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Distribution of Two Alternatively Spliced Variants of the Type II Collagen N-Propeptide Compared With the C-Propeptide in Bovine Chondrocyte Pellet Cultures

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Abstract We have analyzed the distribution of type II collagen N- and C-propeptides in the cell layers and culture medium of bovine articular chondrocyte pellet cultures. Two splice variants of the type II collagen N-propeptide were detected by immunoblotting and immunoassay, using a new anti-peptide antibody, while the C-propeptide was detected using a monoclonal antibody. Type II collagen molecules containing the N-propeptide were detected weakly in cell layers, but not in tissue culture medium of chondrocyte pellet cultures, and both splice variants were observed. Free N-propeptide could not be detected in cell layers or medium. Type II procollagen molecules containing the C-propeptide were detected strongly in cell layers, but not in tissue culture medium, while the free C-propeptide was detected in both cell layers and medium. Since the N- and C-propeptides must be synthesized in a 1:1 molar ratio, we conclude that the N-propeptide is metabolized more quickly than the C-propeptide in this system. Our model can be used to study regulation of procollagen synthesis and propeptidase activity. J. Cell. Biochem. 75:13–21, 1999.

Key words: articular chondrocytes; collagen synthesis; cartilage; chondrocalcin; bovine vitreous

Chondrocytes, the cells of hyaline cartilage, have a phenotype defined partly by the fact that they synthesize predominantly type II collagen, rather than type I collagen [Poole, 1997]. However when chondrocytes are cultured in monolayer, they tend to dedifferentiate to fibroblast-like cells, switching to type I collagen synthesis [Bonaventure et al., 1994; Dharmavaram et al., 1997]. Accurate measurements of type II collagen synthesis can therefore provide very important information in studies of cultured chondrocytes or when these cells are used to engineer new cartilage [Carey et al., 1993; Freed et al., 1998; Riesle et al., 1998]. The traditional approach for the measurement of collagen synthesis has been to load cells with radiolabelled-proline, which then becomes incorporated into the new collagen molecules [Tyler and Benton, 1988]. However, this method is cumbersome, involving ion-exchange chromatography separation of proline and hydroxyproline in each test sample. Furthermore, the method is not specific for any one type of collagen.

Fibrillar collagens are synthesized with N- and C-terminal propeptide domains that are removed rapidly after secretion of the mature molecule from the cell, by specific propeptidases. Levels of propeptides produced by specific cells can be used as a marker of specific collagen synthesis and such assays for type I collagen propeptides have proven to be of significant clinical utility [Heickendorff et al., 1995; Blumsohn et al., 1995; Demers and Kleerekoper, 1994]. The C-terminal propeptide of type II collagen (CpII) was originally purified as chondrocalcin, a large peptide that has the propensity to be retained within the extracellular matrix, through interaction with collagen fibrils and hydroxyapatite [Hinek et al., 1987; Choi et al., 1983]. Radioimmunoassays (RIAs) for CpII have been developed and used to measure levels of this propeptide in human serum and

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synovial fluid samples as well as in cell culture systems [Shinmei et al., 1993; Carey et al., 1997; Poole, 1994]. However, interpretation of these data is difficult because the direct relationship between type II collagen synthesis and free CpII levels may not hold true if some of the propeptide is bound within the cartilage.

The N-propeptide of type II collagen has been less well studied. It can be synthesized as one of two alternatively spliced forms, NpIIA and NpIIB, where NpIIA includes an additional 69 amino acid residues [Ryan et al., 1990; Ryan and Sandell, 1990]. NpIIA is thought to be expressed mainly by chondroprogenitor cells, while NpIIB is the predominant form in adult cartilage tissues [Sandell et al., 1991, 1994a]. Assays for NpII have not previously been described.

A number of in vitro cell culture systems have been developed for the analysis of matrix synthesis and degradation by chondrocytes. Chondrocyte pellet cultures were first used for the study of growth plate chondrocyte maturation [Kato et al., 1988; Jikko et al., 1993; Carey et al., 1993], and we have since adapted the model for use with mature articular chondrocytes [Xu et al., 1996; Oyajobi et al., 1998]. Culturing of chondrocytes on alginate beads has been of particular use for comparisons of pericellular and territorial matrix around chondrocytes [Häuselmann et al., 1992; Bonaventure et al., 1994]. More sophisticated approaches have involved tissue engineering of cartilage by growing chondrocytes on polyglycolic acid scaffolds [Freed et al., 1993, 1994, 1998; Riesle et al., 1998]. Studies using any of these approaches would benefit from accurate measurements of type II collagen synthesis.

The aim of the study reported here was to determine whether the N- and C-propeptides of type II collagen are distributed similarly to each other between extracellular matrix and culture medium or whether there are differences that might aid in the choice of an appropriate assay method. To this end, we have employed the articular chondrocyte pellet culture system as our model, and we have raised a new antibody to NpII for comparison with an existing CpII antibody.

MATERIALS AND METHODS

Isolation of Chondrocytes and Pellet Cultures

Bovine cartilage was obtained from adult animals, shortly after death at a local abattoir. Articular cartilage was removed from the metacarpophalangeal joints and chondrocytes were isolated by sequential enzymatic digestion as follows. Cartilage pieces were incubated at 37°C with 1 mg/ml hyaluronidase for 15 min, 2.5 mg/ml trypsin for 30 min, and 3 mg/ml bacterial collagenase for 19 h (all from Sigma-Aldrich Chemical Co., Poole, Dorset, UK). Once isolated, the chondrocytes were resuspended at 2×10^6 cells/ml in Dulbecco's modified Eagle's medium (DMEM) (Gibco Life Technologies, Paisley, UK) containing 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 10% heat-inactivated fetal calf serum (FCS) (all from Gibco), 10 µg/ml insulin, 10 µg/ml transferrin, and 50 µg/ml ascorbic acid (all from Sigma). To prepare pellet cultures, 1-ml aliquots of the cell suspension were centrifuged at 200*g* for 5 min in 15-ml centrifuge tubes. The resulting cell pellets were cultured at 37°C in a humidified atmosphere of 5%CO_2/95% air. The medium was replaced on days 3 and 5, and cultures were maintained for 7 days. Upon collection, aliquots of medium were immediately added to a cocktail of proteinase inhibitors (10 mM iodoacetamide, 10 mM EDTA, and 10 mM phenylmethylsulfonyl fluoride [PMSF], all from Sigma), incubated at room temperature for 30 min, and then stored at −20°C. At the end of culture the pellets were harvested and stored at −20°C.

Epitope Selection, Peptide Synthesis, and Antibody Generation

Anti-NPII antibodies were raised to synthetic peptide sequences, AH15 and AH16, taken from the known human sequence of NPII. The amino acid sequences of these two epitopes are shown in Figure 1A and their location relative to the epitopes of other antibodies used in this study are shown in Figure 1B. Antibody AH12L3 recognizes peptide epitope AH12 in the triple helical region of the α1(II) chain (L.J. Croucher and A.P. Hollander, submitted), while antibody R160 (a kind gift from Dr. A.R. Poole, Shriner's Hospital, Montreal, Canada) was raised to the C-propeptide of type II collagen [Choi et al., 1983].

Peptides AH15 and AH16 were chosen on the basis of favorable hydrophilicity profiles and on their minimal sequence identity with other procollagen α-chains, as described previously for helical epitopes [Hollander et al., 1994]. The peptides were synthesized, using standard

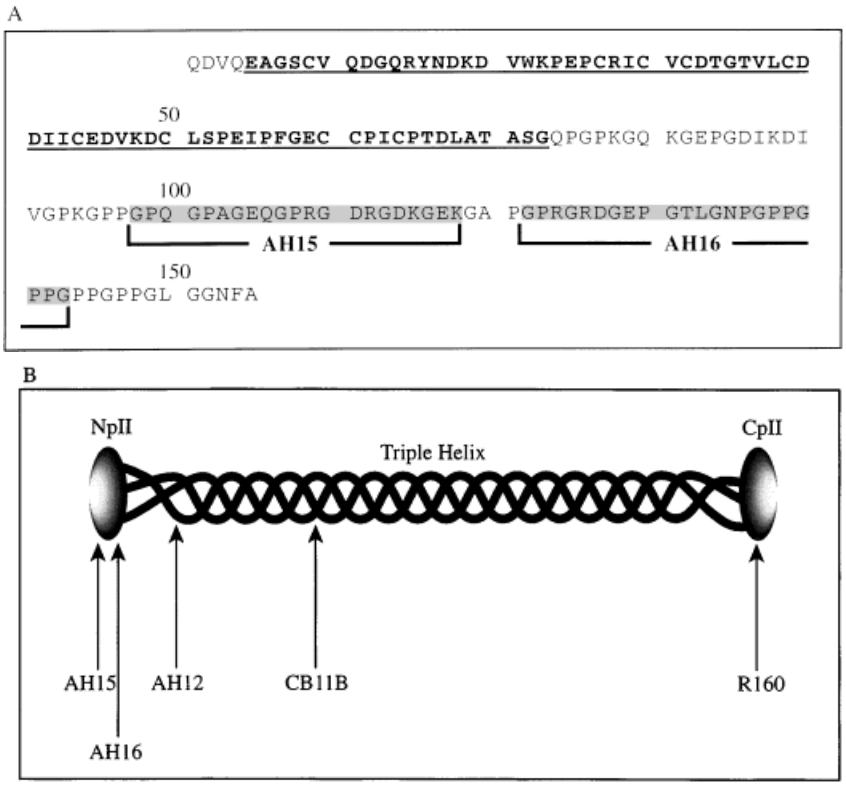

Fig. 1. Identity and location of immunoepitopes. **A:** Amino acid sequence of human type II collagen N-propeptide. Underlined region, additional sequence contained in type IIA collagen but absent from type IIB. The location of peptide epitopes AH15 and AH16 is shown. **B:** Diagrammatic representation of a type II collagen molecule showing the epitopes recognized by various analytical antibodies. CB11B and AH12 are helical epitopes that are exposed on denaturation of the molecule that can be detected using antibodies CoI2–3/4m and AH12L3, respectively. They can be used to detect both pro-type II collagen and the fully processed molecule. AH15 and AH16 (A) are recognized by antibodies AH15N1 and AH16N2, respectively, which can be used to detect the procollagen or free N-propeptide (NpII). R160 is an antibody raised to the whole, purified, C-propeptide (CpII), which can be used to detect procollagen or free CpII.

FMOC chemistry, by Dr. A. Moir, Kreb's Institute, University of Sheffield, UK. They were each made with an additional N-terminal cysteine for conjugation to keyhole limpet hemocyanin (KLH; Calbiochem-Novabiochem Corporation, San Diego, CA). Each peptide was coupled to KLH through its N-terminal cysteine, using the coupling reagent bromoacetic acid N-hydroxysuccinimide ester (Sigma-Aldrich Chemical Co., Poole, Dorset, UK) as previously described [Hughes et al., 1992]. New Zealand white rabbits were immunized subcutaneously with KLH-conjugated peptide, 500 µg per animal, dissolved in phosphate-buffered saline (PBS), and emulsified with an equal volume of complete (first immunization) or incomplete (subsequent immunizations) Freund's adjuvant (Sigma). Blood samples were taken after the fourth immunization and tested concurrently with preimmune sera from the same animals for reactivity with specific epitopes by direct binding enzyme-linked immunosorbent assay (ELISA), as described below.

Direct Binding ELISA

Peptides AH15 and AH16 were prepared in carbonate buffer, pH 9.2, at a concentration of 40 µg/ml, and 50 µl was added to each well of Immulon-2 ELISA plates. The peptides were allowed to passively adsorb to the bottom of the wells at 4°C for 72–96 h, before removal of unbound material with three washes of PBS containing 0.1% v/v Tween 20 (PBS-Tween; Sigma). The wells were blocked with PBS containing 1% w/v BSA (PBS-BSA; Sigma), at 50 µl per well for 30 min at room temperature, before a final wash in PBS-Tween. Test antisera were added to the coated plates at various dilutions in PBS. After incubation at 37°C for 90 min, the plates were washed three times in PBS-Tween. Secondary antibody, alkaline phosphatase-labeled goat-anti-rabbit Ig (Southern Biotechnology Associates, Birmingham, AL), diluted 1:1,000 in PBS containing 1% w/v bovine serum albumin (BSA) and 0.1% v/v Tween 20, was added at 50 µl per well and incubated for a further 90 min at 37°C. Plates were washed as above, with a final wash in distilled water, before the addition of alkaline phosphatase substrate, 0.5 mg/ml disodium p-nitrophenyl phosphate (Sigma), prepared in 8.9 mM diethanolamine, 0.25 mM $MgCl_2$ pH 9.8, 50µl per well. After incubation at ambient temperature for 10–20 min, absorbance was measured at 405 nm on an MRX microplate reader (Dynatech Labs, Billinghurst, West Sussex, UK).

Purification of IgG From Rabbit Anti-Peptide Antibodies

Rabbit antisera were diluted 1:4 in 20 mM sodium-acetate buffer (pH 5.0); 15 ml of this solution was applied to a 5-ml protein G-Sepharose 4 Fast Flow column (Pharmacia Biotech, St. Albans, UK). The flow rate was maintained at 1 ml/min throughout the procedure. An ultraviolet (UV) detector linked to the column outflow was used to monitor protein elution at 280 nm. When all unbound protein had been flushed through the column with sodium-acetate buffer, bound IgG was eluted with 0.1 M glycine-HCl (pH 2.7), collected into 2-ml fractions. These were neutralized at the point of collection with 1–2 drops of 1 M Tris-HCl (pH 9.0). The absorbance of the fractions was determined at 280 nm on a spectrophotometer (Perkin-Elmer Ltd., Beaconsfield, Bucks, UK), and those containing the protein peak were pooled. The IgG was extensively dialysed against PBS and centrifuged to remove any insoluble IgG precipitate, followed by a final determination of IgG concentration by measurement of the absorbance at 280 nm. Aliquots were stored at −20°C.

Preparation of Bovine Vitreous

Eyes were removed from adult cows, shortly after death at a local abattoir. Type II collagen α-chains with or without propeptides still attached were extracted and purified as described previously [Bishop et al., 1994].

Western Immunoblotting Analysis of Type II Procollagen

Samples of bovine vitreous extract, chondrocyte-conditioned medium, and chondrocyte pellets were separated on 6.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels under reducing conditions according to Laemmli [39], and the proteins were transferred to a nitrocellulose membrane for 2 h at 0.25 A. The membrane was then blocked with PBS containing 3% BSA (PBS-BSA-3) for 30 min and washed once in PBS-Tween. The transferred proteins were probed with rabbit antibodies AH15N1, AH16N2, AH12L3 or R160, each prepared at an optimal dilution with PBS-BSA-3, for 2 h at ambient temperature. The final dilutions were 1:10,000 (AH15N1 and AH16N2), 1:10,000 (AH12L3), and 1:3,000 (R160). The membranes were washed three times with PBS-Tween and then incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG, IgM and IgA diluted 1:1,000 in PBS-BSA for 30 min at room temperature. After a further three washes and a final rinse with distilled water, the membranes were incubated with an alkaline phosphate substrate kit (BioRad Laboratories Ltd., Hemel Hempstead, UK), a solution of 5-bromo-4-chloro-3-indolyl phosphate, and nitroblue tetrazolium. After optimal color development, the reaction was stopped by rinsing the membrane in distilled water.

Inhibition ELISA for the Type II Collagen N-Propeptide

The antibody AH15N1 was used to set up an inhibition ELISA for the quantification of type II collagen amino-propeptide. Round-bottomed microtiter plates were used for preincubations. They were pretreated with 100 µl/well of PBS-BSA for 30 min at ambient temperature and washed once with PBS-Tween. The outermost wells were not used to minimise the effects of evaporation. There were six nonspecific binding wells on each plate, which each contained 100 µl of 50 mM Tris-HCl, pH 7.6 (Tris). Maximum binding in the absence of inhibitory epitope was assayed in six wells each containing 50 µl Tris plus 50 µl of polyclonal IgG-purified antibody AH15N1 (diluted 1 in 250 in Tris). Detectable but inhibitable levels of binding were assayed in wells containing 50 µl AH15N1 antibody plus 50 µl standard (the immunising peptide AH15) in concentrations ranging from 0.09 to 200 µM. Samples were assayed in test wells containing 50 µl sample and 50 µl antibody. All standards and samples were tested in duplicate. The plates were sealed with parafilm and incubated in a humidified chamber at 37°C overnight. A multichannel pipette was used to transfer 50 µl of each preincubated sample to the equivalent well of an Immunol-2 ELISA plate, coated with 2 µg/well AH15 peptide and blocked with PBS-BSA as described above. The ELISA plates were incubated for 30 min at room temperature and then washed three times with PBS-Tween. Second antibody, alkaline phosphatase-conjugated goat antirabbit IgG, IgM and IgA diluted 1:1,000 in PBS-BSA-3 was added at 50 µl per well and incubated for 2 h at 37°C. The plates were then washed three times with PBS-Tween and once with distilled water. Alkaline phosphate substrate, disodium p-nitrophenyl phosphate, was prepared fresh

at 0.5 mg/ml in 8.9 mM diethanolamine, 0.25 mM $MgCl_2$, pH 9.8, and 50 µl was added to each well for 5 min at room temperature. The absorbance was measured at 405 nm on an MRX microplate reader.

Determination of Type II Collagen Content

Type II collagen in the bovine vitreous extract was isolated by digestion with 1 mg/ml proteinase K (EC 3.4.21.64; Sigma) at 56°C for 15 h. The extracts were assayed by inhibition ELISA, using a mouse IgG monoclonal antibody to denatured type II collagen, COL2–3/4m, exactly as previously described [Hollander et al., 1994; Kozaci et al., 1997].

RESULTS

Antibody Production and Characterization

Antibody AH15N1 was raised to peptide AH15 and antibody AH16N2 to peptide AH16. IgG was purified from each antiserum as described under Materials and Methods and characterized by direct binding ELISA. Each antibody recognized its own immunizing peptide epitope in a dilution-dependent manner, but neither cross-reacted with the other peptide epitope (Fig. 2).

In Western immunoblotting experiments, antibody AH15N1 was found to recognize type II procollagen in bovine vitreous (Fig. 3B), whereas it did not bind to fully processed type II collagen, the presence of which could be detected separately using antibody AH12L3 (Fig. 3A). Antibody AH15N1 detected four positive bands in the vitreous extract. Preincubation of the antibody with 100 µg/ml of peptide AH15 abolished this immunoreactivity (not shown), indicating that the detection was specific. These bands have been described previously [Bishop et al., 1994] and can be designated as full-length type IIA procollagen (pIIA), full-length type IIB procollagen (pIIB), and type IIA and B collagens lacking the C-propeptide but retaining the N-propeptide (pNIIA and pNIIB).

Antibody AH16N2 only detected bands in the bovine vitreous preparation very weakly (not shown), despite the fact that it reacted well with its immunizing peptide (Fig. 2). Peptides AH15 and AH16 were originally designed on the basis of the human type II procollagen sequence; the bovine type II procollagen sequence is not available. It is likely that the amino acid sequence recognized by the antibody is altered in the bovine protein. Therefore, all further experiments were carried out using antibody AH15N1.

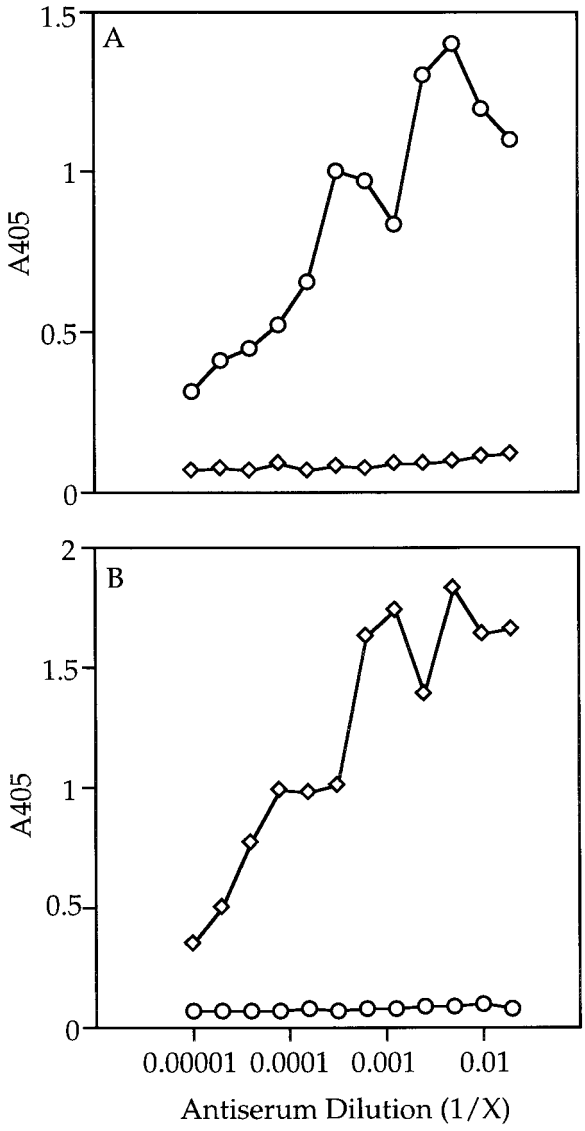

Fig. 2. Direct-binding enzyme-linked immunosorbent assay (ELISA) analysis of the specificity of two new anti-peptide antibodies to NpII epitopes. **A:** Antibody AH15N1. **B:** Antibody AH16N2. The binding of antibody to immobilized antigen is shown for peptide AH15 (*circles*) and peptide AH16 (*diamonds*).

Western Immunoblotting of N-Propeptides in Chondrocyte Pellet Cultures

Cell layers and conditioned medium from chondrocyte pellet cultures were examined separately by Western immunoblotting for the presence of type II procollagen molecules retaining the N-propeptide. A typical result from five

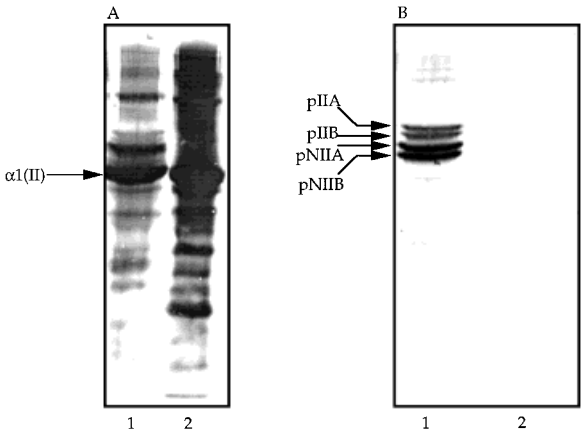

Fig. 3. Western immunoblotting analysis of type II procollagen in bovine vitreous. **A:** Detection of type II collagen α-, β-, and γ-chains using antibody AH12L3. **B:** Detection of procollagen molecules using antibody AH15N1. **Lane 1**, bovine vitreous; **lane 2**, heat-denatured type II collagen. The four forms of type II procollagen detected in vitreous extract are described in more detail in the text. In both cases, the samples were separated by 6.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) before electrotransfer.

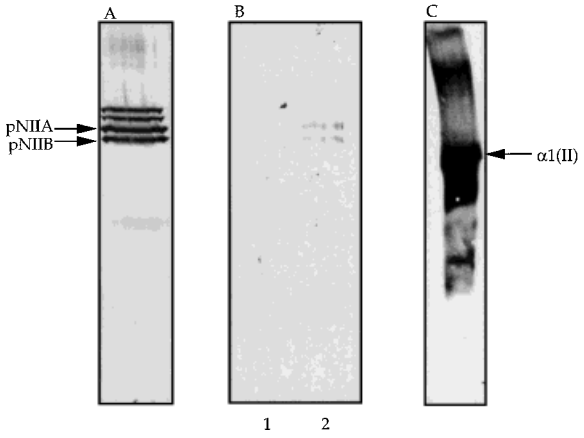

Fig. 4. Western immunoblotting detection of the type II collagen N-propeptide using antibody AH15N1. **A:** Bovine vitreous extract. **B:** Bovine chondrocyte pellet cultures. **Lane 1**, conditioned medium; **lane 2**, cells + matrix. **C:** Type II collagen α-, β-, and γ-chains detected using antibody AH12L3. In all cases the samples were separated by 6.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) before electrotransfer.

separate cultures is shown in Figure 4. Two bands were weakly detected in the cell layer, using antibody AH15N1, while none was detected in the tissue culture medium (Fig. 4B). The two bands had an apparent molecular mass greater than that of fully processed type II collagen (Fig. 4C); indeed, they co-migrated with pNIIA and pNIIB in bovine vitreous extract (Fig. 4A). These results suggest that adult bovine articular chondrocytes can synthesize both type IIA and type IIB collagen in the pellet culture system. Since the free (cleaved) N-propeptides A and B have a molecular mass of approximately 15 kDa and 8 kDa, respectively, it is unlikely that these products would be large enough to be detectable by SDS-PAGE separation and Western blotting.

Western Immunoblotting of C-Propeptides in Chondrocyte Pellet Cultures

Cell layers and conditioned medium from the same chondrocyte pellet cultures as described above were examined separately by Western immunoblotting for the presence of type II procollagen molecules retaining the C-propeptide, as well as for free CpII, which has a molecular mass of approximately 35 kDa, making it large enough to be detected on high-density gels. A typical result from the five separate cultures is shown in Figure 5. A single band migrating more slowly than fully processed type II collagen was clearly detected in the cell layers but not in the tissue culture medium, using antibody R160 (Fig. 5B,C). This band presumably corresponds to type II procollagen retaining the C-propeptide. A band co-migrating with one of two bands in the purified standard free CpII was detected weakly in both cell layers and

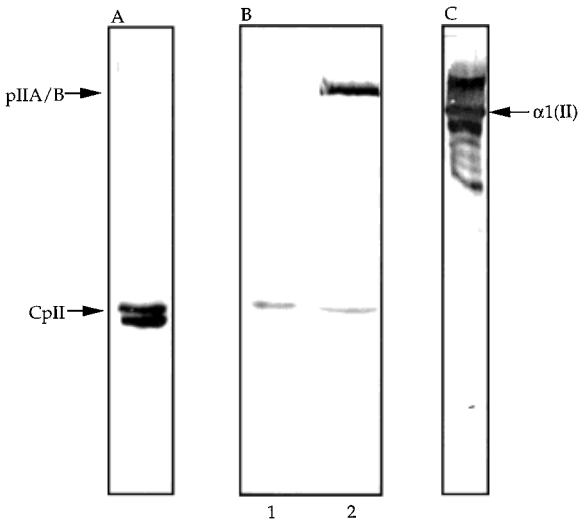

Fig. 5. Western immunoblotting detection of the type II collagen C-propeptide using antibody R160. **A:** Purified free C-propeptide (CpII). **B:** Bovine chondrocyte pellet cultures: **Lane 1**, conditioned medium; **lane 2**, cells + matrix. **C:** Type II collagen α- and β-chains detected using antibody AH12L3. In all cases, the samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) before electrotransfer.

conditioned medium (Fig. 5A,B), using antibody R160. The lower band in standard CpII purified from bovine fetal cartilage was observed previously [Choi et al., 1983]. Its absence in our cultures may be because we used adult bovine cartilage. These observations suggest that procollagen is retained within the extracellular matrix, but free propeptides are released.

Immunoassay of N-Propeptides in Chondrocyte Pellet Cultures

It was important to determine whether the type II collagen N-propeptides were released into the tissue culture medium intact or degraded. Since Western immunoblot analysis of these relatively small propeptides was not possible, we analyzed the tissue culture medium by setting up an inhibition ELISA using antibody AH15N1 and peptide AH15 as a standard. The ELISA standard curve was linear over the range 0.09–200 μM (Fig. 6). Bovine vitreous extract pooled from 25 animals was used as a positive control in the assay, while its type II collagen was measured, for comparison, using antibody Col2–3/4m in an inhibition ELISA. Both NpII and type II collagen itself were detected in the pooled extract, although there was approximately 91 times less propeptide than collagen (Table I). When conditioned tissue culture media from 10 separate chondrocyte pellet cultures were analyzed by immunoassay, no NpII could be detected (Table I). It remains possible that the medium contained low levels of NPII, below the detection limit of the assay.

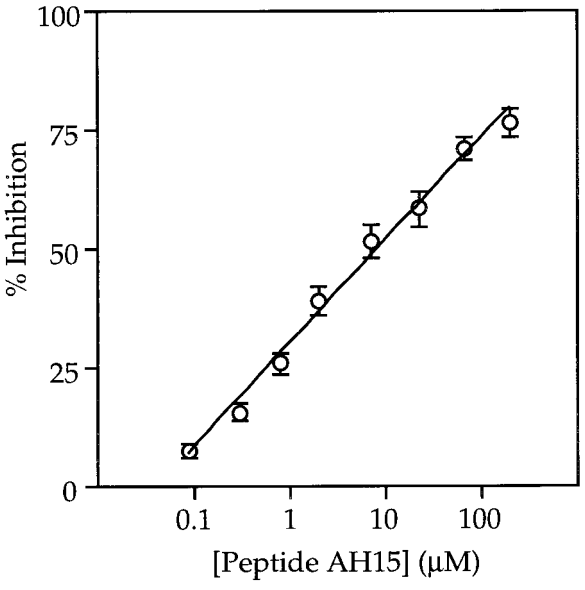

Fig. 6. Standard curve for the detection of type II collagen N-propeptide by inhibition enzyme-linked immunosorbent assay (ELISA), using antibody AH15N and peptide epitope AH15. Results are the mean ±SEM of five separate experiments. Correlation coefficient: r = 0.94, $P < 0.01$.

DISCUSSION

The data reported here suggest that CpII and NpII are metabolized differently to each other in the chondrocyte pellet culture system. Type II collagen bearing CpII is clearly retained in the cell layer, whereas only free CpII is detected in the culture medium. Type II collagen bearing NpII is also retained in the cell layer, although by Western immunoblotting analysis there appeared to be somewhat less of this form of the molecule. Furthermore, free NpII could not be detected by inhibition ELISA using antibody AH15N1. Since CpII and NpII are synthesized in a 1:1 stoichiometric ratio, it is clear that the apparently low levels of epitope AH15 in both cell layer and medium must result from relatively rapid degradation of NpII compared with CpII.

The C-proteinase that removes the C-propeptide from types I and II collagen has been identified as being the same molecule as bone morphogenetic protein-1 [Kessler et al., 1996; Li et al., 1996]. The N-proteinase that removes the N-propeptide from types I and II collagen was recently cloned, expressed, and characterized [Colige et al., 1997]. Both enzymes are zinc-dependent metalloproteinases that are capable of binding to chondrocytes as well as to extracellular matrix proteins [Prockop et al., 1998]. Their synthesis and regulation have not yet been studied well in chondrocytes. It is also unclear which proteinases might result in the

TABLE I. Type II Collagen Metabolism Assays*

Sample	*Np*II (μM)	Type II collagen (μM)
Pooled bovine vitreous	0.31	28.33
Articular chondrocyte conditioned media (n = 10)	<0.09	ND

ND, not determined.

*Type II collagen synthesis was measured as *Np*IIA and *Np*IIB and total type II collagen was measured as epitope CB11B, each by inhibition ELISA as described under Materials and Methods. The lower detection limit for assay of *Np*II was 0.09 μM.

further processing of free propeptides to small fragments after cleavage from the α-chain. The pellet culture system described here, combined with the use of antibodies to N- and C-terminal propeptides and selective proteinase inhibitors, should allow such regulatory studies to be conducted.

Our data also have implications for the development of immunoassays for in vitro work or for clinical studies. Clearly, the AH15N1 inhibition ELISA is not adequate for quantitative studies in our culture model, since epitope AH15 seems to be rapidly degraded. It is likely that small fragments of NpII will survive in the culture medium, and they could be isolated and used to generate new antibodies that will be more effective as reagents for immunoassays. Such assays might also be used to measure NpII in human serum samples in order to detect changes in disease [Shinmei et al., 1993; Carey et al., 1997; Poole, 1994].

Ryan et al. [1990] made the important observation that exon 2 of the COL2A1 gene may be spliced in or out. Chondrocytes from adult articular cartilage splice exon 1 directly to exon 3, resulting in a short NpII domain (type IIB collagen). Juvenile and fetal chondroprogenitor cells splice exon 2 between exons 1 and 3, resulting in the insertion of an additional 69 amino acids in the NpII domain (type IIA collagen) [Sandell et al., 1991, 1994b]. The two procollagens are converted to identical forms of extracellular type II collagen, but different free N-propeptides are generated: NpIIA and NpIIB. The differentially expressed domain includes a series of 10 cysteine residues that are conserved across the fibrillar pro-collagens, as well as in von Willebrand factor and thrombospondin [Ryan and Sandell, 1990]. Type IIA collagen mRNA is expressed not only in chondroprogenitor cells but by cells from a variety of nonchondrogenic fetal tissues during development as well [Sandell et al., 1994a; Hyun-Duck and Upholt, 1991]. Type IIA procollagen protein has been detected in cultures of human juvenile costal chondrocytes, indicating that the mRNA can be transcribed [Sandell et al., 1991]. In adults, the only cells found to express mRNA for type IIA collagen are chondrocytes from the articular cartilage and osteophytes of osteoarthritis patients [Reife et al., 1994]. Normal adult articular chondrocytes do not synthesize type IIA collagen. In the present study, using isolated adult bovine chondrocytes, we have identified two bands of type II procollagen containing the N-propeptide. Their migration positions on SDS-PAGE indicate that they are most likely procollagen IIA and IIB, lacking the C-propeptide, although definitive proof of this would require generating enough of each protein for N-terminal sequencing. These observations indicate that our pellet culture system combined with the use of our antibody to NpII could be used to study the differential regulation of these two forms of collagen under varying culture conditions.

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