severity ranges from mild to lethal

Types of OI

- Type I (Most common, Mild)
    - Mild bone fragility
    - Hearing loss
    - Blue sclerae
- Type II (Most severe)
    - Perinatal lethal
    - Death due to pulmonary complications
    - In utero fractures visible

- Type III (Severe, Non-lethal)
    - Multiple fractures at birth
    - Short stature
    - Spinal curvature (kyphosis)
    - Blue sclerae

Other Features

- Dentinogenesis imperfecta:
    - Defective tooth development
    - Common in OI patients

Treatment

- Treated with bisphosphonates:
    - Inactivate osteoclasts (bone resorbing cells)
    - Increase osteoclast apoptosis
    - Decrease osteoblast apoptosis
    - Result: Inhibition of bone resorption and promotion of bone formation

3. Alport Syndrome

Definition

- A group of inherited disorders of basement membranes

- Affects:

    - Kidney (mainly)
    - Cochlea (inner ear)
    - Eye

Clinical Features

- Glomerulonephritis
- Hematuria
- Proteinuria
- Hypertension
- Progression to End-Stage Renal Disease (ESRD)
- Hearing loss (typically occurs between 2nd-4th decades)

Genetics

- Caused by mutations in Type IV collagen genes
- Genetic frequency: ~1 in 5,000
- Most common form:
    - X-linked dominant inheritance
- Inheritance patterns and symptoms vary depending on specific gene involved

III. Elastin

Overview

- Unlike collagen, elastin has rubber-like properties
- Found in:
    - Lungs
    - Walls of large arteries
    - Elastic ligaments
- Elastic fibers can stretch several times their original length and recoil upon relaxation

A. Structure of Elastin

Tropoelastin

- Precursor to elastin
- Soluble polypeptide (~700 amino acids)
- Composed primarily of small, nonpolar AAs:
    - Glycine
    - Alanine
    - Valine
- Also rich in:
    - Proline
    - Lysine
- Contains very little:
    - Hydroxyproline
    - Hydroxylysine

Extracellular Processing

- Tropoelastin is secreted into the extracellular matrix (ECM)
- Interacts with glycoprotein microfibrils, especially:
  - Fibrillin
  - Acts as scaffold for elastin deposition

Cross-linking

- Lysyl oxidase deaminates lysyl residues → forms allysine
- Desmosine cross-link forms from:
  - 3 allysine residues + 1 lysine residue
  - From same or neighboring tropoelastin molecules
- Result: Insoluble elastin with:
  - Extensive cross-linking
  - High elasticity and flexibility

Clinical Correlation: Marfan Syndrome

Cause

- Mutation in FBN1 gene → abnormal fibrillin-1
- Leads to:
    - Impaired microfibril formation
    - Abnormal fibrillin disrupts normal microfibril function

Clinical Features

- Skeletal abnormalities:
    - Tall stature
    - Long, slender limbs and digits (arachnodactyly)
    - Flexible joints
    - Scoliosis
- Ocular:
    - Lens dislocation (ectopia lentis)

- Cardiovascular:
    - Mitral valve prolapse
    - Aortic aneurysm (risk of dissection)
- Skin may show signs of laxity
- Blue sclerae may be present
    - Also seen in EDS and OI
    - Due to thin connective tissue allowing deeper pigment to be visible

B. α_1-Antitrypsin in Elastin Degradation

Overview of AAT

- α_1-Antitrypsin (AAT) is a protease inhibitor
- Found in blood and body fluids
- Inhibits various proteolytic enzymes (also called:
    - Peptidases
    - Proteases
    - Proteinases)

- Originally named for inhibiting trypsin (from pancreatic trypsinogen)

Primary Function of AAT

- Inhibits neutrophil elastase:
    - A powerful protease
    - Released from activated/degenerating neutrophils
    - Degrades:
        - Elastin in alveolar walls
        - Other structural proteins in multiple tissues

Synthesis of AAT

- Major site: Liver
- Minor sites: Extrahepatic synthesis also occurs

1. Role of AAT in the Lungs

- Alveoli are chronically exposed to low levels of neutrophil elastase

- Elastase, if uncontrolled, can:
    - Destroy elastin in alveolar walls
    - Cause irreversible lung damage (lung tissue can't regenerate)
- AAT is the main inhibitor that prevents this elastin destruction
- Imbalance between elastase and AAT → pulmonary disease

2. α_1-Antitrypsin Deficiency & Emphysema

Epidemiology

- In the U.S., 2–5% of emphysema cases are linked to inherited AAT deficiency

Molecular Defect

- Caused by mutations in the AAT gene

- Most severe and common mutation:
    - Single base mutation: GAG → AAG
    - Results in lysine replacing glutamic acid at position 342
    - This variant is called the Z variant

Pathogenesis of the Z Variant

- Misfolding of AAT protein
- Leads to polymerization and aggregation in the RER of hepatocytes
- Causes:
    - Reduced secretion of AAT into circulation
    - Deficiency of functional AAT
- Classified as a misfolded protein disease

Clinical Consequences

- Unopposed neutrophil elastase damages alveolar walls
  - → Results in early-onset emphysema

- Polymer accumulation in hepatocytes may lead to:
  - Hepatic cirrhosis

Hepatic Consequences of AAT Deficiency

- Misfolded AAT polymer accumulation in hepatocytes leads to:

  - Hepatocellular injury
  - Pediatric end-stage liver failure

- Liver transplantation may be required in severe cases

Effect on Blood AAT Levels

- Due to reduced secretion from liver:
  - Serum AAT levels are decreased
  - Resulting in insufficient AAT reaching lung tissues

Epidemiology & Genetic Inheritance

- AAT mutations most common in:
    - Caucasians of Northern European descent
- Genetic pattern: Autosomal codominant
    - Homozygotes (two abnormal alleles): At risk of emphysema
    - Heterozygotes (one normal, one mutant allele): Usually have:
        - Sufficient AAT levels
        - No lung damage

Biochemical Note – Role of Methionine 358

- Methionine 358 in AAT:
    - Essential for binding to target proteases

- Smoking effect:
    - Causes oxidation of Met-358
    - → Inactivates AAT
    - → Renders AAT unable to neutralize elastase
    - Result:
        - Accelerated lung destruction
        - Worse prognosis in smokers with AAT deficiency

Treatment – Augmentation Therapy

- Weekly intravenous AAT infusion
- AAT diffuses from blood to lungs
    - Achieves therapeutic levels in epithelial lining fluid
- Aims to restore protease-antiprotease balance and slow disease progression