university of groningen

University Medical Center Groningen

University of Groningen

Collagen cross-linking mediated by lysyl hydroxylase 2

Piersma, Bram; Bank, Ruud A

Published in:
Essays in biochemistry

DOI:
10.1042/EBC20180051

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Piersma, B., & Bank, R. A. (2019). Collagen cross-linking mediated by lysyl hydroxylase 2: an enzymatic battlefield to combat fibrosis. *Essays in biochemistry*, *63*(3), 377-387. https://doi.org/10.1042/EBC20180051

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 13-02-2023

Check for updates

Essays in Biochemistry (2019) **63** 377–387
https://doi.org/10.1042/EBC20180051

PORTLAND PRESS

Review Article

Collagen cross-linking mediated by lysyl hydroxylase 2: an enzymatic battlefield to combat fibrosis

Bram Piersma and Ruud A. Bank

Deptartment of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, Groningen 9713 GZ, The Netherlands

Correspondence: Ruud A. Bank (R.A.Bank@umcg.nl)

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

The hallmark of fibrosis is an excessive accumulation of collagen, ultimately leading to organ failure. It has become evident that the deposited collagen also exhibits qualitative modifications. A marked modification is the increased cross-linking, leading to a stabilization of the collagen network and limiting fibrosis reversibility. Not only the level of cross-linking is increased, but also the composition of cross-linking is altered: an increase is seen in hydroxyallysine-derived cross-links at the expense of allysine cross-links. This results in irreversible fibrosis, as collagen cross-linked by hydroxyallysine is more difficult to degrade. Hydroxyallysine is derived from a hydroxylysine in the telopeptides of collagen. The expression of lysyl hydroxylase (LH) 2 (LH2), the enzyme responsible for the formation of telopeptidyl hydroxylysine, is universally up-regulated in fibrosis. It is expected that inhibition of this enzyme will lead to reversible fibrosis without interfering with the normal repair process. In this review, we discuss the molecular basis of collagen modifications and cross-linking, with an emphasis on LH2-mediated hydroxyallysine cross-links, and their implications for the pathogenesis and treatment of fibrosis.

Introduction

It is estimated that fibroproliferative diseases are at the heart of more than one third of all deaths in developed countries [1,2]. So far, there is no effective treatment option that halts or even reverses fibrosis. Thus, there is an urgent need to develop effective therapies, preferably those that can be used in a variety of organs. The hallmark of organ fibrosis is an excessive accumulation of extracellular matrix (ECM), with the fibrillar collagen type I as the most abundant matrix protein. This pathological collagen accumulation ultimately leads to organ failure and subsequent death. So far, relatively little attention has been paid to develop drugs that target the matrix itself, which is remarkable, as the matrix is prominently involved in disease progression [3–7].

Collagen type I is the major constituent of the fibrotic ECM. However, it is not just the quantity of collagen that defines fibrosis. The quality of the collagen, as determined by its post-translational modifications, actively drives the disease progression. Biosynthesis of collagen is a multistep process [8], resulting in modifications throughout the molecule, such as the conversion of proline into hydroxyproline, and the conversion of lysine (Lys) into hydroxylysine (Hyl). The precursor procollagen is secreted out of the cell, its propeptides are cleaved off, and the resulting collagen molecules spontaneously segregate into well-ordered structures (fibrils) in which they are stabilized by means of intermolecular cross-links mediated by lysyl oxidase (LOX) transglutaminase (TG).

Pharmacological therapies have focused on compounds that suppress fibrosis by reduction of disease progression (suppression of inflammation, cell migration, cell proliferation, cell differentiation, viral/bacterial/parasite infections) and on drugs that suppress collagen accumulation [5]. The latter group

Received: 24 April 2019
Revised: 26 June 2019
Accepted: 02 July 2019

Version of Record published:
19 July 2019

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

Essays in Biochemistry (2019) 63 377–387
https://doi.org/10.1042/EBC20180051

consists largely of inhibitors of enzymes involved in the post-translational processing of collagen: procollagen C-proteinase, prolyl 4-hydroxylase, LOX and TG. By inhibiting the action of C-proteinase, procollagens cannot assemble into collagen fibrils and the deposition of collagen in tissues is blocked. Inhibition of prolyl 4-hydroxylase generates unstable collagen that is degraded inside the cell and is not secreted. Inhibition of LOX results in collagen that is less cross-linked, resulting in collagen fibrils that are easier to degrade by proteinases [6]. However, all aforementioned approaches have their drawbacks, as both inflammation and collagen production are essential for adequate tissue repair. This means that although inhibiting inflammation and/or blocking the collagen accumulation may limit fibrosis, it will also limit adequate tissue repair, thus causing deleterious side effects. Thus, an ideal anti-fibrotic drug should not interfere with the repair process, but should selectively prevent abnormal collagen accumulation by interfering with a process that operates in pro-fibrotic cells but not in normal cells. Over the years, such a process has been discovered: the conversion of the Lys of the telopeptides into Hyl by means of the enzyme telopeptide lysyl hydroxylase (LH). This results in a change in cross-link pattern, and in a change of collagen degradability. In this review, we discuss the molecular basis of collagen biosynthesis and post-translational modifications that eventually result in intermolecular cross-linking of collagen molecules within fibers. In particular, we emphasize how LH-mediated cross-linking determines the degradability of the ECM, and what this means for the pathogenesis and treatment of fibrotic diseases.

Lysyl hydroxylation of collagen

Fibrillar collagens consist of a triple helical domain, flanked by telopeptides at both the N- and C-terminal end of the molecule (N-telopeptide and C-telopeptide, respectively). Hydroxylation of Lys occurs both in the triple helix and the telopeptides by the enzyme LH [9]. Only the Lys in the helical sequence Gly-X-Lys is modified; a Lys in the helical sequence Gly-Lys-Y is not hydroxylated. In the last two decades, it has become clear that separate enzymes mediate lysyl hydroxylation of the triple helix and the telopeptides, which makes sense, as the primary sequence in which the hydroxylated telopeptidyl Lys is embedded is entirely different [10]. Three LHs (LH1, lysyl hydroxylase 2 (LH2) and LH3) have been discovered, which are encoded by *PLOD1*, *PLOD2* and *PLOD3*, respectively. The abbreviation PLOD is derived from procollagen-lysine, 2-oxoglutarate 5-dioxygenase (which is the systematic name of LH), while the 1, 2 and 3 indicates the sequence of discovery. LH1 and LH3 exclusively hydroxylate certain Lys residues in the triple helix (helical LHs), where LH2 exclusively hydroxylates the Lys in the telopeptides (hence the name telopeptide LH) [9,10]. In addition, LH3 displays hydroxylysyl galactosyltransferase and galactosylhydroxylysyl glucosyltransferase activities, leading to the formation of glycosylated Hyl residues [9,10].

Collagen cross-linking by lysyl oxidase

Enzymatic collagen cross-linking by means of LOXs is a final step in the biosynthesis of collagen and essential for the physical and mechanical properties of collagen fibrils [11,12]. The formation of these cross-links starts with the oxidative deamination of the ε-amino group of specific Lys or Hyl residues within the C- and N-terminal telopeptides by a family of five LOXs (LOX and LOX-like 1-4 (LOXL1-4)), leading to the formation of the aldehydes allysine (= α-aminoadipic acid-δ-semialdehyde = Lys^{ald}) and hydroxyallysine (= hydroxy-α-aminoadipic acid-δ-semialdehyde = Hyl^{ald}). The reactive aldehyde condensates either with another aldehyde in the same molecule or with Lys, Hyl or histidine (His) residues in neighboring collagen molecules, in order to form intra- and/or intermolecular cross-links. Collagen cross-links can be divided into two classes: (i) cross-links formed via the Lys^{ald} pathway (i.e. derived from a Lys in the telopeptide) and (ii) cross-links formed via the Hyl^{ald} pathway (i.e. derived from a Hyl in the telopeptide) [13,14]. The relative abundance of the two cross-link pathways varies from tissue to tissue, depending on the hydroxylation state of the telopeptide Lys. For example, collagen deposited in skin and cornea is mainly cross-linked via the Lys^{ald} pathway with almost no Hyl^{ald} cross-links present in these tissues [15,16]. In bone, cartilage, tendon, dentin and ligaments, collagen cross-links mainly derive from the Hyl^{ald} pathway [15]. The Lys^{ald} route gives rise to divalent and tetravalent cross-links (recently, it has been proposed that the reported trivalent cross-link histidinohydroxylysinonorleucine is a laboratory artifact [17]), whereas the Hyl^{ald} route gives rise to divalent and trivalent cross-links [13,14] (Figure 1).

The reactive telopeptide Hyl^{ald} can pair with a helical Lys or a helical Hyl to form divalent cross-links. These divalent cross-links further mature either into the respective lysylpyridinoline (LP) or hydroxylysylpyridinoline (HP) cross-links or into the respective deoxypyrrolic (d-PRL) and pyrrolic (PRL) cross-links. The trivalent pyridinoline and pyrrolic cross-links differ with respect to the hydroxylation state of the second telopeptide Lys residue. HP and LP cross-links are derived from two telopeptide Hyl^{ald} residues and a helical Hyl or Lys residue, respectively, whereas

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

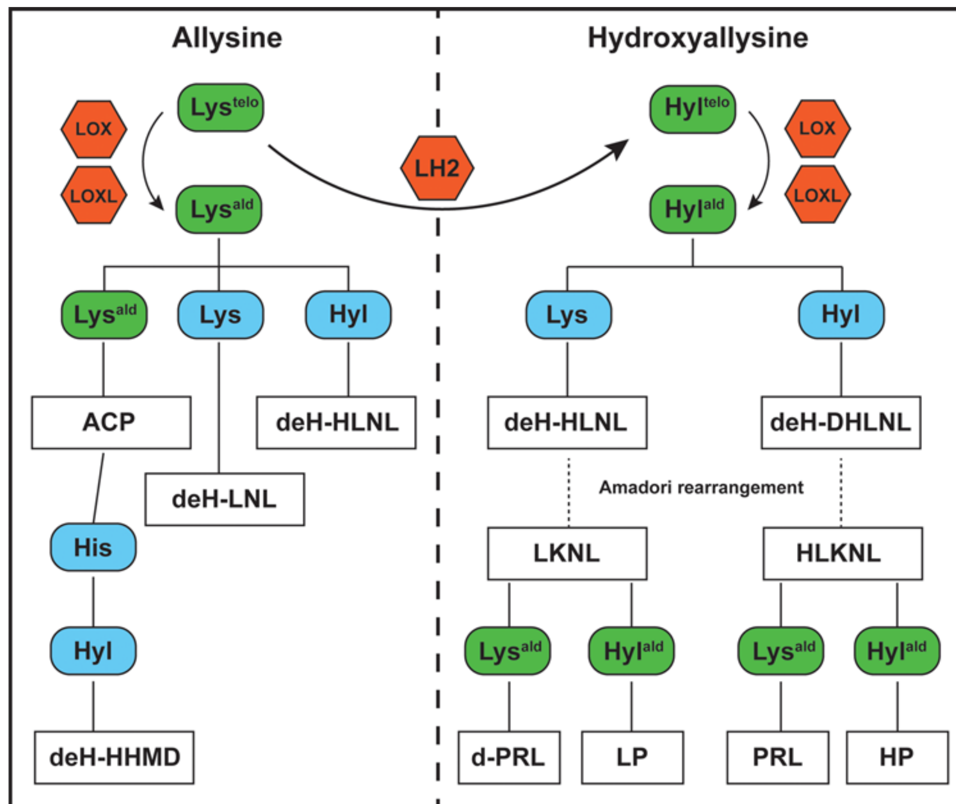

Figure 1. Schematic representation of cross-links derived from the allysine (Lys^{ald}) and hydroxyallysine (Hyl^{ald}) pathway
Amino acids from the telopeptide and the triple helix are marked in green and blue, respectively, enzymes are marked in red, whereas the cross-links are shown in white boxes. The Lys in the telopeptides can be converted into Hyl by lysyl hydroxylase 2 (LH2). The Lys and Hyl in the telopeptides can be converted into the aldehydes Lys^{ald} and Hyl^{ald} by lysyl oxidase (LOX) and/or LOX-like 1-4 (LOXL). The next step for Lys^{ald} is the reaction with another Lys^{ald} from the telopeptides to form an aldol condensation product (ACP), or to react with a Lys or Hyl from the triple helix. The reaction with a Lys or Hyl results in the divalent cross-link dehydro-lysinonorleucine (deH-LNL) and dehydro-hydroxylysinonorleucine (deH-HLNL), respectively, whereas the ACP reacts with a His and finally with a Hyl in the triple helix, leading to the tetravalent cross-link dehydro-histidinohydroxy-merodesmosine (deH-HHMD). The first step for the Hyl^{ald} is the reaction with a Lys or a Hyl in the triple helix. The resulting divalent iminium cross-links deH-HLNL and dehydro-dihydroxylysinonorleucine (deH-DHLNL) undergo a spontaneous Amadori rearrangement, leading to the more stable divalent keto-imines lysino-5-keto-norleucine (LKNL) and hydroxylysino-5-keto-norleucine (HLKNL), respectively. The keto-amines finally react with either a Lys^{ald} or a Hyl^{ald} from the telopeptides, resulting in the trivalent pyrollic cross-links (d-PRL = deoxypyrrole; PRL = pyrrole) or trivalent pyridinoline cross-links (HP, hydroxylysylpyridinoline; LP, lysylpyridinoline).

pyrrolic cross-links are derived from a telopeptide Hyl^{ald}, a telopeptide Lys^{ald}, and a helical Lys or Hyl residue (deoxypyrrole or pyrrole, respectively) [13,14].

Fibrotic tissues show increase Hyl^{ald} cross-link levels

In wound healing of the skin, such as in hypertrophic scarring, large amounts of Hyl^{ald} cross-links are present [18–23]. A predominance of such cross-links is also found in collagen that is produced in the damaged corneal stroma; the resulting scar shows markedly increased levels of Hyl^{ald} derived cross-links at the expense of Lys^{ald} cross-links [24]. The pioneering studies on elevated Hyl^{ald} derived cross-links in abnormal scarring were later confirmed [25–29], followed by reports on increased Hyl^{ald} derived cross-links in other fibrotic disorders, such as various lung diseases (respiratory distress syndrome, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, respiratory bronchiolitis, silicosis and bleomycin-induced lung fibrosis) [30–39], chronic adriamycin nephropathy (an experimental model resulting in non-immunologic glomerulosclerosis and interstitial fibrosis) [40], infarct scar of the myocardium [41,42], joint contractures [43], vessel luminal narrowing [44]; lipodermatosclerosis [45,46], annulo-aortic ectasia [47], fibrotic lesions of Dupuytren's disease [48], skin of patients with lipoid proteinosis [49], diabetes [50,51], skin fibrosis

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

PORTLAND PRESS
Essays in Biochemistry (2019) **63** 377–387
https://doi.org/10.1042/EBC20180051

due to chromoblastomycosis infection [52,53], skeletal muscle abnormalities [54–56], systemic sclerosis [57], uterine fibroids [58], vein graft disease [59] and various liver diseases such as in alveolar echinococcosis (a dense and irreversible fibrosis), hepatocellular carcinoma, alcoholic cirrhosis or cirrhotic livers induced by CCl_4, viral hepatitis or by *Schistosoma mansoni* [60–69].

The presence or absence of LH2 regulates the abundance of Hyl^{ald} cross-links. In recent years, it has been suggested that the relative (and absolute) amount of Hyl^{ald} cross-links are adequate biomarkers for the accumulation of pathological collagen in lung and liver fibrosis [30,32–35,37–39,61–63]. As the same is observed in other fibrotic tissues including skin and kidney, it was actually stated that "It is possible that organ fibrosis is a unique process ultimately associated with a change in cross-linking whereby the proportion of the allysine cross-links decreases in favor of the hydroxyallysine-derived crosslinks" [45]. Thus, Hyl^{ald} cross-links are implicated in the pathogenesis of fibrosis.

Telopeptide LH exhibits two splice variants

The abundance of data on elevated Hyl^{ald} cross-link levels in fibrotic tissues strongly support an up-regulation of telopeptidyl lysyl hydroxylation in fibrotic lesions, which is indeed the case. *PLOD2* encodes two different splice variants, LH2a and LH2b (LH2b contains an extra insert, encoded by exon 13A). LH2b is expressed in a wide variety of tissues and organs, including bone, cartilage and skin, whereas LH2a is only present in the frontal lobe, spleen, kidney, liver and placenta [70–72]. Overexpression of LH2a and LH2b revealed that only LH2b hydroxylates the telopeptides, as overexpression of LH2a does not induce pyridinoline cross-link formation *in vitro* [72], a conclusion that is confirmed by a Bruck syndrome type 2 patient that only expresses LH2a [73]. The inability of LH2a to induce pyridinoline cross-links together with increased levels of LH2b in fibrotic tissues suggests that LH2b is the likely culprit in pathogenic collagen cross-linking [74]. Meanwhile, the exact functions of LH2a remain unclear.

TG-mediated cross-links in fibrosis

The other enzymatically mediated cross-link in collagen is catalyzed by TGs. TGs comprise a family of isozymes that catalyze the formation of covalent bonds between glutamine and Lys in a variety of proteins [75,76]. Increasing the level of γ-glutamyl-ε-lysine cross-links by adding TG to collagen *in vitro*, makes collagen more resistant toward proteinases [77–79]. It is widely believed that TG promotes fibrosis by increasing the number of TG cross-links in collagen [79–84]. However, not a single report quantitatively measured the level of TG cross-links per collagen molecule. Apart from that, TG is far from being specific for collagen, as many other substrates have been identified [85,86]. The only data supporting the hypothesis is the increased staining in fibrotic tissue with an antibody toward TG or TG-mediated cross-links. Paradoxically, this increase always correlates with increased collagen levels. Hence, it is very well possible that the number of TG cross-links in normal and fibrotic collagen molecules is the same. That would be in sharp contrast with Hyl^{ald} cross-links: there is an abundance of data demonstrating elevated Hyl^{ald} cross-links in collagen molecules from fibrotic tissues. Although inhibition of TG can result in an attenuation of fibrosis [76–84], this cannot automatically be attributed to a higher degradability of collagen, as TG is involved in many other intra- and extracellular processes, often in a transamidase-independent manner [75,76]. In fact, studies in TG2 knockout mice showed that TG2 deficiency had no measurable effect on the stability of the fibrotic ECM (e.g. solubility of collagen toward pepsin) or in reversibility of hepatic fibrosis *in vivo* in two hepatotoxin-induced models [87]. Thus, *in vivo* collagen degradability and irreversibility does not seem to be regulated by TG2. Therefore, the concept that TG cross-links are elevated in fibrotic collagen molecules probably needs to be revisited [87,88]. In contrast, inhibition of LOX-mediated collagen cross-linking results in a higher solubility of collagen toward pepsin, and enhances spontaneous fibrosis reversal [89,90].

Collagen containing Lys^{ald} or Hyl^{ald} cross-links show different degradation profiles

An important aspect of fibrosis is the problem of irreversibility of collagen deposition. Remarkably, the effects of the *type* of cross-links on the susceptibility of collagen to proteolytic enzymes has hardly been investigated, despite the fact that the presence of Hyl^{ald} cross-links is indicative for irreversible collagen deposition.

It is generally accepted that an increase in LOX derived collagen cross-links results in a higher resistance toward collagenases [91]. An increase of ~0.1 LOX-mediated cross-link per collagen molecule already results in a 2-3-fold resistance to human collagenase compared with uncross-linked collagen [92]. In fibrosis, we see an increase in the number of Hyl^{ald} and Lys^{ald} cross-links, due to higher LOX activity. In addition, lysyl hydroxylation levels of the telopeptides are increased, which automatically means that the ratio of Hyl^{ald} and Lys^{ald} cross-links is changed, in

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

Essays in Biochemistry (2019) **63** 377–387
https://doi.org/10.1042/EBC20180051

PORTLAND PRESS

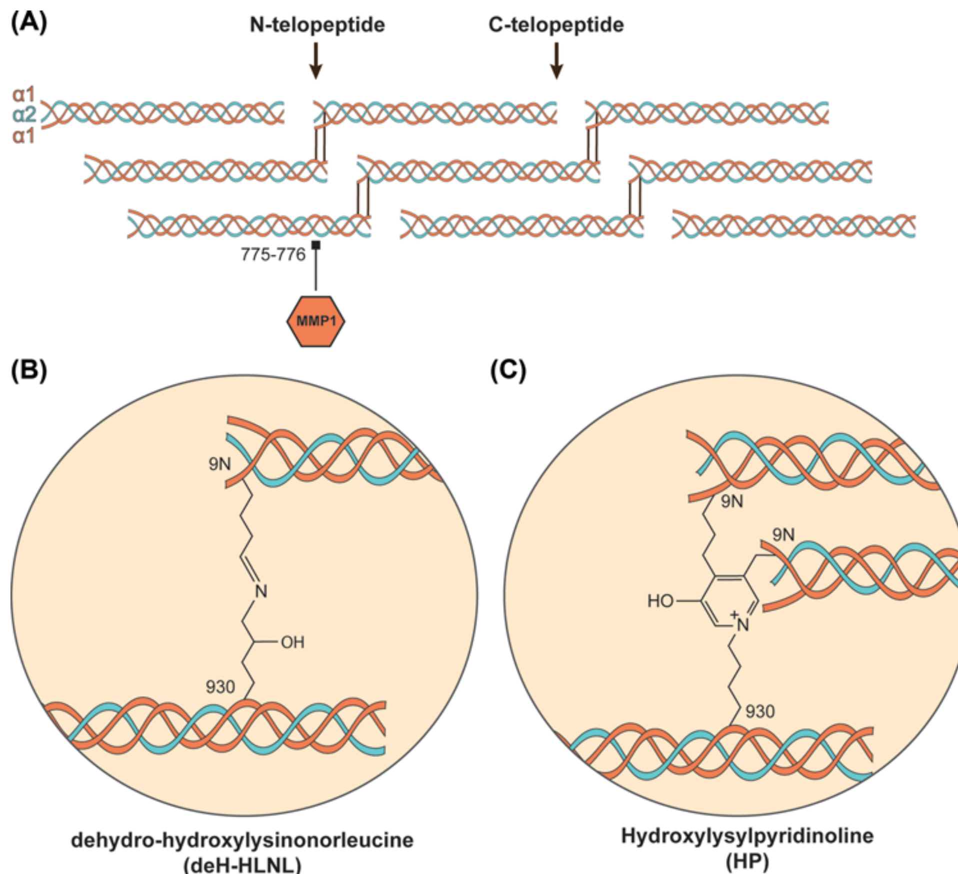

Figure 2. Position of the cross-links derived from the allysine and hydroxyallysine pathway in collagen type I
(**A**) Collagen molecules are packed in a quarter-staggered array in fibrils. Shown are the positions of the cross-links between the N- and C-terminal telopeptides with the triple helix of collagen. The cleavage site of MMP1 (between residue 775 and 776) is opposite the position of the cross-links of an adjacent staggered molecule; the presence of Hyl^{ald} crosslinks inhibits cleavage of the peptide bond between 775 and 776 by MMP1. The residues involved in cross-linking is as follows: the α1(9N) residue from the N-telopeptide (or the 5N residue from the α2 chain) reacts with either the α1(930) or the α2(933) residue from the triple helix. The α1(I) residue 16C from the C-telopeptide reacts with the α1(87) or α2(87) residue from the triple helix; there is no Lys or Hyl present in the C-telopeptide of the α2 chain. (**B**) Structure of the deH-HLNL and the HP cross-link; the position shown is the N-telopeptide. Note that deH-HLNL can be formed between α1(telo) and α1(helix) (shown in the figure), but also between the α1(telo) and α2(helix), or between α2(telo) and α1(helix) or α2(helix). (**C**) Structure of the hydroxylysylpyridinole (HP) cross-link; the position shown is the N-telopeptide. As is the case with figure B, several combinations of α1 and α2 are possible.

favor of Hyl^{ald}. Thus, in fibrosis, we not only see an increase in cross-links, but also a shift in the type of cross-link. A variety of profibrotic cytokines up-regulates both collagen and the three LHs. Interestingly, LH2 levels are increased far more than collagen itself, thus changing the ratio between LH2 and collagen [93,94]. This may explain the increase of Hyl^{ald} cross-links at the expense of Lys^{ald} cross-links.

Collagen cross-linked through Hyl^{ald} is much more difficult to degrade by matrix metalloproteinases (MMPs) than collagen cross-linked by Lys^{ald} [95,96]. The same goes for degradation by non-MMPs, such as pepsin [96]. Indeed, collagen in Bruck syndrome bone lacking Hyl^{ald} cross-links is easily released by pepsin, and these patients present highly osteoporotic bone due to high degradation rates *in vivo* [97]. In long bones with high levels of Hyl^{ald} cross-links, osteoclasts use cathepsin K to degrade collagen, whereas in the calvaria, with low Hyl^{ald} cross-links, osteoclasts use MMPs to degrade collagen [96]. The most probable reason for this observation is that cathepsin K cleaves Hyl^{ald} cross-linked collagen more efficiently than MMPs do, as cathepsin K cleaves collagen at multiple sites [98]. In contrast, MMP1 cleaves collagen only between amino acids 775 and 776, which is sterically in the vicinity of the cross-link sites when collagen molecules are packed into fibrils (Figure 2). In cartilage, collagen type II shows the highest Hyl^{ald} level seen of all fibrillar collagens, and this collagen is hardly degraded (it has an estimated half-life of >100 years) [99]. The

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

Essays in Biochemistry (2019) **63** 377–387
https://doi.org/10.1042/EBC20180051

pathological degradation of collagen type II in osteoarthritis is mainly caused by MMP13 [100,101], which is, as is the case with cathepsin K, able to cleave collagen at more than one position [102]. Finally, in HIF prolyl hydroxylase-2 (PHD2)-deficient mice that have a higher Hyl^{ald} level of collagen type II compared with wild-type mice, the collagen was less susceptible toward MMPs [103]. Taken together, these findings indicate that collagen containing increased Hyl^{ald} cross-links is more difficult to degrade.

Reversibility of fibrosis is hampered by Hyl^{ald} cross-links

The resistance of Hyl^{ald} cross-linked collagen toward degradation by proteinases is an important feature of irreversible collagen accumulation in fibrosis. In fact, Hyl^{ald} cross-link levels might be an important criterion in assessing the irreversibility of fibrosis. The validity of this hypothesis is strengthened by cross-link patterns seen in self-limiting and progressive forms of fibrosis. Collagen produced in response to an injury of skin is initially stabilized by dehydro-dihydroxylysinonorleucine [18–22], a cross-link derived from Hyl^{ald}. In the early stages of wound healing, the collagen of both forms of fibrosis possess dehydro-dihydroxylysinonorleucine as the major cross-link type, but after a few months there is an approximately equal proportion of Lys^{ald} cross-links. Subsequently, self-limiting and progressive fibrosis follow a different course. In hypertrophic scars, a progressive form of skin fibrosis, the 1:1 ratio of the two cross-links is retained. In contrast, the cross-link pattern in the self-limiting form of fibrosis gradually reverts to normal, i.e. there is a virtual disappearance of Hyl^{ald} cross-links and replacement by Lys^{ald} cross-links [20,22,23].

In another set-up, the authors found that osteoarthritis-related fibrosis induced by connective tissue growth factor (CTGF) did not result in increased Hyl^{ald} cross-links, and was transient in nature [104,105]. A bleomycin-induced skin fibrosis model was also transient in nature [95,106], and also this model did not show an increase in Hyl^{ald} cross-links [95]. In contrast, transforming growth factor (TGF-β1) induces persistent osteoarthritis-related fibrosis, which coincides with increased Hyl^{ald} cross-link levels [104,105]. This is in agreement with the observation that the tissue level of pyridinoline correlates to the severity and reversibility of the fibrotic process, where the highest level is found in irreversible fibrosis [53,61–63].

Inhibition of LH 2 to ameliorate fibrosis

The reduced proteolytic turnover of Hyl^{ald} cross-linked collagen explains, at least in part, the irreversibility of collagen deposition. This resistance toward proteolytic enzymes is most probably caused by a changed packing of collagen molecules within the fibrils. Indeed, the type of cross-links dictate the mode of packing of collagen molecules within the fibrils [107–110]. Altogether, the above mentioned data indicate that the type of cross-links provide a mechanism for the regulation of the rate of collagen catabolism: collagen with Hyl^{ald} cross-links are less susceptible to proteolytic degradation than collagen cross-linked by Lys^{ald} residues. Thus, inhibition of LH2 (to enhance the formation of Lys^{ald} cross-links at the expense of Hyl^{ald} cross-links) is an attractive strategy to attenuate fibrosis. Since the up-regulation of LH2 is seen in all organs, a drug that specifically inhibits LH2 activity can be used in a wide range of fibrotic disorders. Furthermore, since LH2 catalyzes only a single reaction downstream in the fibrogenic cascade (namely the formation of unwanted cross-links in collagen of the fibrotic lesions), little (if any) side-effects are expected, as the deposited collagen in the wound area is expected to be normally modified, and thus have the properties (e.g. with respect to tensile strength) required for a normal function of the repaired tissue. In addition, the collagens that normally contain Hyl^{ald} cross-links, such as in tendons, ligaments, bone and cartilage, show a half-life of several years to several decades [111–113]. It is, therefore, unlikely that treatments that even span several months will significantly affect such tissues.

The improved comprehension of the pathogenesis of fibrogenesis in relation to Hyl^{ald} cross-linking opens the way to develop novel therapies. Unfortunately, no specific inhibitors for LH2 currently exist. In order to be active, LH2 needs to form homodimers. Remarkably, mutations in the gene *FKBP10*, encoding the immunophilin FKBP65, gives rise to Bruck syndrome type 1, which shows the same biochemical defect as Bruck syndrome type 2 (in which LH2 is mutated), namely a lack of Hyl^{ald} cross-links in bone [114]. Recently, we found that the mutated FKBP65 prevents the formation of LH2 homodimers [115]. Thus, inhibiting FKBP65 activity is a tool to inhibit LH2 activity, albeit indirectly. Interestingly, inhibition of FKBP65 results in less fibrosis [116,117]. The availability of a 3D model of human LH2 [118] based on the crystal structure of human LH3 [119], might accelerate rational drug design. Equally important, the recent development of a high-throughput assay to measure LH activity enabled a screen of 65000 compounds, identifying ∼30 possible LH2 inhibitors [120]. Thus, steps are currently taken to develop specific anti-fibrotic agents that can slow down or arrest fibrogenesis, or even reverse its progression, by giving matrix-degrading enzymes the opportunity to break down the scars. Interestingly, it has recently been shown that fibrosis also plays an important role in regulating the hallmark features of cancer [121]. Thus, inhibiting the formation of Hyl^{ald} cross-links may also have potential therapeutic value in the field of cancer biology.

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

PORTLAND PRESS

Summary

- A common denominator of fibrotic collagen is an increased level of Hyl^{ald} cross-links in favor of Lys^{ald} cross-links, resulting in collagen that show a high resistance toward MMPs, and as a consequence inhibiting resolution of fibrosis.
- LH2 is responsible for the conversion of Lys into Hyl in the telopeptides of collagen, leading to the formation of Hyl^{ald} cross-links by LOXs.
- Normalizing the cross-link pattern by selectively inhibiting LH2 (and thus Hyl^{ald} cross-linking) alters the balance of collagen degradation; it is expected that this results in reversible fibrosis.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contribution

B.P. and R.B. wrote the manuscript and designed the figures.

Abbreviations

ECM, extracellular matrix; FKBP, FK506-binding protein; HP, hydroxylyslpyridinoline; Hyl, hydroxylysine; Hyl^{ald}, hydroxyallysine; LH, lysyl hydroxylase; LH2, lysyl hydroxylase 2; LP, lysylpyridinoline; LOX, lysyl oxidase; LOXL, LOX-like; Lys, lysine; Lys^{ald}, allysine; MMP, matrix metalloproteinase; PLOD, procollagen-lysine, 2-oxoglutarate 5-dioxygenase; TG, transglutaminase.

References

1 Zeisberg, M. and Kalluri, R. (2013) Cellular mechanisms of tissue fibrosis: 1. Common and organ-specific mechanisms associated with tissue fibrosis. *Am. J. Physiol. Cell Physiol.* **304**, C216–C225, https://doi.org/10.1152/ajpcell.00328.2012
2 Rockey, D.C., Bell, P.D. and Hill, J.A. (2015) Fibrosis – a common pathway to organ injury and failure. *N. Engl. J. Med.* **372**, 1138–1149, https://doi.org/10.1056/NEJMra1300575
3 Karsdal, M.A., Nielsen, S.H., Leeming, D.J., Langholm, L.L., Nielsen, M.J., Manon-Jensen, T. et al. (2017) The good and the bad collagens of fibrosis - their role in signaling and organ function. *Adv. Drug Deliv. Rev.* **121**, 43–56, https://doi.org/10.1016/j.addr.2017.07.014
4 Karsdal, M.A., Manon-Jensen, T., Genovese, F., Kristensen, J.H., Nielsen, M.J., Sand, J.M. et al. (2015) Novel insights into the function and dynamics of extracellular matrix in liver fibrosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **308**, G807–G830, https://doi.org/10.1152/ajpgi.00447.2014
5 Schuppan, D., Ashfaq-Khan, M., Yang, A.T. and Kim, Y.O. (2018) Liver fibrosis: direct antifibrotic agents and targeted therapies. *Matrix Biol.* **68-69**, 435–451, https://doi.org/10.1016/j.matbio.2018.04.006
6 Ricard-Blum, S., Baffet, G. and Théret, N. (2018) Molecular and tissue alterations of collagens in fibrosis. *Matrix Biol.* **68-69**, 122–149, https://doi.org/10.1016/j.matbio.2018.02.004
7 Yamauchi, M., Barker, T.H., Gibbons, D.L. and Kurie, J.M. (2018) The fibrotic tumor stroma. *J. Clin. Invest.* **128**, 16–25, https://doi.org/10.1172/JCI93554
8 Mienaltowski, M.J. and Birk, D.E. (2014) Structure, physiology, and biochemistry of collagens. *Adv. Exp. Med. Biol.* **802**, 5–29, https://doi.org/10.1007/978-94-007-7893-1˙2
9 Yamauchi, M. and Sricholpech, M. (2012) Lysine post-translational modifications of collagen. *Essays Biochem.* **52**, 113–133, https://doi.org/10.1042/bse0520113
10 Gjaltema, R.A.F. and Bank, R.A. (2017) Molecular insights into prolyl and lysyl hydroxylation of fibrillar collagens in health and disease. *Crit. Rev. Biochem. Mol. Biol.* **52**, 74–95, https://doi.org/10.1080/10409238.2016.1269716
11 Cai, L., Xiong, X., Kong, X. and Xie, J. (2017) The role of the lysyl oxidases in tissue repair and remodeling: a concise review. *Tissue Eng. Regen. Med.* **14**, 15–30, https://doi.org/10.1007/s13770-016-0007-0
12 Fang, M., Yuan, J., Peng, C. and Li, Y. (2014) Collagen as a double-edged sword in tumor progression. *Tumour Biol.* **35**, 2871–2882, https://doi.org/10.1007/s13277-013-1511-7
13 Yamauchi, M. and Shiiba, M. (2008) Lysine hydroxylation and crosslinking of collagen. *Methods Mol. Biol.* **446**, 95–108, https://doi.org/10.1007/978-1-60327-084-7˙7
14 Eyre, D.R., Weis, M.A. and Wu, J.J. (2008) Advances in collagen cross-link analysis. *Methods* **45**, 65–74, https://doi.org/10.1016/j.ymeth.2008.01.002
15 Eyre, D.R., Koob, T.J. and Van Ness, K.P. (1984) Quantitation of hydroxypyridinium crosslinks in collagen by high-performance liquid chromatography. *Anal. Biochem.* **137**, 380–388, https://doi.org/10.1016/0003-2697(84)90101-5

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

Essays in Biochemistry (2019) **63** 377–387
https://doi.org/10.1042/EBC20180051

16 Yamauchi, M., Chandler, G.S., Tanzawa, H. and Katz, E.P. (1996) Cross-linking and the molecular packing of corneal collagen. *Biochem. Biophys. Res. Commun.* **219**, 311–315, https://doi.org/10.1006/bbrc.1996.0229
17 Eyre, D.R., Weis, M. and Rai, J. (2019) Analyses of lysine aldehyde cross-linking in collagen reveal that the mature cross-link histidinohydroxylysinonorleucine is an artifact. *J. Biol. Chem.* **294**, 6578–6590, https://doi.org/10.1074/jbc.RA118.007202
18 Forrest, L, Shuttleworth, A., Jackson, D.S. and Mechanic, G.L. (1972) A comparison between the reducible intermolecular crosslinks of the collagen from mature dermis and young dermal scar of the Guinea pig. *Biochem. Biophys. Res. Commun.* **46**, 1776–1781, https://doi.org/10.1016/0006-291X(72)90050-2
19 Bailey, A.J., Bazin, S. and Delauny, A. (1973) Changes in the nature of the collagen during development and resorption of granulation tissue. *Biochim. Biophys. Acta* **328**, 383–390, https://doi.org/10.1016/0005-2795(73)90272-9
20 Bailey, A.J., Bazin, S., Sims, T.J., Le Lous, M., Nicoletis, C. and Delauny, A. (1975) Characterization of the collagen of human hypertrophic and normal scars. *Biochim. Biophys. Acta* **405**, 412–421, https://doi.org/10.1016/0005-2795(75)90106-3
21 Barnes, M.J., Morton, L.F., Bennett, R.C., Bailey, A.J. and Sims, T.J. (1976) Presence of type III collagen in Guinea-pig dermal scar. *Biochem. J.* **157**, 263–266, https://doi.org/10.1042/bj1570263
22 Bazin, S., Le Lous, M. and Delaunay, A. (1976) Collagen in granulation tissues. *Agents Actions* **6**, 272–276, https://doi.org/10.1007/BF01972221
23 Bailey, A.J. and Light, N.D. (1985) Intermolecular cross-linking in fibrotic collagen. *Ciba Found. Symp.* **114**, 80–96
24 Cannon, D.J. and Cintron, C. (1975) Collagen cross-linking in corneal scar formation. *Biochim. Biophys. Acta* **412**, 18–25, https://doi.org/10.1016/0005-2795(75)90335-9
25 Moriguchi, T. and Fujimoto, D. (1979) Crosslink of collagen in hypertrophic scar. *J. Invest. Dermatol.* **72**, 143–145, https://doi.org/10.1111/1523-1747.ep12530609
26 Wan, K.C. and Lewis, W.H.P. (1996) Study of free iron and pyridinoline in hypertrophic scars and normal skin. *Br. J. Biomed. Sci.* **53**, 196–203
27 Uzawa, K., Marshall, M.K., Katz, E.P., Tanzawa, H., Yeowell, H.N. and Yamauchi, M. (1998) Altered posttranslational modifications of collagen in keloid. *Biochem. Biophys. Res. Commun.* **249**, 652–655, https://doi.org/10.1006/bbrc.1998.8955
28 Wan, K.C. and Evans, J.H. (1999) Free radical involvement in hypertrophic scar formation. *Free Radic. Biol. Med.* **26**, 603–608, https://doi.org/10.1016/S0891-5849(98)00245-7
29 Podobed, O.V., Prozorovskii, N.N., Kozlov, E.A., Tsvetkova, T.A., Vozdvizhenskii, S.I. and Del'vig, A.A. (1996) Comparative study of collagen in hypertrophic and keloid matrix. *Vopr. Med. Khim.* **42**, 240–245
30 Gerriets, J.E., Reiser, K.M. and Last, J.A. (1996) Lung collagen cross-links in rats with experimentally induced pulmonary fibrosis. *Biochim. Biophys. Acta* **1316**, 121–131, https://doi.org/10.1016/0925-4439(96)00019-1
31 Reiser, K.M. and Last, J.A. (1986) Collagen crosslinking in lungs of rats with experimental silicosis. *Collagen Rel. Res.* **6**, 313–324, https://doi.org/10.1016/S0174-173X(86)80002-4
32 Reiser, K.M., Tryka, A.F., Lindenschmidt, R.C., Last, J.A. and Witschi, H.R. (1986) Changes in collagen crosslinking in bleomycin-induced pulmonary fibrosis. *J. Biochem. Toxicol.* **1**, 83–91, https://doi.org/10.1002/jbt.2570010109
33 Reiser, K.M., Tyler, W.S., Hennessy, S.M., Dominguez, J.J. and Last, J.A. (1987) Long-term consequences of exposure to ozone. II. Structural alterations in lung collagen of monkeys. *Toxicol. Appl. Pharmacol.* **89**, 314–322, https://doi.org/10.1016/0041-008X(87)90151-7
34 Reiser, K.M. and Last, J.A. (1987) A molecular marker for fibrotic collagen in lungs of infants with respiratory distress syndrome. *Biochem. Med. Metab. Biol.* **37**, 16–21, https://doi.org/10.1016/0885-4505(87)90004-1
35 Last, J.A., King, T.E., Nerlich, A.G. and Reiser, K.M. (1990) Collagen cross-linking in adult patients with acute and chronic fibrotic lung disease. Molecular markers for fibrotic collagen. *Am. Rev. Respir. Dis.* **141**, 307–313, https://doi.org/10.1164/ajrccm/141.2.307
36 Last, J.A., Gerriets, J.E., Armstrong, L.C., Gelzleichter, T.R. and Reiser, K.M. (1990) Hydroxylation of collagen by lungs of rats administered bleomycin. *Am. J. Respir. Cell Mol. Biol.* **2**, 543–548, https://doi.org/10.1165/ajrcmb/2.6.543
37 Last, J.A. and Reiser, K.M. (1989) Biosynthesis of collagen crosslinks. III. In vivo labeling and stability of lung collagen in rats with bleomycin-induced pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* **1**, 111–117, https://doi.org/10.1165/ajrcmb/1.2.111
38 Last, J.A. and Reiser, K.M. (1986) Effects of silica on lung collagen. *Ciba Found. Symp.* **121**, 180–193
39 Last, J.A., Armstrong, L.G. and Reiser, K.M. (1990) Biosynthesis of collagen crosslinks. *Int. J. Biochem.* **22**, 559–564, https://doi.org/10.1016/0020-711X(90)90031-W
40 Di Donato, A., Ghiggeri, G.M., Di Duca, M., Jivotenko, E., Acinni, R., Campolo, J. et al. (1997) Lysyl oxidase expression and collagen cross-linking during chronic adriamycin nephropathy. *Nephron* **76**, 192–200, https://doi.org/10.1159/000190168
41 McCormick, R.J., Musch, T.I., Bergman, B.C. and Thomas, D.P. (1994) Regional differences in LV collagen accumulation and mature cross-linking after myocardial infarction in rats. *Am. J. Physiol.* **266**, H354–H3591
42 González-Santamaría, J., Villalba, M., Busnadiego, O., López-Olañeta, M.M., Sandoval, P., Snabel, J. et al. (2015) Matrix cross-linking lysyl oxidases are induced in response to myocardial infarction and promote cardiac dysfunction. *Cardiovasc. Res.* **109**, 67–78, https://doi.org/10.1093/cvr/cvv214
43 Akeson, W.H, Amiel, D., Mechanic, G.L., Woo, S.L.Y., Harwood, F.L. and Hamet, M.L. (1977) Collagen cross-linking alterations in joint contractures: changes in the reducible cross-links in periarticular connective tissue collagen after nine weeks of immobilization. *Connect. Tissue Res.* **5**, 15–19, https://doi.org/10.3109/03008207709152607
44 Spears, J.R., Zhan, H., Khurana, S., Karvonen, R.L. and Reiser, K.M. (1994) Modulation by β-aminopropionitrile of vessel luminal narrowing and structural abnormalities in arterial wall collagen in a rabbit model of conventional balloon angioplasty versus laser balloon angioplasty. *J. Clin. Invest.* **93**, 1543–1553, https://doi.org/10.1172/JCI117133
45 Brinckmann, J., Açil, Y., Tronnier, M., Notbohm, H., Bätge, B. and Schmeller, W. (1996) Altered X-ray diffraction pattern is accompanied by a change in the mode of cross-link formation in lipodermatosclerosis. *J. Invest. Dermatol.* **107**, 589–592, https://doi.org/10.1111/1523-1747.ep12582991

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

46 Brinckmann, J., Neess, C.M., Gaber, Y., Sobhi, H., Notbohm, H., Hunzelmann, N. et al. (2001) Different pattern of collagen cross-links in two sclerotic skin diseases: lipodermatosclerosis and circumscribed scleroderma. *J. Invest. Dermatol.* **117**, 269–273, https://doi.org/10.1046/j.0022-202x.2001.01414.x

47 Halme, T., Peltonen, J., Sims, T.J., Vihersaari, T. and Penttinen, R. (1986) Collagen in human aorta. Changes in the type III/I ratio and concentration of the reducible crosslink, dehydrohydroxylysinonorleucine in ascending aorta from healthy subjects of different age and patients with annulo-aortic ectasia. *Biochim. Biophys. Acta* **881**, 222–228, https://doi.org/10.1016/0304-4165(86)90007-3

48 Bazin, S., Le Lous, M., Duance, V.C., Sims, T.J., Bailey, A.J., Gabbiani, G. et al. (1980) Biochemistry and histology of the connective tissue of Dupuytren's disease lesions. *Eur. J. Clin. Invest.* **10**, 9–16, https://doi.org/10.1111/j.1365-2362.1980.tb00003.x

49 Harper, J.I., Duance, V.C., Sims, T.J. and Light, N.D. (1985) Lipoid proteinosis: an inherited disorder of collagen metabolism? *Br. J. Dermatol.* **113**, 145–151, https://doi.org/10.1111/j.1365-2133.1985.tb02056.x

50 Buckingham, B. and Reiser, K.M. (1990) Relationship between the content of lysyl oxidase-dependent crosslinks in skin collagen, nonenzymatic glycation, and long-term complications in type I diabetes mellitus. *J. Clin. Invest.* **86**, 1046–1054, https://doi.org/10.1172/JCI114807

51 Reiser, K.M., Crouch, E.C., Chang, K. and Williamson, J.R. (1991) Lysyl oxidase-mediated crosslinking in granulation tissue collagen in two models of hyperglycemia. *Biochim. Biophys. Acta* **1097**, 55–61, https://doi.org/10.1016/0925-4439(91)90024-4

52 Ricard-Blum, S., Esterre, P. and Grimaud, J.A. (1993) Collagen cross-linking by pyridinoline occurs in non-reversible skin fibrosis. *Cell Mol. Biol.* **39**, 723–727

53 Ricard-Blum, S., Hartmann, D.J. and Esterre, P. (1998) Monitoring of extracellular matrix metabolism and cross-linking in tissue, serum and urine of patients with chromoblastomycosis, a chronic skin fibrosis. *Eur. J. Clin. Invest.* **28**, 748–754, https://doi.org/10.1046/j.1365-2362.1998.00335.x

54 Lehto, M., Sims, T.J. and Bailey, A.J. (1985) Skeletal muscle injury – molecular changes in the collagen during healing. *Res. Exp. Med.* **185**, 95–106, https://doi.org/10.1007/BF01854894

55 Huang, Y., Zhao, J.X., Yan, X., Zhu, M.J., Long, N.M., McCormick, R.J. et al. (2012) Maternal obesity enhances collagen accumulation and cross-linking in skeletal muscle of ovine offspring. *PLoS ONE* **7**, e31691, https://doi.org/10.1371/journal.pone.0031691

56 Chapman, M.A., Pichika, R. and Lieber, R.L. (2015) Collagen crosslinking does not dictate stiffness in a transgenic mouse model of skeletal muscle fibrosis. *J. Biomech.* **48**, 375–378, https://doi.org/10.1016/j.jbiomech.2014.12.005

57 Istok, R., Bély, M., Stancíková, M. and Rovenský, J. (2001) Evidence for increased pyridinoline concentration in fibrotic tissues in diffuse systemic sclerosis. *Clin. Exp. Dermatol.* **26**, 545–547, https://doi.org/10.1046/j.1365-2230.2001.00886.x

58 Kamel, M., Wagih, M., Kilic, G.S., Diaz-Arrastia, C.R., Baraka, M.A. and Salama, S.A. (2017) Overhydroxylation of lysine of collagen increases uterine fibroids proliferation: roles of lysyl hydroxylases, lysyl oxidases, and matrix metalloproteinases. *Biomed. Res. Int.* **2017**, 5316845, https://doi.org/10.1155/2017/5316845

59 Kahle, B., Schmidtke, C., Hunzelmann, N., Bartels, C., Sievers, H.H., Steenbock, H. et al. (2016) The extracellular matrix signature in vein graft disease. *Can. J. Cardiol.* **32**, 1008–1017.e11, https://doi.org/10.1016/j.cjca.2015.11.014

60 Herbage, D., Chevalier, O., Hassanein, H. and Janin, B. (1983) Collagen solubility and cross-linking in normal and fibrotic human livers. *Contr. Microbiol. Immunol.* **7**, 237–244

61 Ricard-Blum, S., Bresson-Hadni, S., Vuitton, D.A., Ville, G. and Grimaud, J.A. (1992) Hydroxypyridinium collagen cross-links in human liver fibrosis: study of alveolar echinococcosis. *Hepatology* **15**, 599–602, https://doi.org/10.1002/hep.1840150408

62 Ricard-Blum, S., Ville, G. and Grimaud, J.A. (1992) Pyridinoline, a mature collagen cross-link, in fibrotic livers from Schistosoma mansoni-infected mice. *Am. J. Trop. Med. Hyg.* **47**, 816–820, https://doi.org/10.4269/ajtmh.1992.47.816

63 Ricard-Blum, S., Liance, M., Houin, R., Grimaud, J.A. and Vuitton, D.A. (1995) Covalent cross-linking of liver collagen by pyridinoline increases in the course of experimental alveolar echinococcosis. *Parasite* **2**, 113–118, https://doi.org/10.1051/parasite/1995022113

64 Ricard-Blum, S., Bresson-Hadni, S., Guerret, S., Grenard, P., Volle, P.J., Risteli, L. et al. (1996) Mechanism of collagen network stabilization in human irreversible granulomatous liver fibrosis. *Gastroenterology* **111**, 172–182, https://doi.org/10.1053/gast.1996.v111.pm8698196

65 Hayasaka, A., Iida, S., Suzuki, N., Kondo, F., Miyazaki, M. and Yonemitsu, H. (1996) Pyridinoline collagen cross-links in patients with chronic viral hepatites and cirrhosis. *J. Hepatol.* **24**, 692–698, https://doi.org/10.1016/S0168-8278(96)80265-0

66 Grenard, P., Blanquier, B. and Ricard-Blum, S. (1997) Urinary excretion of the collagen cross-link pyridinoline increases during liver fibrosis. *J. Hepatol.* **26**, 1356–1362, https://doi.org/10.1016/S0168-8278(97)80472-2

67 Ricard-Blum, S., Bresson-Hadni, S., Grenard, P., Humbert, P., Carbillet, J.P., Risteli, L. et al. (1998) The level of the collagen cross-link pyridinoline reflects the improvement of cutaneous lesions in one case of skin alveolar echinococcosis. *Parasitol. Res.* **84**, 715–719, https://doi.org/10.1007/s004360050475

68 Perepelyuk, M., Terajima, M., Wang, A.Y., Georges, P.C., Janmey, P.A., Yamauchi, M. et al. (2013) Hepatic stellate cells and portal fibroblasts are the major cellular sources of collagens and lysyl oxidases in normal liver and early after injury. *Am. J. Physiol. Gastrointest. Liver Physiol.* **304**, G605–G614, https://doi.org/10.1152/ajpgi.00222.2012

69 Schilter, H., Findlay, A.D., Perryman, L., Yow, T.T., Moses, J., Zahoor, A. et al. (2019) The lysyl oxidase like 2/3 enzymatic inhibitor, PXS-5153A, reduces crosslinks and ameliorates fibrosis. *J. Cell. Mol. Med.* **23**, 1759–1770

70 Yeowell, H.N. and Walker, L.C. (1999) Tissue specificity of a new splice form of the human lysyl hydroxylase 2 gene. *Matrix Biol.* **18**, 179–187, https://doi.org/10.1016/S0945-053X(99)00013-X

71 Salo, A.M., Sipilä, L., Sormunen, R., Ruotsalainen, H., Vainio, S. and Myllylä, R. (2006) The lysyl hydroxylase isoforms are widely expressed during mouse embryogenesis, but obtain tissue- and cell-specific patterns in the adult. *Matrix Biol.* **25**, 475–483, https://doi.org/10.1016/j.matbio.2006.08.260

72 Mercer, D.K., Nicol, P.F., Kimbembe, C. and Robins, S.P. (2003) Identification, expression, and tissue distribution of the three rat lysyl hydroxylase isoforms. *Biochem. Biophys. Res. Commun.* **307**, 803–809, https://doi.org/10.1016/S0006-291X(03)01262-2

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

Essays in Biochemistry (2019) **63** 377–387
https://doi.org/10.1042/EBC20180051

73 Puig-Hervás, M.T., Temtamy, S., Aglan, M., Valencia, M., Martínez-Glez, V., Ballesta-Martínez, M.J. et al. (2012) Mutations in PLOD2 cause autosomal-recessive connective tissue disorders within the Bruck syndrome–osteogenesis imperfecta phenotypic spectrum. *Hum. Mutat.* **33**, 1444–1449, https://doi.org/10.1002/humu.22133

74 van der Slot, A.J., Zuurmond, A.M., van den Bogaerdt, A.J., Ulrich, M.M., Middelkoop, E., Boers, W. et al. (2004) Increased formation of pyridinoline cross-links due to higher telopeptide lysyl hydroxylase levels is a general fibrotic phenomenon. *Matrix Biol.* **23**, 251–257, https://doi.org/10.1016/j.matbio.2004.06.001

75 Eckert, L., Kaartinen, M.T., Nurminskaya, M., Belkin, A.M., Colak, G., Johnson, G.V. et al. (2014) Transglutaminase regulation of cell functions. *Physiol. Rev.* **94**, 383–417, https://doi.org/10.1152/physrev.00019.2013

76 Steppan, J., Bergman, Y., Viegas, K., Armstrong, D., Tan, S., Wang, H. et al. (2017) Tissue transglutaminase modulates vascular stiffness and function through crosslinking-dependent and crosslinking-independent functions. *J. Am. Heart Assoc.* **6**, e004161, https://doi.org/10.1161/JAHA.116.004161

77 Zhou, X., Jamil, A., Nash, A., Chan, J., Trim, N., Iredale, J.P. et al. (2006) Impaired proteolysis of collagen I inhibits proliferation of hepatic stellate cells. Implications for regulation of liver fibrosis. *J. Biol. Chem.* **281**, 39757–39765, https://doi.org/10.1074/jbc.M605621200

78 Jones, R.A., Kotsakis, P., Johnson, T.S., Chau, D.Y.S., Ali, S., Melino, G. et al. (2006) Matrix changes induced by transglutaminase 2 lead to inhibition of angiogenesis and tumor growth. *Cell Death Differ.* **13**, 1442–1453, https://doi.org/10.1038/sj.cdd.4401816

79 Johnson, T.S., Skill, N.J., El Nagas, A.M., Oldroyd, S.D., Thomas, G.L., Douthwaite, J.A. et al. (1999) Transglutaminase transcription and antigen translocation in experimental renal scarring. *J. Am. Soc. Nephrol.* **10**, 2146–2157

80 Philp, C.J., Siebeke, I., Clements, D., Miller, S., Habgood, A., John, A.E. et al. (2018) Extracellular matrix cross-linking enhances fibroblast growth and protects against matrix proteolysis in lung fibrosis. *Am. J. Respir. Cell. Mol. Biol.* **58**, 594–603, https://doi.org/10.1165/rcmb.2016-0379OC

81 Wang, Z., Stuckey, D.J., Murdoch, C.E., Camelliti, P., Lip, G.Y.H. and Griffin, M. (2018) Cardiac fibrosis can be attenuated by blocking the activity of transglutaminase 2 using a selective small-molecule inhibitor. *Cell Death Dis.* **9**, 613, https://doi.org/10.1038/s41419-018-0573-2

82 Penumatsa, K.C., Toksoz, D., Warburton, R.R., Kharnaf, M., Preston, I.R., Kapur, N.K. et al. (2017) Transglutaminase 2 in pulmonary and cardiac remodeling in experimental pulmonary hypertension. *Am. J. Physiol. Lung Cell Mol. Physiol.* **313**, L752–L762, https://doi.org/10.1152/ajplung.00170.2017

83 Lin, C.H.S., Chen, J., Zhang, Z., Johnson, G.V.W., Cooper, A.J.L., Feola, J. et al. (2016) Endostatin and transglutaminase 2 are involved in fibrosis of the aging kidney. *Kidney Int.* **89**, 1281–1292, https://doi.org/10.1016/j.kint.2016.01.030

84 Johnson, T.S., El Koraie, A.F., Skill, N.J., Baddour, N.M., El Nahas, A.M., Njloma, M. et al. (2003) Tissue transglutaminase and the progression of human renal scarring. *J. Am. Soc. Nephrol.* **14**, 2052–2062, https://doi.org/10.1097/01.ASN.0000079614.63463.DD

85 Tatsukawa, H., Otsu, R., Tani, Y., Wakita, R. and Hitomi, K. (2018) Isozyme-specific comprehensive characterization of transglutaminase-crosslinked substrates in kidney fibrosis. *Sci. Rep.* **8**, 7306, https://doi.org/10.1038/s41598-018-25674-4

86 Tatsukawa, H., Tani, Y., Otsu, R., Nakagawa, H. and Hitomi, K. (2017) Global identification and analysis of isozyme-specific possible substrates crosslinked by transglutaminases using substrate peptides in mouse liver fibrosis. *Sci. Rep.* **7**, 45049, https://doi.org/10.1038/srep45049

87 Popov, Y., Sverdlov, D.Y., Sharma, A.K., Bhaskar, K.R., Li, S., Freitag, T.L. et al. (2011) Tissue transglutaminase does not affect fibrotic matrix stability or regression of liver fibrosis in mice. *Gastroenterology* **140**, 1642–1652, https://doi.org/10.1053/j.gastro.2011.01.040

88 Iredale, J.P. and Pellicoro, A. (2011) "It is a good morning exercise for a research scientist to discard a pet hypothesis every day before breakfast: it keeps him young" (Konrad Lorenz, 1903-1989). *Gastroenterology* **140**, 1395–1398, https://doi.org/10.1053/j.gastro.2011.03.025

89 Liu, S.B., Ikenaga, N., Peng, Z.W., Sverdlov, D.Y., Greenstein, A., Smith, V. et al. (2016) Lysyl oxidase activity contributes to collagen stabilization during liver fibrosis progression and limits spontaneous fibrosis reversal in mice. *FASEB J.* **30**, 1599–1609, https://doi.org/10.1096/fj.14-268425

90 Ikenaga, N., Peng, Z.W., Vaid, K.A., Liu, S.B., Yoshida, S., Sverdlov, D.Y. et al. (2017) Selective targeting of lysyl oxidase-like 2 (LOXL2) suppresses hepatic fibrosis progression and accelerates its reversal. *Gut* **66**, 1697–1708, https://doi.org/10.1136/gutjnl-2016-312473

91 Robins, S.P., Milne, G., Duncan, A., Davies, C., Butt, R., Greiling, D. et al. (2003) Increased skin collagen extractability and proportions of collagen type III are not normalized after 6 months healing of human excisional wounds. *J. Invest. Dermatol.* **121**, 267–272, https://doi.org/10.1046/j.1523-1747.2003.12373.x

92 Vater, C.A., Harris, Jr, E.D. and Siegel, R.C. (1979) Native cross-links in collagen fibrils induce resistance to human synovial collagenase. *Biochem. J.* **181**, 639–645, https://doi.org/10.1042/bj1810639

93 van der Slot, A.J., van Dura, E.A., de Wit, E.C., DeGroot, J., Huizinga, T.W.J., Bank, R.A. et al. (2005) Elevated formation of pyridinoline cross-links by profibrotic cytokines is associated with enhanced lysyl hydroxylase 2b levels. *Biochim. Biophys. Acta* **1741**, 95–102, https://doi.org/10.1016/j.bbadis.2004.09.009

94 Brinckmann, J., Kim, S., Wu, J., Reinhardt, D.P., Batmunkh, C., Metzen, E. et al. (2005) Interleukin 4 and prolonged hypoxia induce a higher gene expression of lysyl hydroxylase 2 and an altered cross-link pattern: important pathogenetic steps in ealry and late stage of systemic scleroderma? *Matrix Biol.* **24**, 459–468, https://doi.org/10.1016/j.matbio.2005.07.002

95 van der Slot-Verhoeven, A.J., van Dura, E.A., Attema, J., Blauw, B., DeGroot, J., Huizinga, T.W.J. et al. (2005) The type of collagen cross-link determines the reversibility of experimental skin fibrosis. *Biochim. Biophys. Acta* **1740**, 60–67, https://doi.org/10.1016/j.bbadis.2005.02.007

96 van den Bos, T., Speijer, D., Bank, R.A., Brömme, D. and Everts, V. (2008) Differences in matrix composition between calvaria and long bone in mice suggest differences in biomechanical properties and resorption: special emphasis on collagen. *Bone* **43**, 459–468, https://doi.org/10.1016/j.bone.2008.05.009

97 Bank, R.A., Robins, S.P., Wijmenga, C., Breslau-Siderius, L.J., Bardoel, A.F.J, van der Sluijs, H.A. et al. (1999) Defective collagen crosslinking in bone, but not in ligament or cartilage, in Bruck syndrome: indications for a bone-specific telopeptide lysyl hydroxylase on chromosome 17. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1054–1058, https://doi.org/10.1073/pnas.96.3.1054

98 Kafienah, W, Brömme, D, Buttle, DJ, Croucher, LJ and Hollander, A (1998) Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. *Biochem. J.* **331**, 727–732, https://doi.org/10.1042/bj3310727

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society

99 Verzijl, N., DeGroot, J., Thorpe, S.R., Bank, R.A., Shaw, J.N., Lyons, T.J. et al. (2000) Effect of collagen turnover on the accumulation of advanced glycation end products. *J. Biol. Chem.* **275**, 39027–39031, https://doi.org/10.1074/jbc.M006700200
100 Rose, B.J. and Kooyman, D.L. (2016) A tale of two joints: the role of matrix metalloproteases in cartilage biology. *Dis. Markers* **2016**, 4895050, https://doi.org/10.1155/2016/4895050
101 Mort, J.S., Beaudry, F., Théroux, K., Emmott, A.A., Richard, H., Fisher, W.D. et al. (2016) Early cathepsin K degradation of type II collagen in vitro and in vivo in articular cartilage. *Osteoarthritis Cartilage* **24**, 1461–1469, https://doi.org/10.1016/j.joca.2016.03.016
102 Vankemmelbeke, M., Dekeyser, P.M., Hollander, A.P., Buttle, D.J. and Demeester, J. (1998) Characterization of helical cleavages in type II collagen generated by matrixins. *Biochem. J.* **330**, 633–640, https://doi.org/10.1042/bj3300633
103 Stegen, S., Laperre, K., Eelen, G., Rinaldi, G., Fraisl, P., Torrekens, S. et al. (2019) HIF-1α metabolically controls collagen synthesis and modification in chondrocytes. *Nature* **565**, 511–515, https://doi.org/10.1038/s41586-019-0874-3
104 Remst, D.F.G., Blaney Davidson, E.N., Vitters, E.L., Blom, A.B., Stoop, R., Snabel, J.M. et al. (2013) Osteoarthritis-related fibrosis is associated with both elevated pyridinoline cross-link formation and lysyl hydroxylase 2b expression. *Osteoarthritis Cartilage* **21**, 157–164, https://doi.org/10.1016/j.joca.2012.10.002
105 Remst, D.F.G., Blaney Davidson, E.N. and van der Kraan, P.M. (2015) Unravelling osteoarthritis-related synovial fibrosis: a step closer to solving joint stiffness. *Rheumatology (Oxford)* **54**, 1954–1963, https://doi.org/10.1093/rheumatology/kev228
106 Zigrino, P., Brinckmann, J., Niehoff, A., Lu, Y., Giebeler, N., Eckes, B. et al. (2016) Fibroblast-derived MMP-14 regulates collagen homeostasis in adult skin. *J. Invest. Dermatol.* **136**, 1575–1583, https://doi.org/10.1016/j.jid.2016.03.036
107 Mechanic, G.L., Katz, E.P., Henmi, M., Noyes, C. and Yamauchi, M. (1988) Locus of a histidine-based, stable trifunctional, helix to helix collagen cross-link: stereospecific collagen structure of type I skin fibrils. *Biochemistry* **26**, 3500–3509, https://doi.org/10.1021/bi00386a038
108 Yamauchi, M., Chandler, G.S., Tanzawa, H. and Katz, E.P. (1996) Cross-linking and the molecular packing of corneal collagen. *Biochem. Biophys. Res. Commun.* **219**, 311–315, https://doi.org/10.1006/bbrc.1996.0229
109 Yamauchi, M. and Mechanic, G.L. (1988) Cross-linking of collagen. In *Collagen. Volume 1: Biochemistry* (Nimni, M.E., ed.), pp. 157–172, CRC Press, Boca Raton
110 Yamauchi, M. and Katz, E.P. (1993) The post-translational chemistry and molecular packing of mineralizing tendon collagens. *Connect. Tissue Res.* **29**, 81–98, https://doi.org/10.3109/03008209309014236
111 Sivan, S.S., Wachtel, E., Tsitron, E., Sakkee, N., van der Ham, F., DeGroot, J. et al. (2008) Collagen turnover in normal and degenerate human intervertebral discs as determined by the racemization of aspartic acid. *J. Biol. Chem.* **283**, 8796–8801, https://doi.org/10.1074/jbc.M709885200
112 Heinemeier, K.M., Schjerling, P., Heinemeier, J., Magnusson, S.P. and Kjaer, M. (2013) Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb 14C. *FASEB J.* **27**, 2074–2079, https://doi.org/10.1096/fj.12-225599
113 Gineyts, E., Cloos, P.A.C., Borel, O., Grimaud, L., Delmas, P.D. and Garnero, P. (2000) Racemization and isomerization of type I collagen C-telopeptides in human bone and soft tissues: assessment of tissue turnover. *Biochem. J.* **345**, 481–485, https://doi.org/10.1042/bj3450481
114 Schwarze, U., Cundy, T., Pyott, S.M., Christiansen, H.E., Hegde, M.R., Bank, R.A. et al. (2013) Mutations in FKBP10, which result in Bruck syndrome and recessive forms of osteogenesis imperfecta, inhibit the hydroxylation of telopeptide lysines in bone collagen. *Hum. Mol. Genet.* **22**, 1–17, https://doi.org/10.1093/hmg/dds371
115 Gjaltema, R.A., van der Stoel, M.M., Boersema, M. and Bank, R.A. (2016) Disentangling mechanisms involved in collagen pyridinoline cross-linking: the immunophilin FKBP65 is critical for dimerization of lysyl hydroxylase 2. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 7142–7147, https://doi.org/10.1073/pnas.1600074113
116 Staab-Weijnitz, C.A., Fernandez, I.E., Knüppel, L., Maul, J., Heinzelmann, K., Juan-Guardela, B.M. et al. (2015) FK506-binding protein 10, a potential novel drug target for idiopathic pulmonary fibrosis. *Am J. Respir. Crit. Care Med.* **192**, 455–467, https://doi.org/10.1164/rccm.201412-2233OC
117 Liang, X., Chai, B., Duan, R., Zhou, Y., Huang, X. and Li, Q. (2017) Inhibition of FKBP10 attenuates hypertrophic scarring through suppressing fibroblast activity and extracellular matrix deposition. *J. Invest. Dermatol.* **137**, 2326–2335, https://doi.org/10.1016/j.jid.2017.06.029
118 Scietti, L., Campioni, M. and Forneris, F. (2019) SiMPLOD, a structure-integrated database of collagen lysyl hydroxylase (LH/PLOD) enzyme variants. *J. Bone Miner. Res.*, https://doi.org/10.1002/jbmr.3692
119 Scietti, L., Chiapparino, A., De Giorgi, F., Fumagalli, M., Khoriauli, L., Nergadze, S. et al. (2018) Molecular architecture of the multifunctional collagen lysyl hydroxylase and glycosyltransferase LH3. *Nat. Commun.* **9**, 3163, https://doi.org/10.1038/s41467-018-05631-5
120 Devkota, A.K., Veloria, J.R., Guo, H.F., Kurie, J.M., Cho, E.J. and Dalby, K.N. (2019) Development of a high-throughput lysyl hydroxylase (LH) assay and identification of small-molecule inhibitors against LH2. *SLAS Discov.* **24**, 484–491
121 Yamauchi, M., Barker, T.H., Gibbons, D.L. and Kurie, J.M. (2018) The fibrotic stroma. *J. Clin. Invest.* **128**, 16–25, https://doi.org/10.1172/JCI93554

Downloaded from https://portlandpress.com/essaysbiochem/article-pdf/63/3/377/844130/ebc-2018-0051c.pdf by University of Groningen user on 18 February 2020

© 2019 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society