Isolation of bovine type X collagen and immunolocalization in growth-plate cartilage

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Type X collagen was extracted with 1 M-NaCl and 10 mM-dithiothreitol at neutral pH from fetal-bovine growth cartilage and purified to homogeneity by using f.p.l.c. gel filtration on a Superose 12 column, followed by ion-exchange chromatography on a Mono Q column. The purified protein migrates in SDS/polyacrylamide gels with an apparent $M_r$ of 58000 under reducing conditions and as a high-$M_r$ oligomer in its unreduced form. The amino acid composition is similar to the published composition of chick type X collagen. Pepsin digestion at 4°C decreases the $M_r$ of the monomer to 43000; purified bacterial collagenase digests most of the molecule, leaving a non-collagenous domain of apparent $M_r$, 15000, which probably represents the C-terminal globular domain. The IgG fraction from a rabbit antiserum raised against purified bovine type X collagen was specific for this collagen by the criteria of e.l.i.s.a. and immunoblotting after immunoabsorption with collagen types I, II, IX and XI. Immunofluorescence localization of type X collagen in sections of fetal-bovine and human cartilage was possible after acetone fixation of sections and hyaluronidase treatment. Type X collagen was restricted to the zone of hypertrophic and calcified cartilage inside the bone spicules of the growth plate.

INTRODUCTION

During long-bone development, chondrocytes differentiate along a series of discrete developmental stages which can be defined not only by morphological criteria, but also by their pattern of collagen types synthesized at each stage (for review, see von der Mark, 1980, 1986). Chondrocyte precursor cells in the undifferentiated limb mesenchyme synthesize type I collagen, this changing to type-II-collagen synthesis with the onset of chondrogenesis (Linsenmayer et al., 1973; von der Mark et al., 1976). The discovery of type X collagen in the culture medium of chondrocytes (Gibson et al., 1982; Capasso et al., 1982) and in the zone of growth cartilage (Schmid & Conrad, 1982) opened the possibility for unequivocal identification of hypertrophic chondrocytes in situ and in vitro with biochemical, recombinant DNA or immunological tools (Gibson et al., 1983; Schmid & Linsenmayer, 1983, 1985a,b; Capasso et al., 1984; for review see Schmid & Linsenmayer, 1987). Thus it has been possible to monitor precisely the differentiation in cell culture of embryonic-type-II-collagen-producing chick chondrocytes ("stage I chondrocytes"; Castagnola et al., 1986) to type-X-collagen-producing hypertrophic chondrocytes ("stage II chondrocytes") using SDS/polyacrylamide-gel electrophoresis (Gibson et al., 1983, 1984; Castagnola et al., 1986, 1987) or Northern hybridization (Castagnola et al., 1988).

With monoclonal antibodies (Schmid & Linsenmayer, 1985a,b; Summers et al., 1988) and cDNA probes (Ninomiya et al., 1986) type X collagen was located in hypertrophic cartilage of growth plates, in the calcifying cartilage of sternal and rib cartilage, but also in the developing notochord (Linsenmayer et al., 1986) and in bone trabecules of chick calvaria (Schmid & Linsenmayer, 1985a). Less information is available on the distribution of type X collagen in mammalian cartilage. Endochondral ossification in mammalian bone has rather different anatomical structures; however, the available chick type-X-collagen antibodies and probes fail to cross-react with the corresponding mammalian collagen or RNA respectively. Thus the preparation of antibodies and cDNA probes recognizing mammalian type X collagen has been gaining considerable interest, particularly in view of several genetic disorders that may involve defects in chondrocyte hypertrophy, e.g. thanatophoric dysplasia or other forms of chondrodysplasia and dwarfism.

Bovine type X collagen has been identified in organ culture of hypertrophic cartilage after radioactive labeling as a disulfide-linked high-$M_r$ component that, after reduction, gives rise to $\alpha$-chains of $M_r$ 63000 (Grant et al., 1985). By comparison, chicken type X collagen has been isolated from culture medium both as a non-disulfide-linked monomer with $\alpha$-chains of $M_r$ 64000 (Capasso et al., 1982, 1984; Gibson et al., 1982; Schmid & Linsenmayer, 1983) and as a disulfide-linked dimer of $M_r$ 120000 (Kwan et al., 1986). Analysis of the chicken type-X-collagen gene structure indicated the presence of cysteine residues in the C-terminal region of the primary transcript, which, however, are apparently lost during processing of the molecule (Ninomiya et al., 1986). Similar to chicken type X collagen, bovine type X is converted into a 46000-$M_r$ peptide after limited proteolysis with pepsin (Grant et al., 1985). Ayad et al. (1987) reported the extraction of both 59000- and 49000-$M_r$ forms of type X collagen from bovine growth plate by guanidine, suggesting processing of the 59000-$M_r$ molecule in situ.

Up until now purification of a mammalian type X collagen has been hampered by the high $M_r$ of the molecule and its tendency to form high $M_r$ oligomers even in the presence of reducing agents.
collagen to homogeneity such as to permit amino acid sequence analysis or the preparation of antibodies has not been achieved. Here we report on the extraction of type X collagen from fetal-bovine growth-plate cartilage with 1 m-NaCl and reducing agents, and describe chromatographic procedures for the purification of the molecule. With the purified protein we were able to raise in rabbits specific antibodies that could be used to localize type X collagen in the extracellular matrix of bovine growth-plate cartilage.

MATERIALS AND METHODS

Extraction of type X collagen

Epiphyseal cartilage from the long bones of calf fetuses (30–60 cm in length) was carefully cleaned from adhering connective tissue and homogenized in an ice bath in 1 m-NaCl/0.05 m-Tris/HCl, pH 7.4, containing proteinase inhibitors (1 mM-EDTA, 1 mM-phenylmethylsulphonyl fluoride and 1 mM-N-ethylmaleimide), 10 mM-dithiothreitol and 0.1 mM-β-aminopropionitrile using an Ultra-Turrax homogenizer (Jahnke & Kunkel, Staufen, Germany). The homogenate was centrifuged for 1 h at 4 °C at 40,000 g and the supernatant was saturated to 33% with (NH₄)₂SO₄. The precipitate containing type X collagen was spun down as described above. Alternatively, the 1 m-NaCl extract was dialysed directly against DEAE-cellulose starting buffer (0.2 m-NaCl/0.05 m-Tris/HCl, pH 7.4).

Chromatographic procedures

The 1 m-NaCl extract was applied to a 40 ml column of DEAE-cellulose (Whatman DE 52). Collagen samples were eluted at 4 °C from the column with the same buffer, followed by the elution of proteoglycans with 1 m-NaCl/0.05 m-Tris/HCl, pH 7.4.

For gel filtration, the DEAE-cellulose-purified collagens were concentrated by ultrafiltration, denatured at 50 °C and applied in 2 ml aliquots to a preparative Superose 12 (Pharmacia, Uppsala, Sweden; particle size 10–40 μm) column, using an f.p.l.c. unit (Pharmacia). The column was run at 32 °C and eluted with 0.2 m-NaCl/0.05 m-Tris/HCl, pH 7.4, at 1 ml/min under a constant pressure of 500 kPa.

Fractions from the Superose column were dialysed against 50 mM-Tris/HCl, pH 7.5, and applied to a Mono Q column (Pharmacia) equilibrated in the same buffer. The column was eluted under a pressure of 2 MPa using a linear gradient between 0 and 0.5 m-NaCl over 50 ml.

SDS/polyacrylamide-gel electrophoresis

Samples were dissolved in 3% SDS/sample buffer (Laemmli, 1970), with or without dithiothreitol, denatured at 95 °C for 5 min and analysed by electrophoresis in 10% polyacrylamide mini-slab gels. Gels were stained with Coomassie Blue or silver as described by Oakley et al. (1980).

Enzymic digestions

Collagen samples were digested with pepsin (0.2 mg/ml; twice-crystallized; Serva, Heidelberg, Germany) in 0.5 M-acetic acid for 24 h at 4 °C, then neutralized, dialysed and freeze-dried. For collagenase digestions, 10 μg samples were taken up in 0.1 m-NaCl/5 mM-CaCl₂/50 mM-Tris/HCl (pH 7.5)/0.1 mM-N-ethylmaleimide, heat-denatured for 30 min at 50 °C and incubated with 145 munits of proteinase-free purified collagenase (Advance Biofacture, Lynbrook, NJ, U.S.A.) for 4 h at 37 °C.

Amino acid analysis

Collagen samples were hydrolysed in 6 M-HCl at 110 °C under N₂, evaporated to dryness and dissolved in 100 μl of 0.2 m-NaHCO₃, pH 9.0. A 100 μl portion of dimethylaminobenzensulphonyl chloride (dabsyl chloride) (2 mg/ml in acetonitrile) was added and the reaction mixture was heated to 70 °C for 15 min, dried under vacuum and dissolved in 0.5 ml of 70% ethanol. The derivatized amino acids were separated by h.p.l.c. on a Lichrospher 100 CDH-18 column (Merck, Darmstadt, Germany) and the Beckman h.p.l.c. system Gold. Elution was achieved with a gradient between 0.045 m-sodium acetate, pH 4.13, and acetonitrile/tetrahydrofuran (4:1, v/v).

Antibody preparation

Rabbits were immunized subcutaneously with 0.2 mg of purified bovine type X collagen coupled to haemocyanin, dissolved in phosphate-buffered saline (0.137 mM-NaCl/2.7 mM-KCl/1.45 mM-KH₂PO₄/8.0 mM-NaHPO₄, pH 7.2) and emulsified in complete Freund’s adjuvant. After two booster injections with the same amount of type X collagen in incomplete Freund’s adjuvant, blood was collected from the ear vein, and the antiserum was absorbed with bovine collagen types II, IX and XI, human collagen type I and fibronectin, each coupled to Sepharose CL6B (Pharmacia). From the absorbed serum the IgG fraction was obtained by f.p.l.c. on Protein A–Superose (Pharmacia). Antibody titres were tested by using an e.l.i.s.a. (Rennard et al., 1980) with 3,3',5,5'-tetramethylbenzidine as colour substrate. Microtitre dishes were coated with 50 μg of collagen/well.

Immunoblotting

Samples were blotted on to nitrocellulose filters (Bio-Rad, Richmond, CA, U.S.A.) after electrophoresis on 10% (w/v) polyacrylamide gels as described by Towbin et al. (1979). After blocking with low-fat milk protein, blotted proteins were immunostained with rabbit anti-collagen type X) IgG fraction (0.1 μg/ml) using peroxidase-conjugated goat anti-rabbit Ig (Bio Yeda, Rehovot, Israel) in a 1:1000 dilution as second antibody and α-chloronaphthol as colour substrate.

Immunofluorescence staining

Frozen sections of fetal-bovine epiphyseal cartilage were collected on polystyrene-coated glass slides, fixed in acetone and digested with testicular hyaluronidase (2 mg/ml; Serva, Heidelberg, Germany) in phosphate-buffered saline, pH 5.0. Fluorescence staining with rabbit anti-(collagen type X) IgG (10 μg/ml), followed by fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Behringwerke, Marburg), was performed as described by von der Mark et al. (1976). Sections were viewed under a Leitz Diaplan microscope equipped for epifluorescence.
RESULTS

Isolation and purification of type X collagen

Sequential extractions of fetal-bovine growth-plate cartilage with (1) 1 M-NaCl at neutral pH, (2) 4 M-guanidinium chloride, (3) acetic acid and (4) pepsin, and analysis of the extracts on SDS/polyacrylamide gels under reducing conditions revealed a collagenase-susceptible 58000-\(M_r\) molecule predominantly in the 1 M-NaCl extract (results not shown). The yield of this molecule, which was later identified as type X collagen (see below), was considerably enhanced in the presence of 10 mM-dithiothreitol, suggesting increased solubilization of type X collagen by the cleavage of intermolecular cystine bonds without reducing intramolecular bonds.

The native type X collagen was separated from type II collagen and other neutral-salt-soluble cartilage-matrix components by f.p.l.c. gel filtration on a Superose 12 column at 32 °C (Fig. 1). The fraction eluted in the void volume was rechromatographed on Mono Q ion-exchange columns (Fig. 2); at 0.4 M-NaCl, type X collagen was eluted in a single peak which was analysed by SDS/polyacrylamide-gel electrophoresis (Fig. 3). Without reduction, type X collagen hardly penetrated into the gel, indicating an \(M_r\) considerably higher than 180000, which would correspond to \([\alpha 1(X)\]a.\] After reduction, the rechromatographed material appeared as a single band of \(M_r\) 58000 ± 4000.

Characterization as type X collagen

The amino acid composition of the purified material correlated well with the published analyses of chicken type X collagen obtained by protein hydrolysis (Quarto et al., 1985) or deduced from the cDNA sequence (Ninomiya et al., 1986) (Table 1). The collagenous nature of the protein was confirmed by its susceptibility to

Fig. 1. F.p.l.c. molecular-sieve chromatography on Superose 12 (Pharmacia) of bovine collagens extracted from fetal growth plates with 1 M-NaCl under mild reducing conditions

After removing proteoglycans by DEAE-cellulose chromatography (see the Materials and methods section), the extract was dialysed into 0.2 M-NaCl/0.05 M-Tris/HCl, pH 7.4, denatured at 50 °C and applied to a preparative column of Superose 12 (1.5 cm x 40 cm) equilibrated in the above buffer. Elution was carried out at 32 °C. Fraction A contained type X collagen.

Fig. 2. F.p.l.c. anion-exchange chromatography of type X collagen on Mono Q

Fraction A (Fig. 1) was dialysed into 0.05 M-Tris/HCl and applied to a Mono Q column equilibrated in the same buffer. The column was eluted with a linear NaCl gradient of 0–0.5 M over 50 ml.

Fig. 3. SDS/polyacrylamide-gel electrophoresis and immunoblotting of purified bovine type X collagen

Bovine type X collagen purified by Mono Q chromatography (see Fig. 2) was applied to SDS/10 %-(w/v)-polyacrylamide gels without reduction (a) and after reduction with dithiothreitol (DTT) (b). The \(M_r\) of the non-reduced form is above 300000. The gel was stained with Coomassie Blue. (c) Immunoblotting of lane b with a rabbit antiserum raised against the purified bovine type X collagen. The arrow on the left indicates the type X collagen monomer. The arrowhead on the right indicates the type X collagen oligomer.
puriﬁed bacterial collagenase, which degraded the 58 000-\(M_r\) component completely, leaving smaller fragments of \(M_r\) 15 000 (Fig. 4). These components probably represent the non-collagenous domains of type X collagen (Ninomiya et al., 1986).

Digestion of the native molecule with pepsin decreased the apparent \(M_r\) of the 50 000-\(M_r\) \(\alpha\) chain to 43 000 on reducing SDS/polyacrylamide gels (Fig. 5), which is consistent with the result obtained after pepsin treatment of chick type X collagen and previous reports on bovine type X collagen (Grant et al., 1985; Ayad et al., 1987). Without reduction, bovine type X collagen migrated as a high-\(M_r\) component on top of SDS/polyacrylamide gels also after pepsin treatment, indicating that pepsin did not remove the cysteine bridges from the molecule [see also Grant et al. (1985) and Ayad et al. (1987)].

Preparation of rabbit antibodies against type X collagen

Rabbits were immunized with puriﬁed bovine type X collagen coupled to haemocyanin, and the antisera were tested for titres against several collagens by e.l.i.s.a. After absorption of the antiserum with ﬁbronectin and collagen types I, II, IX, and XI, the IgG fraction was isolated by chromatography on protein A–Superose. The IgG fraction was positive for type X collagen in the e.l.i.s.a. at concentrations of 0.1 \(\mu\)g/ml (half-maximal absorbance), and negative for all other cartilage collagens tested, as well as for ﬁbronectin (Fig. 6). In the immunoblot, the antibody speciﬁcally labelled the 59 000-\(M_r\) band of bovine type X collagen (Fig. 3) at concentrations of 0.1 \(\mu\)g of IgG/ml.

Immunofluorescence localization of bovine type X collagen

Frozen sections of fetal-bovine cartilage required ﬁxa-
tion with acetone and subsequent digestion with testicular

### Table 1. Amino acid composition of bovine and chick type X collagen

<table>
<thead>
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<th>Amino acid</th>
<th>Species… Form…</th>
<th>Bovine 59 000 (M_r)</th>
<th>45 000 (M_r)*</th>
<th>Chick 59 000 (M_r)†</th>
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* From Quarto et al. (1985).
† From Ninomya et al. (1986) and Lu Valle et al. (1988).

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**Fig. 4. Collagenase digestion of bovine type X collagen**

Puriﬁed bovine type X collagen was denaturation at 50 °C for 30 min and then incubated with proteinase-free puriﬁed bacterial collagenase at 37 °C for 4 h in 0.1 M-NaCl/0.05 M-Tris/HCl, pH 7.5, containing 5 mM-CaCl₂ and 0.1 mM-N-ethylmaleimide (a) or with buffer only (b). The SDS/18 %–polyacrylamide gels were silver-stained. C, collagenase; the arrow shows uncleaved 58 000-\(M_r\)-type X collagen; the small arrowhead shows the possible non-collagenous domain of type X collagen.

**Fig. 5. Pepsin digestion of bovine type X collagen**

(a) Pepsin digestion at 4 °C without reduction leaves the molecule as a high-\(M_r\) component (arrowhead 1) of \(M_r\) similar to that of the non-pepsin-treated material (Fig. 3), indicating the presence of intramolecular disulphide bonds within the triple helix. After reduction with dithiothreitol (b), most of the pepsin-digested material migrated with an apparent \(M_r\) of 43 000 (arrowhead 3). The 10 %–polyacrylamide/SDS gel was Coomassie Blue-stained. (c) Indicates \(M_r\) markers. The arrowhead 2 indicates undigested type X monomers.
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Fig. 6. E.L.I.S.A. of the IgG fraction from a rabbit anti-(bovine type X collagen) serum after absorption with types I, II, IX and XI collagen and fibronectin

Microtitre dishes were coated with 50 μg/ml of pepsin-extracted collagen type I (human) (□), types II (●), IX (◆), X (◇) and XI (■) (bovine) and fibronectin (□) from human plasma. The concentration of the IgG solution was 50 μg/ml.

hyaluronidase to reveal type X collagen by immunofluorescence. The rabbit antibody located the protein exclusively in the zone of columnar hypertrophic cartilage beneath the cartilage–bone erosion zone (Fig. 7). It was also present in remnants of calcified cartilage within bone trabecules of endochondral bone (Fig. 8). Zones of resting cartilage and other tissues did not stain with anti-(collagen type X) antibody.

DISCUSSION

Although numerous studies on the biosynthesis, isolation, protein and gene structure and immunolocalization of chicken type X collagen have led to a complete characterization of this collagen type (for review, see Schmid & Linsenmayer, 1987), relatively little progress has been made in the isolation and characterization of mammalian collagen type X.

The existence of a collagen in mammalian growth-plate cartilage with properties similar to those of chicken type X collagen was first demonstrated in organ culture of fetal-bovine growth-plate cartilage after metabolic labelling with radioactive precursors (Grant et al., 1985). From the zone of hypertrophic cartilage in the costochondral junction, a molecule of Mr 63 000 was extracted, which was converted into a 46 000-Mr form by mild pepsin digestion. In contrast with chick type X collagen, however, without reduction both forms migrated in SDS/PAGE as high-Mr components above β-chains of type II collagen, indicating the presence of intrachain and intermolecular cystine bridges (Grant et al., 1985). Ayad et al. (1987) reported the extraction of the smaller, processed, 49 000-Mr form of type X collagen with 4 M-guanidinium chloride or with pepsin from fetal-bovine growth-plate cartilage, besides an unprocessed 59 000-Mr form. No significant amounts of bovine type X collagen

Fig. 7. (a) Immunofluorescence localization of type X collagen in the hypertrophic zone of a fetal-calf distal tibial growth plate and (b) phase-contrast picture of the same section (magnification × 480)

Frozen sections were fixed with acetone, digested with testicular hyaluronidase and stained with 10 μg of absorbed rabbit anti-(bovine type X collagen) IgG/ml, followed by fluorescein isothiocyanate-labelled goat anti-rabbit IgG. Only the hypertrophic zone was positive. The zone of resting cartilage did not stain (not shown).
were extractable with 1 M-NaCl (Ayad et al., 1987), and from fetal-chick sternal cartilage, type X collagen was only extractable by pepsin digestion (Quarto et al., 1985).

Here we show that substantial amounts of unprocessed native type X collagen could be extracted from fetal growth-plate cartilage with 1 M-NaCl at neutral pH under mild reducing conditions. Addition of 10 mM-dithiothreitol without denaturing agents was apparently sufficient to reduce intermolecular cystine bonds while leaving intramolecular disulphide bonds intact. Additional amounts of 59000-M_r, type X collagen were obtained in subsequent guanidinium chloride extracts. We did not observe processed forms of M_r, 43000-49000 (Ayad et al., 1987), either in the 1 M-NaCl extract or in subsequent guanidinium chloride extracts.

The neutral-salt-soluble form of type X collagen was purified to homogeneity by using three conventional chromatographic procedures. The material was readily soluble and did not reveal any hydrophobic characteristics. A hydrophobic nature of type X collagen has been reported by Kwan et al. (1986) and Ayad et al. (1987) and would be consistent with a previously reported 29-amino-acid-long hydrophobic domain at the C-terminal end of the chicken type X collagen (Ninomiya et al., 1986).

The identification of the purified 59000-M_r molecule as type X collagen is based on the following criteria: (i) like chicken type X collagen it is converted into a 43000-M_r form by limited digestion with pepsin; (ii) purified collagenase digests the molecule and leaves a 15000-M_r fragment intact, presumably the non-collagenous C-terminal domain; (iii) the amino acid composition is similar to that of chicken type X collagen, with the exception of two additional cysteine residues; (iv) antibodies to bovine type X collagen located the molecule in the zone of hypertrophic and calcified cartilage in the growth plate of fetal-bovine cartilage.

The processing of type X collagen is the subject of some controversy. In cell and organ culture studies on chicken growth-plate cartilage (Schmid & Conrad, 1982; Summers et al., 1988), no processing of the 59000–63000-M_r form to the 49000-M_r form was found; similarly, pulse-chase experiments using organ cultures of bovine rib cartilage did not show conversion of the synthesized 63000-M_r form of type X collagen (Grant et al., 1985). Capasso et al. (1984), however, reported the conversion of the 64000-M_r form of type X collagen into a 30000-M_r fragment in pulse-chase-labelled chondrocytes, whereas Jimenez et al. (1986) found a 70000-M_r precursor form of type X collagen in long-term cultures of hypertrophic chicken chondrocytes. Ayad et al. (1987) reported the extraction of a smaller, processed, 49000-M_r form of type X collagen with 4 m-guanidinium chloride from fetal-bovine growth-plate cartilage, besides the unprocessed 59000-M_r form, and concluded that processing of type X collagen takes place in cartilage. We could not detect significant amounts of a 49000-M_r form of type X collagen in guanidinium chloride extracts of fetal-bovine growth cartilage.

In contrast with the developing chicken long bone, in which the zone of hypertrophic and calcifying cartilage stretches in an irregular manner from the mid diaphysis to the epiphysis (Schmid & Linzenmayer, 1985a,b), the corresponding region in mammalian long bones or ribs is confined to an anatomically rather narrow zone (Grant et al., 1985). Immunofluorescence analysis of 20–30-week-old fetal-bovine epiphyseal cartilage with the affinity-purified antibody to type X collagen located this collagen specifically in that zone. Intense reactions were also observed in remnants of calcified cartilage in enchondral-bone trabecules. Preliminary studies have shown that the antibodies described can also be used for immunostaining of human tissues (T. Kirsch, K. von der Mark & G. Weseloh, unpublished work). Thus the availability of antibodies recognizing both bovine and human type X collagen offers new potential not only for studies on cartilage development, but also for studies on growth defects involving growth-plate cartilage.

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REFERENCES

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