Biochemical and Biophysical Research Communications 461 (2015) 445–449

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Localization of the dominant non-enzymatic intermolecular cross-linking sites on fibrous collagen

Hiroko Chiue [a, b], Tsutako Yamazoye [a], Sueo Matsumura [a, c, *]

[a] Department of Biophysics, Kobe University Graduate School of Health Sciences, Suma, Kobe 654-0142, Japan
[b] Laboratory of Food Chemistry, Faculty of Nutrition, Kobe Gakuin University, Nishi, Kobe 651-2180, Japan
[c] Department of Nutrition Management, Faculty of Health Sciences, Hyogo University, Hiraoka, Kakogawa 675-0195, Japan

ARTICLE INFO

*Article history:*
Received 21 March 2015
Available online 17 April 2015

*Keywords:*
Rat tail tendon
Collagen
Non-enzymatic cross-linking site
Telopeptides

ABSTRACT

Previous studies have shown that fibrous collagen undergoes intermolecular cross-linking at multiple sites of the elongated triple-helical regions among adjacent juxtaposed collagen molecules on incubation with a very high concentration of reducing sugar such as 200 mM ribose, and the similarity of the changes in its physicochemical properties to that of senescent collagen aged in vivo has been emphasized. In the present study, however, it was found that when incubated with less than 30 mM ribose, fibrous collagen underwent intermolecular cross-linking primarily between the telopeptide region of a collagen molecule and the triple-helical region of another adjacent collagen molecule, and intermolecular cross-linking between the triple-helical regions of adjacent collagen molecules was very small. Physiological significance of the previous studies thus needs to be reevaluated.



## 1. Introduction

Fibrous collagen normally undergoes a series of age-related changes characterized by browning, decreasing solubility, increasing stiffness and increasing resistance to enzymatic digestion [1], and these changes are known to be accelerated in diabetes mellitus [1–6]. These age-related and diabetes-accelerated changes in fibrous collagen are supposed to be brought about by sugar-derived cross-links recognized also as advanced glycation end products (AGE), which are supposed to be produced through a series of non-enzymatic reactions between the side chains of collagen polypeptides and glucose or its metabolites [3–10]. Pentosidine, a fluorescent imidazopyridinium compound including a lysine and an arginine cross-linked by a derivative of pentose, has previously been proposed to be a major senescence cross-link responsible for the age-related and diabetes-related changes in the physical properties of collagen [7–10], but is now recognized as only a minor cross-link, since its content in senescent tissues was less than 0.1 mol per mole collagen [11]. Recent studies suggest that "glucosepane", a 1,4-dideoxy-5,6-glucosone-derived lysine–arginine cross-link, is now recognized as a major protein cross-link in the senescent human extracellular matrix, since its content amounts to 0.6 mol per mole collagen [12,13].

Numbers of early studies have shown that incubation of fibrous collagen in vitro with a very high concentration of reducing sugar such as 1 M glucose or 0.2 M ribose gives rise to a number of changes on fibrous collagen that resembled those occurred on senescent fibrous collagen aged in vivo as described above [7–11,14–20]. Although the changes that occurred on fibrous collagen in vitro and in vivo are supposed to resemble each other, it is not known whether these changes are identical or not. Available evidence indicates that there are large differences between the changes on these collagens. For example, fibrous collagen that had been incubated with 0.2 M ribose in vitro was shown to incorporate more than 45 mol of ribose per mole collagen [20], while the contents of so far identified cross-links in senescent fibrous collagen aged in vivo were reported to be less than 1 mol cross-link per mole collagen [11]. The cross-links generated in vitro were reported to localize on the elongated triple helical regions of the collagen molecules [14–20], while the location of the cross-links in senescent collagen has not been examined precisely. In the present study fibrous collagen was incubated in vitro with a low concentration of ribose to gain insight into the mechanism underlying cross-link formation in vivo and its effects on fibrous collagen. In

*Abbreviations:* SDS, sodium dodecyl sulfate; CB, cyanogen bromide.

* Corresponding author. Department of Nutrition Management, Faculty of Health Sciences, Hyogo University, Hiraoka, Kakogawa 675-0195, Japan. Fax: +81 79 427 5112.
*E-mail address:* matsumur@hyogo-dai.ac.jp (S. Matsumura).

http://dx.doi.org/10.1016/j.bbrc.2015.04.011
0006-291X/

contrast to previous observations, cross-link formation was found to proceed primarily at a site between the telopeptide region of a collagen molecule and the triple-helical region of another juxtaposed collagen molecule.

## 2. Materials and methods

### 2.1. Preparation of collagen samples

Tail tendons dissected from 8-week-old rats were washed with phosphate-buffered saline (PBS; 20 mM sodium phosphate, 0.15 M NaCl, pH 7.5) and distilled water, and then lyophilized. The tendons were minced into fine pieces and used for the present studies as native fibrous collagen. Acid-solubilized and reconstituted, loosely packed fibrous collagen (loose fibrous collagen) was prepared from the tendon pieces as described by Miller and Rhodes [21]. The tendon pieces were suspended in 10 volumes of 0.5 M acetic acid at 4 °C with constant stirring overnight. The suspensions were centrifuged and the supernatants were dialyzed against distilled water and lyophilized. This collagen yields loosely packed fibrils of collagen on suspension in neutral aqueous solution and used as loose fibrous collagen. Truncated collagen, which lacked the telopeptide regions at both N-terminal and C-terminal ends of the collagen chains, was prepared as follows. The loose fibrous collagen was incubated in 0.5 M acetic acid with 1/50 weight of pepsin at room temperature for 2 days and centrifuged for 1 h at 40,000× *g*, and the supernatant was dialyzed against distilled water, lyophilized and used as truncated collagen. In a previous study [20], this truncated collagen was termed as "soluble collagen", since it is monomeric and soluble in neutral aqueous medium at and below 30 °C, but It immediately becomes fibrous when incubated at 37 °C [20].

### 2.2. Glycation and cross-linking of collagen

A collagen sample, tendons (native fibrous collagen), acid soluble collagen (loose fibrous collagen) or truncated collagen, each approximately 10 mg, was incubated at 37 °C for 5, 10, or 15 days in 2 ml of PBS containing D-ribose (0–100 mM), 1 mM EDTA and 0.02% sodium azide. At an indicated time, the reaction mixtures were centrifuged at 10,000× *g* for 10 min, and the insoluble collagen pellets were washed with distilled water by centrifugation as above and then lyophilized. A part of the lyophilized samples was boiled with 2% SDS solution and subjected to SDS-PAGE as described below. Another part of the glycated samples was digested with pepsin or cyanogen bromide as described below, and then subjected to SDS-PAGE.

### 2.3. Pepsin digestion and cyanogen bromide cleavage of glycated collagen

A part of the glycated collagen samples was digested with 1/30 weight of pepsin in 0.5 M acetic acid for 3 days at 30 °C and then subjected to SDS-PAGE. Another part of the glycated collagen samples was suspended in 70% formic acid (10 mg/ml) and flushed with nitrogen gas, and an equal to twice weight of cyanogen bromide (CB) was quickly added. The reaction was allowed to proceed for 4 h at 30 °C. The reaction was terminated by dilution of the reaction mixture with 10 volumes of cold distilled water and then lyophilized.

### 2.4. SDS-PAGE

SDS-PAGE was carried out as described by Laemmli [22]. Proteins were stained with Coomassie Brilliant Blue R-250. The stained gels were scanned in EPSON GT8200UF scanner and analyzed by Scion Image software.

## 3. Results

### 3.1. Cross-linking of rat tail tendon collagen by incubation with ribose and pepsin digestion of the collagen

Washed and minced rat tail tendon collagen (native fibrous collagen) was incubated with 0–30 mM ribose at 37 °C for 5 days and boiled with SDS to dissociate the collagen chains and then subjected to SDS-PAGE (Fig. 1A). Control collagen that had been incubated without ribose contained a large amount of non-cross-linked monomeric α1 chain and small amounts of monomeric α2 chain and cross-linked dimeric β chains. The amounts of the monomeric α1 and α2 chains and cross-linked β chains decreased with increasing concentrations of ribose, and highly cross-linked polymeric collagen chains at the top of the gel increased, indicating that the monomeric collagen chains and dimeric β chains were cross-linked to highly cross-linked polymeric chains by incubating with increased concentrations of ribose. It was noted that incubation with a higher concentration of ribose resulted in formation of heavily cross-linked huge polymeric collagen chains that did not enter the gel and were not visible on the gel shown.

Pepsin, an acid protease, is known to cleave only the small non-helical telopeptide regions at the N-terminal and C-terminal ends of the type I collagen molecules at and below 30 °C but not the elongated triple-helical regions. To locate the cross-links on the collagen molecules, rat tail tendon collagen that had been incubated with increasing concentrations of ribose as above was digested with pepsin, neutralized with sodium hydroxide, boiled with SDS to dissociate the collagen chains and then subjected to

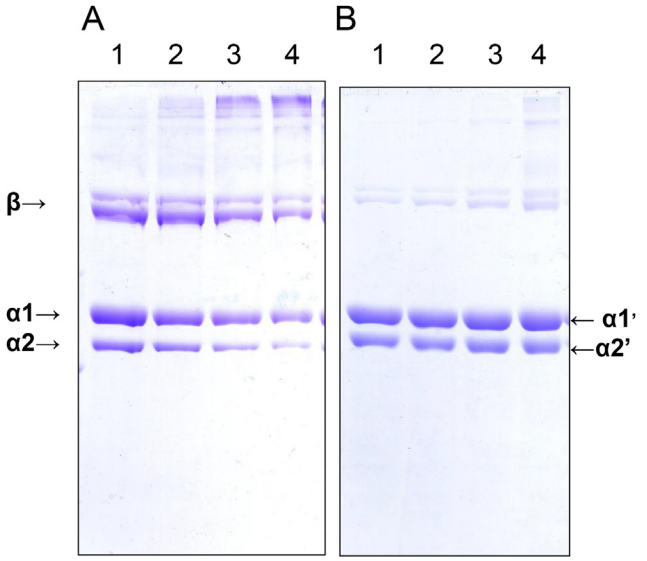


**Fig. 1.** SDS-PAGE of rat tail tendon collagen cross-linked by incubation with ribose (A), and of its pepsin digests (B). (A) Rat tail tendon collagen was incubated with 0–30 mM ribose for 5 days at 37 °C, boiled with SDS, and then subjected to SDS-PAGE. Ribose concentration was; 0 mM (lane 1), 10 mM (lane 2), 20 mM (lane 3), 30 mM (lane 4). The contents of monomeric α-1 and α-2 collagen chains decreased due to formation of cross-linked polymeric collagen chains by incubation with ribose. (B) Rat tail tendon collagen cross-linked by as in A was digested first with 1/30 weight of pepsin for 3 days at 30 °C, boiled with SDS and then subjected to SDS-PAGE. Ribose concentration for the lanes 1–4 was the same as in A. The monomeric α-1 and α-2 collagen chains once disappeared as in A due to formation of cross-linked polymeric collagen chains reappeared as α-1′ and α-2′ collagen chains due to cleavage of cross-linked telopeptide regions by pepsin.

SDS-PAGE (Fig. 1B). Each pepsin digest from the glycated samples was found to contain similar and each large amount of monomeric collagen chains designated as α1′ and α2′ chains, which were slightly smaller than the intact α1 and α2 chains due to the loss of the N-terminal and C-terminal telopeptide regions cleaved by pepsin. The contents of α1′ and α2′ chains in the pepsin digests from the collagen samples that had been pre-incubated with 10–30 mM ribose were much larger than that of the respective α1 and α2 chains in the collagen samples prior to pepsin digestion. The contents of dimeric or polymeric collagen chains in the respective pepsin digests were very small compared to that of the respective collagen samples prior to pepsin digestion. These results indicated that large quantities of polypeptide chains constituting the triple-helical regions were liberated from the cross-linked polymeric collagen molecules by losing the cross-linked telopeptide regions by pepsin digestion. It was apparent that at least one part of the most intermolecular cross-links was located at the telopeptide regions of collagen, and the other part of the cross-links was likely to be on the triple-helical regions.

### 3.2. Cyanogen bromide digestion of cross-linked collagen

Evidence that the intermolecular cross-linking of collagen proceeds initially at the telopeptide regions at an early stage and then at the triple-helical regions later was obtained by another analytical procedure, i.e. cyanogen bromide (CB) digestion, for the collagen pre-incubated with higher concentrations of ribose. Fibrous collagen was incubated with 0–80 mM ribose for 5, 10 or 15 days and then subjected to CB digestion. SDS-PAGE of control CB digests from collagen samples pre-incubated without ribose revealed numbers of polypeptides designated as CB1-1~8 and CB2-1~5 (Fig. 2A). The CB polypeptide patterns from the control digests did not change appreciably during even prolonged time of incubation. On the other hand the CB digests from the collagen samples pre-incubated with 40 or 80 mM ribose revealed that the contents of polypeptide CB1-6, which derived from the C-terminal end of the α1 collagen chain containing the small telopeptide region, decreased obviously at 5-day incubation, while the contents of other polypeptides did not change appreciably, indicating that CB1-6 polypeptide was cross-linked preferentially to another polypeptide to form a larger polypeptide at an early stage (Fig. 2A, lanes 1–3). SDS-PAGE of the CB digests from the collagen incubated with 40 or 80 mM ribose for 10 and 15 days revealed that the contents of almost all other polypeptides decreased obviously and then disappeared from the gel, indicating that cross-linking of collagen chains occurred at multiple sites on the triple-helical regions to form large polypeptides (Fig. 2A, lanes 5, 6, 8, 9). In another set of experiments, fibrous collagen that had been pre-incubated with 40 or 80 mM ribose for 5, 10 or 15 days was digested at first with pepsin for 3 days at 30 °C to cleave the telopeptide regions and then subjected to CB digestion. SDS-PAGE of the pepsin/CB digests from the collagen incubated for 5 days with 40 or 80 mM ribose revealed that the contents of nearly all polypeptides including the CB1-6′ polypeptide did not change to an appreciable extent, indicating that cross-linking of collagen chains did not occur at additional sites except the telopeptide regions (Fig. 2B, lanes 1–3). The pepsin/CB digests from the collagen pre-incubated for 10 days with 40 or 80 mM ribose revealed that the content of CB1-6′ polypeptide decreased appreciably and the contents of other polypeptides also decreased slightly, indicating that cross-linking of collagen chains proceeded at multiple sites on the triple-helical regions too (Fig. 2B, lanes 4, 5). The pepsin/CB digests from the collagen pre-incubated for 15 days with 40 or 80 mM ribose revealed larger extents of decreases in all polypeptides and progression of cross-linking at numerous sites (Fig. 2B, lanes 8, 9).

### 3.3. Cross-linking of loose fibrous collagen and truncated collagen

The results described above showed that intermolecular cross-linking of collagen chains by ribose-derived AGEs proceeds preferentially at the telopeptide regions during incubation with a low concentration of ribose and during a short period of incubation with a higher concentration of ribose. To examine whether a change in the state of intermolecular configuration of the collagen fibrils affects the intermolecular cross-linking of collagen or not, loose fibrous collagen, i.e. acid-solubilized and reconstituted collagen fibrils, which are supposed to possess looser intermolecular configuration as compared with the native tendon collagen fibrils, was incubated with increasing concentrations of ribose, and the rate and extent of intermolecular cross-linking of the collagen chains were studied by comparing with that of the native fibrous

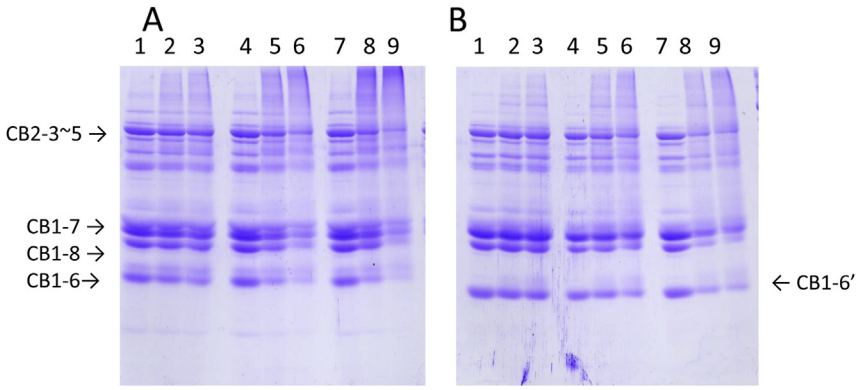


**Fig. 2.** SDS-PAGE of cyanogen bromide (CB) digests of rat tail tendon collagen cross-linked by incubation with ribose (A) and of the CB digests of the pepsin digests of the former cross-linked collagen (B). (A) Rat tail tendon collagen was incubated with 0 mM ribose for 5 days (lane 1), 10 days (lane 4), and 15 days (lane 7), with 40 mM ribose for 5 days (lane 2), 10 days (lane 5), and 15 days (lane 8), and with 80 mM ribose for 5 days (lane 3), 10 days (lane 6), and 15 days (lane 9), and digested with CB, and then subjected to SDS-PAGE. With increased concentration of ribose and prolonged time of incubation, contents of a few to several and then all CB peptides, especially CB1-6 peptide, decreased due to formation of highly cross-linked CB-resistant polymeric collagen chains that did not enter the gel. CB1-6 peptide derives from the C-terminal end region of α-1 collagen chain containing the small telopeptide. (B) Rat tail tendon collagen was glycated with ribose as in A, digested first with 1/30 weight of pepsin for 3 days at 30 °C, digested with CB and then subjected to SDS-PAGE. The lanes in B are numbered as in A so that the concentration of ribose and the time of incubation are the same as those in A. The contents of CB peptides, especially CB1-6′, did not decrease appreciably at 5 days of incubation even with 80 mM ribose since the terminal telopeptide region had been lost from CB1-6 by prior pepsin digestion, but became very low at 15 days due to formation of polymeric collagen cross-linked at the triple-helical regions.

collagen. As shown in Fig. 3(A and B), both control collagen and loose fibrous collagen contained similar amounts of monomeric α1 and α2 chains and dimeric β chains, and these collagen chains were lost by incubation with 10–30 mM ribose. The rate and extent of loss of the collagen chains from the control collagen and loose fibrous collagen were nearly identical to each other, indicating that a change in the state of intermolecular configuration of collagen fibrils did not affect the intermolecular cross-linking of collagen. In addition, to examine whether the triple-helical region of collagen undergoes by itself intermolecular cross-linking or not, truncated collagen which lacked the telopeptides and consisted only of the triple-helical regions was prepared by digesting tendon collagen with pepsin as described in Materials and Methods, and incubated with increasing concentrations of ribose, and then the rate and extent of intermolecular cross-linking of the collagen chains were also studied (Fig. 3C). The truncated collagen contained two species of monomeric collagen chains, designated as α1′ and α2′ chains, each being slightly smaller than the control α1 and α2 chains, respectively, and having slightly larger electrophoretic mobility as compared with the control α1 and α2 chains, respectively, owing to the loss of the telopeptide regions from the control α1 and α2 chains. The contents of the collagen chains, α1′ and α2′ chains in the truncated collagen did not decrease by incubation with 10–30 mM ribose. This result indicates that the truncated collagen did not undergo intermolecular cross-linking at and below 30 mM ribose. It was noted that the electrophoretic mobility of the truncated collagen chains, α1′ and α2′ chains, was retarded by incubation with ribose. This change was supposed to be due to glycation of the collagen chains by ribose. The truncated collagen chains were supposed to contain 5–10 mol of ribose per mole of collagen chain as estimated from a previous study carried out for the truncated collagen, which was termed formerly as “soluble collagen” [20]. In the previous study and in the present study, the truncated collagen was found to undergo intermolecular cross-linking when incubated with 100 mM ribose at 37 °C (data not shown).

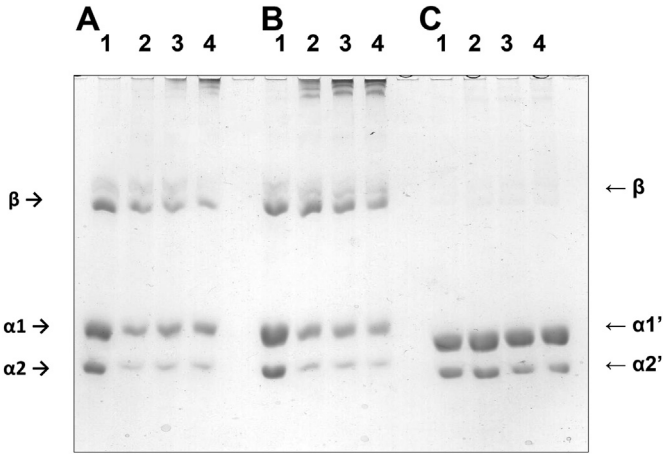


**Fig. 3.** SDS-PAGE of native fibrous collagen, loose fibrous collagen and truncated collagen cross-linked by incubation with ribose. Rat tail tendon collagen (native fibrous collagen) (A), loose fibrous collagen (acid-soluble and reconstituted collagen) (B) or truncated collagen (pepsin-digested collagen) (C) was incubated with 0–30 mM ribose for 10 days at 37 °C, boiled with SDS and subjected to SDS-PAGE. The concentration of ribose was 0 mM (lanes 1), 10 mM (lanes 2), 20 mM (lanes 3), and 30 mM (lanes 4). The contents of monomeric α-1and α-2 collagen chains and of dimeric β collagen chains of native fibrous collagen (A) and loose fibrous collagen (B) decreased similarly due to formation of cross-linked polymeric collagen chains by incubation with ribose, while the contents of α-1′ and α-2′ collagen chains of truncated collagen (C) did not decrease appreciably since the collagen chains were not cross-linked to polymeric collagen chains due to lack of the telopeptides.

## 4. Discussion

Previous studies have shown that fibrous collagen undergoes non-enzymatic intermolecular cross-linking of collagen molecules at their triple-helical regions [7–11,14–20]. These previous studies did not appear to pay attention to the presence or absence of non-enzymatic cross-links at the telopeptide regions, or appeared to have excluded the presence of the cross-links at the regions for a few reasons. First, fibrous collagen in most mature animal tissues is known to contain enzymatically formed cross-links named as pyridinoline and hydroxypyridinone at the telopeptide regions of the constituent collagen molecules [23]. To exclude the enzymatically pre-formed cross-links from the non-enzymatically post-formed cross-links, the telopeptide regions should have been taken off from the triple-helical regions by pre-incubating with pepsin in advance [16]. In the present study, however, we have shown that fibrous collagen undergoes non-enzymatic cross-linking primarily at a site linking the telopeptide region and the triple-helical region on incubation with a low concentration of ribose. Therefore, it remains to be examined whether senescent human collagen aged in vivo contains non-enzymatic cross-links at the telopeptide regions or not. Second, the cross-links introduced into the elongated triple-helical regions were supposed to affect the physical properties of fibrous collagen more effectively than that introduced into the short telopeptide regions and thus were supposed to account well for the changes in physical properties of senescent collagen. It is of interest to examine how the non-enzymatic cross-links introduced into the telopeptide regions affect the physical properties of collagen and to examine whether they are sufficient to account for the changes occurred in vivo on senescent collagen or not.

Non-enzymatic intermolecular cross-linking of fibrous collagen is proposed to occur between a lysine residue of a constituent collagen molecule and an arginine residue of another adjacent juxtaposed collagen molecule in the fibrils [7–13]. The first step of the cross-linking reaction is proposed to be glycation by a reducing sugar of ε-amino residue of a lysine residue of a collagen molecule [7–10]. Following two steps of rearrangement reactions, the sugar moiety of the glycated lysine residue on a collagen chain of the collagen molecule reacts with an arginine residue on a collagen chain of another adjacent juxtaposed collagen molecule. To enable the cross-linking reaction to proceed, it is essential that the glycated lysine residue and the arginine residue are sterically close or in appropriately spaced to each other at least for a short moment. In the present study we have shown that non-enzymatic intermolecular cross-linking occurs primarily between a site, an arginine residue, on the telopeptide region of a collagen molecule and the other site, a lysine residue, on the triple-helical region of another adjacent collagen molecule, when fibrous collagen was incubated with a low concentration of ribose. The former site on the telopeptide region and the latter site on the triple-helical region are likely to be sterically close or in appropriately spaced to each other. In addition, the telopeptide regions are unfolded and highly mobile, enabling the arginine residue easily accessible to the glycated lysine residue on the triple-helical region of an adjacent collagen molecule. Consistently with the location of one part of the cross-linking site on the telopeptide region, the truncated collagen which lacked the telopeptide regions did not undergo non-enzymatic cross-linking on incubation with a low concentration of ribose to a significant extent.

On the other hand, the triple-helical regions of intact collagen and the truncated collagen were found to undergo intermolecular cross-linking, when incubated with a high concentration of reducing sugar [20]. This intermolecular cross-linking between the triple-helical region to triple helical region may occur as follows.

Collagen contains numbers of lysine residues. Rat type I collagen contains approximately 30 lysine residues on both α-1 and α-2 collagen chains. In the presence of a low concentration of reducing sugar, only a limited number of the lysine residues are glycated. Under such a condition, only one or two of the glycated lysine residues may be able to react with an arginine residue on the telopeptide region of an opposite collagen molecule. In the presence of a high concentration of reducing sugar, many lysine residues are supposed to be glycated. Rat type I collagen contains approximately 50 arginine residues including 2 arginine residues at the carboxyl ends [23]. Although many of the individual glycated lysine residues and arginine residues in opposite collagen molecules may not be sterically closely localized, some limited number of the glycated lysine residues happens to be made to accessible to some arginine residues, enabling cross-linking reaction to proceed.

The present study in vitro indicates that in the presence of a low concentration of reducing sugar fibrous collagen undergoes intermolecular cross-linking primarily between a telopeptide region of a collagen molecule and a triple-helical region of another adjacent collagen molecule and that intermolecular cross-linking between a triple-helical region and another triple helical region is small. The present study suggests that in vivo where the concentration of the reducing sugar, glucose, is usually about 5 mM and less than 8 mM, and even in the cases of severe untreated diabetes it is less than 25 mM, intermolecular cross-linking of fibrous collagen is likely to occur only at the telopeptide regions, and cross-linking at the triple-helical region to triple-helical region is likely to be little. On other hand, since collagen is a very long-lived extracellular protein, it likely to receive glycation at many sites during very long passage of time, such as 40 years. Under such a situation, intermolecular cross-linking at the triple-helical region to triple-helical region may occur.

## Conflict of interest

None.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.011.

## References

[1] R.R. Kohn, Principles of Mammalian Aging, second ed., Prentice-Hall, Englewood Cliffs, New Jersey, 1978.
[2] C.R. Hamlin, R.R. Kohn, J.H. Luschin, Apparent accelerated aging of human collagen in diabetics mellitus, Diabetes 24 (1975) 902–904.
[3] T.T. Andreassen, K. Seyer-Hansen, A.J. Bailey, Thermal stability, mechanical properties and reducible cross-links of rat tail tendon in experimental diabetes, Biochim. Biophys. Acta 677 (1981) 313–317.
[4] S.L. Schnider, R.R. Kohn, Effects of age and diabetes mellitis on the solubility and nonenzymatic glucosylation of human skin collagen, J. Clin. Invest. 67 (1981) 1630–1635.
[5] S.L. Schnider, R.R. Kohn, Effects of age and diabetes mellitus on the solubility of collagen from human skin, tracheal cartilage and dura matter, Exp. Gerontol. 17 (1982) 185–194.
[6] D.K. Yue, S. McLennan, L. Delbridge, D.J. Handelsman, T. Reeve, J.R. Turtle, The thermal stability of collagen in diabetic rats: correlation with severity of diabetes and non-enzymatic glycosylation, Diabetologia 24 (1983) 282–285.
[7] D.R. Sell, V.M. Monnier, Structure elucidation of a senescence cross-link from human extracellular matrix: implication of pentoses in the aging process, J. Biol. Chem. 264 (1989) 21597–21602.
[8] S.K. Grandhee, V.M. Monnier, Mechanism of formation of the Maillard protein cross-link pentosidine: glucose, fructose, and ascorbate as as pentosidine precursors, J. Biol. Chem. 266 (1991) 11649–11653.
[9] D.G. Dyer, J.A. Blackledge, S.R. Thorpe, J.W. Baynes, Formation of pentosidine during nonenzymatic browning of proteins by glucose: identification of glucose and other carbohydrates as possible precursors of pentosidine in vivo, J. Biol. Chem. 266 (1991) 11654–11660.
[10] M. Brownlee, Advanced protein glycosylation in diabetes and aging, Annu. Rev. Med. 46 (1995) 223–234.
[11] A.J. Bailey, R.G. Paul, L. Knott, Mechanisms of maturation and ageing of collagen, Mech. Ageing Dev. 106 (1998) 1–56.
[12] K.M. Biemel, D.A. Friedl, M.O. Lederer, Identification and quantitation of major Maillard cross-links in human serum albumin and lens protein; evidence for glucosepasne as the dominant compound, J. Biol. Chem. 277 (2002) 24907–24915.
[13] D.R. Sell, K.M. Biemel, O. Reihl, M.O. Lederer, C.M. Strauch, V.M. Monnier, Glucosepane is a major protein cross-link of the senescent human extracellular matrix: relationship with diabetes, J. Biol. Chem. 280 (2005) 12310–12315.
[14] H.F. Bunn, P.J. Higgins, Reaction of monosaccharides with proteins: possible evolutionary significance, Science 213 (1981) 222–224.
[15] S. Tanaka, G. Avigad, B. Brodsky, E.F. Eikenberry, Glycation induces expansion of the molecular packing of collagen, J. Mol. Biol. 203 (1988) 495–505.
[16] S. Tanaka, G. Avigad, E.F. Eikenberry, B. Brodsky, Isolation and partial characterization of collagen chains dimerized by sugar-derived cross-links, J. Biol. Chem. 263 (1988) 17650–17657.
[17] M.J. Kent, N.D. Light, A.J. Bailey, Evidence for glucose-mediated covalent cross-linking of collagen after glycosylation in vitro, Biochem. J. 225 (1985) 745–752.
[18] M.X. Fu, K.J. Wells-Knecht, J.A. Blackledge, T.J. Lyons, S.R. Thorpe, J.W. Baynes, Glycation, glycoxidation, and cross-linking of collagen by glucose: kinetics, mechanism, and inhibition of late stages of the Maillard reaction, Diabetes 43 (1994) 676–683.
[19] P. Bai, K. Phua, T. Hardt, M. Cernandes, B. Brodsky, Glycation alters collagen fibril organization, Connect. Tissue Res. 28 (1992) 1–12.
[20] S.F. Tian, S. Toda, H. Higashino, S. Matsumura, Glycation decreases the stability of the triple-helical strands of fibrous collagen against proteolytic degradation by pepsin in a specific temperature range, J. Biochem. 120 (1996) 1153–1162.
[21] E.J. Miller, R.K. Rhodes, Preparation and characterization of the different types of collagen, Meth. Enzymol. 82 (1982) 33–64.
[22] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature (London) 227 (1970) 680–685.
[23] P. Bornstein, W. Traub, The chemistry and biology of collagen, in: H. Neurath, R.L. Hill (Eds.), The Proteins, third ed., Academic Press, New York, vol. 4, pp. 411–632.