Characterization of Collagen Types XII and XIV from Fetal Bovine Cartilage*

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The structurally related type XII-like collagen molecules TL-A and TL-B were recently identified in fetal bovine epiphyseal cartilage and subsequently shown to be collagen types XII and XIV, respectively. By indirect immunofluorescent staining of cartilage using monoclonal antibodies to the NC3 domains of each molecule, it was shown that type XII collagen was present predominantly around cartilage canals, the articular surface, subperichondrial margins, and the perichondrium, was less so in the remaining cartilage matrix, and was absent from the growth plate region. In the permanent cartilage of trachea, type XII stained somewhat more intensely in the margins beneath the loose connective tissue. Type XIV collagen localized more uniformly throughout the articular cartilage and was also absent from the growth plate region, whereas in tracheal cartilage, its distribution was similar to type XII. We have characterized the structure of these cartilage molecules and compared them with those from fetal bovine skin. Extraction of cartilage with 1 M NaCl and differential NaCl precipitation yields a fraction enriched for these two collagens. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting with monoclonal antibodies to the large amino-terminal non-triple-helical domain, NC3, revealed the presence in cartilage of two forms of type XII collagen: type XIIB, the molecule previously identified in chick and bovine tissues, and type XIIA, a much larger form equivalent to the molecule recently identified in WISH-transformed epithelial cell culture medium (Lunstrum, G. P., McDonough, A. M., Marinkovich, M. P., Keene, D. R., Morris, N. P., and Burgeson, R. E. (1992) *J. Biol. Chem.* 267, 20087–20092). Digestion with bacterial collagenase shows that the increased mass is present in the NC3A domain. Additional purification by velocity sedimentation and observation of rotary-shadowed images demonstrates molecules with extended non-triple-helical arms approximately 80 nm in length analogous to the WISH cell molecules. Electrophoretic mobilities of bands corresponding to type XIIA, but not type XIIB, are sensitive to chondroitinase ABC, indicating that type XIIA is a chondroitin sulfate proteoglycan and that modification occurs predominantly within the NC3A domain distal to NC3B. Neither type XIIB from skin nor type XIIA from WISH cells are chondroitinase-sensitive. By similar analysis, a portion of the type XIV collagen chains in cartilage was also sensitive to chondroitinase digestion. Chondroitin sulfate is apparently not located on its NC3 domain. As in skin, collagen types XII and XIV have subtly different distributions within cartilage and type XII may have a tissue-specific structure.