Presence of pro-forms of decorin and biglycan in human articular cartilage

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The proteoglycans decorin and biglycan in extracts of human articular cartilage were analysed by SDS/PAGE and immunoblotting, using antisera raised to peptide sequences present in the pro-regions and the mature core proteins. In adult cartilage, both pro-forms and mature processed forms of the proteoglycan core protein were observed for both decorin and biglycan. In the case of biglycan, it was also shown that additional proteolytic processing takes place after removal of the propeptide and that this accounts for the presence of non-glycanated forms of the molecule. For both decorin and biglycan, the relative abundance of the pro-forms was much less in the juvenile than the adult. Different adult connective tissues, including meniscus, tendon and intervertebral disc were also examined for the presence of pro-forms of the proteoglycans. While the mature form of decorin was present at a similar level in extracts of all tissues examined, the pro-form was only detected in the articular cartilage. In the case of biglycan, the abundance of the mature form was more varied, with high levels in articular cartilage, intermediate levels in meniscus and the annulus fibrosus of the intervertebral disc, low levels in the nucleus pulposus of the intervertebral disc, and non-detectable levels in the patellar tendon. The pro-form of biglycan was detected in the disc tissue extracts, albeit at a lower level than in articular cartilage, but was not detected in the meniscus or tendon. The proportion of the pro-form relative to the mature form of biglycan was, however, higher in the nucleus pulposus of the intervertebral disc than in articular cartilage. Thus, the persistence of pro-forms of both decorin and biglycan is a feature of the extracellular matrix of some connective tissues, although their abundance is both tissue- and age-dependent, with adult articular cartilage being a particularly rich source.

INTRODUCTION

The proteoglycans decorin and biglycan are components of the extracellular matrix of many connective tissues. Their core proteins belong to the family of leucine-rich repeat (LRR) proteins, which are characterized by the presence of numerous adjacent leucine-rich regions bearing the consensus sequence LXXLXLXXNXL [1]. Together with fibromodulin and lumican, decorin and biglycan form the family of LRR proteoglycans, with each of their core proteins possessing ten central leucine-rich repeats [2]. Decorin and biglycan differ from fibromodulin and lumican in possessing chondroitin sulphate or dermatan sulphate rather than keratan sulphate. The presence of dermatan sulphate on the decorin and biglycan of bovine articular cartilage led to the original names of DS-PGII and DS-PGI, respectively [3]. The composition of the dermatan sulphate chains varies considerably with site, age and species [4,5], as does the relative degree to which the two proteoglycans are synthesized [6]. In the case of biglycan it was shown that the core protein can exist in a non-glycanated form devoid of dermatan sulphate chains [7,8]. Such non-glycanated biglycan could arise by either proteolytic processing of the N-terminal region of the core protein in which dermatan sulphate substitution occurs, or by a lack of dermatan sulphate substitution during synthesis [9].

To date the complete cDNA sequence of the decorin gene has been described in the human [10], bovine [11], mouse [12], rat [13], rabbit [14] and chicken [15]. The sizes of the primary translation products vary between 354 and 360 amino acids, and in each case a common region of 30 amino acids precedes the expected start site of the mature proteoglycan core protein, as determined by amino acid sequence analysis of the proteoglycan isolated from connective tissue matrices [4,5,16]. In each case this common region is composed of a 16-residue signal peptide [17,18] and a 14-residue pro-region. The cleavage site between the pro-region and the mature core protein is well conserved, having a consensus sequence of ML/IE-DEA/G. In the case of the biglycan gene the complete cDNA sequence has been described in human [19], bovine [20], mouse [21] and rat [22], with the size of the primary translation product being either 368 or 369 amino acids. In each case a common region of 37 amino acids precedes the expected start site of the mature proteoglycan core protein [4,5,16], being composed of a 16-residue signal peptide and a 21-residue pro-region. The cleavage site between the pro-region and the mature core protein is again well-conserved, having a consensus sequence of MM/LN-DEE. At present it is not clear whether removal of the pro-region from decorin and biglycan is an intracellular or extracellular event, and whether pro-forms of the proteoglycan may persist within the extracellular matrix of various connective tissues.

The purpose of the present work was to determine whether pro-forms of the decorin and biglycan core proteins could be detected within the extracellular matrix of human connective tissues, particularly articular cartilage, and if so to determine whether their presence was age-dependent.

MATERIALS AND METHODS

Source of tissue

Various human connective tissues were collected at autopsy within 20 h of post mortem, from individuals who had no clinical record of a connective-tissue disorder. The individuals included two newborns (aged 1 and 3 months) and three adults (aged 37, 60 and 64 years). Articular cartilage from the femoral condyles was collected from all individuals, and in addition meniscus, patellar tendon, skin and intervertebral disc (from the L4–L5 spinal segment) were collected from the adults. In the case of the intervertebral disc the tissue was dissected to separate the annulus fibrosus from the nucleus pulposus. All tissues were stored at −20 °C until used for extraction.

Abbreviation used: LRR, leucine-rich repeat.

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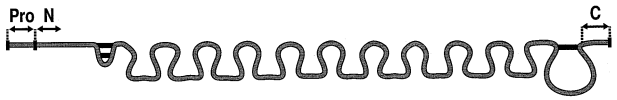

DECORIN

Pro	GPFQQRGLFGGC
C	CGYVRSAIQLGNYK

BIGLYCAN

Pro	LPFEQRGFWGGC
C	CGTDRLAIQFGNYKK
N	DEEASGADTSGGKARAKGGC
Pro/C	LPFEQRGFWGGCGTDRLAIQFGNYKK

Figure 1 Peptide sequences used for the generation and analysis of anti-peptide sera against decorin and biglycan

The core proteins of decorin and biglycan are represented pictorially with ten central LRRs flanked by disulphide-bonded domains and extended terminal regions. The N-terminal region includes the propeptide. Sites selected for peptide synthesis within the pro-region (Pro), the N-terminal region of the mature core protein (N), and the C-terminal region (C) are indicated. The sequences of the corresponding peptides are indicated below. Each peptide possesses a linker region and a cysteine residue (boxed) for use in coupling to ovalbumin.

Preparation of tissue extracts

Frozen tissues were mounted in a cryostat for sectioning to 20 μm. The resulting tissue sections were extracted with 10 vol. of 4 M guanidinium chloride/0.1 M sodium acetate, pH 6.0, containing proteinase inhibitors, for 48 h at 4 °C with continuous stirring [23]. The extracts were then separated from the tissue residues by centrifugation, and used directly for immunoblotting analysis without further purification. In this way no bias is created by the selective fractionation of glycanated and non-glycanated forms of the proteoglycans that would result during subsequent purification.

SDS/PAGE analysis and immunoblotting

Prior to electrophoretic analysis all extracts were dialysed into 0.1 M Tris/HCl, 0.1 M sodium acetate, pH 7.3, and then incubated with 0.1 unit/ml chondroitinase ABC (ICN) for 4 h at 40 °C. In this manner decorin and biglycan are converted into their non-glycanated core proteins, which can readily be resolved by SDS/PAGE. Chondroitinase treatment also results in the degradation of the chondroitin sulphate component of aggrecan in cartilagenous tissues, which considerably reduces the viscosity of the extracts so facilitating analysis. The chondroitinase-treated extracts were analysed on 10 % polyacrylamide slab gels under reducing conditions, using the procedures described by Laemmli [24]. After electrophoresis the fractionated proteins were electrophoretically transferred to nitrocellulose membranes [25], which were subsequently blocked with BSA [7]. The core proteins of decorin and biglycan were then detected using specific first-step anti-peptide antibodies raised in rabbits, followed by a second-step goat anti-(rabbit IgG) antibody conjugated to alkaline phosphatase. Visualization then involved reaction with Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate [7].

Generation of anti-peptide sera

Peptides corresponding to sequences within the pro-region and the N-terminal and C-terminal regions of the decorin and biglycan core proteins (Figure 1) were synthesized by fluorenylmethoxycarbonyl (Fmoc) chemistry on an Applied Biosystems model 431A peptide synthesizer, and purified by HPLC. The peptides possessed a terminal cysteine residue, that could be used for subsequent coupling to ovalbumin, and in most cases the cysteine residue was separated from the authentic sequence by one or two spacer glycine residues. For the acidic N-terminal peptide of biglycan, a basic amino acid segment was added to the spacer region to ensure solubility. An additional peptide possessing sequences from both the pro-region and the C-terminal region of biglycan was also synthesized. In this case the cysteine residue used for coupling and its adjacent spacer glycine residues were located in the centre of the peptide. The peptides were coupled to ovalbumin [26], using *N*-hydroxysuccinimidyl bromoacetate [27]. The peptide–ovalbumin conjugates were then used to generate polyclonal antisera in rabbits [7]. All specific antisera were used at a 1:200 dilution. The specificity of each antiserum was assessed by immunoblotting following preincubation with the immunizing peptide (1.5 mg of peptide/ml of serum). In all cases this procedure abolished immunoreactivity.

Purification of anti-peptide antibodies

To facilitate immunoprecipitation of the decorin and biglycan core proteins, the specific anti-peptide antibodies present in the various antisera were purified by affinity chromatography using the immobilized immunizing peptide. The peptide was bound to Sulfolink coupling gel (Pierce), following the manufacturer's instructions. Coupling involves interaction between the free thiol present on the terminal cysteine residue of the peptide and the iodoacetyl group present on the resin. For affinity purification, the antiserum (typically 20 ml) was passed through the peptide-conjugated resin (5 ml) that had been equilibrated in PBS. The resin was then washed, first with PBS (40 ml) and subsequently with PBS containing 1 M NaCl (40 ml), to remove non-specifically bound material. The specific immunoglobulin was then eluted with 0.1 M glycine, pH 2.8, and fractions were immediately neutralized by the addition of 1 M Tris/HCl, pH 9.5 (200 μl per 4 ml fraction). Protein content of the fractions was monitored by absorbance at 280 nm, and fractions containing the eluted antibodies were pooled and dialysed into PBS for storage.

Immunoprecipitation and protein sequencing

Decorin and biglycan core proteins, present in cartilage extracts after treatment with chondroitinase ABC, were purified by immunoprecipitation using the affinity-purified antibodies to the C-terminal regions of the core proteins. For immunoprecipitation the extract (200 μl) was mixed with an equal volume of the antibody (40 μg) in PBS. After 30 min at room temperature, Protein A–Sepharose (100 μl of a 50 % slurry) was added and the mixture left for an additional 30 min with continuous mixing to allow interaction with immune complexes and immunoglobulins. The resin was then recovered by centrifugation, washed with PBS containing 1 M NaCl and 0.1 % Tween 20, and subsequently with PBS alone. The resin was then mixed with SDS/PAGE sample buffer (50 μl) and the mixture incubated at 100 °C for 3 min. The supernatant was again recovered by centrifugation and subjected to SDS/PAGE analysis under non-reducing conditions. The absence of a reducing agent ensures that the intact immunoglobulins are well separated from the proteoglycan core proteins. The resolved proteins were then electrophoretically transferred to poly(vinylidene difluoride) membranes (Transblot, Bio-Rad) for direct N-terminal amino acid sequence analysis [28]. Proteins were identified on the blots by brief staining with Coomassie Brilliant Blue R250, and the excised membrane was analysed on an Applied Biosystems model 473A protein sequencer.

RESULTS

Extracts of adult articular cartilage that had been digested with chondroitinase ABC were analysed for the presence of decorin core proteins by SDS/PAGE and immunoblotting (Figure 2A). Two antisera were used in the analysis, one recognizing a peptide sequence at the C-terminus of the core protein and the other recognizing the propeptide region. The antiserum recognizing the C-terminal region revealed two core protein components with a size difference of about 2 kDa. The antiserum recognizing the pro-region revealed a similar pattern, but with components that were about 1 kDa larger than those observed with the other antiserum. In both cases no core protein components were observed if chondroitinase pretreatment of the extracts was omitted (results not shown). These data are consistent with the presence of both pro-forms and mature forms of decorin in the adult cartilage extracts, and with both forms of the molecule existing entirely as proteoglycans.

The same extracts were also used for analysis of the presence of biglycan core proteins (Figure 2B). In this case, three antisera were used. The first recognized a peptide sequence at the C-terminus of the core protein, the second recognized a peptide sequence at the N-terminus of the mature core protein and the third recognized the propeptide region. The antiserum recognizing the C-terminal region revealed three core protein components, with that of largest size being seen only after chondroitinase treatment, as described previously [7]. The antiserum recognizing the N-terminal region of the mature core protein revealed only a single component corresponding in size to that of the largest component revealed by the antiserum recognizing the C-terminal region. This component was only detected if the extract had been treated with chondroitinase (results not shown). The antiserum recognizing the pro-region also revealed a single core protein component, but with a size that was about 2 kDa larger than that observed with the antibody recognizing the N-terminal region. These data are consistent with the presence of both pro-forms and mature forms of biglycan in the adult cartilage extracts, and with the pro-form existing only as a proteoglycan. In addition, the data demonstrate that only the proteoglycan form of the mature biglycan possesses an intact core protein.

To verify the presence of the pro-forms of decorin and biglycan, the core proteins were purified for N-terminal amino acid sequence analysis by immunoprecipitation. The antibodies used for this purpose where those recognizing the C-terminal regions of the core proteins. As there is no evidence for proteolytic processing in this region, the antibodies should recognize both mature and pro-forms of the molecules. When the anti-decorin antibody was used, the immunoprecipitated protein gave rise to two N-terminal sequences, DEAXG IGPEV PDDR … and GPFQQ RGLFD FMLE …, with the former being present at about four times the molar abundance of the latter. These sequences correspond to those of the mature and pro-forms of the decorin core protein, respectively, and confirm that both forms of decorin are indeed present within the extracellular matrix of the adult cartilage. It is likely that the size of the decorin pro-region is insufficient to allow clear resolution of the pro-form and mature form of the decorin core proteins upon SDS/PAGE analysis (Figure 2A).

When the anti-(biglycan C-terminus) antibody was used for immunoprecipitation a somewhat surprising result was obtained, as only a single amino acid sequence was detected corresponding to that of the mature core protein, DEEAX GADX … While the N-terminus of the pro-form of the core protein could be selectively blocked, thus preventing sequence analysis by Edman degradation, it is also possible that the C-terminal antibody does not recognize the biglycan core protein possessing the propeptide. The increased size of the biglycan pro-region should allow distinct resolution of the pro-form and mature forms of the core proteins on immunoblotting, yet no evidence for the presence of the pro-form was obtained by SDS/PAGE analysis (Figure 2B). This raises the possibility that the pro-form of biglycan could be present in very low amounts, but that the antibody recognizing the pro-region is very efficient in its detection, whereas that recognizing the C-terminal region is not. To address this point a peptide possessing both biglycan epitopes was coupled to ovalbumin and the resulting conjugate, which therefore contains equal amounts of the two epitopes, was analysed by SDS/PAGE and immunoblotting (Figure 3). The conjugate was recognized by the antiserum recognizing the propeptide epitope and by that recognizing the C-terminal epitope with similar efficiency. This suggests that unlike the situation with decorin, the C-

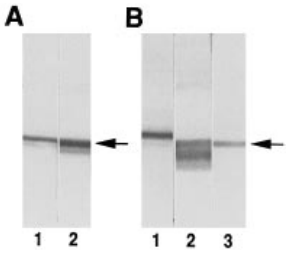

Figure 2 Immunoblotting analysis of decorin and biglycan in adult human articular cartilage

Cartilage extracts that had been treated with chondroitinase ABC were analysed by SDS/PAGE and subsequent immunoblotting. Equal volumes of extract were analysed using antisera raised against decorin (**A**) or biglycan (**B**) core protein sequences. The protein sequences used were present in the propeptide region (lanes 1), the C-terminal region (lanes 2), and the N-terminal region of the mature core protein (lane 3). The migration position of ovalbumin is indicated (←).

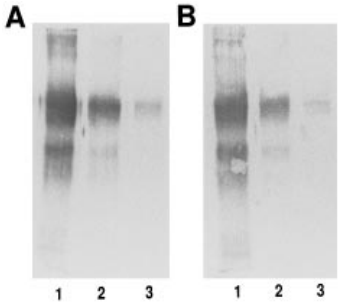

Figure 3 Immunoblotting analysis of a protein conjugate possessing equal concentrations of the epitopes in the C-terminal region and pro-region of biglycan

Ovalbumin, to which had been coupled a peptide corresponding to the propeptide region of biglycan and its C-terminal region separated by a central cysteine residue (Pro/C in Figure 1), was analysed by SDS/PAGE and subsequent immunoblotting. The conjugate was analysed in decade dilutions of (1) 50 ng, (2) 5 ng and (3) 0.5 ng, using purified antibodies recognizing the propeptide region of biglycan (**A**) or its C-terminal region (**B**). Both antibody preparations were used at a concentration of 1 μg/ml for the first step of immunoblotting and the blots were then developed under identical conditions. Some degradation of the ovalbumin-conjugates is apparent in the blots.

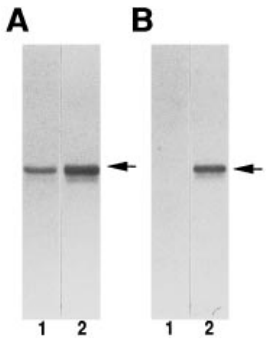

Figure 4 Immunoblotting analysis of decorin in human articular cartilage of different ages

Cartilage extracts that had been treated with chondroitinase ABC were analysed by SDS/PAGE and subsequent immunoblotting. Equal volumes of extract were analysed using antisera raised against the C-terminal region of the decorin core protein (**A**) or the propeptide region (**B**). Extracts were prepared from juvenile (lanes 1) and adult (lanes 2) cartilage. The migration position of ovalbumin is indicated (←).

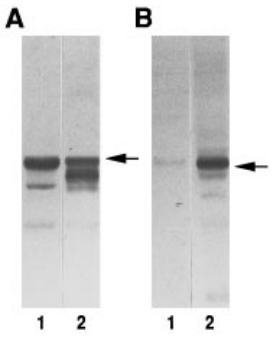

Figure 5 Immunoblotting analysis of biglycan in human articular cartilage of different ages

Cartilage extracts that had been treated with chondroitinase ABC were analysed by SDS/PAGE and subsequent immunoblotting. Equal volumes of extract were analysed using antisera raised against the C-terminal region of the biglycan core protein (**A**) or the propeptide region (**B**). Extracts were prepared from juvenile (lanes 1) and adult (lanes 2) cartilage. The migration position of ovalbumin is indicated (←).

terminal epitope of the biglycan core protein is not accessible for antibody recognition in the pro-form of the molecule.

To determine whether the pro-forms of decorin and biglycan are present in articular cartilage at different ages, tissue extracts from adult cartilage were compared with those from juvenile cartilage by immunoblotting. The antiserum recognizing the C-terminal region of the decorin core protein revealed the presence of decorin in the extracts from both age groups (Figure 4), though the abundance was greater in the adult. In contrast, the antiserum recognizing the pro-region of the decorin core protein was not able to detect the pro-form of the proteoglycan in the juvenile extracts. A similar situation was observed for biglycan, where the antiserum to the pro-region of the core protein revealed barely detectable levels of the pro-form of the proteoglycan in the juvenile (Figure 5). As expected the antiserum to the C-terminal region of the biglycan core protein showed biglycan to be present in the extracts from both age groups, with proteolytic processing of the mature core protein being increased in the adult. Thus, the pro-forms of decorin and biglycan are not present at similar abundances in articular cartilage of all ages,

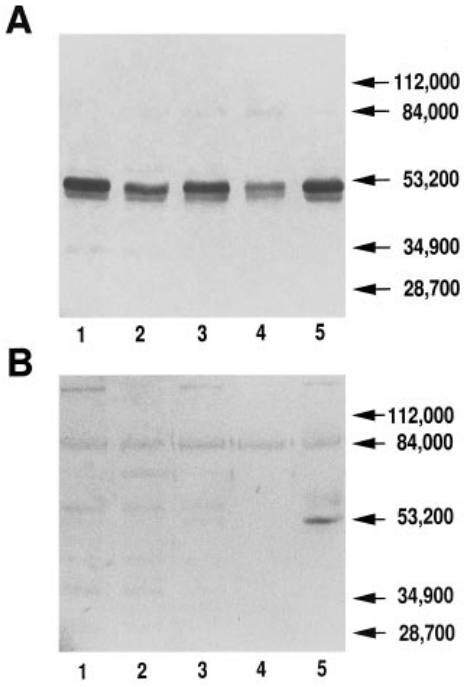

Figure 6 Immunoblotting analysis of decorin and prodecorin in different adult human connective tissues

Tissue extracts that had been treated with chondroitinase ABC were analysed by SDS/PAGE and subsequent immunoblotting. Equal volumes of extract were analysed using an antiserum raised against the C-terminal region of the decorin core protein (**A**) or its propeptide region (**B**). Extracts were obtained from meniscus (lane 1), patellar tendon (lane 2), annulus fibrosus (lane 3), nucleus pulposus (lane 4) and articular cartilage (lane 5). The positions of reference molecular-mass markers are indicated.

and high levels appear to be a feature of adult but not juvenile tissue.

It was also of interest to ascertain whether the pro-forms of decorin and biglycan were present in connective tissues other than cartilage. To this end adult tissue extracts from meniscus, patellar tendon and intervertebral disc were analysed by immunoblotting. Using the antiserum recognizing the C-terminal region of the decorin core protein revealed that this proteoglycan was present at similar levels in all the tissues (Figure 6A). In contrast, the antiserum to the pro-region of the decorin core protein exhibited immunoreactivity only with the cartilage extracts (Figure 6B). Analysis of biglycan with the antiserum raised against the C-terminal region of its core protein did not show a similar widespread distribution, as observed for decorin. While the level of biglycan observed in meniscus and the annulus fibrosus of the intervertebral disc approached that observed in articular cartilage, much lower levels were detected in the nucleus pulposus of the intervertebral disc and no immunoreactive material was detected in the tendon (Figure 7A). The antiserum raised against the pro-region of the biglycan core protein did not reveal any immunoreactive material in the meniscus or tendon extracts, but did exhibit detection in the intervertebral disc extracts (Figure 7B). Detection was higher in the nucleus pulposus than the annulus fibrosus, but levels were still lower than those observed in articular cartilage. However, when one takes into account the low level of the mature forms of biglycan present in the nucleus pulposus, it is possible that this tissue may exhibit a higher proportion of unprocessed biglycan than articular car-

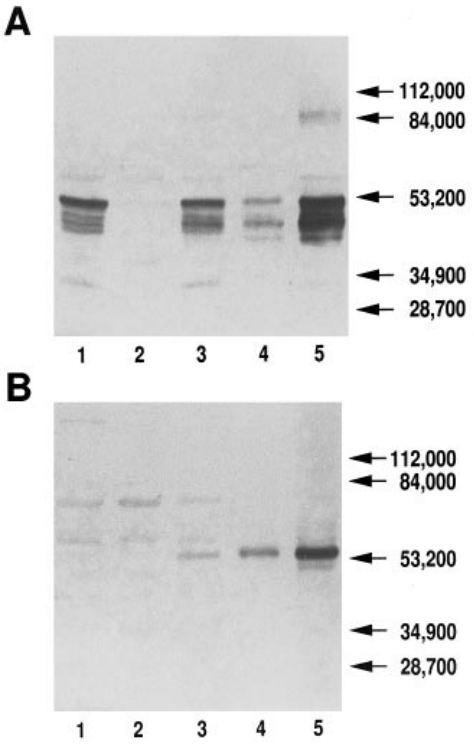

Figure 7 Immunoblotting analysis of biglycan and probiglycan in different adult human connective tissues

Tissue extracts that had been treated with chondroitinase ABC were analysed by SDS/PAGE and subsequent immunoblotting. Equal volumes of extract were analysed using an antiserum raised against the C-terminal region of the biglycan core protein (**A**) or its propeptide region (**B**). Extracts were obtained from meniscus (lane 1), patellar tendon (lane 2), annulus fibrosus (lane 3), nucleus pulposus (lane 4) and articular cartilage (lane 5). The positions of reference molecular-mass markers are indicated.

tilage. It is thus apparent that the presence and relative abundance of the pro-forms of decorin and biglycan are not a consistent feature of all connective tissues in which these proteoglycans are present.

DISCUSSION

The data presented in this study demonstrate that both decorin and biglycan residing in the extracellular matrix of human articular cartilage may possess core proteins that commence with either the amino acid sequence previously reported for the mature core protein [4,5,16] or with an amino acid sequence corresponding to the core protein in which at least part of the putative pro-region remains. Such pro-forms of the proteoglycans are particularly abundant in adult cartilage, but not juvenile cartilage or other adult connective tissues where the proteoglycans occur in high abundance. In the case of decorin the additional amino acid sequence is of 14 amino acids and corresponds to the intact pro-region. In the case of biglycan, it is not clear whether the pro-region is intact, although the difference in electrophoretic mobility between the pro-form and mature forms of the molecules would be compatible with the presence of all 21 amino acids that comprise the intact pro-region.

The presence of a pro-form of biglycan has previously been described as a product of bovine and rat smooth muscle cells in culture [29], and in this case the secreted proteoglycan appeared to be entirely in the pro-form. Amino acid sequence analysis of the secreted proteoglycan revealed a single sequence beginning with the first amino acid of the putative 21 residue propeptide. It is, however, possible that the retention of the propeptide in this case reflects a culture anomaly, as the amino acid sequence of biglycan isolated from bovine aorta indicated the presence of only the mature form of the core protein [30]. Immunohistochemical studies support this conclusion, as the pro-form of biglycan was only detectable intracellularly in rat and bovine aortic tissue [31]. One might speculate that under the culture conditions the proteinase activity responsible for removal of the propeptide is not present. It is also of interest to note that a biglycan core protein possessing part of the propeptide region has been isolated from rat myeloid cell cultures [32]. In this case, however, the pro-region was shorter than expected for removal of only signal peptide, with processing having occurred between amino acid residues 7 and 8 of the intact propeptide sequence. Again, it is not clear whether such partial processing represents an anomaly of culture or an *in vivo* event. In contrast, there have been no reports in the literature confirming the secretion of pro-forms of decorin, either by cells in culture or *in vivo*. The existence of the pro-form of the decorin core protein within chondrocytes has, however, been described as an intermediate in the intracellular processing of the proteoglycan during its synthesis [33]. Thus, the current work represents the first description of the pro-forms of decorin and biglycan as actual components of a connective-tissue matrix *in vivo*.

While the current study provides evidence that processing of the pro-forms of biglycan and decorin to their respective mature forms may be incomplete in some connective tissues, it does not give information on where such processing is occurring. Theoretically, it could be occurring during intracellular processing and transport, or extracellularly following secretion. Irrespective of the processing site, it is likely that the same proteinase is responsible for processing the two proteoglycans to their mature forms, as the peptide sequences being cleaved show considerable structural similarity: … ML/IE-DEA/G … for decorin and … MM/LN-DEE for biglycan in all species so far examined. The nature of the amino acids at the P3, P2, P1′ and P2′ subsites [34] are well conserved in both molecules. Variation in the degree of processing between the pro-forms of decorin and biglycan in a given tissue could then reflect differences between the amino acids at the P1 and P3′ subsites. Alternatively, they could reflect variation in the accessibility of the proteinase to the different proteoglycans. Certainly, within the extracellular matrix it is known that decorin and biglycan are located at different sites [35], probably reflecting the functional ability of decorin but not biglycan to interact with collagen fibrils [36]. It is unlikely that the degree of processing observed reflects an artefact of the extraction protocol, as proteinases should not be active in 4 M guanidinium chloride, particularly when proteinase inhibitors for each proteinase subgroup are also present. Furthermore, the site at which processing occurs prior to an acidic aspartate residue is not compatible with the specificity of the currently known repertoire of either lysosomal proteinases or matrix metalloproteinases.

It is also not apparent from the present work whether the processing of the pro-forms of decorin and biglycan to their mature forms is a necessary or a fortuitous event. The former scenario implies some functional significance, whereas the latter does not. There is, however, no evidence as to whether the pro-form of decorin, for example, binds as well to collagen fibrils as does the mature form. It is, however, interesting to note that the two other LRR proteoglycans, fibromodulin and lumican, which also interact with collagen fibrils [37,38], do not appear to be further proteolytically processed following removal of their

signal peptides. This initially processed form corresponds to that detected in the extracellular matrix [39,40]. This might be interpreted as evidence to support the removal of the propeptides of decorin and biglycan as being fortuitous and of no functional consequence.

The inability to detect the pro-form of the biglycan core protein by amino acid sequence analysis following immunoprecipitation with the antibody to the C-terminal region was somewhat surprising. A number of possible explanations could account for this, relating to problems with either the sequencing or the immunoprecipitation. Sequencing problems would be due to blocking of the N-terminus of the protein, though there is no easy way of explaining why such blocking should selectively affect the propeptide of biglycan and not the mature form of biglycan or both forms of decorin. Immunoprecipitation problems could arise if proteolytic processing of the core protein were occurring within the C-terminal region of the core protein, so destroying the epitope required for immune reaction. However, the antiserum recognizing the N-terminus of the mature core protein shows a single component, suggesting that processing at the C-terminus is not occurring. Furthermore, the observed size on immunoblotting of the biglycan core protein bearing the pro-region is compatible with its being intact. However, the immunoblotting experiments do suggest that this pro-core protein is not recognized by the antiserum raised against the C-terminal region. One could therefore speculate that when the pro-region is present the biglycan core protein adopts a conformation such that its C-terminal region eludes immunological recognition. It is not clear why this should occur, or whether it would be of any functional consequence.

The apparent absence of proteolytic processing in the C-terminal region of the biglycan core protein is in contrast to the situation occurring within the N-terminal region, which accounts for all the small forms of the biglycan core protein observed on immunoblotting. This supports the concept that these non-glycanated forms of biglycan [7] arise by proteolytic removal of the N-terminal region bearing the dermatan sulphate chains, rather than by an inability of the cells to substitute the core protein with the glycosaminoglycan chains. It is unlikely that such processing involves the same proteolytic agent responsible for removal of the pro-region of the core protein, as the two events exhibit opposing age-related trends.

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