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TUTORIAL

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# BIOCHEMISTRY OF CONNECTIVE TISSUE BIOCHEMISTRY OF MIXED SALIVA

Edited by A.I. Glukhov, E.V. Babchenko

Министерство образования и науки РФ

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# Part 1
# BIOCHEMISTRY OF CONNECTIVE TISSUE

**Main subjects:**
1.1. Glycosaminoglycans
1.2. Collagens
1.3. Elastin
1.4. Adhesive proteins
1.5. Mineralized connective tissue
1.6. Mineral composition and structure of apatites in the hard tissue
1.7. Organic matter of mineralized tissues
1.8. Bone tissue remodeling
1.9. Regulation of remodeling, growth and development of bone tissue
1.10. Markers of bone tissue metabolism
1.11. Features of dental tissue structure and metabolism

Human tissues are composed not only of cells. Most mammalian cells are located in tissues, where they are surrounded by a complex **extracellular matrix.** The matrix includes different polysaccharides and proteins spontaneously organized to form ordered structures. The tissue in which extracellular matrix occupies a considerably larger volume than cells is often referred to as "**connective tissue**" (Fig. 1.1).

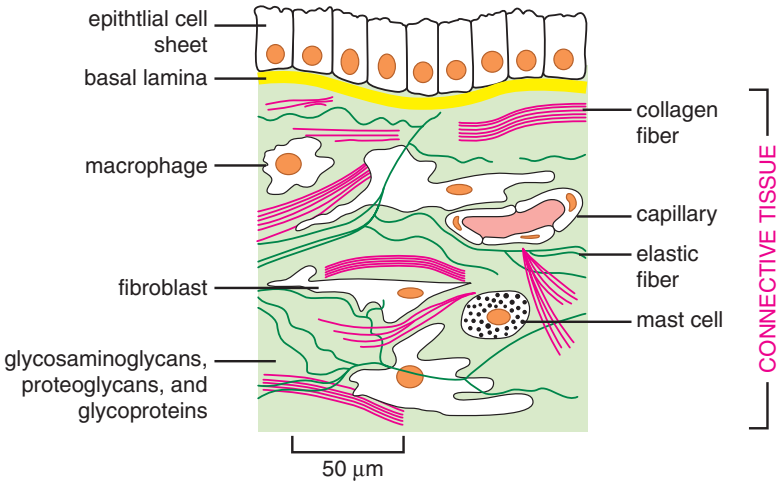



**Fig. 1.1. Organization: extracellular matrix-basal membrane-epithelium**

The connective tissue contacts with the layer of epithelial cells. The basal membrane (very thin and stiff film) resides between epithelium and extracellular matrix.

**Connective tissue provides:**

- active exchange of metabolites and ions between the blood and tissues;
- formation of the structure of organs and tissues during embryogenesis and the postnatal period;
- flexibility of interacting surfaces of joints because it forms cartilage;
- protection against external influence by regulation of the functional activity of phagocytes and immune system cells;
- regeneration and replacement of imperfection by stimulating functional activity and proliferation of tissue cells.

The share of connective tissue varies in different organs: in bones and skin it is the main component. Connective tissue may be mineralized forming hard bone or tooth structures; and also form transparent matter of eye cornea, or a cord-like structure making tendons resistant to rupture.

The macromolecules of extracellular matrix are mainly synthesized and secreted by cells located there. In most connective tissue types, fibroblasts are the main participants in these processes. In some specialized tissues, such as cartilage and bone, this function is performed by other cells, e.g., cartilage is formed by chondroblasts; bone — by osteoblasts.

The extracellular matrix of non-mineralized tissues is formed by two classes of macromolecules: **polysaccharides** — glycosaminoglycans (GAG), present in free condition and covalently bonded to protein; two types of **fibrillar proteins** — collagen and elastin (structural); and fibronectin, laminin and nidogen (adhesive).

The glycosaminoglycan and proteoglycan molecules forma hydrated, gel-like medium in which fibrillar proteins are immersed. The aqueous phase of polysaccharide gel provides diffusion of nutrients, metabolites and hormones between the blood and tissue cells. Collagen fibers organise order and reinforce matrix; and rubber-like elastic structures impart resilience. Adhesive proteins interacting with cells, collagen and GAG afford integration of the matrix components.

## 1.1. GLYCOSAMINOGLYCANS

These heteropolysaccharides, glycosaminoglycans (GAG), are a family of linear polymers composed of repeating disaccharide units -(A-B)n- (Table 1.1.). One of the two monosaccharides in the unit is always either N-acetylglucosamine (G1cNAc) or N-acetylgalactosamine (Ga1NAc); in most cases the other one is uronic acid, usually D-glucuronic or L-iduronic acid. In some glycosaminoglycans, one or more of the hydroxyls of the amino sugar are esterified with sulfate. A combination of sulfate and carboxylic groups of the uronic acid residues gives a very high density of negative charge to glycosaminoglycans.

All sugars are synthesized from glucose. Depending on the structure of carbohydrate residues, the nature of bonds between them, the number and position of sulfate groups, four main GAG groups are distinguished: hyaluronic acid; chondroitin sulfate and dermatan sulfate; heparin sulfate and heparin; and keratan sulfate.

## Glycosaminoglycan synthesis

All glycosaminoglycans (except hyaluronic acid) are attached to extracellular proteins to form proteoglycans. The protein part of these compounds accounts for no more than 5%. The polypeptide chains (core protein) of proteoglycans are synthesized on ribosomes of ER of fibroblasts. GAGs are mainly formed in Golgi apparatus. Polypeptide synthesis is preceded by a special linker trisaccharide (–xylose–galactose–galactose) acting as a primer for GAG growth being bonded to serine residue in the protein (Fig. 1.2). The chain of GAG composed of repeating disaccharide units (A and B) is synthesized by successively binding carbohydrate residues (Fig. 1.3).

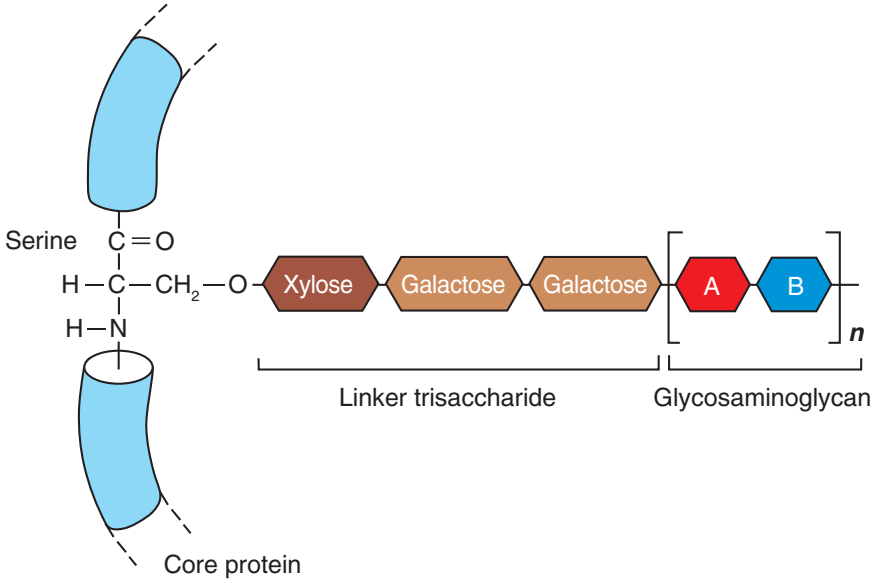
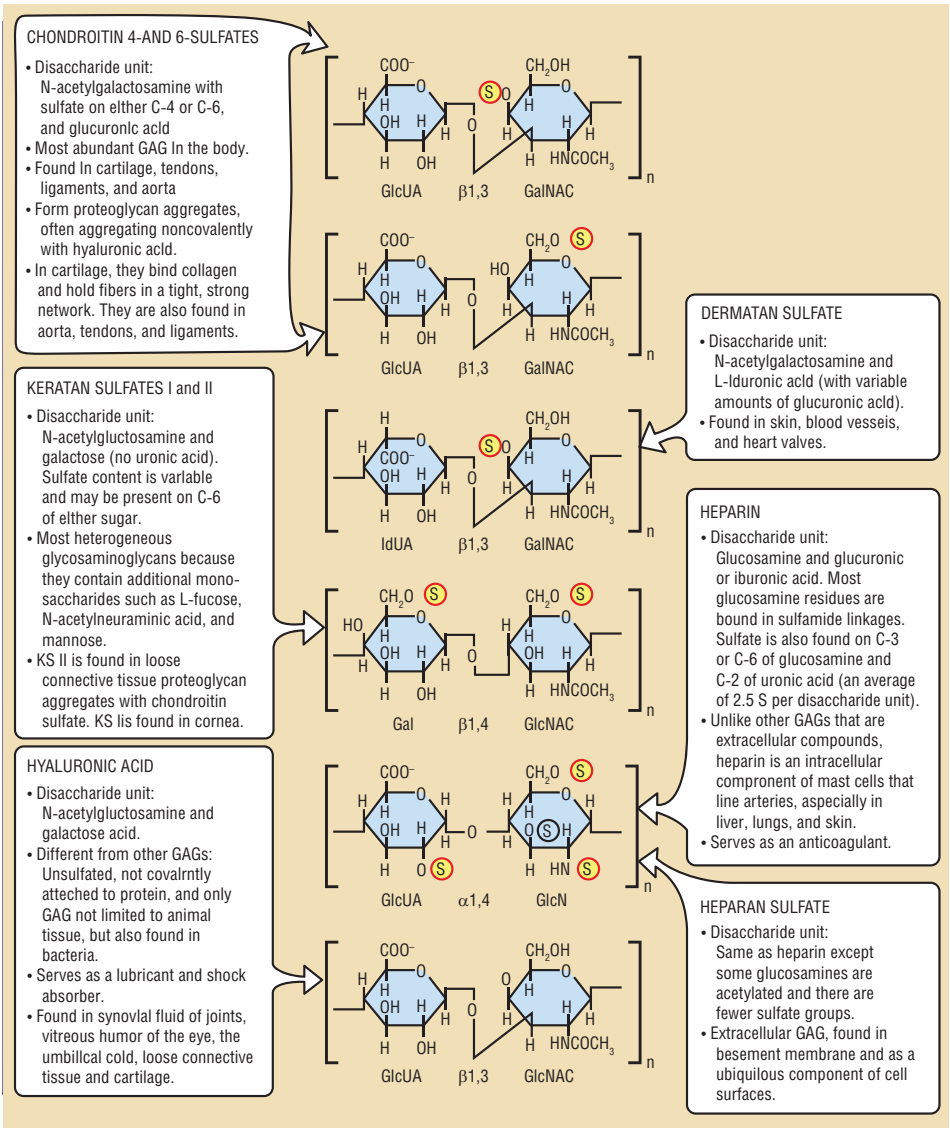



**Fig. 1.2. The link between GAG linker and serine of core protein**

Hyaluronic acid is synthesized by **hyaluronate synthetase** linked to the internal surface of plasma membrane. As the polymer chain lengthens, hyaluronic acid exteriorizes through the membrane on the external surface. Outside the cell, hyaluronic acid can interact with hyaluronate-linking proteins and participate in **aggrecan** formation.

Each reaction of trisaccharide linker formation and subsequent chain growth is catalyzed by specific glycosyltransferases, with sugars preactivated earlier:

**Table 1.1.** Glycosaminoglycan structure and distribution




Upon completion of synthesis, the proteoglycan molecule leaves the cell (Fig. 1.4). In the extracellular space some proteoglycans may form proteoglycan aggrecans (PGA), giant supramolecular assemblies of many core proteins all bound to a single hyaluronate molecule by link protein (Fig. 1.5).

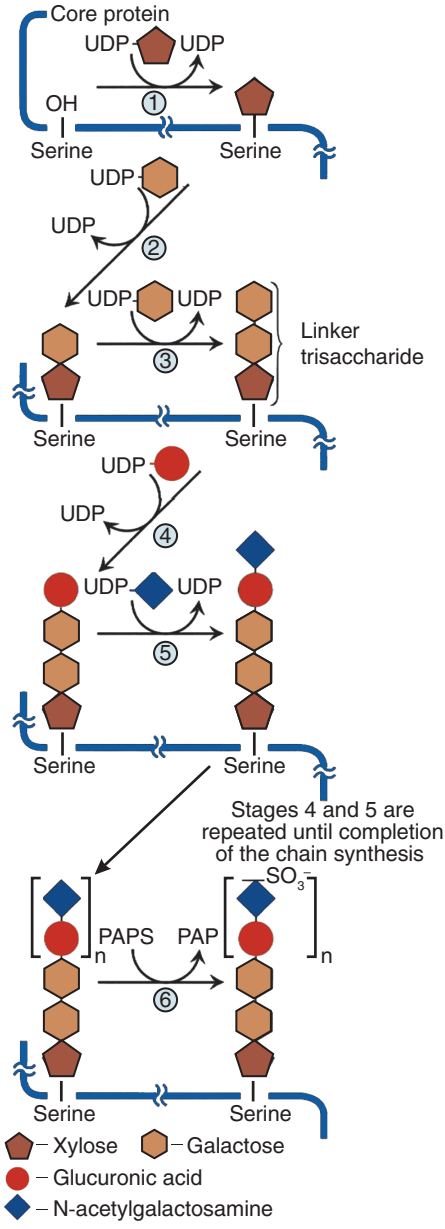



**Fig. 1.3. Chondroitin sulfate synthesis**

As the chain is lengthened, many carbohydrate residues are modified by sulfation and epimerization (transfer of functional groups in a saccharide molecule). The source of $-SO_3^-$ groups is phosphoadenosylphosphosulfate (PAPS). Attachment of sulfate group increases the negative charge of proteoglycans considerably.

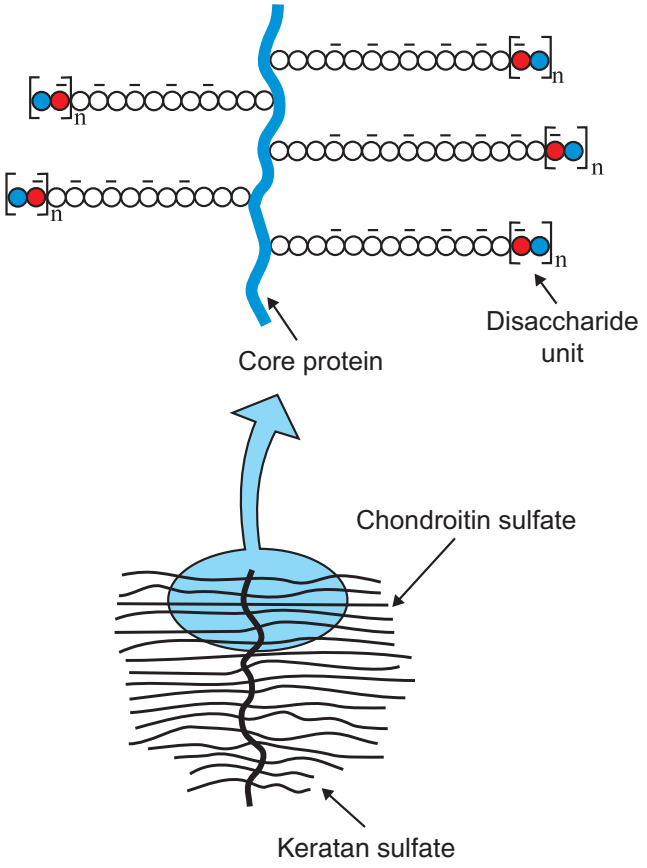



**Fig. 1.4. Proteoglycan structure resembling a bottle brush**

Due to the high density of negative charges, polysaccharide chains bind many osmotically active ions: $Na^+$, $Ca^{2+}$, and $K^+$. The high ion concentration retains water in the extracellular matrix causing it to swell to solidity. A hyaluronate molecule can bind 200 to 500 molecules of water. The specific structure and capacity for hydration of the extracellular matrix determine the degree of rigidity with elasticity and resilience.

Therefore the major amount of proteoglycans and PGA is contained in the intercellular substance of intervertebral disks and cartilages, menisci, tendons, ligaments and other anatomic structures which experience mechanical load and deformation. These disks are compressed during the day time and restore elasticity at night but are prone to become deformed with age (Fig. 1.6).

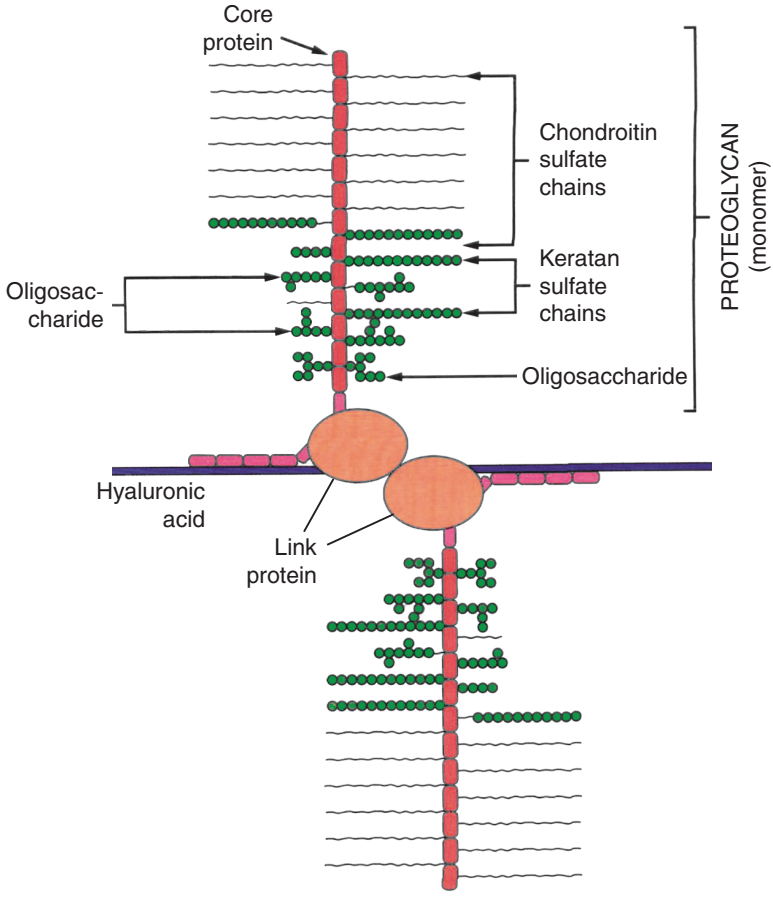



**Fig. 1.5. Aggrecan structure of cartilaginous matrix**

The structure formed may include more than a hundred proteoglycan monomers non-covalently bonded to a single hyaluronic acid molecule. The complex is stabilized by link proteins which are simultaneously bound to the core protein and the hyaluronic acid chain. The molecular mass of this structure may exceed $10^8$ Da and occupy a volume equal to that of a bacterial cell. Such a structurally complex proteoglycan as aggrecan is the main structural element of the cartilaginous matrix. It contains about 100 chondroitin sulfate chains and about 60 keratan sulfate chains.

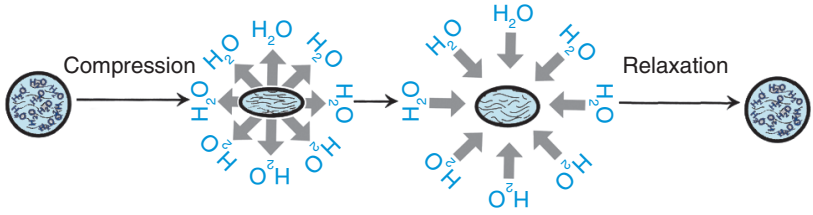



**Fig. 1.6. GAG spring function**

## Catabolism of glycosaminoglycans

Degradation of proteoglycans and PGA occurs in lysosomes. The protein components of molecules are hydrolyzed by lysosomal proteases, and GAG chains are degraded by a number of acidic glycosidases and sulfatases. In the absence of one of the enzymes involved in catabolism the degradation process becomes disrupted. GAG molecule fragments are accumulated in lysosomes. **Lysosomal storage disorders (resulting from GAG accumulation) are referred to as mucopolysaccharidoses** (the original name for GAG is mucopolysaccharide).

# 1.2. COLLAGENS

Collagens are a family of very similar proteins with some variations depending on tissue localization. They are mainly synthesized and secreted by connective tissue cells. Collagens constitute approximately 30% of all human proteins. All types of collagen have a triple helical structure: formed by folding three separate polypeptide chains called $\alpha$-chains which are coiled coil ($\alpha$-chains are wound around each other).

## Amino acid composition of collagen α-chains

The number of amino acids in each polypeptide chain may vary between 600 and 3000. The amino acid sequence in collagen is generally a repeating tripeptide unit, Gly–X–Y, where X is often Pro, and Y is often 4-Hyp (Fig. 1.7). The presence of glycine in each triplet (amino acid with no radical) affords tight junctions between the individual $\alpha$-chains.

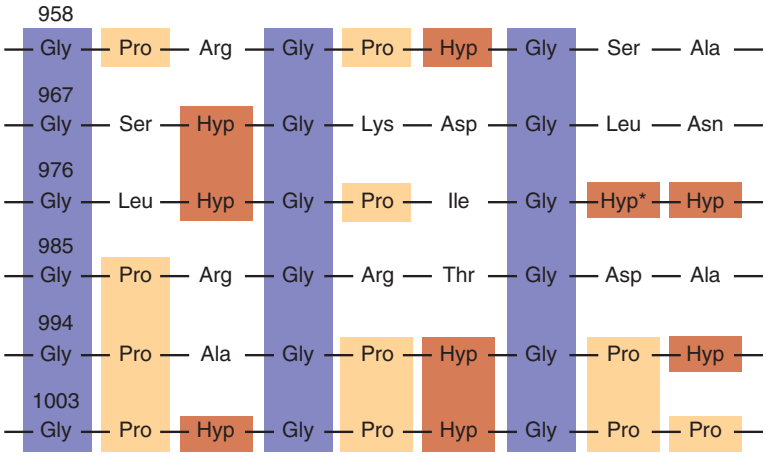



**Fig. 1.7. Amino acid composition of a fragment of collagen α-chain**

Collagen generally includes non-essential amino acids and is a low-value protein. For example, the polypeptide chain contains neither tryptophan nor cysteine; it includes a small amount of methionine, tyrosine and histidine. In addition to hydroxyproline, there is another nonstandard amino acid — hydroxylysine. These derivatives are formed by incorporation of a hydroxyl group into the amino acid residues of proline and lysine.

## Synthesis and formation of collagen fibrils

The process of polypeptide chain formation on ribosomes from the beginning to fiber formation occurs in two stages. The first stage proceeds in connective tissue fibroblasts and is referred to as **intracellular stage**. Protein synthesis occurs on the ribosomes of endoplasmic reticulum. Simultaneously, many molecules of collagen pre-pro-α-chains are formed (Fig. 1.8).

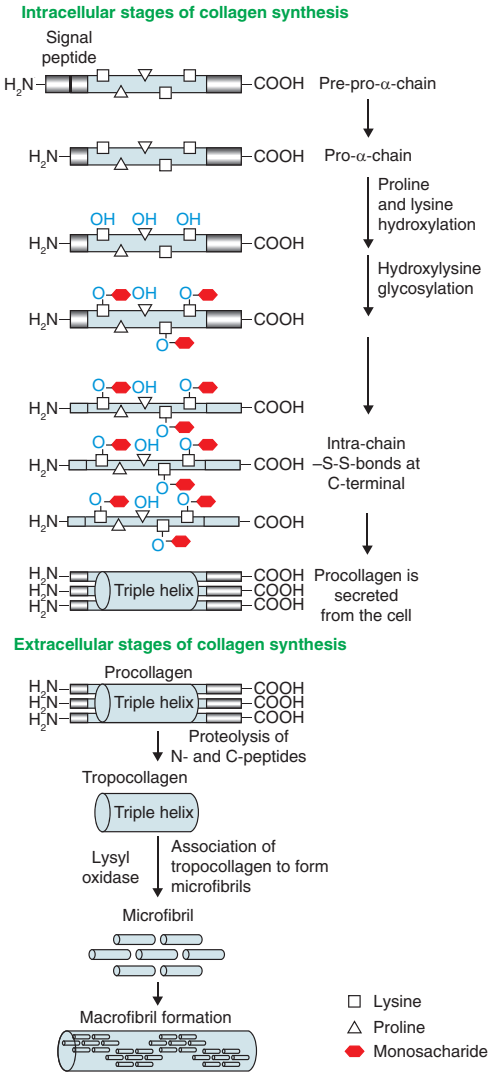



**Fig. 1.8. Collagen chain modification and the stages of tropocollagen synthesis**

There is a hydrophobic signal peptide composed of 100 amino acids at the N-terminal of the growing pre-pro-α-chain. The function of this peptide is to facilitate protein transport into ER lumen. After signal peptide splitting the pro-α-chain is formed.

In the ER cavity, proline and lysine residues are hydrolyzed to form hydroxyproline and hydroxylysine (Fig. 1.9). These reactions are catalyzed by iron-containing ($Fe^{2+}$) enzymes **prolyl hydroxylase and lysyl hydroxylase**. $Fe^{2+}$ stabilization requires the presence of reductant — ascorbic acid (vitamin C).

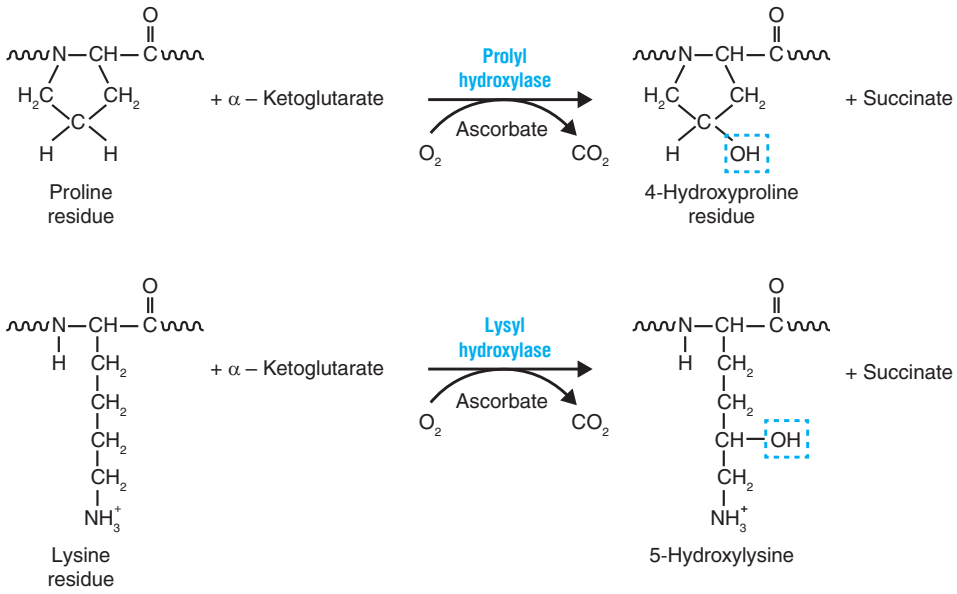



**Fig. 1.9. Proline and lysine residue hydroxylation in pre-pro-collagen in ER**

Hydroxyproline residues participate in formation of hydrogen bonds which stabilize the triple collagen structure. Lysine and hydroxylysine are necessary for covalent bond formation in collagen fibril assembly.

The following posttranslational modification — hydroxylysine glycosylation — proceeds simultaneously with triple structure formation and ends when spiralization has been completed. Specific glycosyltransferases can bind galactose, galactose and glucose, as well as mannose (at C-terminal) residues to hydroxylysine –OH groups (Fig. 1.10).

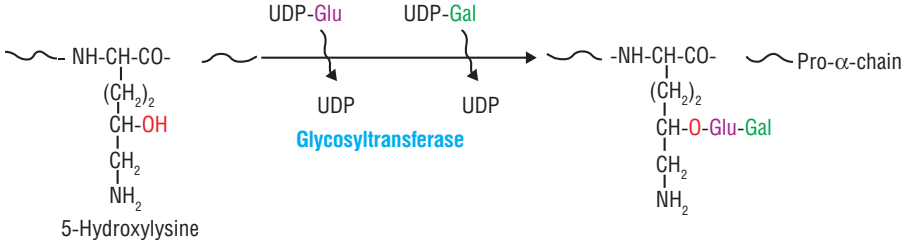



**Fig. 1.10. Glycosylation of hydroxylysine amino acid residue**

Correct orientation of chains in relation to each other is provided by N- and C-terminal fragments of pro-α-chain, which have a globular structure and contain cysteine residues. The N-terminal segment forms only intrachain disulfide bonds and prevents spiralization of the fragment of 100 amino acid residues. The intra- and interchain disulfide bonds in the C-terminal fragment of 250 amino acid residue provide for a correct pro-α-chain orientation while at the same time interfering with spiralization of this segment. They also prevent formation of large collagen fibrils in the cells, which would otherwise disturb the functions of these cells and connective tissue.

Structures composed of 3 pro-α-chains enter the secretory granules and are released into the extracellular space. The first modification of the **extracellular stage** is partial proteolysis of N- and C-nonspiralized terminals. Peptide bond hydrolysis is catalyzed by specific peptidases resulting in formation of a tropocollagen molecule.

**Tropocollagen** is a rod-shaped molecule. Three separate polypeptide chains are wrapped around each other and are of equal lenth. Each chain includes about 1000 amino acid residues.

The assembly of tropocollagen into microfibrils — fibrillogenesis — is preceded by another modification of lysine residues within a three-spiral molecule. Lysyloxidase, extracellular enzyme containing $Cu^{2+}$, catalyzes deamination of lysine and hydroxylysine. This leads to formation of allysine (lysine aldehyde) and hydroxyallysine (hydroxylysine aldehyde), which are highly reactive (Fig. 1.11).

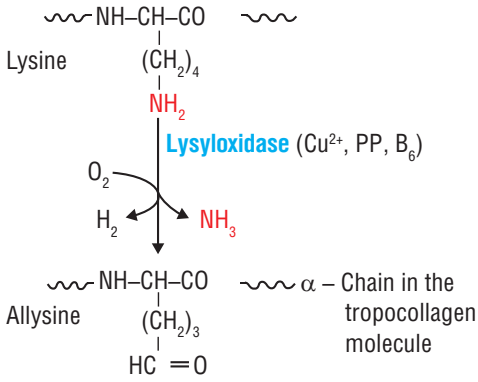



**Fig. 1.11. Extracellular modification of lysine radicals**

## Fibrillogenesis

Formation of microfibrils is a spontaneous process. Tropocollagen molecules are arranged in parallel rows (Fig. 1.12).

The fibrils are stabilized by spontaneous formation of intramolecular covalent bonds between the lysine, allysine, hydroxylysine or hydroxyallysine groups (Fig. 1.13).

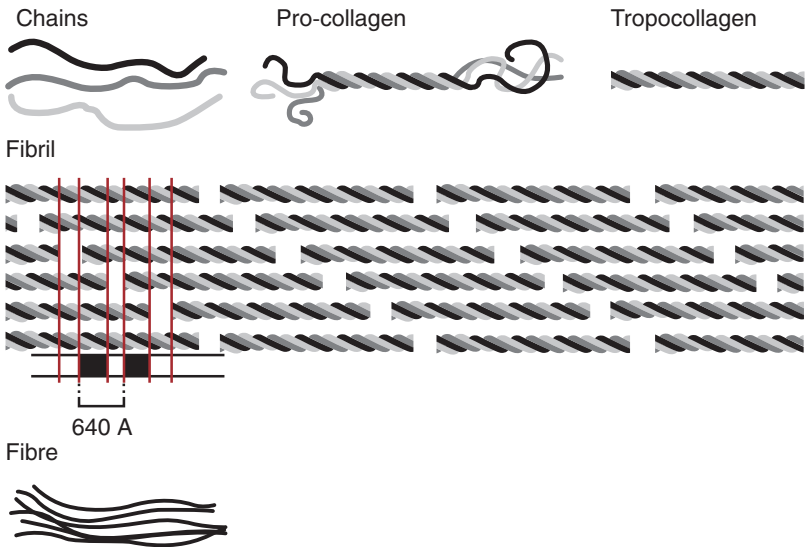



**Fig. 1.12. Arrangement of tropocollagen in collagen fibers**

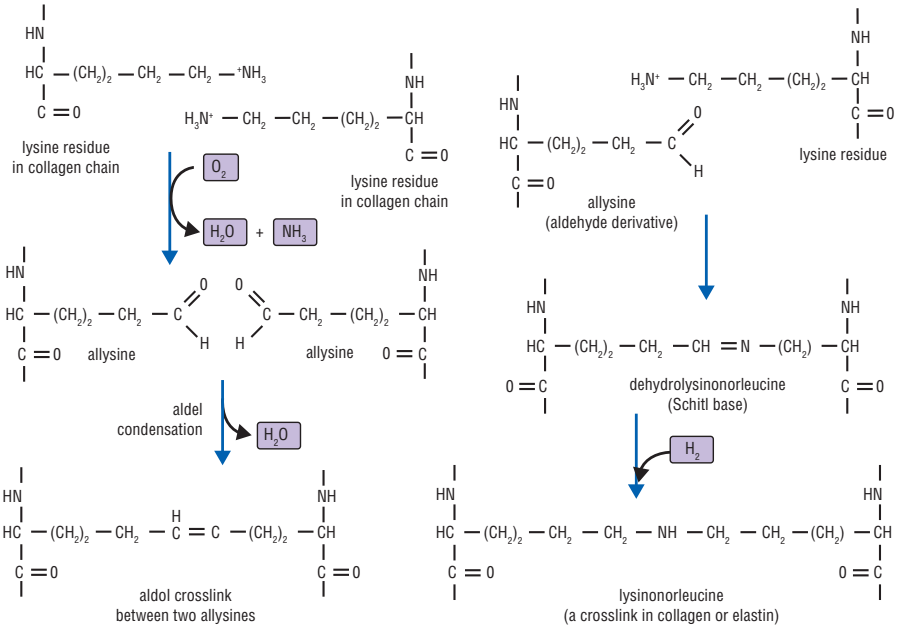



**Fig. 1.13. Formation of intrachain covalent bonds which stabilize the structure of collagen microfibrils**

Covalent bonds of this type occur in collagen and elastin only. The amount and type of these cross–links are different in different tissues. Formation of a larger or lesser amount of these cross–links depends on tensile strength of tissue. For example in the Achilles tendon the tensile strength of the fibers is important, and these links are multiple. The

strength of collagen fibers is determined by hydrogen bonds between the peptide chains of collagen, the triple helix structure, multiple covalent bonds between tropocollagen molecules, association of these triple helical units into a "quarter staggered" alignment so that each one is displaced longitudinally from its neighbor by one-quarter of its length.

Collagen microfibrils differ in thickness and structural organization in different tissues. For example, they are arranged like twigs in wicker furniture and resist loading in all directions. In tendons they are assembled into parallel bundles stacked along the main axis (Fig. 1.14). In mature bone tissue and cornea they are arranged like alternating layers in plywood: the fibrils of each layer are packed parallel to each other and at right angles to the adjacent lavers.

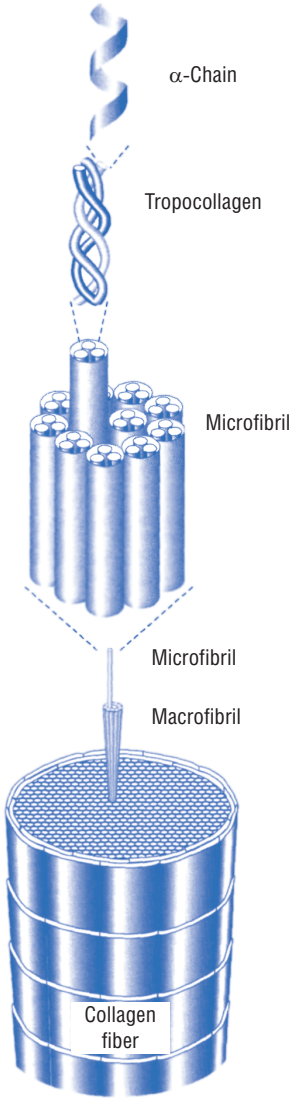



**Fig. 1.14. Collagen fiber formation**

The size, structure, and arrangement of collagen fibrils are determined by connective tissue cells. They can express one or several genes encoding different types of pre-pro-α-chains, thus influencing all the subsequent stages of intra- and extracellular modifications as well as packing of collagen fibers.

## Collagen types

Collagens are polymorphic proteins. Twenty different collagen α-chains, each of them encoded by an individual gene, have been identified. Type I, II, III and IV collagens are the most widespread ones. Type I, II and III are fibril proteins; the commonest among them is type I collagen. Type IV collagen molecules occur only in the basement membrane where it forms a mesh-like network.

The triple helix of type II and type III collagens includes 3 identical chains whose structure can be written as $[\alpha_1(II)]_3$ and $[\alpha_1(III)]_3$. Type I and type IV collagens are formed by two different α-chains and written as $[\alpha_1(I)]_2\alpha_2(I)$ and $[\alpha_1(IV)]_2\alpha_2(IV)$.

## Collagen catabolism

Collagen is a slowly metabolizable protein. Its half-life takes weeks and even months. The enzyme **collagenase** (metalloproteinase) cleaves all three tropocollagen polypeptide chains at ¼ length from C-end between glycine and leucine (isoleucine). The resulting fragments are dissolved in water and hydrolysed further by different proteases to di- and tripeptides (Fig. 1.15).

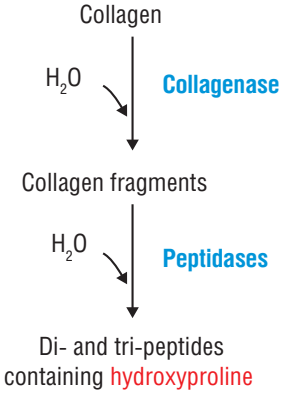



**Fig. 1.15. Collagen catabolism**

The $Ca^{2+}$, $Zn^{2+}$-dependant collagenase (optimal pH 8.5) is inactive within tissue cells. Fibroblasts and macrophages synthesize and secrete the enzyme. The rate of enzyme synthesis is regulated at the transcription stage.

Extracellular matrix macromolecules can be damaged, for example, as a result of glycosylation (non-enzymatic attachment of glucose residues to protein amino acid residues) or proteolysis. Such changes disrupt the interaction and arrangement of connective tissue elements. Metalloproteinases are activated at the sites of injury; they degrade incorrectly orientated molecules. Fibroblast receptors respond to alteration of microenvironmental structures by increased synthesis and secretion of matrix components. The activity of collagen catabolism is evidenced by the amount of hydroxyproline excreted with urine over 24 hours as the amino acid occurs in collagen only. In a healthy human daily excretion of hydroxyproline is 15–20 mg.

**Aging.**

The processes of growth, development and aging are accompanied by considerable alterations in connective tissue. Biochemical changes caused by aging are as follows:

- collagen metabolism decreases;
- the number of cross-links between tropocollagen molecules increases, which makes them rigid and fragile;
- the GAG/collagen ratio is decreased;
- the amount of bound water decreases, which results in dry skin, changes in the mechanical properties of cartilages and tendons, and decreased corneal transparency.

## 1.3. ELASTIN

The elasticity required for the functioning of blood vessels, lungs, tendons and skin is provided by the extracellular matrix fibers in these tissues. Rubber-like properties are determined by features of elastin composition and structure. Unlike the collagen family, the elastin structure is encoded by a single gene.

The protein is composed of 750 amino acid residues. The molecules contain many amino acids with hydrophobic radicals: glycine, valine, alanine, and proline account for 70% of the total number of polypeptide amino acids. The chain includes a small amount of hydroxyproline and no hydroxylysine or carbohydrate fragments. Polypeptide molecules have no strictly definite conformation, as, for example, in collagen fibrils. Tensile strength increases the structural order.

### Elastin synthesis and formation of polymer structures

Elastin is synthesized as a soluble 70 kDa monomer called tropoelastin. Some of the prolines in tropoelastin are hydroxylated to hydroxyproline by prolyl hydroxylase in endoplasmic reticulum.

After secretion from the cell, lysyl oxidase catalyzes the formation of allysine residues at the sites with -Lys-Ala-Ala-Lys- and Lys-Ala-Ala-Ala-Lys- sequences. Chemically active aldehyde reacts with other lysine and allysine residues. Four radicals form **desmosine** (pyridinoline) and **isodesmosine** (isopyridinoline) with a similar structure (Fig. 1.16). Two, three, or four tropoelastin molecules may take part in formation of these structures (Fig 1.17). In addition to desmosines, **lysinenorleucine** cross-links formed by two lysine radicals are formed in elastin.

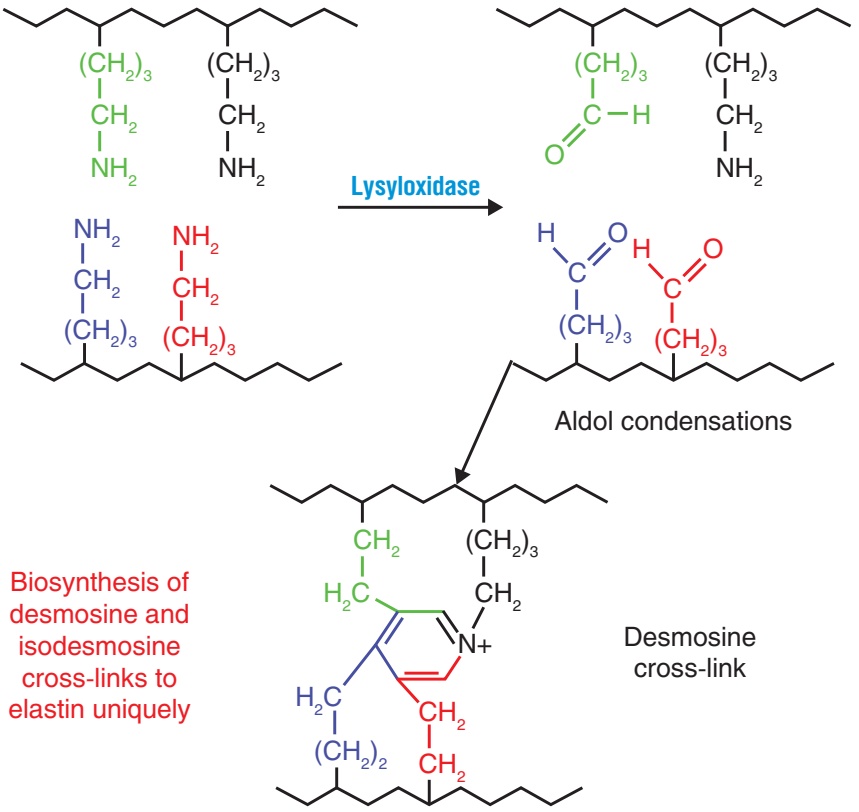



**Fig. 1.16. Desmosine and isodesmosine are interchain linkage in the elastine molecule**

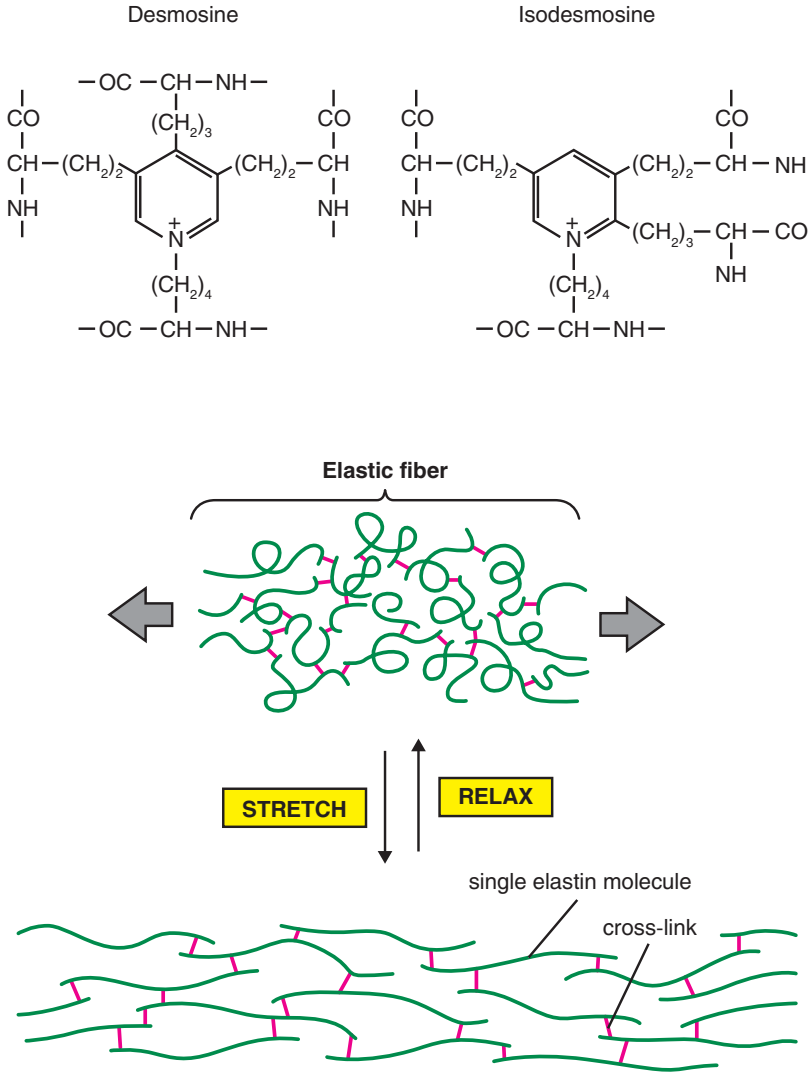



**Fig. 1.17. Intrachain bonds forming the elastin structure**

Cross-linked and randomly curved elastin fibers can stretch and shrink in two directions like rubber.

## Elastin catabolism

The half-life of elastin is about 75 years. During one's lifetime only half of elastin is renewed. Protein is hydrolyzed by elastase **secreted by neutrophils** into the extracellular space. Elastase is active in slightly alkaline medium (pH optimum 7.5–8.5); it does not have absolute specificity and can also hydrolyze collagen, immunoglobulins, proteoglycans, hemoglobin, and other proteins.