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Cartilage oligomeric matrix protein (COMP) is an abundant component of tendon

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Abstract An abundant matrix protein was purified under native conditions from adult bovine tendon and identified as cartilage oligomeric matrix protein (COMP) by immunochemical crossreaction, amino acid sequence identity of tryptic peptides derived from both N- and C-terminal regions, and structure revealed by electron microscopy. Immunohistochemistry showed age-dependent differences in distribution of COMP in tendon.

Key words: COMP; Thrombospondin; Extracellular matrix; Aging; Bovine tendon

1. Introduction

Tendons function to transmit the force of muscle contraction to bone and are composed of collagen, proteoglycans, non-collagenous proteins, and a relatively few cells. The major molecular constituent is type I collagen which makes up 80–90% of the dry weight. The collagen molecules combine in a quarter-stagger arrangement into microfibrils, which are laterally joined into subfibrils and fibrils. This structural organization of the collagen molecules infers high tensile strength to the tissue. Proteoglycans and non-collagenous proteins in the tendon matrix may serve to regulate collagen fibril size, as links between the collagen fibrils and/or other matrix constituents, and in mediating cell–matrix interactions. In areas where tendons pass over an articular joint, large compressive stresses are produced. These parts show a cartilage-like phenotype, including the expression of the large cartilage proteoglycan aggrecan [1].

Cartilage oligomeric matrix protein (COMP), a pentameric non-collagenous glycoprotein, has recently been isolated and characterized in a native form from the Swarm rat chondrosarcoma [2] as well as from bovine [3,4] and human articular cartilage [5]. Sequence determination of COMP revealed it to be a member of the thrombospondin protein family [6]. COMP has been found in a variety of cartilages but could not be detected in a panel of extracts of other tissues when analysed by inhibition enzyme linked immunosorbent assay (ELISA), leading to the assumption that COMP is a cartilage-specific matrix protein [3].

2. Materials and methods

Adult bovine tendon (flexor tendons which pass over the fetlock joint, 200 g wet weight) was obtained fresh from the slaughterhouse. Tendon pieces were homogenized on ice with a Polytron homogenizer at full speed in (500 ml) prechilled neutral salt buffer 0.15 M NaCl, 50 mM Tris-HCl, pH 7.4, containing the proteinase inhibitors 1 mM phenylmethanesulfonyl fluoride (PMSF), 2 mM *N*-ethylmaleimide (NEM) and 0.025 mg/ml leupeptin. The homogenate was centrifuged for 30 min at 8,000 rpm in a Beckman JA 20 rotor at 4°C. The supernatant was saved and the pellet resuspended in the same buffer by brief homogenization on ice. The sequential extractions were all performed with proteinase inhibitors and consisted of two extractions with neutral salt buffer and two extractions with neutral salt buffer containing, in addition, 10 mM EDTA.

The first extract obtained with EDTA-containing buffer was diluted with an equal volume of cold distilled water and immediately applied to an ion exchange column of (50 × 2.6 cm) of DEAE Sepharose (Pharmacia) equilibrated at 4°C with 10 mM Tris-HCl, pH 7.4, containing 10 mM EDTA, 1 mM PMSF, and 2 mM NEM. After washing with equilibration buffer, the column was eluted with a linear gradient from 0 to 0.5 M NaCl (1000 ml) in the same buffer. COMP-containing fractions were pooled, concentrated by ultrafiltration (YM 10 filter, Amicon) and applied to a gel filtration column (100 × 2.6 cm) of Sepharose CL 4B (Pharmacia) eluted with 0.15 M NaCl, 50 mM Tris-HCl, pH 7.4, containing 2 mM EDTA, 1 mM PMSF, and 2 mM NEM. COMP-containing fractions were identified by SDS-PAGE, pooled, and further purified by separation on a Superose 6 column eluted in the same buffer.

SDS-PAGE was performed according to the protocol of Laemmli [7]. Reduction of disulfide bonds was achieved by addition of 7 μl/ml 2-mercaptoethanol to the sample buffer. Purified bovine tendon COMP was submitted to SDS-PAGE under reducing conditions and electrophoretically transferred to an Immobilon-P^{SQ} membrane (Millipore). The band corresponding to COMP subunits was cut out, digested with trypsin and fragments sequenced on an Applied Biosystems sequencer after separation by microbore reversed-phase HPLC.

Antisera against COMP, purified from bovine and human articular cartilage and Swarm rat chondrosarcoma, as well as an antiserum against bovine type I collagen, were raised in rabbits. ELISA was performed in plates coated with purified bovine tendon or articular cartilage COMP at 5 μg/ml. Serial dilutions of rabbit antisera to bovine, human, or rat COMP, and bovine type I collagen were added and binding detected using a secondary antibody to rabbit IgG conjugated with peroxidase (DAKO) and 5-amino-2-hydroxy benzoic acid as a substrate. Immunohistochemistry was done on chondroitinase ABC-treated cryosections using the antiserum to bovine COMP and a peroxidase-coupled second antibody [8].

3. Results and discussion

An extract of bovine tendon, obtained with EDTA-containing buffer, contained enriched amounts of a protein with similar electrophoretic mobility and sensitivity to reduction as COMP (apparant molar mass 450 kDa before and 110 kDa after reduction), a major matrix protein of bovine articular cartilage. This protein was isolated by sequential ion exchange chromatography and gel filtration (Fig. 1). ELISA titration of

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the purified bovine tendon protein showed strong recognition by antisera both to human and bovine cartilage COMP, and to a lesser, but significant, extent by an antiserum to Swarm rat chondrosarcoma COMP. The antiserum to bovine cartilage COMP showed the same affinity in ELISA titration to the tendon protein as to purified bovine cartilage COMP (Fig. 2). Final confirmation of the identity of tendon COMP was achieved by amino acid sequence analysis from one N- and two C-terminal tryptic fragments of tendon COMP (Fig. 3). The three peptide sequences of bovine tendon COMP showed

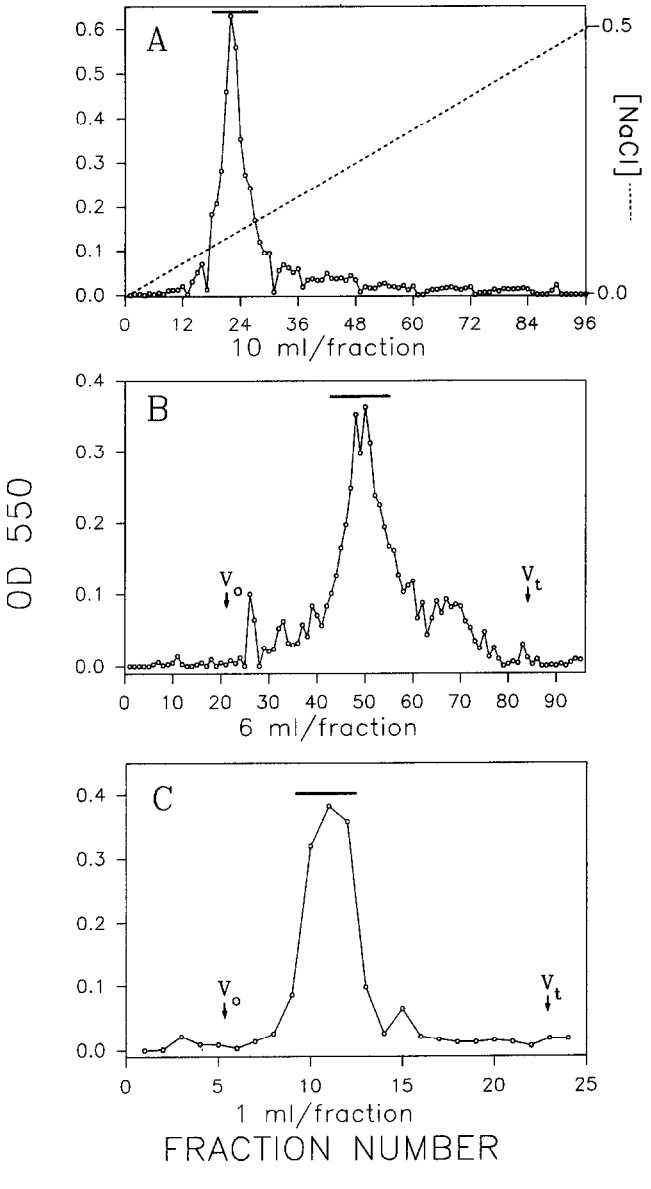

Fig. 1. Purification of bovine tendon COMP. The first EDTA containing extract was applied to a DEAE-Sepharose column (A) and eluted with a linear gradient of 0–0.5 M NaCl. COMP containing fractions (bar) were pooled, concentrated by ultrafiltration, and passed over a Sepharose CL-4B molecular sieve (B). The COMP pool (bar) from the CL-4B chromatography was further concentrated and rechromatographed on FPLC Superose 6 HR 10/30 (C) molecular sieve. Purified COMP (bar) from tendon had the same electrophoretic motility as COMP from both bovine articular cartilage and Swarm rat chondrosarcoma (not shown).

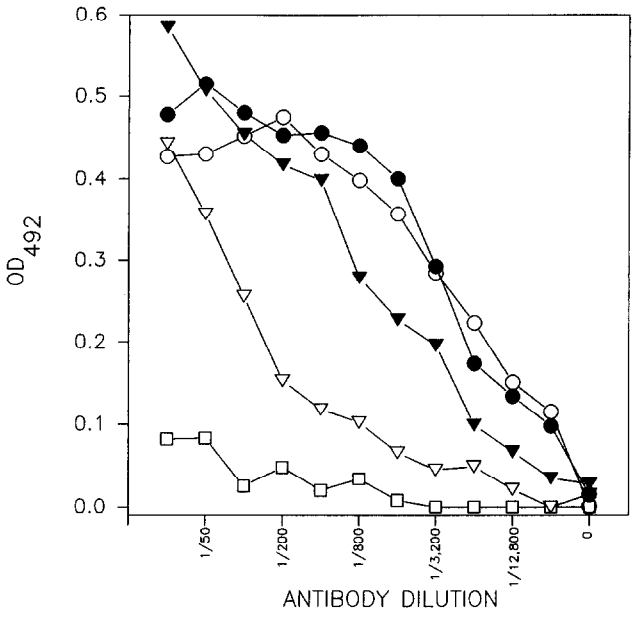

Fig. 2. ELISA titration of purified tendon COMP and COMP from bovine articular cartilage. All antibodies to cartilage COMP recognized tendon COMP. Antibodies to bovine cartilage COMP recognized bovine tendon COMP with the same affinity as their original antigen. Polyclonal antibodies to bovine (○) articular cartilage COMP (anti-BAC) on bovine articular cartilage COMP; (●) anti-BAC to tendon COMP (T-COMP); (▼) antibodies to human articlar cartilage COMP on T-COMP; (▽) antibodies to Swarm rat chondrosarcoma COMP to T-COMP; (□) antibodies to type I collagen on T-COMP.

marked homology to the corresponding sequences of rat and, when known, bovine COMP [6]. Electron microscopic evaluation of bovine tendon COMP revealed particles identical to those of bovine articular cartilage COMP [4] with five globular end units connected by thin flexible strands to a central domain (not shown). Immunostaining on cryosections of adult and fetal bovine tendon with antibodies to bovine cartilage COMP showed intense staining in the areas around the collagen fibers and the connective tissue surrounding the tendon bundles (Fig. 4). In adult tendon an increased punctuate staining in the areas around collagen fibers was observed.

The protein purified from tendon agreed in electrophoretic mobility, immunological reactivity, electron microscopic structure, and amino acid sequence with bovine articular cartilage derived COMP. We therefore conclude that this tendon protein is identical to cartilage COMP and thus represents another constituent of the cartilaginous extracellular matrix, in addition to aggrecan, which is present in tendon.

Tendon extracellular matrix consists predominantely of type I collagen, but non-collagenous proteins are present and may play important structural and/or functional roles. Tendons appear to express a cartilage-like phenotype particularly when subjected to compressive loads [1]. The nature of the phenotypic change is not well understood but believed to be due to either a proliferation of cartilage-like cells or a switch in synthetic expression of the tendon fibroblasts. It is probable that synthesis of a cartilage-like matrix provides a structural modification in the tendon to enable it to adapt to altered mechanical requirements.

Fragment 1:

rat cartilage COMP	... - R^{54} - V - K - E - I - T - F - L - K - N - T - V^{65} - ...
bov. tendon COMP	...- E - I - T - F - L - K - ...

Fragment 2:

rat cartilage COMP	... - S^{679} - Y - R - W - F - L - Q - H - R - P - Q - V - G - Y - I - R^{694} - ...
bov. cartilage COMP	... - S - Y - R - W - F - L - Q - H - R - P - Q - V - G - Y - I - R - ...
bov. tendon COMP	... - W - F - L - Q - H - R - G - Q - V - G - ...

Fragment 3:

rat cartilage COMP	... - R^{694} - V - R - F - Y - E - G - P - E - L - V - A - D - S - N - V - V - L - D - T - A^{714} - ...
bov. cartilage COMP	... - R - V - R - F - Y - E - G - P - E - L - V - A - D - S - N - V - I - L - D - T - T - ...
bov. tendon COMP	... - F - Y - E - G - P - E - L - V - A - D - X - N - V - I - L - ...

Fig. 3. Amino acid sequence of tryptic fragments of bovine tendon COMP. The protein sequences of the three tryptic fragments for bovine tendon COMP are highly homologous with the corresponding cDNA-derived sequences for rat and, when available, bovine cartilage COMP. Pro^{688} (rat cartilage COMP) in the cDNA derived sequences of rat and bovine cartilage COMP was detected as Gly in the protein sequence of bovine tendon COMP. In the protein sequence of fragment 3 no amino acid derivative was recovered in the step corresponding to Ser^{707} in the rat sequence indicating that this residue carries an O-linked oligosaccharide. The numbering of residues in the rat sequence includes the signal peptide.

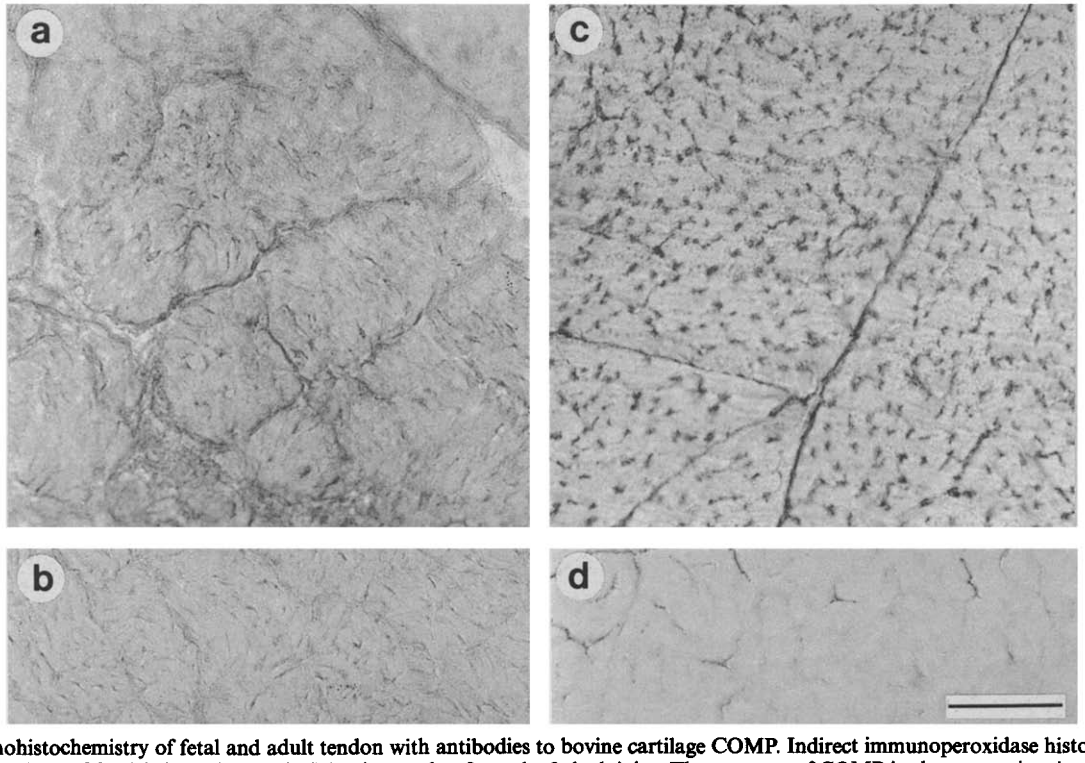

Fig. 4. Immunohistochemistry of fetal and adult tendon with antibodies to bovine cartilage COMP. Indirect immunoperoxidase histochemistry was done on cryosections of fetal (a,b) and adult (c,d) bovine tendon from the fetlock joint. The presence of COMP in the connective tissue surrounding tendon bundles and in a punctuate manner between the collagen fibers is clearly detected (c). The staining both around and within tendon bundles is seen also in fetal tendon (a), but is more diffuse. Control sections were treated with preimmune serum at the same dilution (b,d). Bar = 50 μm.

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