INTRODUCTION

The collagen framework of hyaline cartilage is a highly cross-linked unique heteropolymer. In essence the bulk type II collagen is polymerized on a template of type XI collagen and type IX collagen covalently decorates the surface type II molecules of nascent fibrils. All three collagen subunits, II, IX, and XI, are heavily cross-linked in the same fibril through a lysyl oxidase-mediated mechanism (1). In adult human articular cartilage, type III collagen can also be detected (2-4). In a study to understand better the structural role of type III collagen in cartilage, we find that type III collagen molecules are present in the extracrystalline matrix of adult human articular cartilage as covalently cross-linked polymers extensively cross-linked to type II collagen.

METHODS

Human knee joints were obtained from the Northwest Tissue Bank from donors aged 20-75. Articular cartilage was sliced from the load-bearing surfaces of the femoral chondyles and from an equivalent site in a 3 yr cow (bovine) knee. Minced tissue was extracted in 4M guanidine HCl, 0.05M Tris-HCl, pH 7.4 containing protease inhibitors, at 4°C for 24h to remove proteoglycans and other matrix proteins. The guanidine-insoluble tissue residue was then washed thoroughly with water and freeze-dried. Cross-linked collagens were solubilized by digesting the washed residue with pepsin at 4°C. Different collagen fractions were then precipitated from the acid solution at 0.7M, 1.2M and 2.0M NaCl (5). Collagen fractions were analyzed by SDS-PAGE and Western blotting using two mouse monoclonal antibodies to human type III collagen. MAb 4G9 is specific to a conformation epitope in the globular domain of the N-propeptide. MAb 2C3 is specific to a prolylcyte neoepitope at the C-terminus of the α1(III) N-telopeptide sequence YDVKSGVAVGG, where K is a cross-linked lysine.

RESULTS

Using interrupted SDS-PAGE we were able to identify type III collagen in pepsin-solubilized material from most adult articular cartilage samples examined (Fig.1). Chain identities, indicated by their migration on SDS-PAGE, were established beyond doubt by in-gel trypsin digestion, and micobore LC/mass spectrometry with database matching and by N-terminal protein sequence analysis.

Fig. 2 shows a Western blot analysis using mAb 2C3 to probe type II collagen chains in extracts of adult human and bovine articular cartilage for covalently attached type III collagen N-telopeptide. The 2C3 antibody is specific to human collagen III and does not cross-react with bovine collagen. The results reveal that α1(III) N-telopeptide can be detected on α1(II) chains. In addition to the signal from the main α and β chains of type II collagen in the pepsin digest, 2C3 also binds to a band at 160 kDa not visible by Coomassie staining (Fig. 2). This band also reacted with mAb 4G9 and mAb 1C10. From these properties, this component appears to be an α1(II) chain cross-linked to an α1(III) N-telopeptide that still has an α1(III) N-propeptide trimer domain attached. Presumably this reflects a pepsin partial cleavage product from the matrix. The findings also imply that relatively large amounts of the N-propeptide domain of type III collagen are present in the extracrystalline matrix of adult cartilage. The presence of collagen III N-propeptides in articular cartilage was confirmed by mAb 4G9 ELISA assay of molecular-sieve column fractions eluted from a CNBr digest of the 4M guanidine HCl insoluble residues of adult cartilage.

DISCUSSION

The results reveal a pool of type III collagen covalently linked to type II collagen in articular cartilage of adult joints. The site of linkage is likely to be the C-helix (K930) of α1(II) to the α1(III) N-telopeptide cross-linking lysine. In earlier work we showed that collagen III also forms cross-links to type II collagen C-telopeptides (3). In skin and other tissues immunogold electron microscopy showed that collagen III with retained N-propeptides is present on the surface of type I collagen fibrils (6). Similar findings were also reported for human articular cartilage (4). Taken together, type III collagen molecules accumulate in mature human articular cartilage cross-linked to the surface of type II collagen fibrils. The amount presumably varies between individuals, sampling site and tissue microanatomy dependent on history of injury and wear and tear of normal joint function, trauma and perhaps aging. Collagen III content seems to be significantly higher in OA cartilage. It is known that type III collagen is prominent in fibrous repair tissue in skin and other tissues. Therefore, it is possible that type III collagen is synthesized as a modifier of existing fibril networks in response to tissue and matrix injury.

REFERENCES


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