Analysis of collagen types synthesized by rabbit ear cartilage chondrocytes *in vivo* and *in vitro*

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(Received 31 January 1984/Accepted 16 March 1984)

This study compares the collagen types present in rabbit ear cartilage with those synthesized by dissociated chondrocytes in cell culture. The cartilage was first extracted with 4M-guanidinium chloride to remove proteoglycans. This step also extracted type I collagen. After pepsin solubilization of the residue, three additional, genetically distinct collagen types could be separated by fractional salt precipitation. On SDS (sodium dodecyl sulphate)/polyacrylamide-gel electrophoresis they were identified as type II collagen, (1 α ,2 α ,3 α) collagen and M-collagen fragments, a collagen pattern identical with that found in hyaline cartilage. Types I, II, (1 α ,2 α ,3 α) and M-collagen fragments represent 20, 75, 3.5, and 1% respectively of the total collagen. In frozen sections of ear cartilage, type II collagen was located by immunofluorescence staining in the extracellular matrix, whereas type I collagen was closely associated with the chondrocytes. Within 24 h after release from elastic cartilage by enzymic digestion, auricular chondrocytes began to synthesize type III collagen, in addition to the above-mentioned collagens. This was shown after labelling of freshly dissociated chondrocytes with [³H]proline 1 day after plating, fractionation of the pepsin-treated collagens from medium and cell layer by NaCl precipitation, and analysis of the fractions by CM(carboxymethyl)-cellulose chromatography and SDS/polyacrylamide-gel electrophoresis. The 0.8M-NaCl precipitate of cell-layer extracts consisted predominantly of type II collagen. The 0.8M-NaCl precipitate obtained from the medium contained type I, II, and III collagen. In the supernatant of the 0.8M-NaCl precipitation remained, both in the cell extract and medium, predominantly 1 α -, 2 α -, and 3 α -chains and M-collagen fragments. These results indicate that auricular chondrocytes are similar to chondrocytes from hyaline cartilage in that they produce, with the exception of type I collagen, the same collagen types *in vivo*, but change their cellular phenotype more rapidly after transfer to monolayer culture, as indicated by the prompt onset of type III collagen synthesis.

The major structural macromolecules of hyaline-cartilage matrix, chondroitin sulphate proteoglycan, type II collagen and other minor collagen types, and several cartilage-specific glycoproteins, are well characterized (for reviews see Hascall & Hascall, 1981; Mayne & von der Mark, 1983). The so-called 'minor' collagen molecules include (i) collagen molecules composed of 1 α -, 2 α -, and 3 α -

chains (Burgeson & Hollister, 1979; Reese & Mayne, 1981; Burgeson *et al.*, 1982; von der Mark *et al.*, 1982), henceforward referred to as '(1 α ,2 α ,3 α) collagen', and (ii) a disulphide cross-linked collagen molecule of M_r 300 000 called 'pM collagen' (von der Mark *et al.*, 1984), which after pepsin treatment gives rise to a larger fragment of M_r 130 000–140 000 and a smaller fragment of M_r 35 000. Such fragments, referred to as 'M-collagen' fragments in the present study, have been described in pepsin extracts of pig hyaline cartilage ('CF1' and 'CF2'; Shimokomaki *et al.*, 1980, 1981),

Abbreviations used: CM-, carboxymethyl-; SDS, sodium dodecyl sulphate; IgG, immunoglobulin G.

in bovine and human cartilage ('CPS-1' and 'CPS-2'; Ayad *et al.*, 1981, 1982), and in chick cartilage ('HMW' and 'LMW', or 'M1' and 'M2' respectively; Reese & Mayne, 1981; von der Mark *et al.*, 1982). Both ($1\alpha, 2\alpha, 3\alpha$) collagens and pM collagen constitute about 5–10% of the total collagen in hyaline cartilage (for a review see Mayne & von der Mark, 1983). The macromolecular organization of these collagens in the cartilage matrix is, however, still unknown. In addition to these collagens, a short-chain collagen of M_r 59000 was found to be synthesized by chick chondrocytes under certain culture conditions (Gibson *et al.*, 1982, 1983; Schmid & Conrad, 1982a), and by hypertrophic chick chondrocytes in organ culture (Schmid & Conrad, 1982b). This collagen, also called 'G-collagen' (Gibson *et al.*, 1983), has not been identified so far in cartilage *in situ*. It is not disulphide-cross-linked and gives rise to a 45000- M_r fragment after pepsin treatment.

In addition to type II collagen (Eyre & Muir, 1975) and chondroitin sulphate proteoglycan (Rokosova & Bentley, 1973), elastic cartilage contains elastin as a major constituent. Elastin is apparently responsible for the increased elasticity of ear cartilage in comparison with hyaline cartilage. No information is available on the presence of collagens other than type II in elastic cartilage. However, in the present study we demonstrate the presence of type I, II, ($1\alpha, 2\alpha, 3\alpha$), and pM collagen in rabbit ear cartilage.

Chondrocytes isolated from elastic cartilage have also been shown to synthesize elastin (Moskalewski, 1976) and cartilage-type proteoglycans (Madsen & Lohmander, 1979). Recently, we have analysed the proteoglycans synthesized in auricular chondrocytes of various donor ages and found that, in all age groups, cartilage-type chondroitin sulphate proteoglycans are synthesized that are able to form large aggregates with hyaluronic acid (Madsen *et al.*, 1983). In the present study we analysed the collagen types synthesized by auricular chondrocytes of 4-week-old rabbits in cell culture and compare the results with the collagen types found in whole ear cartilage. It is known from cultures of hyaline chondrocytes that such cells may change their phenotype, expressed as an alteration in the synthesis of collagen and other matrix molecules (Schiltz *et al.*, 1973; Mayne *et al.*, 1976; Müller *et al.*, 1977; Benya *et al.*, 1977). Therefore chondrocytes were analysed only in short-term culture, i.e. 1 day after plating, assuming that the collagen types identified *in vitro* correlated with the collagen types present in ear cartilage *in situ*. Interestingly enough, after 1 day in culture these cells had already begun to synthesize type III collagen, in addition to the types synthesized *in vivo*.

Materials and methods

Cartilage

New Zealand White rabbits, aged 4 weeks, were obtained from a local dealer. The ear cartilage was dissected free of adhering tissue and perichondrium as described previously (Madsen *et al.*, 1983). For cell culture, the cartilage was digested with 0.1% (w/v) bacterial collagenase (type IA, Sigma) overnight, and the dissociated chondrocytes were treated as described previously (Madsen *et al.*, 1983). Cartilage was also frozen in liquid N_2 directly after dissection and freeze-dried for subsequent collagen analysis.

Cell cultures

Cells were cultured in F-12 medium supplemented with 10% (v/v) newborn-calf serum (Flow Laboratories), ascorbic acid (50 μ g/ml), L-glutamic acid (30 μ g/ml), and gentamycin (50 μ g/ml) at 4×10^6 cells per 60-mm-diameter culture dish. The day after plating the medium was changed to proline-free F-10 medium containing 100 μ g of β -aminopropionitrile fumarate/ml, 100 μ g of ascorbic acid/ml and 50 μ g of gentamycin/ml, and cells were labelled with L-[5- 3 H]proline (37 Ci/mmol; Amersham International) for 24 h in the absence or presence of 10% calf serum. Pilot experiments revealed only minor differences between the fractions obtained with or without serum, and they were therefore pooled in the subsequent analyses.

Extraction of collagens from whole cartilage

Freeze-dried ear cartilage was homogenized in 4M-guanidinium chloride with a Polytron homogenizer and stirred at 4°C overnight. The homogenate was centrifuged and the resulting pellet was digested with pepsin (1:100, w/w) in 0.5M-acetic acid at 4°C for 24 h. Pepsin was inactivated at pH 8, and the solubilized collagens were fractionated at pH 3 by fractional salt precipitation with 0.8M-, 1.2M- and 2.0M-NaCl as described by von der Mark *et al.* (1982). The guanidinium extract was dialysed against water and freeze-dried. Quantification of collagen was performed by hydroxyproline analysis of the different extracts. A 1 mg portion of the freeze-dried material was hydrolysed with 6M-HCl at 108°C for 24 h, and the hydrolysate was analysed for hydroxyproline on a Durrum amino acid analyser as described by von der Mark *et al.* (1982).

Extraction of collagens from cell cultures

After labelling had been performed, the medium was removed, mixed with an equal volume of 1M-NaCl containing proteinase inhibitors (0.05M-EDTA; 0.1M-6-aminohexanoic acid; 5mM-

benzamidinehydrochloride; 1 mM-phenylmethanesulphonyl fluoride and 10 mM-*N*-ethylmaleimide). Cell layers were extracted for 24 h at 4°C with 1 M-NaCl containing proteinase inhibitors as described above. Media and cell-layer extracts were dialysed against 0.5 M-acetic acid, titrated to pH 2 with formic acid, and digested with pepsin (0.3 mg/ml) (twice crystallized, from pig stomach; Serva) for 6 h at 15°C. Samples were neutralized with NaOH, dialysed against distilled water and then against 0.1 M-acetic acid.

Fractional salt precipitation of cell-culture material

A 10 mg portion of lathyrictic-rat skin collagen was added to each sample, which was subsequently dialysed against 0.8 M-NaCl in 0.1 M-acetic acid. The resulting precipitate was pelleted by centrifugation at 15000g for 30 min. The pellet was dissolved in 0.1 M-acetic acid and dialysed against CM-cellulose-chromatography buffer. The supernatant was mixed with 10 mg of lathyrictic-rat skin collagen and also dialysed against the same buffer.

CM-cellulose chromatography and SDS/polyacrylamide-gel electrophoresis

Samples were denatured and separated at 42°C on 1.5 cm × 15 cm water-jacketed columns containing CM-cellulose (CM 52; Whatman), equilibrated in 0.04 M-sodium acetate/6 M-urea, pH 4.8 (von der Mark *et al.*, 1982). Samples were eluted with a linear salt gradient between 0 and 0.12 M-NaCl over a total volume of 500 ml. Fractions containing ³H radioactivity were dialysed against distilled water and freeze-dried. Electrophoresis on 6% (w/v)-polyacrylamide slab gels in the presence of SDS was performed as described by von der Mark *et al.* (1982).

Immunofluorescence techniques

Rabbit antibodies specific for bovine types I and III collagen were kindly provided by Dr. Manfred Wiestner (Max-Planck-Institut für Biochemie). Rabbit and guinea-pig antibodies to chick type II collagen were prepared and tested for specificity as described by von der Mark *et al.* (1976).

Cryostat sections of ear cartilage were treated with testicular hyaluronidase (1 mg/ml; Serva, Heidelberg, Germany) and fluorescently labelled with antibodies as described by von der Mark *et al.* (1976).

Monolayer cultures (8 days) were fixed with ethanol, air-dried and 'stained' with antibodies as described by von der Mark *et al.* (1977). Fluorescein-conjugated goat anti-(rabbit IgG) and rabbit anti-(guinea-pig IgG) sera (Behring Werke, Marburg, Germany) were used in a 1:20 dilution.

Results

Collagen types of elastic cartilage in situ

Elastic cartilage was dissected from 4-week-old rabbit ears, special care being taken to remove completely the perichondrium. The cartilage was extracted with 4 M-guanidinium chloride and analysed for collagen by SDS/polyacrylamide-gel electrophoresis and amino acid analysis. On the basis of hydroxyproline content, the freeze-dried extract contained about 30% collagen, which was identified as type I collagen by SDS/polyacrylamide-gel electrophoresis (Fig. 1e). The collagens from the insoluble residue were solubilized with pepsin under native conditions (von der Mark *et al.*, 1982). After inactivation of pepsin, the solubilized collagens were fractionated by NaCl precipitation, and the resulting fractions were analysed by SDS/polyacrylamide gel electrophoresis. As shown in Fig. 1, this fractionation procedure resulted in three homogeneous fractions: type II

Fig. 1. SDS/polyacrylamide-gel electrophoresis of collagens from ear elastic cartilage of 4-week-old rabbits. Cartilage was solubilized with pepsin and fractionated with NaCl in 0.5 M-acetic acid. Samples were separated on 6% (w/v)-polyacrylamide gels, the buffer system of Laemmli (1970) being used. (a, b) 2.0 M-NaCl precipitate (M1-collagen fragment): (a) reduced with mercaptoethanol, (b) unreduced; (c) 1.2 M-NaCl precipitate [(1 α ,2 α ,3 α) collagen]; (d) 0.8 M-NaCl precipitate (type II collagen); (e) 4 M-guanidinium chloride extract. Protein bands were stained with Coomassie Blue.

collagen, precipitating at 0.8M-NaCl (Fig. 1d), ($1\alpha, 2\alpha, 3\alpha$) collagen, precipitating at 1.2M-NaCl (Fig. 1c) and an M-collagen fragment precipitating at 2.0M-NaCl (Fig. 1b). This fragment corresponded in migration on SDS/polyacrylamide-gel electrophoresis to the larger M-collagen fragment of pig hyaline cartilage (Shimokomaki *et al.*, 1981) and was reducible to small-*M*, material migrating close to the electrophoretic front (Fig. 1a). Fig. 1(d) also demonstrates the absence, from elastic cartilage, of type III collagen, which should co-precipitate with type II collagen. The relative amounts of collagen types in ear cartilage were estimated by hydroxyproline analysis of the individual collagen fractions Type I, II, ($1\alpha, 2\alpha, 3\alpha$) and M-collagen fragments represented 20, 75, 3.5, and 1% respectively of the total collagen content.

Fig. 2. Immunofluorescence staining of lateral sections through ear cartilage from a 4-week-old rabbit, after removal of perichondrium

Frozen sections were treated with testicular hyaluronidase before antibody staining. The sections were incubated with guinea-pig antibodies to type II collagen (a) and with rabbit antibodies to type I collagen (b). Subsequently they were stained with fluorescein isothiocyanate-conjugated rabbit anti-(guinea-pig IgG) (a) or goat anti-(rabbit IgG) (b) sera. Magnification $\times 330$.

Immunofluorescence localization of types I and II collagen in rabbit ear cartilage

The unexpected finding of type I collagen in rabbit ear cartilage prompted an investigation of its localization in the cartilage. Therefore frozen sections of ear cartilage from 4-week-old rabbits were examined for the presence of types I and II collagen by the immunofluorescence technique. Type I collagen was localized with specific antibodies within, or closely associated with, chondrocytes in the centre of the ear cartilage (Fig. 2b),

Fig. 3. CM-cellulose chromatography of ^3H -labelled collagen from the cell layer and culture medium of rabbit ear chondrocyte cultures

Cultures were labelled 1 day after plating with [^3H]-proline, treated with pepsin under native conditions and precipitated with 0.8M-NaCl at pH 3. Chromatography was performed at 42°C on CM-cellulose equilibrated in 0.04M-sodium acetate/6M-urea, pH 4.8. Collagens were eluted with a linear salt gradient between 0 and 0.12M-NaCl over a total volume of 500ml, starting at 'G'. —, A_{228} profile of added carrier type I collagen from lathyrus-rat skin; ---●—, ^3H radioactivity.

whereas the absence of type I collagen from the perichondrial region confirmed the complete removal of perichondrial tissue by the dissecting procedure. Interestingly, even after treatment with testicular hyaluronidase, type I collagen was not found within the extracellular matrix, where abundant type II collagen was localized by the same staining procedure (Fig. 2a).

Collagen types synthesized by chondrocytes of elastic cartilage in cell culture

Rabbit ear chondrocytes were labelled for 24 h with [^3H]proline 1 day after enzymic release from the cartilage. The collagens secreted into the culture medium and extracted from the cell layer with 1M-NaCl were digested with pepsin under native conditions in order to convert procollagens into collagens. The ^3H -labelled collagens were fractionated by precipitation with 0.8M-NaCl at acidic pH, which precipitates types I, II and III

collagen and leaves ($1\alpha, 2\alpha, 3\alpha$) collagen and M-collagen fragments in the supernatant (Burgeson & Hollister, 1979). Both fractions were then subjected to CM-cellulose chromatography, allowing the separation of $\alpha 1(\text{II})$ -, $\alpha 2(\text{I})$ - or $\alpha 1(\text{III})$ -chains in the 0.8M-NaCl precipitate, and of ($\alpha 1, \alpha 2, \alpha 3$) collagen and M-collagen fragments in the 0.8M-NaCl supernatant fraction (von der Mark *et al.*, 1982; Herrmann & von der Mark, 1975).

Collagens precipitating at 0.8M-NaCl

When the collagen extracted from the cell layer was precipitated with 0.8M-NaCl and separated by CM-cellulose chromatography, the radioactivity elution profile demonstrated a prominent peak that is co-eluted with the carrier $\alpha 1(\text{I})$ -chains (Fig. 3a). When subjected to electrophoresis in SDS/polyacrylamide gels, this fraction was found to consist predominantly of $\alpha 1(\text{II})$ -chains (results not shown).

The 0.8M-NaCl precipitate of the pepsin-digested culture medium was separated by CM-cellulose chromatography into two fractions (Fig. 3b). Fraction a consisted of a mixture of $\alpha 1(\text{I})$ - and $\alpha 1(\text{II})$ -chains, which co-migrate on SDS/polyacrylamide-gel electrophoresis (Fig. 4a). Fraction b contained three bands migrating in the positions of 1α -, $\alpha 1$ - and $\alpha 2(\text{I})$ -chains, and a γ -component (Fig. 4b), which after reduction of cystine bridges migrated in the position of $\alpha 1(\text{III})$ -chains (Fig. 4c). This indicated that the 0.8M-NaCl precipitate of the medium fraction consisted predominantly of

Fig. 4. SDS/polyacrylamide-gel electrophoresis of ^3H -labelled collagen chains from the 0.8M-NaCl precipitate of the culture medium (fluorograph)

The material was first separated by CM-cellulose chromatography (Fig. 3b), and the fractions were analysed by electrophoresis on 6% (w/v)-polyacrylamide gels in the presence of SDS. (a) peak a; (b) peak b; (c) peak b reduced with mercaptoethanol.

Fig. 5. CM-cellulose chromatography of the cell layer collagens in the 0.8M-NaCl supernatant

Chromatographic conditions were as in Fig. 3. Fractions a, b and c were further analysed by SDS/polyacrylamide-gel electrophoresis (not shown) and found to contain 3α -chains (a), 1α -chains and M1-collagen fragments (b), and 2α -chains (c). The commencement of the gradient elution is indicated by 'G'.

type II collagen, together with about 30% type III collagen and some type I collagen.

Collagens remaining in the supernatant after precipitation with 0.8M-NaCl

The supernatant from the cell-layer extract was subjected to CM-cellulose chromatography and revealed an elution profile typical for a mixture of ($1\alpha, 2\alpha, 3\alpha$) collagen (Burgeson & Hollister, 1979) and M-collagen fragments (von der Mark *et al.*, 1982) (Fig. 5).

Fig. 6. SDS/polyacrylamide-gel electrophoresis of the ^3H -labelled culture medium collagens in the 0.8M-NaCl supernatant (fluorograph)

The material was freeze-dried and directly applied to the gel. (a) Unreduced; (b) reduced with mercaptoethanol. 'MR2' refers to the major reduced fragment of M1-collagen (von der Mark *et al.*, 1982).

The 0.8M-NaCl supernatant fraction of the medium collagens was subjected to SDS/polyacrylamide-gel electrophoresis. It contained 1α -, 2α - and 3α -chains as well as the M1- and M2-collagen fragments (Fig. 6), and gave rise to an elution pattern (results not shown) on CM-cellulose similar to that depicted in Fig. 5.

Immunofluorescence localization of types I, II, and III collagen in cultured chondrocytes

For further support of type I, II and III collagen synthesis by auricular chondrocytes in culture, 8-day monolayer cultures were fixed with ethanol and stained with antibodies specific for collagen types I, II, and III. As shown in Figs. 7(a) and 7(b), most cells stain intracellularly with antibodies to type II collagen, as do chondrocytes from hyaline cartilage. In addition, about 10–20% of the cells, predominantly those of hypertrophic appearance, showed a cytoplasmic reaction with antibodies to types I (Fig. 7c) and III (Fig. 7d) collagen. This observation suggests that synthesis of type I and III collagen does not occur in all cells concomitantly in monolayer conditions, but may be restricted to distinct populations of the chondrocytes.

Discussion

In the present study we have compared the pattern of collagen types present in rabbit articular cartilage *in situ* with that produced by freshly dissociated chondrocytes from the same tissue *in vitro*. With the exception of type I collagen, the collagen types found in elastic cartilage *in situ* were identical with those described in hyaline cartilage: type II collagen, ($1\alpha, 2\alpha, 3\alpha$) collagen and M-collagen fragments. Interestingly, immunofluorescence examination of the ear cartilage indicated that type I collagen, in contrast with type II collagen, is not a constituent of the cartilage matrix, but located within the chondrocytes or in the pericellular space. The fact that type I collagen was solubilized by guanidinium chloride, whereas the other cartilage collagens required pepsin treatment for solubilization, supports the notion that type I collagen is not an integral constituent of the extracellular matrix in elastic cartilage. The functional role of type I collagen in this location remains to be shown. Immunoelectron-microscopic studies on the precise location of this collagen type in relation to the cartilage cell and of the matrix constituents may eventually lead to better understanding of this unexpected finding.

Surprisingly, after only 1 day in monolayer culture, dissociated chondrocytes synthesize, in addition to the described collagens, significant amounts of type III collagen, which is secreted to the medium. No type III collagen was detected in

Fig. 7. Immunofluorescence staining of rabbit ear chondrocytes after 8 days in monolayer culture. The cells were fixed in ethanol and air-dried. Staining was done with rabbit antibodies to chick type II collagen (a), guinea-pig antibodies to chick type II collagen (b), rabbit antibodies to bovine type I collagen (c), and rabbit antibodies to bovine type III collagen (d). Magnification $\times 900$.

the analysis of the whole cartilage sample, which was prepared in the same manner as the cartilage sample used as source for chondrocyte cultures.

The immunofluorescence studies on cell cultures indicated that not all chondrocytes produce type I and III collagen, which is a characteristic of fibroblastic cells. However, contamination of the chondrocyte cultures with cells from the perichondrium, in numbers sufficient to account for the amounts of type III collagen found by CM-cellulose chromatography, can be excluded, since immunofluorescence examination of ear cartilage sections indicated the complete removal of perichondrium. Overgrowth by a few residual perichondrial cells can also be excluded, since cell cultures were analysed only 1 day after plating. Furthermore, cells that stained for type III collagen showed a round-to-polygonal morphology characteristic of hypertrophic chondrocytes. It is possible that type III collagen synthesis begins in cells derived from the more mature, hypertrophic region of the ear cartilage, in a similar manner to the G- or '59k collagen', which is predominantly synthesized by hypertrophic chondrocytes (Schmid & Conrad, 1982b).

Therefore the onset of type III collagen synthesis

in cultures of auricular chondrocytes appears to reflect a change in the phenotypic expression primarily affecting chondrocytes in an advanced stage of differentiation. It may be caused by the extended exposure of the chondrocytes to collagenase during digestion of the cartilage tissue and transfer of the released cells to monolayer culture. Similar changes in the collagen pattern are well established in cultures of hyaline chondrocytes (Schiltz *et al.*, 1973; Mayne *et al.*, 1976; Müller *et al.*, 1977; Benya *et al.*, 1977). However, these changes usually occur after several days in monolayer culture and are completed within several weeks, depending on the culture conditions. It may be noted that proteoglycan synthesis in rabbit ear chondrocyte cultures seems to be more stable than collagen biosynthesis, as these cultures continue to produce cartilage-type proteoglycans for at least 2 weeks (Madsen & Lohmander, 1979).

The rapid onset of type III collagen synthesis in elastic chondrocytes resembles the initiation of fibronectin synthesis in freshly dissociated sternal chondrocytes (Dessau *et al.*, 1978), or the immediate change in the collagen phenotype observed after release of chick corneal fibroblasts from the stroma (Conrad *et al.*, 1980). The latter cells also

begin to synthesize type III collagen immediately in cell culture, although this collagen type is absent from the intact corneal stroma. Similarly, chick tendon fibroblast cultures show an increasing number of type III-collagen-producing cells with time in culture and increasing passage number (Herrmann *et al.*, 1980). It remains to be established whether the onset of type III collagen synthesis in an elastic chondrocyte is also associated with a simultaneous cessation of the production of type II collagen and other cartilage collagens, as was observed in hyaline chondrocytes (von der Mark *et al.*, 1977).

In the present investigation the collagen chains were identified on the basis of their elution profile on CM-cellulose and band pattern on SDS/polyacrylamide-gel electrophoresis, both in relation to standard rat skin type I collagen. In case of type I, II, III collagen, and ($1\alpha, 2\alpha, 3\alpha$) collagen, the identity was unequivocal. Rabbit types I and II collagen were eluted on CM-cellulose chromatography in the same positions as related collagens from chick or rat. Rabbit type III collagen was eluted together with $\alpha 2(I)$ -chains, similar to chick type III collagen (Herrmann & von der Mark, 1975), but different from human or bovine type III collagen (Epstein, 1974). On the other hand, monomeric $\alpha 1(III)$ -chains from rabbit ear cartilage migrated on SDS/polyacrylamide-gel electrophoresis together with $\alpha 1(I)$ - or $\alpha 1(II)$ -chains, whereas chick $\alpha 1(III)$ -chains migrate between $\alpha 1(I)$ - and $\alpha 2(I)$ -chains.

In the case of the M-collagen fragments, further identification by peptide mapping will be necessary. Disulphide-linked M-collagen fragments vary considerably in M_r , according to the species. The large- M_r -component, which we identified as an M1-collagen fragment of rabbit elastic cartilage, migrates close to the 130000- M_r M-collagen fragment found in pig hyaline cartilage (Shimokomaki *et al.*, 1980). A smaller disulphide-linked M-collagen fragment similar to CPS-2 (Ayad *et al.*, 1981), CF2 (Shimokomaki *et al.*, 1981), or M2 (von der Mark *et al.*, 1982) was also observed. Conceivably, it may overlap with the G-collagen fragment (Gibson *et al.*, 1982, 1983), which, however, does not change migration rate after reduction.

We are indebted to Ms. G. Carlsson and Ms. M. Engström for skilful technical assistance. We also thank Dr. K. Kühn for support of this work and constructive criticism of the manuscript. Financial support was obtained from the Swedish Medical Research Council (Project 03355), the Department of Agriculture, the King Gustaf V 80th Birthday Fund, from the funds of Karolinska Institutet, and from the Deutsche Forschungsgemeinschaft (Ma 534/6).

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