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Breakthroughs and Views

Collagen overglycosylation: A biochemical feature that may contribute to bone quality

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Abstract

Skeletal ability to resist mechanical stress is determined by bone amount and quality, which relies on macro- and micro-architecture, turnover, bone matrix, and mineralisation; the role of collagen has not been clearly elucidated. Numerous post-translational steps are involved in collagen type I biosynthesis, including residue hydroxylation and glycosylation catalysed by enzymes that work until the protein folds forming the triple helix; therefore, folding rate regulates these processes. Overglycosylated hydroxylysines are poor substrates for ε-amino group deamination which initiates cross-link formation. Three clinical conditions associated with fractures may relate collagen overglycosylation with bone quality: (i) *Osteogenesis Imperfecta*, in which genetic mutations distort triple helix conformation and slow folding rate favouring overglycosylation; (ii) diabetes mellitus, with collagen overglycosylation by AGE accumulation; and, (iii) menopause, according to experimental studies demonstrating ovariectomy-related trabecular bone collagen overglycosylation preventable by 17β-estradiol or tamoxifen. Specific actions on collagen of drugs used for bone protection should be explored in future studies.

Keywords: Glycosylation; Collagen; Osteoporosis; Bone quality; *Osteogenesis Imperfecta*; Diabetes mellitus; Menopause

The function of the skeleton, as an antigravity scaffold capable of resisting mechanical stress, is determined by the amount of bone and by the type of tissue structure able to best exert bone resistance, generally defined as "bone quality." Bone quality relies on different parameters, such as the macro-architecture, micro-architecture, turnover rate, bone matrix, and mineralisation [1]. While each of these is essential for a good quality of bone, the crucial role of collagen, the main component of the bone matrix upon which all of the above-mentioned parameters depend to some extent, has not been clearly elucidated.

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Collagen biosynthesis and fibril formation

A large number of intracellular post-translational processing steps are involved in the biosynthesis of type I procollagen. They include hydroxylation of prolyl residues, hydroxylation of lysyl residues, and glycosylation of some hydroxylysine residues with galactose and glucosylgalactose [2]. All the reactions are catalysed by specific enzymes that work until the protein folds into the triple-helical conformation. As a consequence, the degree of modification of the protein is regulated by its rate of folding [3] (see Fig. 1).

Once the mature molecules are formed, they assemble into fibrils, the functional supramolecular structural framework. In fibrils, molecules of collagen are parallel to each other and the three strands forming the triple helix

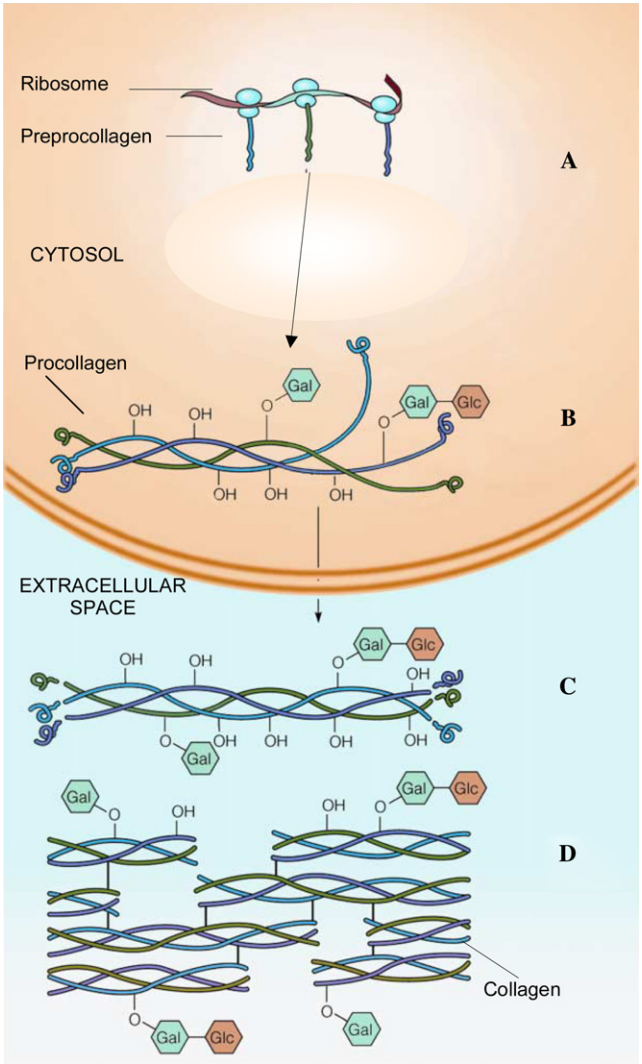

Fig. 1. Biosynthesis of collagen molecule. The process of collagen synthesis is schematically presented as a consecutive series of steps from (A) to (D). Steps (A) and (B) take place in the cytosol while steps (C) and (D) are extracellular processes. (A) Procollagen biosynthesis. (B) After the release of α-1 and α-2 chains from the ribosome, the formation of the triple helix occurs through a zipper-like mechanism initiating at the COOH-terminus of the protein and propagating to the NH_2 terminus. Hydroxylation of prolyl and lysyl residues, and glycosylation of hydroxylysine residues take place and its extent depends on the rate of folding. (C) Extracellular transport, and amino- and carboxy-terminal propeptide cleavage by specific proteases leaving the triple helical domain molecule which assembles into fibril. (D) Oxidative deamination of ε-amino groups of lysines and hydroxylysines, forming allolysines and hydroxyallolysines, catalysed by the ε-amino lysyl-hydroxylysyl deaminase for the subsequent and spontaneous formation of nonreducible pyridinoline and deoxypyridinoline cross-links.

overlap by multiples of 67 nm. Each strand is 300 nm long and there is a 40 nm gap between the end of one and the beginning of the successive one in the triple helix. In bone, the hydroxyapatite crystals lie in the 40 nm gaps between successive collagen molecules forming the fibril [2].

To detail the effects of the extent of lysine hydroxylation and hydroxylysine glycosylation on fibril formation, the group of Kivirikko studied in vitro the formation of fibrils of a recombinant human type II collagen produced in insect cells. The experimental model provided conclusive evidence in favour of the hypothesis that the degree of glycosylation determines fibril diameter, orientation, organisation, and resistance to mechanical stress [4].

In addition to the diameter as a determinant of the mechanical resistance and the tensile strength of the fibrils, another very important determinant is the type and the number of cross -links within and between the collagen molecules forming the fibril. The last post-translational modification of the collagen molecule, which occurs extracellularly, is the oxidative deamination of the ε-amino groups of lysines and hydroxylysines, forming allolysines and hydroxyallolysines, catalysed by the ε-amino lysyl-hydroxylysyl deaminase [5]. This is the only enzymatic step necessary for the formation of the nonreducible cross-links pyridinoline and deoxypyridinoline; the rest of the reactions of the pathway occur spontaneously until the pyridinium ring is formed. Interestingly, the hydroxylysines that are linked to a sugar are poor substrates for the deaminase. Therefore, the higher the glycosylation, the lower the pyridinium cross-links and in turn, the lower the tensile strength of the fibril. Hence, an overglycosylation of the collagen molecule results in a lower diameter and a lower cross-link number, with as a consequence a lower stabilisation of the fibril and an impaired resistance of the structural framework (see Fig. 1).

The *Osteogenesis Imperfecta* model: "the disease of brittle bone"

Osteogenesis Imperfecta is a genetic disease due to a mutation in the coding sequences of either pro-α1 gene or pro-α2 gene. Over 50 mutations that substitute amino acid residues with bulkier side chains for glycine residues have been defined in patients with various forms of *Osteogenesis Imperfecta* [6]. Replacement of a glycine residue (one Gly every third position makes the event very probable) with a bulkier amino acid distorts the conformation of the triple helix, because each of the glycine residues in the repetitive Gly-X-Y sequence of collagen is located in the sterically hindered position in the centre of the triple strand. The consequence of the mutation, in terms of the amount of post-translational modification, depends upon the position on the primary structure and upon the amino acid that substitutes the glycine. This can lead to three situations: (i) the structural abnormality can prevent the complete folding of the three chains in the triple helix, with the consequence that the chains will be degraded

intracellularly; (ii) the mutation can prevent a proper fibril assembly making a weaker fibril; and (iii) triple helix and fibril formation is not prevented but the mutation might slightly modify the structural characteristic of the fibrils thus affecting their mechanical properties. The consequences are that mutations determine the gravity of the form of *Osteogenesis Imperfecta*, from mild, with nonfrequent fractures, to severe that is not compatible with life. As a consequence of an overmodified collagen molecule in *Osteogenesis Imperfecta*, the characteristic alignment of the single molecules is also altered, and the ''holes'' formed by the gaps between collagen molecules, where the hydroxyapatite crystals lie, are positioned differently with respect to normal bone. In this model, crystals occur in various shapes and sizes, and are oriented and aligned with respect to collagen in a manner which is distinct from that found in normal calcified tissues [7]. As a result of the altered matrix, mineralisation occurs by forming crystals within the organic matrix with poor mechanical properties. Altogether, this leads to a tissue with ultimately a low strength, which makes *Osteogenesis Imperfecta* ''the disease of brittle bone.''

The model of diabetes

Type 2 diabetes, a chronic condition in which the hallmark is hyperglycaemia, has been associated with increased risk of fracture of the hip, proximal humerus, and foot in recent epidemiological studies [8–11]. Evidence from observational studies and animal models of diabetes suggests that bone has poorer quality [12–14], not accounted for by lower density. Wang et al. [15] found that a higher concentration of advanced glycation end products (AGEs) was associated with decreased strength in human cadaver femurs.

In diabetes, overglycosylation is the consequence of hyperglycaemia. In fact, another way to glycosylate lysine and hydroxylysine residues is the nonenzymatic attachment of glucose to their ε-amino group, according to the mass-action law. The nonenzymatic stable Amadori early glycated products undergo to a series of additional re-arrangements, resulting in irreversible AGEs that accumulate continuously over the life span of the protein. The consequences on the fibril are the same as described above: although the sugar is linked by N-glycosidic bond, rather than by O-glycosidic linkage, to the δ-hydroxyl group of hydroxylysines, it is reasonable to infer that both, a lower fibril diameter and a lower number of cross-links occur. The reason for that is: (i) because the steric hindrance caused by glucose, the fibril diameter will be smaller, and (ii) the nonavailability of free ε-amino groups of hydroxylysines to be oxidated as allolysines will cause a lower number of pyridinoline cross-links.

Collagen glycosylation and post-menopausal alteration of bone quality

Experimental data have suggested a possible role of collagen glycosylation in the alterations of bone quality related to menopause. Data on this issue are provided by castration experiments in rat. Ovariectomy (as well as orchidectomy, with minor differences) in this animal model is accompanied by an increased glycosylation only of the trabecular bone collagen, which is prevented by the administration of 17β-estradiol or tamoxifen [16]. Such an observation integrates the well-known observation that estrogen deprivation increases the bone turnover rate and bone loss, only in the trabecular compartment, just after castration, and that the effects are prevented by 17β-estradiol or tamoxifen administration. This kind of biochemical event was accompanied by the well-known increased osteoclastic activity and behaves in a similar manner of such a cellular event. Therefore folding may be a key factor for understanding the relation between collagen glycosylation and bone quality after menopause. It requires a number of proteins acting into the endoplasmic reticulum of the cell, where the C-propeptide domains of three polypeptide α-chains fold individually, and then interact and trimerise to initiate the folding of the triple helical region. This highly complex process requires the coordinated action of a large number of endoplasmic reticulum-resident enzymes and molecular chaperones.

Conclusions

An altered quality of bone, such as observed in *Osteogenesis Imperfecta*, diabetes, and perhaps in menopause are associated to an altered collagen fibril caused by the overglycosylation of collagen molecule. The same type of reasoning may perhaps be applied to other conditions associated with chronic hyperglycaemia, such as hypercortisolaemia (endogenous or exogenous), or with increased protein glycosylation, such as ageing, to help explain the increased bone fragility and frequency of fractures. Many questions are still unresolved and would require future studies to be answered. For example, are estrogens controlling the expression of chaperones involved in the process of collagen folding? How do bisphosphonates affect collagen quality and is this action related to their action decreasing the risk of fractures? It is reasonable to believe that for a better understanding of the action of drugs on bone protection and bone quality, future studies should take into consideration their action on collagen.

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