Med Learn

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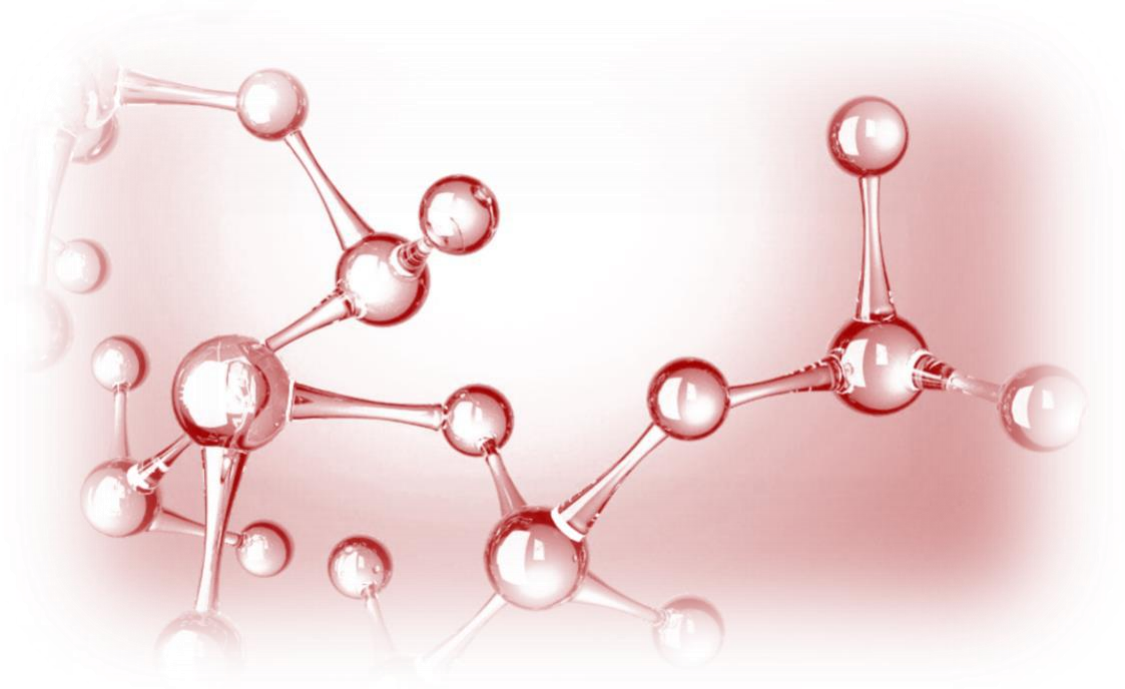
Biochemistry

Sheet 11

Fibrous proteins & Immunoglobulin



❖ Proteins:

- **Biological Functions of Proteins**
  - Enzymes → catalysts for reactions
  - Contractile/motion → myosin; actin
  - Structural → collagen, keratin, actin
  - Defense → antibodies
  - Signaling → hormones, receptors
  - Toxins → diphtheria; enterotoxins
  - Transport molecules → hemoglobin; lipoproteins, channel proteins

- **Proteins can be divided into two groups according to Tertiary 3D structure:**
  - **Fibrous** → elongated fiber-like proteins with a uniform secondary-structure only (like Alpha helix)
    - ✓ Examples: Collagen, Elastin & Keratins
  - **Globular** → globe-like with 3D compact structures
    - ✓ Examples: Myoglobin, Hemoglobin & Immunoglobulin

- **Extracellular matrix:** A space largely filled by an intricate network of macromolecules such as proteins and polysaccharides that assemble into an organized meshwork closely associated with cell surface
  - The function of ECM is to **regulate interaction between cells** and this function is mediated by proteins mainly

❖ Collagens

- The collagens are a family of fibrous proteins with 25 different types found in **all multicellular animals**
- They are the **most abundant proteins in mammals** (constituting 25% of the total protein mass in them)
- Collagen molecules are named as type I collagen, type II collagen, type III collagen, and so on
- The main function of collagen molecules is to **provide structural support** to tissues
- Hence, the primary feature of a typical collagen molecule is its **stiffness**

- It is a **triple-stranded**, **helical** protein, in which three collagen polypeptide chains called **α-chains** → are wound around one another in a ropelike superhelix → making the molecule stronger (more non-covalent interactions)
- This basic unit of collagen is called **tropocollagen**
- Compared to the α-helix, the collagen helix is much **more extended with 3.3** residues per turn

- **Composition of collagens**
- Collagens are rich in **glycine (33%) and proline (13%)**
  - Glycine allows the three helical α chains to pack tightly together to form the final collagen superhelix
  - Proline creates the kinks and stabilizes the helical conformation in each a chain

- It is also unusual in containing **hydroxyproline (9%) and hydroxylysine**
  - Hydroxylation **increases** the chance of **hydrogen bonding** → increasing solubility, rigidity & strength
  - Hydroxyproline importance:
    - ✓ Stabilizing collagen ➔ Without hydrogen bonds between hydroxyproline residues, the collagen helix is unstable and loses most of its helical content at temperatures above 20 °C
    - ✓ Normal collagen is stable even at 40 °C
  - Hydroxylysine serves as attachment sites of polysaccharides making collagen a glycoprotein
    - ✓ Sugars allow collagen to **recognize and interact** with cell surface receptors

- Every third residue is glycine → the preceding residue being **proline or hydroxyproline** in a repetitive fashion as follows:
  - Gly – pro – Y
  - Gly – X – hydroxyproline

- Some of the lysine side chains are **oxidized** to **allysine** (aldehyde derivatives) by **lisyl oxidase**
- Covalent **aldol cross-links** form between hydroxylysine residues and lysine or another oxidized lysine
  - These cross-linking can be intramolecular (inside the same molecule) or intermolecular (between different moleculase)

- **Function of cross-linking**
- These cross-links **stabilize the side-by-side packing** of collagen molecules and generate a **strong fibril**
- If cross-linking is inhibited, the tensile strength of the fibrils is drastically reduced; collagenous tissues become fragile, and structures such as skin, tendons, and blood vessels tend to tear
- The amount of **cross-linking in a tissue increases with age** → That is why meat from older animals is tougher than meat from younger animals and this is why we have wrinkles

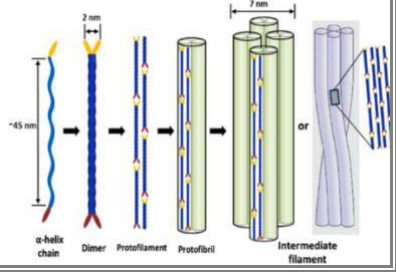
- **Formation of collagen fibers**
- **Protocollagen (Tropocollagen):** a unit of 3 coiled polypeptide chains
- **5 protocollagen** polymerize into a **microfibril** → Microfibrils align with each other forming larger collagen **fibrils** (strengthened by the formation of covalent Aldehyde cross-links between lysine residues) → Microfibrils assemble into collagen **fibers**
- **Scurvy:** a disease is caused by a dietary deficiency of ascorbic acid (vitamin C)
  - Deficiency of vitamin C prevents proline hydroxylation
  - The defective pro-α chains fail to form a stable triple helix and are immediately degraded within the cell → **Blood vessels become extremely fragile**, and **teeth become loose in their sockets**

❖ Elastins

- They are strong & elastic fibrous proteins (flexible)
- Tissues such as skin, blood vessels, and lungs, need to be both **strong and elastic** in order to function
  - A network of elastic fibers in the extracellular matrix of these tissues gives them the required resilience so that they can recoil after transient stretch (stretch and relax)
  - **Long, inelastic collagen fibrils are interwoven with the elastic** fibers to limit the extent of stretching and prevent the tissue from tearing
- The main component of elastic fibers is **elastin**, which is a highly hydrophobic protein and is rich in proline and glycine, It contains some hydroxyproline, **but no hydroxylysine** → it is **not glycosylated**
- The primary component, **tropoelastin** molecules, is cross-linked between lysines to one another
- **Elastin structure:**
- The elastin protein is composed largely of two types of short segments that alternate along the polypeptide chain:
  - **Hydrophobic segments** → which are responsible for the elastic properties of the molecule → by reducing the chance of making hydrogen bonds making it more elastic)
  - **Alanine- and lysine-rich α-helical segments** → which form cross-links between adjacent molecules (Lysine is the one making the cross-linking of course, but it was noticed that there are repeated units of alanine and lysine)
- **Three allysyl** side chains plus one unaltered **lysyl** side chain form a **desmosine crosslink**

❖ Keratin

- Keratin is a major component of **intermediate filaments** which exist **inside** the cell
- Collagen & elastin present in the **ECM**
- Two important classes of proteins that have similar amino acid sequences and biological function are called **α-and β-keratins**, which act as members of a broad group of intermediate filament proteins
- **α-keratin** is the major proteins of hair and fingernails as well as animal skin
  - α-keratin has an unusually **high content of cysteine**



- **α-keratin structure:**
- **2 helical α-keratin** molecules interwind **forming a dimer** (**protofilaments**)
- **2 dimers** twist together to form a 4-molecule **protofibril**

- **Eight protofibrils** combine to make **one microfibril**
- **Hundreds of microfibrils** are cemented into a **microfibril**

- α-keratin in **fingernails (not hair**) can be hardened by the introduction of **disulfide cross-links**

- **Why does curly hair become straight when wet?**
  - Because keratin molecules in hair will **make hydrogen bonds with water** rather than with each other so the alpha helices straighten up → when the water dries up, they reform the hydrogen bonds together

- **Having a hair design?**

  - **Temporary Wave**

When hair gets wet, water molecules disrupt some of the **hydrogen bonds**, which help to keep the alpha-helices aligned. When hair dries up, the hair strands are able to maintain the new curl in the hair for a short time.

  - **Permanent wave**

A reducing substance (usually **ammonium thioglycolate**) is added to reduce some of the disulfide cross-links. The hair is put on rollers or curlers to shift positions of alpha-helices. An oxidizing agent, usually hydrogen peroxide, is added to reform the **disulfide bonds** in the new positions until the hair grows out

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❖ Immunoglobulins

- **Types of immunity**
  - **Innate** → you're born with these mechanisms, such as:
    - ✓ **Surface barriers** (skin & mucus) & **internal defense** (phagocytes, Fever, NK (natural killer) cells, antimicrobial proteins and inflammation)
  - **Adaptive** → you develop these mechanisms as you grow up
    - ✓ **Humoral immunity (B cells)** → involve antibodies (immunoglobulins)
    - ✓ **Cellular immunity (T cells)** → killing infected cells

- **How do B cells work?**
  - B cells **secrete immunoglobulins** (antibodies)
  - Immunoglobulins have three roles:

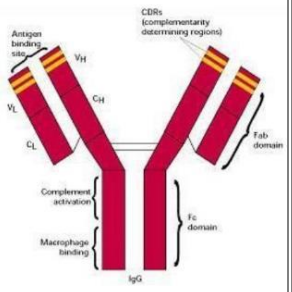
1) Antibodies bind to pathogens and induce their **phagocytosis into immune cells**
2) Antibodies bind to viruses and microbial toxins **neutralizing (destroying) them**
3) Antibodies **recruit white blood cells** and a system of **blood proteins** to lyse (get rid of) pathogens (complement system)

- **Is this an antigen, pathogen or normal (harmless) molecule. How do cells know?**
  - When B cell is activated by antigen, it proliferates and differentiates into an antibody-secreting effector cell → Such cells make and secrete large amounts of soluble (rather than membrane-bound) antibody at a rate of about 2000 molecules per second
  - Recognition depends on interaction between glycoproteins on the surface of the foreign body & the antibody

❖ Structure of antibodies

- Antibodies are **large Y-shaped** molecules consisting of **two identical heavy** chains and **two identical light** chains held together by **disulfide bonds**
  - The four polypeptide chains are held together by covalent disulfide (-S-S-) bonds
  - Within each of the polypeptide Chains there are also intra-chain disulfide bonds.

- They (antibodies) are **glycoproteins**, with oligosaccharides linked to their heavy chains



- **A light chain** consists of one **variable domain** (V_L) → differs between antibodies + **one constant domain** (C_L) → same between different antibodies
- **The heavy chain** consists of **one variable region** (V_H) + **three constant regions** (C_{H1}, C_{H2}, and C_{H3})
  - ➢ Each one of the C domains in the heavy chain (C_{H1}, C_{H2}, and C_{H3}) performs a certain function
    - ✓ C_{H2} is responsible for complement system activation
    - ✓ C_{H3} is responsible for phagocyte activation
- The domains of the heavy chain are bound to the domains of the light chain
- **CDRs** → they are hyper variables, the **most sites** that the structure of the protein can **differ** are these sites and they are within the variable domain of light and heavy chain

• Constant regions

- They are **uniform from** one antibody to another **within the same isotype**
  - The Fc domain (part of heavy chain) of antibodies are important for binding to phagocytic cells allowing for antigen clearance (Activate complementary system, activate phagocytes)

• Variable regions

- The variable region is found at the tips of the Y and is the part of the antibody that binds to part of the antigen **(epitope)**
- Each antibody can bind to two antigens **But** each B cell produces only one kind of antibodies
- The **primary sequences** of the variable regions among different antibodies are **quite distinct**
  - ➢ About 7-12 amino acids in each one that contribute to the antigen-binding site

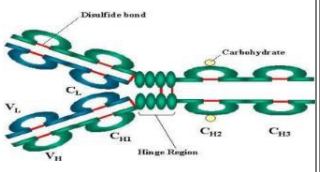
• Hypervariable regions

- Also called **Complementarity Determining Regions (CDRs)** are found within the variable regions of both the **heavy** and **light** chains → vary in their primary structure (affecting secondary & tertiary)
- They recognize & bind specifically to antigen with high affinity (dissociation constant Kd 10^{-12}-10^{-7})

• Immunoglobulin fold

- The hypervariable regions exist as a specialized domain called "Immunoglobulin fold", which is a motif (super secondary structure) that is present in every immunoglobulin
  - ➢ It consists of a sandwich of **two anti- parallel β sheets** held together by a disulfide bond making a shape of a barrel, hence known as "beta barrel"
- The **hypervariable regions** are specifically in **three loops** connecting the β sheets to each other

- **Hinge region:**
- A hinge region exists where the arms of the antibody molecule form a Y in the constant region of the heavy chain
- It adds some **flexibility to the molecule**



• Diversity

- Antigen-antibody binding is mediated by **noncovalent interactions** (but also with high-affinity)
- The enormous diversity of antigen-binding sites can be generated by changing only the lengths and amino acid sequences of the hypervariable loops
- The overall three-dimensional structure necessary for antibody function remains constant
- Each individual is capable of producing more than 10^{11} different antibody molecules

- **Producing this huge number of (diverse) antibodies from the same genes is done via:**
  - **DNA rearrangement**
    - ✓ **recombination of exons**, variable & joining regions
  - **Imprecise joining of regions**
    - ✓ Any change in the joining of regions → Producing different antibodies
  - **Addition/deletion of nucleotides during rearrangement**
    - ✓ Will cause a change in the amino acid sequence → primary structure → different antibody
  - **Somatic hypermutation**
    - ✓ Many mutations

- **We have 5 classes of antibodies:**
  - There are **two light chains** (lambda or kappa)
  - There are **five heavy chains** (alpha, delta, gamma, epsilon or mu) that make five types of immunoglobulins known as immunoglobulins **isotype** (IgA, IgD, IgG, IgE, IgM)

Isotype	Structure	Notes
IgM		Contain mu heavy chains Expressed on the surface of B-cells Found primarily in plasma cells The first antibodies produced in significant quantities against an antigen Promote phagocytosis and activate the complement system that leads to cell killing Appear usually as pentamers
IgG		Contains Gamma chains Monomers Most abundant immunoglobulins in sera (600-1800 mg/dL) Promote phagocytosis and activate the complement system Only kind of antibodies that can cross the placenta
IgD		Contains delta heavy chains Present on surface of B-cell that have not been exposed to antigens
IgE		Heavy chains type epsilon A monomer Plays an important role in allergic reactions
IgA		Contain alpha chains Found mainly in mucosal secretion The initial defense in mucosa against pathogen agents Appear usually as dimers

- **Class switching**
- **Changing the class** of the antibody but having the **same variable region**
  - First, before binding antigen, B cells contain **IgM molecules only**
  - Following antigen binding, class switching occurs
  - Class switching refers to a DNA rearrangement changing the heavy chain constant gene
  - That causes production of IgG, IgA, and IgE

- **Idiotype vs. isotypes vs. allotypes**
  - Immunoglobulin molecules that have different variable domains of both their light (VL) chains and heavy (VH) chains and are said to share an **idiotype** → the **change is in the variable region**
  - The different classes of immunoglobulins are determined by their different CH regions and called **isotypes** → the **change is in the constant region**
  - Immunoglobulins of the same class but different among individuals of the same species due to different genetics are called **allotypes** → the **same constant & variable regions** → but small differences between individuals

- **Hybridoma and monoclonal antibodies**
- **Normally** we produce **polyclonal** antibodies → directed against (recognize) a number of different epitopes on the antigen (these epitopes are proteins & part of the same antigen [usually])
- **Myeloma** (cancer B cells) produce **monoclonal antibodies**

- Sometimes we need monoclonal antibodies
  - We hybridize a B cell with a cancer B cell (myeloma) → producing immortal B cell that produces a single monoclonal antibody (**Hybridoma**) → so we get monoclonal B cells that can proliferate fast and endlessly
- Monoclonal antibodies **made in mice** can be humanized by attaching the CDRs onto appropriate sites in a human immunoglobulin molecule

- **Benefits of monoclonal antibodies**
  - **Measure the amounts of many individual proteins and molecules** (such as plasma proteins, steroid hormones)
  - **Determine the nature of infectious agents** (such as types of bacteria)
  - **Used to direct therapeutic agents to tumor cells**
  - **Used to accelerate removal of drugs from the circulation when they reach toxic levels**