Enzymatic and non-enzymatic functions of the lysyl oxidase family in bone

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Abstract

Advances in the understanding of the biological roles of the lysyl oxidase family of enzyme proteins in bone structure and function are reviewed. This family of proteins is well-known as catalyzing the final reaction required for cross-linking of collagens and elastin. Novel emerging roles for these proteins in the phenotypic development of progenitor cells and in angiogenesis are highlighted and which point to enzymatic and non-enzymatic roles for this family in bone development and homeostasis and in disease. The explosion of interest in the lysyl oxidase family in the cancer field highlights the need to have a better understanding of the functions of this protein family in normal and abnormal connective tissue homeostasis at fundamental molecular and cellular levels including in mineralized tissues.

Introduction

Determinants of bone strength

Bone strength depends not only on bone mineral density, but also on determinants of bone quality. The structure of the organic components of bone, of which about 90% consists of type I collagen synthesized by osteoblasts, is now understood to determine the quality and material properties of bone. Strength measurements of bones in which the bone mineral density is minimally altered and the organic phase is significantly altered support this understanding [1–6]. Major determinants of the functional integrity of the organic phase are the degree and nature of enzymatic collagen cross-links, and the negative effects of non-enzymatic collagen glycation which can be considered in a competitive relationship with enzyme-derived cross-linking. The current review will focus on aspects of enzyme-dependent biosynthetic cross-linking in bone with emphasis on the lysyl oxidases.

Lysyl hydroxylase and lysyl oxidase

Enzymes and reactions responsible for collagen cross-linking

Enzyme-dependent collagen cross-linking depends on a combination of intracellular modifications of procollagen alpha chains by lysyl hydroxylases, and on extracellular modifications by lysyl oxidases. Lysyl hydroxylases are endoplasmic reticulum-associated oxidases which require α-ketoglutarate and ascorbate. These cofactors are also required for collagen prolyl hydroxylases. Lysyl hydroxylases catalyze hydroxylation of the penultimate carbon atom on some lysine residues of procollagen alpha chains. Lysyl hydroxylase 2b catalyzes the hydroxylation of some lysine residues in the telopeptide region of fibrillar collagens, while lysyl hydroxylase 1 performs this reaction on some lysine residues in the triple helical regions [7–9]. Only a subset of lysine residues is hydroxylated, and the degree of hydroxylation is tissue-specific, as recently reviewed [4]. These hydroxyl groups further serve as sites of

attachment of the carbohydrate moieties of collagen, and only a subset of these hydroxyl groups become glycosylated. Glycosylation of collagens is different from the well-known N- and O-glycosylation pathways which occur on asparagine and serine/threonine residues of other proteins. Collagen glycosylations are catalyzed by hydroxylysyl galactosyltransferase, followed by galactosylhydroxylysyl glucosyltransferase to produce glucosylgalacosyl hydroxylysine. Interestingly, galactosylhydroxylysyl glucosyltransferase activity has been shown to be carried out by lysyl hydroxylase 3 [10,11].

The final enzyme reaction required for cross-link formation is catalyzed by the lysyl oxidase family which is made up of five members: lysyl oxidase (LOX) and the four lysyl oxidase like enzymes, lysyl oxidase like 1–4 (LOXL1–LOXL4). These secreted extracellular enzymes can all catalyze the oxidation of the lysine and hydroxylysine side chains of collagens in the telopeptide regions of fibrillar procollagens to form peptidyl α-aminoadipic-δ-semialdehyde residues or peptidyl-δ-hydroxy-α-aminoadipic-δ-semialdehyde residues. These residues are also known as allysine and hydroxyallysine, respectively [12,13]. Substrate preferences for LOXL1 for elastin [14] and LOXL2 for type IV collagen [15] are apparent. However, direct comparative analyses of substrate specificities among the 5 purified LOX isoforms have not been performed. Substrate sequence requirements for purified bovine aorta LOX using synthetic peptides mimicking relevant collagen sequences have been determined [16]. For example, G_4EKG_6 was a more efficient substrate than G_5KEG_4, while DEK sequences within peptides, which therefore have two negatively charged residues on the N-terminal side of lysine, were unfavorable. Consistent with this understanding is the fact that EK sequences are oxidized in the C-terminal telopeptide region of fibrillar collagens, while KE sequences are not, determined by structure analysis of actual collagen cross-links [16]. It is clear, however, that collagen fibrils are better LOX substrates than denatured collagen and collagen peptides. The physical chemical basis for this is only partially understood [17]. This observation does not exclude the fact that LOX can oxidize soluble substrates, but the enzyme may be less efficient than with insoluble or semi-soluble substrates. The concept was put forth that LOX may bind to one collagen chain in the triple helical domain to oxidize a lysine residue in the neighboring juxtaposed telopeptide. This notion has not been further investigated to our knowledge, but would provide an explanation for identification of cross-links throughout collagen fibrils, rather than only on the surface of fibrils, while also being consistent with the observation that insoluble or semi-soluble collagen fibrils more readily undergo LOX oxidation [16]. In this model, LOX would bind to a procollagen molecule in the triple helical domain before fibril formation, and then act on a neighboring collagen molecule telopeptide lysine residue as fibril formation commenced and progressed.

The importance of an insoluble or semi-soluble substrate for optimal LOX activity is also true for elastin [18]. Synthetic peptide elastin-like copolymers with the same sequences around lysine residues but which form coacervates are superior substrates to those copolymer peptides that do not coacervate [19]. Thus, semi-soluble structures consistently provide an optimum environment for LOX activity [19]. The physical state of protein structures is a major determinant of the optimal potential for lysyl oxidase catalyzed oxidative deamination, while the primary sequence surrounding the lysine residues to be oxidized appears to be of secondary importance. Electrostatic charge is, in addition, an important determinant of substrate potential. Soluble cationic proteins generally are good substrates for lysyl oxidase in vitro [20]. This finding may be of considerable biological importance because many regulatory proteins such as growth factors are cationic, and some of these have been shown to serve as substrates for lysyl oxidase in vitro, thereby attenuating their ability to initiate signaling [21,22].

As noted, lysyl oxidases act on lysine and hydroxylysine residues exclusively in the telopeptide regions of fibrillar collagens. These aldehydes are highly reactive and result in the formation of the normal immature and mature biosynthetic collagen cross-links over time without apparent involvement of LOX or other enzymes [23–25]. Cross-links form within collagen trimers at each telopeptide end, and between some modified lysine residues in the telopeptide region and non-modified lysine residues located in neighboring juxtaposed triple helical regions. These cross-links stabilize collagen structure and confer tensile strength of connective tissues. The microenvironment and favorable juxtapositions of modified and non-modified lysyine- and hydroxylysine residues determine the actual cross--links that can form. Thus, lysyl oxidases catalyze the final enzyme-mediated reactions required for biosynthetic collagen cross-linking, but lysyl oxidases do not directly cross-link collagen.

Hydroxylysine reactivity

The structures of collagen cross-links have been reviewed elsewhere and will not be repeated in detail here [4,13,26,27]. However, an important point is that the chemical reactivity of respective lysine residues is modulated by whether or not lysyl hydroxylation has occurred. For example the pKa, of hydroxylysine residues is 8.5 while that of lysine residues is more typically about 9.5 [28]. Thus, hydroxylysine residues are more nucleophilic and chemically reactive than non-modified lysine