Interaction between Proteoglycan Subunit and Type II Collagen from Bovine Nasal Cartilage, and the Preferential Binding of Proteoglycan Subunit to Type I Collagen

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We studied the interaction of proteoglycan subunit with both types I and II collagen. All three molecular species were isolated from the ox. Type II collagen, prepared from papain-digested bovine nasal cartilage, was characterized by gel electrophoresis, amino acid analysis and CM-cellulose chromatography. By comparison of type I collagen, prepared from papain-digested calf skin, with native calf skin acid-soluble tropocollagen, we concluded that the papain treatment left the collagen molecule intact. Interactions were carried out at 4°C in 0.06 M-sodium acetate, pH 4.8, and the results were studied by two slightly different methods involving CM-cellulose chromatography and polyacrylamide-gel electrophoresis. It was demonstrated that proteoglycan subunit, from bovine nasal cartilage, bound to cartilage collagen. Competitive-interaction experiments showed that, in the presence of equal amounts of calf skin acid-soluble tropocollagen (type I) and bovine nasal cartilage collagen (type II), proteoglycan subunit bound preferentially to the type I collagen. We suggest from these results that, although not measured under physiological conditions, it is unlikely that the binding *in vivo* between type II collagen and proteoglycan is appreciably stronger than that between type I collagen and proteoglycan.

In addition to the presence of type II collagen [$\alpha_1(\text{II})$]₃, in bovine nasal cartilage (Miller & Lunde, 1973), Lee-Own & Anderson (1975*a,b*) demonstrated that it also contained some type I collagen, [$\alpha_1(\text{I})$]₂ α_2 . The latter was isolated as a complex with proteoglycan, which led us to suggest that such a complex might have some functional importance *in vivo*. However, this hypothesis was only supported by the isolation of the complex and by the observation that under certain conditions proteoglycan subunit had a preference for binding to α_2 and β_{12} rather than to α_1 and β_{11} components of type I collagen. This latter finding presumably reflected stronger binding between proteoglycan and type I collagen owing to the more basic α_2 chain. Various other reports (Mathews & Decker, 1968; Öbrink & Wasteson, 1971; Lowther & Natarjan, 1972; Öbrink, 1973; Greenwald *et al.*, 1975) have also described interactions between type I collagen and proteoglycans, but as yet there has been no characterization of the interaction between a type II cartilage collagen and proteoglycan from the same tissue. The work presented in the present paper demonstrates an interaction between type II collagen

and proteoglycan subunit, both isolated from bovine nasal cartilage, and the preferential combination of proteoglycan with type I collagen when added to a mixture of types I and II collagens.

Materials and Methods

All reagents were of analytical grade except for glucuronolactone and carbazole. Papain (papaya latex, Sigma P 13525, lot 43C 8190) was from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K., and CM-cellulose (CM-52) was obtained from Whatman Biochemicals, Maidstone, Kent, U.K. Proteoglycan subunit was prepared from bovine nasal cartilage by the method of Hascall & Sajdera (1969) as described by Lee-Own & Anderson (1975*b*), and calf skin acid-soluble tropocollagen was prepared by the method of Steven & Jackson (1967).

Analytical methods

Cartilage collagen was hydrolysed in 6M-HCl at 110°C for 16h under N₂. Acid was removed by repeated rotary evaporation and the sample was then used for hydroxyproline assay by the method of Woessner (1961) and for amino acid analysis on a JEOL JLC-6AH amino acid analyser. Cartilage collagen (100mg) was digested by a highly purified

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proteinase-free bacterial collagenase (400 µg), prepared as described by Lee-Own & Anderson (1975c), in 0.05 M-Tris/HCl/0.005 M-CaCl₂, pH 7.5, (5 ml) at 37°C for 24 h. The mixture was dialysed, and portions (0.5 ml) were then assayed for hexuronic acid by the method of Bitter & Muir (1962) with glucuronolactone as standard. Analytical polyacrylamide-gel electrophoresis was performed on 5% (w/v) polyacrylamide in the presence of sodium dodecyl sulphate and urea by the method of Furthmayr & Timpl (1971). CM-cellulose chromatography was carried out by the method of Piez *et al.* (1963) as described by Lee-Own & Anderson (1975b). The column eluate was continually monitored at 230 nm with a Beckmann DB spectrophotometer, and the absorbance was automatically recorded on a Vitatron lin-log converter. Peaks of absorbance eluted in the gradient were pooled, dialysed, freeze-dried and subjected to polyacrylamide-gel electrophoresis.

Preparation of collagens after papain digestion

Type II collagen. Type II collagen was prepared from bovine nasal cartilage by a modification of the papain digestion method of Strawich & Nimni (1971). Bovine nasal septa, excised from cattle within 2 h of death, were dissected free of adhering tissue and perichondrium, frozen at -20°C, and shredded with a Stanley Surform (Stanley Tools, Sheffield, S. Yorks., U.K.). Shredded cartilage (wet wt. 50 g) was dispersed in 0.02 M-NaH₂PO₄/0.02 M-cysteine/0.003 M-EDTA, pH 7.2, (200 ml) at 4°C and papain (200 mg) was added. The mixture was left at 4°C for 48 h without stirring, and then the enzyme was inactivated by the addition of 0.003 M-iodoacetamide in 0.15 M-NaCl (200 ml). After being stirred for 2 h at 4°C, the residue was separated from the soluble digest by centrifugation at 35000g for 1 h at 4°C. The soluble digest was discarded and the residue dispersed in 0.5 M-NaCl/0.05 M-Tris/HCl, pH 7.5, (500 ml) and stirred at 4°C for 96 h. The solution was centrifuged at 35000g for 1 h at 4°C and the residue was discarded. The collagen in the soluble extract was precipitated by the addition of solid NaCl to 20% (w/v) and after standing for 2 h at 4°C the extract was centrifuged at 2000g for 45 min at 4°C. The precipitated collagen was twice dissolved in 0.5 M-acetic acid (500 ml) and salted out with solid NaCl (added to 5%, w/v), dissolved in 0.5 M-acetic acid (500 ml) and dialysed against 0.5 M-NaCl/0.02 M-NaH₂PO₄, pH 7.2, (4 litres). The collagen was precipitated from the non-diffusible material by the addition of ethanol (previously cooled to -20°C) to 15% (v/v) and, after standing for 2 h at 4°C, was centrifuged at 2000g for 45 min at 4°C. Finally, the collagen was dissolved in 0.5 M-acetic acid (350 ml), dialysed against 4 vol. of distilled water and freeze-dried. The collagen was

characterized by amino acid analysis, CM-cellulose chromatography and gel electrophoresis.

Type I collagen. All the hair and most of the epidermis were removed from a piece of calf skin with a sharp scalpel, and the loose connective tissue was scraped off the inner surface. Type I collagen was then prepared by the procedure described above.

Interactions between collagen and proteoglycan subunit

Interactions were studied by gel electrophoresis and CM-cellulose chromatography of the interaction mixture. The materials for interaction were dissolved in 0.06 M-sodium acetate, pH 4.8, at 4°C; the two solutions were mixed and adjusted to give a concentration of 2 mg of collagen/ml of 0.06 M-sodium acetate, pH 4.8. The mixture was stirred gently at 4°C for 15 h and then analysed by one of the following methods.

Method 1. The precipitate resulting from the interaction procedure was centrifuged down at 1000g for 10 min. The supernatant was removed, denatured by incubation at 50°C for 1 h and subjected to CM-cellulose chromatography and gel-electrophoretic analysis as described by Lee-Own & Anderson (1975b).

Method 2. The interaction mixture was analysed as described originally by Lee-Own & Anderson (1975b). It was denatured by incubation at 50°C for 45 min and any precipitate was removed by centrifugation at 1000g for 10 min. The supernatant was heated for a further 10 min at 50°C, then analysed by the CM-cellulose chromatography and gel-electrophoresis procedures.

Results

Preparation of type II and type I collagens after papain digestion of tissues

The product from 50 g (wet wt.) of nasal cartilage was 710 mg (dry wt.). It had a hydroxyproline content of 9.2% and an amino acid analysis as shown in Table 1. The hexuronic acid content, determined after digestion by collagenase, was 0.09%. The banding pattern on polyacrylamide-gel electrophoresis is shown in Plate 1 together with that of calf skin acid-soluble tropocollagen. Plates 1(a) and 1(b) compare the CM-cellulose chromatography profiles of the two samples.

Although the hydroxyproline content of the type II collagen was low for a collagen, its amino acid analysis compared favourably with that reported by Strawich & Nimni (1971), and indicated that the sample was not significantly contaminated by any other protein. The low hydroxyproline content was probably due to underhydroxylation of proline. The

Table 1. *Amino acid composition of cartilage collagens*

The values given are expressed as amino acid residues/1000 residues.

Amino acid	Bovine nasal cartilage	Bovine articular cartilage (Strawich & Nimni, 1971)
Hyp	94	95
Asp	45	51
Thr	22	19
Ser	23	28
Glu	90	82
Pro	135	115
Gly	321	330
Ala	100	98
Val	17	19
Met	9	9
Ile	9	13
Leu	25	27
Tyr	1	2
Phe	13	14
Hyl	21	29
Lys	17	15
His	3	5
Arg	55	48

low hexuronic acid content established that the collagen did not contain more than 0.4% proteoglycan. The behaviour of the sample on polyacrylamide-gel electrophoresis and CM-cellulose chromatography was consistent with that expected for a type II collagen, and the absence of α_2 and β_{12} components showed that there was no type I collagen present.

CM-cellulose chromatography and polyacrylamide-gel electrophoresis indicated that the main component of the cartilage collagen preparation was the $\alpha_{1(III)}$ chain. Calf skin was digested with papain to check that the enzyme was not acting like pepsin (Miller, 1972) in depolymerizing polymeric collagen by cleavage of the telopeptide regions, which bear the cross-links. The residue was processed as for nasal cartilage, and the collagen was isolated. Polyacrylamide-gel electrophoresis and CM-cellulose separations of the collagen prepared from the papain-digested calf skin are shown in Plate 1(c). A control sample of calf skin acid-soluble tropocollagen, produced by the method of Steven & Jackson (1967), is also shown. The most significant difference between the two samples was that the type I collagen prepared from the papain-digested calf skin had a greater ratio of β_{11} chains to α_1 chains. Calf skin acid-soluble tropocollagen was not the ideal control, but the comparison served to demonstrate that papain treatment did not produce a greater proportion of α chains than was present in a non-enzymically derived collagen. These results were consistent with

those of Strawich & Nimni (1971) who reported that collagen was not susceptible to papain treatment at 4°C if it was in the fibrillar form.

Study of interactions between collagens and proteoglycan subunit

Proteoglycan subunit was allowed to interact with type II collagen in the absence and presence of type I collagen [calf skin acid-soluble tropocollagen produced by the method of Steven & Jackson (1967)]. The two methods for the detection and analysis of interactions (methods 1 and 2) differed in that method 1 analysed interactions of proteoglycan with native collagen, whereas method 2 detected interaction with the constituent α and β components.

Interaction of proteoglycan subunit with type II collagen. It was found that under the conditions of method 1, 0.7mg of proteoglycan subunit precipitated 14mg of type II collagen. After interaction the precipitate was centrifuged down and CM-cellulose chromatography of the denatured supernatant showed that there were no collagen constituents present. This result establishes that native collagen and proteoglycan from the same tissue do have a marked affinity for each other.

The results of allowing type II collagen (7mg) to interact with increasing amounts of proteoglycan subunit by method 2 are shown in Plate 2. With 0.7mg of proteoglycan subunit there was a slight precipitate after interaction and denaturation, and only a small alteration in the chromatogram profile was observed (Plate 2b). An increased amount of proteoglycan subunit (1.5mg) produced a marked depression of the chromatogram profile (Plate 2c), and a precipitate of 3mg was obtained. Both precipitate and material eluted from the column were shown to contain collagen by polyacrylamide-gel electrophoresis. The precipitate appeared richer in β components, indicating that proteoglycan may bind preferentially to cross-linked components of type II collagen.

Although polyacrylamide-gel electrophoresis and CM-cellulose chromatography showed that collagen isolated after papain digestion of calf skin did not have an increased content of α chains, it was important to establish that this collagen behaved in the same way as non-enzymically produced collagen on interaction with proteoglycan. When type I collagen (7mg), prepared after papain digestion of calf skin, was left to interact with proteoglycan subunit (0.7mg), and the method 2 procedure was followed, the collagen behaved in the same way as calf skin acid-soluble tropocollagen. The chromatogram profile of the denatured interaction mixture eluted from CM-cellulose (Plate 1d) showed that α_2 and β_{12} components had been selectively removed from the precipitate (Lee-Own & Anderson, 1975b). This experiment established that papain digestion had not

EXPLANATION OF PLATE 1

CM-cellulose chromatography profiles of bovine nasal cartilage collagen (a), calf skin acid-soluble tropocollagen (b), collagen prepared by papain digestion of calf skin (c) and collagen (prepared by papain digestion of calf skin) left to interact with proteoglycan subunit (d)

CM-cellulose chromatography was carried out at 42°C by the method of Piez *et al.* (1963). Solutions or interaction mixtures were denatured at 50°C for 1 h and supernatants were applied to a column (15 cm × 1 cm) of CM-cellulose equilibrated with 0.06 M-sodium acetate, pH 4.8. Elution was carried out at 42°C at a rate of 27 ml/h with a linear gradient of NaCl (0.0–0.1 M, total volume 240 ml) in the above buffer. Effluent was monitored at E_{230} , and fractions (3.9 ml) were collected. Electrophoretic separations on 5% (w/v) polyacrylamide gels were carried out by the method of Furthmayr & Timpl (1971). Portions (20 μ l) of sample solutions (2.5%, w/v) were electrophoresed for 4 h at 6 mA/tube, and the gels were stained with Coomassie Blue. (a) Sample of bovine nasal cartilage collagen (7 mg): gel (1), bovine nasal cartilage collagen; gel (2), calf skin acid-soluble tropocollagen. (b) Calf skin acid-soluble tropocollagen (7 mg). (c) Collagen prepared by papain digestion of calf skin (7 mg). (d) Collagen (7 mg), prepared by papain digestion of calf skin, left to interact with proteoglycan subunit (0.7 mg). Precipitate remaining after denaturation at 50°C was subjected to gel electrophoresis (gel 4); gel (1), reference sample of calf skin acid-soluble tropocollagen; gel (2), reference sample of collagen prepared by papain digestion of calf skin; gel (3), material from fractions indicated by (3) on the chromatogram.

EXPLANATION OF PLATE 2

Elution from CM-cellulose of bovine nasal cartilage collagen allowed to interact with increasing amounts of proteoglycan subunit

CM-cellulose chromatography was carried out at 42°C on the supernatants from denatured interaction mixtures as described in the Materials and Methods section and for Fig. 1. Electrophoretic separations on 5% polyacrylamide gels were performed under the conditions described for Fig. 1, by the method of Furthmayr & Timpl (1971). (a) Control sample of bovine nasal cartilage collagen (7 mg). (b) Bovine nasal cartilage collagen (7 mg) allowed to interact with proteoglycan subunit (0.7 mg). (c) Bovine nasal cartilage collagen (7 mg) allowed to interact with proteoglycan subunit (1.5 mg). Precipitate remaining after denaturation (3 mg) at 50°C was subjected to polyacrylamide-gel electrophoresis (gel 3); gel (1), reference sample of bovine nasal cartilage collagen; gel (2), fractions indicated by (2) on the chromatogram.

EXPLANATION OF PLATE 3

Elution from CM-cellulose of a mixture of equal parts of bovine nasal cartilage collagen and calf skin acid-soluble tropocollagen allowed to interact at 4°C with increasing amounts of proteoglycan subunit

Interaction mixtures were stirred at 4°C overnight, centrifuged and the supernatants were denatured at 50°C and examined by CM-cellulose chromatography as described in the Materials and Methods section and for Plate 1. Electrophoretic separations on 5% polyacrylamide gels were performed under the conditions described for Plate 1, by the method of Furthmayr & Timpl (1971). (a) Control sample of a mixture of bovine nasal cartilage collagen (7.0 mg) and calf skin acid-soluble tropocollagen (7.0 mg). (b) Mixture of bovine nasal cartilage collagen (7.0 mg) and calf skin acid-soluble tropocollagen (7.0 mg) allowed to interact with proteoglycan subunit (0.25 mg). Precipitate obtained at 4°C was subjected to polyacrylamide-gel electrophoresis (gel 5); gel (1), reference sample of calf skin acid-soluble tropocollagen; gel (2), reference sample of bovine nasal cartilage collagen; gels (3) and (4) are the fractions (3) and (4) indicated on the chromatogram. (c) Mixture of bovine nasal cartilage collagen (7.0 mg) and calf skin acid-soluble tropocollagen (7.0 mg) allowed to interact with proteoglycan subunit (0.35 mg). Precipitate obtained at 4°C was subjected to polyacrylamide-gel electrophoresis (gel 4). Gel (1), reference sample of calf skin acid-soluble tropocollagen; gel (2), reference sample of bovine nasal cartilage collagen; gel (3), fraction (3) as indicated on the chromatogram.

EXPLANATION OF PLATE 4

Elution from CM-cellulose of a mixture of equal parts of bovine nasal cartilage collagen and calf skin acid-soluble tropocollagen allowed to interact with increasing amounts of proteoglycan subunit at 4°C, before denaturation at 50°C

CM-cellulose chromatography was carried out at 42°C on the supernatants from denatured interaction mixtures as described in the Materials and Methods section and for Plate 1. Electrophoretic separations on 5% polyacrylamide gels were performed under the conditions described for Plate 1, by the method of Furthmayr & Timpl (1971). The chromatograph profile for a control sample of a mixture of bovine nasal cartilage collagen (7.0 mg) and calf skin acid-soluble tropocollagen is shown in Plate 3(a). (a), (b) and (c), mixtures of bovine nasal cartilage collagen (7.0 mg) and calf skin acid-soluble tropocollagen (7.0 mg) allowed to interact with proteoglycan subunit (a, 0.7 mg; b, 1.5 mg; c, 3.0 mg). Precipitates (b, 4 mg; c, 7.0 mg) remaining after denaturation at 50°C were subjected to polyacrylamide-gel electrophoresis (gel 4; 4A is an enlargement of bands). Gel (1), reference sample of calf skin acid-soluble tropocollagen, 1A is an enlargement of bands; gel (2), reference sample of bovine nasal cartilage collagen; gel (3), fraction (3) as indicated on the chromatograms.

affected the manner in which type I collagen interacted with proteoglycan subunit, and thus supported the contention that the collagen was native.

Interaction of proteoglycan subunit with type II collagen in the presence of type I collagen. To assess the relative affinity of proteoglycan subunit for types I and II collagen, equal amounts (7mg) of each collagen were allowed to compete for increasing amounts of proteoglycan subunit.

Method 1. The CM-cellulose chromatography profile of the collagen mixture is shown in Plate 3(a). When 0.25mg of proteoglycan subunit was allowed to interact with the collagen mixture the CM-cellulose chromatography profile (Plate 3b) showed a large peak due to combined $\alpha_{1(I)}$, $\alpha_{1(II)}$, $\beta_{11(I)}$ and $\beta_{11(II)}$ components and a decreased peak corresponding to β_{12} and α_2 components. Gel electrophoresis showed that the precipitate (3.5mg) contained both α_1 and α_2 chains, and the peaks of absorbance eluted from the CM-cellulose column migrated as expected for a mixture of $\alpha_{1(I)}$, $\alpha_{1(II)}$, $\beta_{11(I)}$, $\beta_{11(II)}$, β_{12} and α_2 chains. With an increased amount of proteoglycan subunit (0.35mg) CM-cellulose chromatography (Plate 3c) showed that the peak due to the α_2 and β_{12} components had almost completely disappeared, indicating that the material eluted from the column (therefore not associated with proteoglycan) was only type II collagen. This conclusion was supported by gel electrophoresis (Plate 3c), and the latter also revealed that the α_2 and β_{12} components were in the precipitate, which therefore contained the type I collagen. Finally, 0.7mg of proteoglycan subunit was sufficient to produce complete precipitation of the collagens from solution. It is therefore concluded that, under the conditions used, native type I collagen combines preferentially with proteoglycan subunit in the presence of an equal quantity of native type II collagen.

Method 2. Lee-Own & Anderson (1975b) showed that under the conditions of method 2, 0.7mg of proteoglycan subunit preferentially complexed the α_2 and β_{12} constituents of 7mg of type I collagen, after interaction and denaturation of the interaction mixture. This provided a means for investigation of the interaction of proteoglycan with chains from both types I and II collagens. When a mixed solution of calf skin acid-soluble tropocollagen (7mg) and type II collagen (7mg) was allowed to interact with a solution of proteoglycan subunit (0.7mg), CM-cellulose chromatography showed that there was a complete absence of α_2 and β_{12} components from the supernatant after interaction and denaturation. Further, gel electrophoresis demonstrated that the precipitated material consisted largely of these two components (Plate 4a) and it was apparent from the CM-cellulose profile that the peak due to the combined $\alpha_{1(I)}$, $\alpha_{1(II)}$, $\beta_{11(I)}$ and $\beta_{11(II)}$ components was not significantly decreased in area. However, with an

increased amount of proteoglycan subunit (1.5mg) the peak area on the CM-cellulose chromatogram due to the combined components (Plate 4b) was significantly decreased. Polyacrylamide-gel electrophoresis of the precipitate (4mg) gave a banding pattern identical with that of type I collagen (Plate 4b) whereas the peak eluted from the CM-cellulose resembled type II collagen. Finally, the amount of proteoglycan subunit was increased to 3.0mg. The CM-cellulose profile was drastically decreased (Plate 4c) and polyacrylamide-gel electrophoresis showed that the precipitate (7mg) now contained a predominance of α_1 chains, indicating the co-precipitation of types I and II collagen. The material eluted from the column appeared to consist solely of α chains, suggesting again that proteoglycan may be binding preferentially to cross-linked components of type II collagen. We therefore conclude that, with a small amount of proteoglycan subunit (0.7mg), only α_2 and β_{12} constituents are precipitated. As the amount of proteoglycan is increased (1.5mg) the precipitate also includes $\alpha_{1(I)}$ and $\beta_{11(I)}$ components, as proved by the identity in polyacrylamide-gel profile with type I collagen. Finally, a still larger quantity of proteoglycan subunit (3.0mg) precipitates type II collagen. Thus method 2 also demonstrates that proteoglycan subunit complexes preferentially with type I collagen. We do not think that the presence of a very small amount of proteoglycan in our type II collagen preparation (28 μ g of proteoglycan in 7mg of collagen) would lead to grossly erroneous results in these competitive experiments.

Discussion

Both method 1 and method 2 showed that our type II collagen preparation interacted with proteoglycan subunit.

Our competitive experiments demonstrated that whether the interaction mixtures were analysed by method 1 or method 2, the interpretation of the results was similar: type I collagen was complexed by proteoglycan subunit in preference to type II collagen. As method 1 assesses the binding between proteoglycan and native collagen, whereas method 2 assesses the binding with α and β components, our observations imply that binding does not depend on the complex secondary, tertiary and quaternary structure of the native collagen molecule. However, only 0.7mg of proteoglycan subunit was necessary to precipitate completely a mixture of 7mg of type I collagen and 7mg of type II collagen at 4°C, but more than 3mg of proteoglycan subunit was required to prevent release of denatured collagen components when the precipitate was incubated at 50°C. This difference probably reflects the ability of one proteoglycan molecule to complex many native collagen

molecules, but that when the non-covalent bonds holding collagen components together are broken on heating, more proteoglycan molecules are necessary to form a complex. We have not investigated whether heating for 45–60 min has any effect on proteoglycan subunit.

The question that remains to be answered is whether our conclusions are applicable to physiological conditions. For theoretical reasons (Lee-Own & Anderson, 1975b) the charge situation governing the electrostatic binding will not alter greatly between pH 4.8 and 7.2. However, the higher physiological ionic strength has been shown to weaken interactions between type I collagen and proteoglycan (Obrink & Wasteson, 1971), but whether this effect is also observed with type II collagen is unknown. Ananthanarayanan & Nimni (1975) showed that, under physiological conditions, a very dilute solution of bovine nasal cartilage proteoglycan caused a sharp increase in viscosity of a very dilute solution of bovine articular cartilage collagen, but was without effect on a similar solution of rat skin collagen. The latter non-interaction might be due to the different species of origin of the two molecules; it is therefore desirable to derive all material from the same species for this type of experiment.

In the light of our results, it seems unlikely that the insolubility of cartilage collagen is due to very strong association with proteoglycan (Strawich & Nimni, 1971), but is probably the result of cross-linking of type II collagen (Miller, 1971, 1972). Seyer *et al.* (1974a,b) found that initial extraction of cartilage with 9M-LiCl selectively solubilized the type I collagen, but type II collagen was only extractable after pepsin digestion of the residue. Further, if severe lathyrism is induced, normally insoluble cartilage collagen and proteoglycan are completely extracted in low-ionic-strength solutions (Glimcher *et al.*, 1969; Campo, 1974). Our results indicate that proteoglycan subunit appears to bind preferentially to β components of type II collagen, suggesting a higher affinity for cross-linked collagen than for α -chains. This evidence implies that the cross-linking of collagen is important in the interaction of collagen with matrix proteoglycan.

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