INTRODUCTION

Type II collagen, the major structural protein of cartilage, is essential for normal embryonic skeletal development and the mechanical properties of articular cartilage. Mutations in the gene Col2a1 cause chondrodysplasia and osteoarthritis in mice and humans. The type II collagen monomer, a homotrimer, is synthesized by chondrocytes as procollagen molecules with extension peptides at the N- and C-terminal ends. The process by which the newly synthesized chains associate and fold in the rough endoplasmic reticulum is not well characterized for type II procollagen.

The Disproportionate micromelia (Dmm) mouse carries a three nucleotide deletion in Col2a1 in the region encoding the C-propeptide resulting in the substitution of one amino acid, Asn, for two amino acids, Lys-Thr, in the wild-type gene (1). This presumably causes a structural alteration in a domain that is highly conserved within type I, II and III collagens. We have used the Dmm mouse to investigate the role of the C-propeptide in the assembly of stable triple helical type II collagen. Here we report on the consequences of this mutation on type II collagen protein expression in the cartilage of homozygous (D/D) and heterozygous (D/+).

METHODS

Tissue acquisition and genotype determination.

Day 19 fetuses were obtained from matings of heterozygous mice. The genotype of the fetuses was determined by PCR amplification of a region of the Col2a1 gene followed by restriction enzyme analysis.

Collagen extraction.

Rib plates from control (+/+), homozygous (D/D) and heterozygous (D/+) mice were prepared. Collagen was extracted from the frozen, acetone-fixed rib cartilage of +/+, D/D, and D/+ mice. Note the absence of extracellular matrix staining and the presence of cell-associated deposits of wild-type and heterozygous mice but absent from that of homozygous mice.

The lack of any type II collagen in the matrix of the homozygotes explains the severity of the matrix pathology which results in neonatal death of the Dmm mouse. The homozygous mouse, however, is viable and shows a normal phenotype at birth. Our finding that stable type II collagen is present in the heterozygote matrix, albeit at reduced levels, suggest that the normal allele is capable of rescuing the phenotypic effects of the Dmm allele. However, the heterozygote does develop mild chondrodysplasia 4 weeks postnatally and joint pathology resembling osteoarthritis (3). Our findings are consistent with an insufficiency of type II collagen in the extracellular matrix of cartilage from the heterozygote during growth.

In this study, we establish the role of C-propeptide binding for proper assembly of type II procollagen molecules. The one for two amino acid substitution in the C-propeptide of the α1(II) collagen chain in the Dmm mouse shortens and alters the net charge in a conserved domain. We speculate that this is sufficient to change the secondary structure and prevent one of the two intrachain disulfide bonds in the C-propeptide from forming. This in turn prevents chain association so that triple-helical molecules cannot assemble from purely mutant chains in the homozygotes. Analysis of procollagen extracts of cartilage supports this conclusion.

RESULTS AND DISCUSSION

Pepsin solubilizes type II collagen from cross-linked fibrils by cleaving telopeptides but leaving the triple-helical domains intact. Figure 1 shows the detection of α1(II) chains from wild-type and heterozygous tissue but not from homozygous mouse cartilage samples. Type II collagen appears, therefore, to be completely absent from the D/D fetal cartilage matrix.

Immunohistochemical localization of type II collagen in rib cartilage using Mab 1C10 (Figure 2) clearly shows that cartilage from the homozygous mutant mouse lacks type II collagen staining in the extracellular matrix (compare D/D with +/+). Cartilage from the heterozygous mouse showed less intense matrix staining for type II collagen than the cartilage of wild-type mice (compare D/+ and +/+). This was even more clear than in the cartilage from the homozygous mouse (compare D/+ and D/D). Type II collagen was detected as patchy deposits only in the cell lacunae of cartilage from the homozygous mouse. Some cells in the cartilage of the heterozygous mouse showed similar staining but this was not evident in the cartilage of the wild-type mouse.

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REFERENCES