Collagen - the Ultrastructural Element of the Bone Matrix

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There is an increased interest and more studies highlight the fact that bone strength depends not only on bone tissue quantity, but also on its quality, which is characterized by the geometry and shape of bones, trabecular bone microarchitecture, mineral content, organic matrix and bone turnover. Fibrillar type I collagen is the major organic component of bone matrix, providing form and a stable template for mineralization. The biomedical importance of collagen as a biomaterial for medical and cosmetic purposes and the improvement of the molecular, cellular biology and analytical technologies, led to increasing interest in establishing the structure of this protein and in setting of the relationships between sequence, structure, and function. Bone collagen crosslinking chemistry and its molecular packing structure are considered to be distinct features. This unique post-translational modifications provide to the fibrillar collagen matrices properties such as tensile strength and viscoelasticity. Understanding the complex structure of bone type I collagen as well as the dynamic nature of bone tissues will help to manage new therapeutic approaches to bone diseases.

Keywords: bone matrix, structural protein, type I collagen, cross-links, osteoporosis

The skeleton is an active metabolic organ that exerts important functions such as: locomotion, soft tissues support and protection, pool of calcium and phosphorus in the body, and the site of bone marrow storage [1,2]. From the ultrastructural point of view, the bone is a heterogeneous material, being composed of mineral phase (hydroxyapatite crystals-Ca10(PO4)6(OH)2), organic matrix and cellular elements [3]. The organic material of bone consists of proteins (~ 95%), lipids (~2%) and water [3,4]. The protein composition of bone was classically determined by demineralization of the tissue, with isolation and classification based on its configuration in: structural proteins (collagen, fibronectin) and non-collagenous proteins, with specialized function (collagen fibril regulation molecules, enzymes, growth factors, signaling molecules and proteoglycans including: decorin, biglycan, lumican, osteoaderin) [3,4].

Type I collagen structure and synthesis

Collagen is the dominant insoluble fibrous protein of the extracellular matrix in the human body, representing ~30% of the total proteins [5-7]. At least 29 genetically distinct types of collagen molecules encoded by at least 44 genes, have been identified so far [5,7]. They are incorporated into several subgroups according to the molecular structure and assembly mode. Fibril-forming collagens represent the largest subgroup, and includes types I, II, III, V, XI, XXIV and XXVII molecules [6,7].

Of all types of collagens, fibrillar type I collagen is the major structural protein in bone, and also in tendon, ligaments, dentin, interstitial tissues, and skin, assuring tissues form, stability and connectivity [6-8]. The molecular structure was determined based on several methods as studies of chemical composition, physicochemical analysis of solutions, X-ray crystallography, electron microscopy, and a variety of spectroscopy techniques [5].

Type I collagen is a 300-nm thin protein of 1.5-nm in diameter, composed of three subunits: two chains $\alpha 1$ and one α 2, assembled in the form of a right-handed triple helix [5-8]. Each chain contains 1050 amino acids arranged in a characteristic repeat sequence, characterized by the presence of a glycine (Gly) residue at every third amino acid (Xxx-Yyy-Gly) [3,6,9]. In the Xxx and Yyy position can be any amino acids, but more often are encountered (2S)-proline (Pro, 28%) and (2S,4R)-4-hydroxyproline (Hyp, 38%), making the Pro-Hyp-Gly sequence the most common triplet (10%) in collagen molecule [5,9]. This peptide (Pro-Hyp-Gly) has an important role in triple helix stability by inter-chain hydrogen bonds. The basic features are the direct hydrogen bonds between amino group (NH) of a Gly residue with a carbonyl group (CO) at the X position in an adjacent polypeptide, but also a second hydrogen bond between NH (X position) to the CO of a Gly in a neighboring chain. This second link is a direct bond or is mediated by water [6,10].

The biosynthesis of collagen is a complex process involving chain synthesis, association, procollagen processing, secretion, self-assembly and a series of post-translational modification with progressive cross-linking [7].

The collagen synthesis starts with transcription of pro-á chains genes into messenger RNAs at the ribosome levels with the formation of polypeptide precursors (pre-pro-αchains). These precursors exhibit a special amino acid sequence at their N-terminal ends that act as a signal facilitating the binding of ribosomes to the rough endoplasmic reticulum (RER) [12]. In the RER lumen, the precursors chains undergo a series of post-translational processing reactions generating the procollagen molecules [6,7,11,12]:

1.Hydroxylation of proline and lysine residues, found in the Y-position of the Gly-X-Y-sequence, is catalyzed by membrane-bound hydroxylase [11, 12].

The proline hydroxylation is mediated by the action of prolyl-4-hydroxylases and prolyl-3-hydroxylase. This step

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is critical for the triple helix conformation, especially for thermal stabilization [6,7,9].

The lysine hydroxylation is more variable in contrast to proline hydroxylation, and vary between 15–90%, with tissue specificity and may depend on the physiological/ pathological condition of the tissue. There were identified three genes encoding isoforms of lysyl hydroxylase: LH1, LH2 with two spliced forms: LH2a and H2b; LH3. Lysine hydroxylation may have an important role in determining bone quality, since studies on osteoblastic cell-culture showed that altered levels of LH2b cause defective cross-linking patterns with alteration in collagen fibrillogenesis and matrix mineralization [7].

These proline and lysine hydroxylation reactions require the presence of: molecular oxygen, 2-oxoglutarate, Fe^{2+}, and ascorbic acid (vitamin C), as a reducing agent [7, 11]. In the absence of these co-factors, the hydroxylating enzymes are unable to function, thus the stability of the chains are impaired and the non-hydroxylated procollagen chains are degraded within the cell. This is the clinical situation of scurvy [6].

2.Glycosylation: glucose and galactose residues are added to some of the hydroxylysine residues in the helical domain prior to triple-helix formation [6, 7]. The reactions are catalyzed by glycosyl transferase and glucosyl-galactosyl transferase using uridine diphosphate galactose (UDP-galactose) and uridine diphosphate glucose (UDP-glucose) as carbohydrate donors, in the presence of a free epsilon-amino group of the hydroxylysine residue, and Mn^{2+} as cation cofactor [7].

The association of alterations of bone matrix type I collagen glycosylation with some diseases are described, like: osteogenesis imperfecta, postmenopausal osteoporosis, osteosarcoma, osteofibrous dysplasia, suggesting the role of collagen glycosylation in bone mineralization [7].

3.Disulfide-bond formation: formation of interchain disulfide bonds between the C-terminal extensions of the pro-á chains brings the three á chains in position for helix formation [6,11].

After this post-translational steps taking place in RER, the pro-á chains are connected near the C-terminus of the chains, generating the triple helix structure towards the N-terminus, in a zipper-like fashion forming the procollagen molecule [6,7].

The procollagen molecules are directed to the Golgi apparatus, where they are incorporated into secretory vesicles and will be secreted into the extracellular space by exocytosis [6,11].

The collagen biosynthesis process continues outside the cell with the removal of terminal N- and C-propeptides by enzymatic cleavage generating the tropocollagen molecules [6,7,11]. This reaction is catalyzed by a group of metalloproteinases (the ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs and BMP1: bone morphogenetic protein 1)/Tolloid-like proteinases), and generates the mature type I collagen molecule [7, 12]. This molecule is made of three domains: the N-terminal domain or N-telopeptide, the central triple helical domain which constitutes more than 95% of the molecule and the C-terminal domain or C-telopeptide [7].

In the extracellular matrix, collagen molecules suffer a self-assembly process being packed in parallel way and arranged in a staggered pattern, giving rise to a fibrillar structure [7,11]. The overlap between the molecules correspond to a length approximately three-quarters of a molecule [7, 13]. This gaps and overlaps of the fibrils generate a striated pattern by electron microscopy, known as the D- period with a length of 67 nm [12].

Post-translational modifications of type I collagen

Fibronectin and integrins, such as the collagen-binding $\alpha 2\beta 1$ integrin, play an essential role in the organization and deposition of fibrillar collagen in the extracellular space [13].

During fibrillogenesis, the final stage of the collagen biosynthesis process is achieved by formation of covalent bonds between the collagen fibrils and the generation of a mature and stable collagen fiber [7,11,12]. In this stage, the lysine and hydroxylysine residues in the N and C-telopeptides regions are oxidative delaminated resulting peptidyl aldehydes. These can react with another aldehyde or with the å-amine groups derived from the unmodified lysine and hydroxylysine residues to form covalent intra and inter molecular cross-links [7,8,12].

Collagen crosslinking plays an important role in tissue differentiation and biomechanical properties assuring elasticity, stability of the extracellular matrix, support for mineral deposition and binding of other macromolecules [3,14]. Compositional and conformational changes of bone collagen have profound influence on the bone properties, especially with regard to mechanical performance [15]. In case of bone fractures, the method of osteosynthesis, the integrity of the periosteum [16,17], but also the normal function of other organs such as kidneys or liver may influence the collagen formation and the bone properties, such as resistance or fragility and all this have an impact on the bone and adjacent tissues healing [18-22].

Chemical analyses of the collagen crosslinks have demonstrated two types of crosslinks: one is enzymatic and the other occur non-enzymatic, by glycation [3, 14].

Enzymatic crosslinks are catalyzed by lysyl oxidase (LOX). The reaction is $RCH2NH2 + H2O + O2 \rightarrow RCH = O + NH3 + H2O2$, where the amine group is derived from lysine and hydroxylysine [7]. LOX is a copper-dependent amine oxidase and requires pyridoxal phosphate (vitamin B6) and a covalently integrated organic molecule: lysine tyrosylquinone as cofactors. [7,23]. There are some factors that influence the transcription and activation of LOX as: transforming growth factor beta (TGF β), insulin-like growth factor I (IGF-I), estrogens and calcitriol, which are positive enzyme regulators. Instead, factors such as fibroblast growth factor (FGF) and high dose of prostaglandin E2 and Tumor Necrosis Factor á inhibit LOX expression [23].

Initially only two amino acids are connected, forming immature divalent enzymatic crosslinks consisting of dihydroxylysinornorleucine (DHLNL) and hydroxylysinonorleucine (HLNL) molecules [23]. Some of them are subsequently converted into mature trivalent crosslinks that connect three amino acids and include pyridinolines, pyrroles, and histidine-derived crosslinks [14,23-25]. Divalent enzymatic cross-links and their maturation to trivalent crosslinking is regulated by bone collagen turnover, by lysyl hydroxylases and lysyl oxidases expression, and may also be influenced by interaction with proteoglycans and collagen-binding proteins [25].

Non-enzymatic cross-links involve interaction by glycation, oxidation or glycoxidation between the helical Lys or Hyl residue and oxo group of sugars (glucose, fructose, ribose or other metabolic intermediate: glyoxal, methylglyoxal, and 3-deoxyglucosone) creating a glycosyl-Lys via Schiff base formation. The hexosyl Lys or Hyl is

then stabilized by spontaneous Amadori rearrangement, which ultimately generates advanced glycation end products (AGEs) such pentosidine and glucosepane [14,23,26,27].

AGEs formed in bone are divided into two types, those making crosslinks between collagen molecules and those that are non-crosslinked [23]. AGEs cross-links are formed by reaction of the Amadori adduct with amino-acids such as helical Lys or arginine (Arg) in adjacent collagen molecules [26, 27]. Non-crosslink types of AGEs, such as carboxymeythyllysine, can interact with RAGE (receptor for AGEs), a member of the immunoglobulin receptor superfamily expressed by bone cells, impairing the homeostasis and activity of osteoblasts and osteoclasts, decreasing bone turnover [23, 25].

The process of bone AGEs formation in collagen fibers is non-regulated and accumulative, supported by the increased collagen half-life, that varies between 1 to 2 years [25,28]. This low biological turnover of collagen confers susceptibility to interaction with other metabolites, like glucose [28]. Excessive accumulation of AGEs cross-links is considered to be responsible for collagen bone matrix stiffening, making its fibrils to dissipate less energy. This collagen stiffening would allow the formation and propagation of micro-damage with an increased rate, throughout bone matrix [25-27]. Also, the collagen glycation may affect the interaction between collagen and other molecules such as proteoglycans, enzymes (e.g., collagenase) and cell integrins [28].

A clinical situation where AGEs may be particularly relevant is type 2 diabetes [29]. Bone tissue of this patient accumulate pentosidine and other AGEs including vesperlysine, methylglyoxal-derived lysine dimer, glyoxal-derived lysine dimer, imidazolone and N(epsilon)-carboxymethyllysine. This situation is supported by chronic hyperglycaemia associated to alteration of glycemic metabolism [23]. Studies conducted in these patients reported a strong association between increased serum or urinary pentosidine levels and prevalent and incident vertebral fractures, independent of confounding factors such as levels of glycated hemoglobin, bone mineral density and renal function [23,29,30]. Chronic kidney disease and hormonal disorders interfere with calcium metabolism which may lead to a less stabile collagen bone matrix [31,32].

In addition to enzymatic and non-enzymatic crosslinks, bone collagen can undergo spontaneous isomerization and racemization of the aspartic acid residues within the C-telopeptide (CTX), forming two isomers: α (newly formed collagen) and β (matured isomerized collagen) CTX. Again, this conversion is favored by low turnover of bone collagen and alters the properties of the collagen molecule [23]. In some diseases such as acromegaly or tumoral pathology, thicker collagen bone matrix may develop in the extremities [33-37].

Conclusions

Bone is a complex and dynamic tissue, which may be pathologically affected in the sense of bone mineral density alterations and / or impairment of bone quality, the latter being underdiagnosed by bone densitometry assessment. Fibrillar type I collagen is the major ultrastructural element of bone organic matrix with an important role in providing bone strength. Relate to their weight, it is considered that collagen fibers are stronger than steel. In order to gain these characteristic features, collagen undergo post-translational modifications during biosynthesis. Impaired enzymatic crosslinking and/or increasing of AGEs are considered a major cause of bone fragility associated with aging, as well as in a number of pathological states such: primary osteoporosis or in case of secondary osteoporosis: diabetes mellitus, chronic kidney disease, inflammatory bowel disease, hyperhomocysteinemia , pharmacological glucocorticoid treatment.

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