Collagen II Containing a Cys Substitution for Arg-α1–519

HOMOTRIMERIC MONOMERS CONTAINING THE MUTATION DO NOT ASSEMBLE INTO FIBRILS BUT ALTER THE SELF-ASSEMBLY OF THE NORMAL PROTEIN*

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A recombinant system was used to prepare human type II procollagen containing the substitution of Cys for Arg at α1–519 found in three unrelated families with early onset generalized osteoarthritides together with features of a mild chondrodysplasia probably best classified as spondyloepiphyseal dysplasia. In contrast to mutated procollagens containing Cys substitutions for obligatory Gly residues, the Cys substitution at α1–519 did not generate any intramolecular disulfide bonds. The results were consistent with computer modeling experiments that demonstrated that the α carbon distances were shorter with Cys substitutions for obligatory Gly residues than with Cys substitutions in the Y position residues in repeating -Gly-Y- sequences of the collagen triple helix. The mutated collagen did not assemble into fibrils under conditions in which the normal monomers polymerized. However, the presence of the mutated monomer in mixtures with normal collagen II increased the lag time for fibril assembly and altered the morphology of the fibrils formed.

Collagen II is the major structural component of cartilage, in that it accounts for about 30% of the dry weight of developing cartilage and about 60% of the dry weight of adult articular cartilage (1, 2). The fibrils of collagen II are distended by the presence of water, proteoglycans, and other matrix components such as collagens IX and XI that bind to the surface of collagen II fibrils (3, 4). Like other fibrillar collagens, collagen II is first synthesized as a procollagen in which the three α chains of the monomer are extended by the presence of N- and C-propeptides. The processing of procollagen II to collagen II requires a procollagen N-proteinase to cleave the three chains of the NH2-terminal propeptide and a separate procollagen C-proteinase to cleave the three chains of the C-propeptide (5). After processing of the procollagen by the two proteases, the resulting collagen II spontaneously forms fibrils. Over 50 mutations in the gene coding for collagen II (COL2A1) have been reported in patients with the heterogeneous cartilage disorders referred to as chondrodysplasias (5–10). Also, transgenic mice expressing mutated collagen II genes develop phenotypes similar to human chondrodysplasias (11–14). About half of the mutations in the COL2A1 gene causing human chondrodysplasias are single-base substitutions that convert codons for obligate glycine residues in the repeating -Gly-Y- sequence of the collagen triple helix to codons for amino acids with bulkier side chains (6, 9, 10). The remaining mutations are premature termination codons, RNA-splicing mutations, partial gene deletions, sequence insertions, or sequence duplications.

One special class of mutations found in the human COL2A1 gene are single base substitutions that converted codons for Arg residues in the Y position of the repeating -Gly-Y- sequence of the triple helix to codons for Cys. The first Arg to Cys substitution was seen in a family with early onset generalized osteoarthritis together with features of a mild chondrodysplasia probably best classified as a spondyloepiphyseal dysplasia (15). Subsequently, four additional families with a similar phenotype were seen with the same mutations in the codon for Arg-α1–519 (16). Of the total of five families with the Arg-α1–519 mutations, three may be related through an early Icelandic founder, and the other two are apparently unrelated (17). Three additional probands with similar phenotypes were reported with a Cys for Arg substitution at α1–75 (18, 19). In contrast, a similar Cys for Arg substitution at α1–789 was found in two unrelated probands with a severe phenotype of spondyloepiphyseal dysplasia (20, 21). The Cys for Arg substitutions in collagen II are of special interest because they are the first amino acid substitutions in the Y position of the repeating -Gly-Y- sequences of a collagen shown to cause a phenotype. They are also of interest because they are among the most frequently recurrent mutations in collagen genes among the more than 200 such mutations now reported (6–10). In addition, since the Arg-α1–519 and Arg-α1–75 mutations produce relatively mild phenotypes, they may be representative of mutations that cause relatively common disorders of connective tissue.

Previously, we expressed the human COL2A1 gene in HT-1080 cells, a human tumor cell line that synthesizes the collagen IV found in the basement membrane but not any fibrillar collagen (22, 23). As was shown previously, these cells are a very useful tool for producing properly folded and posttranslationally modified procollagen II (24).

Here we have prepared recombinant human procollagen II containing the substitution of Cys for Arg at α1–519 found in three unrelated families with a mild cartilage disorder (15–17). In contrast to mutated procollagens containing Cys substitutions for obligatory Gly residues (6–10), the Cys in the Y position at α1–519 in collagen II did not generate any intramolecular disulfide bonds. Also, the mutated collagen did not assemble into fibrils under conditions in which the normal
monomers readily polymerized. However, the presence of the mutated monomer in mixtures with normal type II collagen increased the lag time for fibril assembly and altered the morphology of the fibrils formed.

**MATERIALS AND METHODS**

The COL1A1/COL2A1 Hybrid Gene Construct—Previously we prepared recombinant procollagen II by using a COL1A1/COL2A1 hybrid construct in which expression of the COL1A1 gene was driven by a promoter of the COL1A1 gene (25). To obtain a fragment of the COL2A1 gene coding for Cys at position 1–519, genomic DNA was isolated from cultured skin fibroblasts (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis associated with type II collagen (26) from a proband with primary generalized osteoarthritis.

To assemble a COL1A1/COL2A1 hybrid construct containing the mutated a1–519 codon, the complete gene was assembled from four fragments: a 1.9-kb SphI-HindIII fragment from the 5′-end of the human COL1A1 gene (25–30), the 12-kb SphI-SphI fragment containing the 3′-end of the a1–519 codon; and two additional SalI fragments of 14 and 3.5 kb (25). The construct contained 476 base pairs of the promoter, the first exon, and most of the first intron of the COL1A1 gene extending up to +1,445 base pairs. The COL1A1 fragment was linked to 29.5 kb of sequences of the human COL2A1 gene that was extended from the SphI site in the 3′-end of the second intron of the gene at a 1:249 base pairs to about 3.5 kb beyond the major polyadenylation signal of the gene.

For the first step of assembly, the SphI site in the 5′-end of the 1.9-kb SphI-HindIII fragment and the SphI site in the 3′-end of the 3.5-kb SphI-SphI fragment were converted to SalI sites. The HindIII site in the 3′-end of the 1.9-kb SphI-HindIII fragment was converted to a SphI site. The two SalI-SphI and SphI-SalI fragments were then assembled into a SalI site in a modified cosmid vector by three-way ligation (27). In the second step, the 14-kb SphI-SphI fragment with the wild type sequence and the 12-kb fragment containing the mutated a1–519 codon were inserted by three-way ligation into the SphI site of the construct obtained in the first step.

Expression of the Normal and Mutated COL1A1/COL2A1 Hybrid Genes and Protein Purification—To express the normal and mutated COL1A1/COL2A1 genes, the constructs were stably transfected into the human cell line HT-1080 by calcium phosphate precipitation, and clones were selected with G418 for expression of the co-transfected gene extending up to 1–519 codon; and two additional SalI fragments of 14 and 3.5 kb (25). The construct contained 476 base pairs of the promoter, the first exon, and most of the first intron of the COL1A1 gene extending up to +1,445 base pairs. The COL1A1 fragment was linked to 29.5 kb of sequences of the human COL2A1 gene that was extended from the SphI site in the 3′-end of the second intron of the gene at a 1:249 base pairs to about 3.5 kb beyond the major polyadenylation signal of the gene.

Fibril Formation from Normal and Mutated Collagen II—For the studies on fibril formation, mutated pC-collagen II was 13C-labeled, but normal pC-collagen II was not. The pC-collagens and C-proteinase were dialyzed separately against fibril formation buffer (34) and then stored in parafilm-sealed tubes under an atmosphere of 10% CO2 and 90% air at 20 °C. To initiate fibril formation, pC-collagen and C-proteinase were mixed at 4 °C in a 250-μl polypropylene tube in a total volume of 20 μl to give a final concentration of 150 μg/ml pC-collagen and 50 units/ml C-proteinase. To study fibril formation in the presence of mutated collagen, normal and mutated pC-collagens were mixed to provide solutions in which the concentration of normal pC-collagen II was 150 μg/ml but mutated pC-collagen II was added to give ratios of normal:mutated that were 1:2, 1:3, and 1:4. Therefore, the total concentrations of pC-collagen varied from 188 to 300 μg/ml. The concentration of C-proteinase was increased so that the time required for complete cleavage was about 60 min. After preparing the mixtures, the tubes were briefly flushed with water-saturated 10% CO2 and 90% air. The tubes were capped and incubated from 0.5 to 24 h at 37 °C. The formed fibrils were separated by centrifugation at 13,000 × g for 10 min. The supernatant was removed and transferred to a dialysis tube. The pellet was resuspended in 20 μl of fibril formation buffer. Concentrated electrophoresis sample (5 ×) buffer was added to each sample to give a final concentration of 2% SDS, 2% glycerol, and 0.01% bromphenol blue in 62 mM Tris-HCl buffer, pH 6.8 at 20 °C. The samples were heated at 100 °C for 3 min and separated by electrophoresis on a 7.5% polyacrylamide gel in SDS. The gels were stained with colloidal Brilliant Blue G (Sigma), destained, and air-dried between two sheets of cellophane. Relative amounts of stained a1(II) chains were assayed with a laser densitometer (Ultrascan XL; Pharmacia). With 13C-labeled samples, the gels were also assayed with a phosphor storage plate (PhosphorImager 400S; Molecular Dynamics) for the pixel counts in the protein bands. The assay made it possible to detect as little as 0.1 μg of a1(II) chains in either the supernatant or pellet fractions.

**Microscopy of Fibrils—** For dark-field light microscopy, mixtures of pC-collagen and C-proteinase (40 μl final volume) were placed in sealed chambers formed by gluing a plastic ring to a microscopic slide and capping the chamber with a coverslip (35). Fibrils were photographed using a light microscope (model 9901; Zeiss).

In electron microscopy, a hanging drop system for fibril assembly was used to minimize the distortion of the fibrils (36). Aliquots of 5 μl of a mixture containing pC-collagens and C-proteinase were transferred onto a Teflon-insulated wire mounted inside a screw cap to form a hanging drop. Then the drop on the wire was suspended above a small amount of fibril formation buffer and the tube was tightly closed. The tube was submerged in the water bath at 37 °C and incubated for 24 h. The whole drop was then transferred to a carbon-coated electron mi-
a codon of -TGT- for Cys.

Molecular Modeling of Collagen Molecule—Molecular modeling was performed on a Silicon Graphics (Onyx) computer system using the SYBYL software package, version 6.1 (Tripos, Inc., St. Louis, MO). Models of the collagen II triple helix were created as described by Chen et al. (37). The parent model was generated with a 36-amino acid peptide in which the Arg at α1–519 was in the middle of the wild type sequence: GFP GER GSP GAQ GLO GPR GLP GTP GTD GPR GAS GPA. To minimize end group effects, the amino terminus was substituted with an N-acetyl group and the carboxyl terminus with -NHCH3.

The parent molecule was then modified to generate three mutated molecules in which a Cys residue was substituted at α1–518, α1–519, and α1–520. All the models were energy minimized using a conjugate gradient method and subject to repeating cycles of molecular dynamics using Kollman force fields and united atoms (38). Two hundred-picosecond dynamic trajectory runs were saved and analyzed for low energy conformers.

RESULTS

Expression of the Mutated Procollagen II—To obtain a construct of the mutated COL2A1 gene, a 12-κb SphI-SphI fragment was isolated from a cosmId library prepared with genomic DNA from a proband previously found to have primary generalized osteoarthritis associated with a mild chondrodysplasia (15). The proband was heterozygous for a single base mutation that converted the codon for Arg at α1–519 to a codon for Cys and that was co-inherited with the phenotype in his large family. The 12-κb SphI-SphI fragment was assembled into a COL1A1/ COL2A1 hybrid construct in which expression of the COL2A1 gene was driven by a COL1A1 promoter (see Ref. 25). The construct was assembled in two steps, each of which required a three-way ligation. The gene construct was then used to prepare stable transfectants of the mammalian cell HT-1080 (24, 31, 36). A clone secreting the mutated procollagen II was isolated and used for preparation of the recombinant protein. To confirm the presence of the mutation in the expressed gene, total RNA was isolated from the clone, the RNA was reverse-transcribed, the resulting cDNA was amplified with a primer paired specific for the region containing the mutation, and the PCR product was sequenced. As indicated in Fig. 1, the results demonstrated that the codon of -CGT- for Arg at α1–519 was converted to -TGT-, a codon for Cys.

To obtain adequate amounts of the recombinant mutated procollagen II for functional assays, 36 liters of medium from cultures of the clone were concentrated, and the protein was purified to homogeneity by ion exchange chromatography. The protein was shown to be homogeneous by electrophoresis on a SDS-polyacrylamide gel stained with colloidal Brilliant Blue G (not shown).

Assays for Thermal Stability and Disulfide Bonds—To assay the thermal stability of the triple helix of the mutated collagen
II, the protein was assayed by brief digestion with mixture of chymotrypsin and trypsin. As indicated in Fig. 2, there was no apparent difference in thermal stability between the mutated collagen II and normal collagen II.

Of note was that the band below \( \alpha_1(II) \) chains seen previously with the sample of recombinant normal collagen II digested with a mixture of chymotrypsin and trypsin (39) was also present in mutated recombinant collagen II. The same band was seen after digestion of collagen II from chick sternum cartilage (not shown) with the chymotrypsin/trypsin mixture. Sokolov et al. (40, 41), who presented evidence that the band arose from cleavage of an NH\(_2\)-terminal fragment in 20–30% of the molecules.

To assay the mutated procollagen for the presence of intramolecular disulfide bonds within the triple helix, the purified protein was digested with trypsin and chymotrypsin at 25 °C to remove the N- and C-propeptides. The resulting collagen was then assayed by electrophoresis in polyacrylamide gels in SDS under nonreducing conditions. No dimers of \( \alpha_1(II) \) chains were detected (not shown). Under the same conditions, a series of mutated procollagens I containing cysteine substitutions for obligate glycine residues were previously shown to form interchain disulfide bonds (see Ref. 42). Although no interchain disulfide bonds were detected in the mutated collagen II, a small fraction of disulfide-linked \( \alpha_1(II) \) chains was detected in the supernatant of samples that were incubated at 37 °C for 20 h or longer (Fig. 3, B and C). The relative migration of the protein bands without reduction indicated that they were dimers of \( \alpha_1(II) \) chains. Assay of the \(^{14}\text{C}\)-labeled pro\( \alpha_1(II) \) chains by pixel counts indicated that the disulfide-bonded dimers accounted for about 3% of the total \( \alpha_1(II) \) chains.

**Computer Modeling of a Collagen Triple Helix Containing Cys Substitutions**—To explore why the Arg-\( \alpha_1-519 \) mutation did not form intramolecular disulfide bonds, a series of modeling experiments was carried out. For the modeling experiments, an amino acid sequence containing 36 amino acids spanning the Arg at \( \alpha_1-519 \) was used to define the normal conformation of the triple helix. Three mutated versions of the model were then generated (Fig. 4). The distances separating identical \( \alpha \)-carbons were measured between pairs of chains that are staggered by 1 amino acid in the collagen triple helix (see Ref. 3). In the model with the normal sequence, the \( \alpha \)-carbon distances between the Gly-\( \alpha-520 \) residues were shorter than the \( \alpha \)-carbon distances between the Arg-\( \alpha-519 \) residues (Table I). The distances separating identical \( \alpha \)-carbons in the Gly-\( \alpha-520 \) residues were also shorter than the \( \alpha \)-carbon distances between the pro-\( \alpha-518 \) residues. With the three mutated sequences in

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**Fig. 4.** Computer simulations of a 36-amino acid peptide spanning the sequence found at \( \alpha_1-519 \). Only the peptide backbone and the skeletons of side chains of Arg and Cys are shown. The side chain nitrogen of the Arg residues are in blue. The sulfhydryl groups of the Cys residues are in yellow. Left panel, side view. Right panel, end-on view, NH\(_2\)- to COOH-terminal. A, normal sequence. B, same sequence with the substitution of Cys for Arg at \( \alpha_1-519 \). C, same sequence with the substitution of Arg for Gly at \( \alpha_1-520 \). D, same sequence with the substitution of Cys for Pro at \( \alpha_1-517 \).
Fibril Assembly—To examine assembly of the mutated protein into fibrils, the isolated procollagen II was converted to pC-collagen II by digestion with procollagen N-proteinase, and the pC-collagen II was reisolated on a gel filtration column. The pC-collagen II was then used as a substrate for fibril assembly under the conditions that were previously used with both pC-collagen II and pC-collagen I (24, 31, 34–36). With samples containing 150 μg/ml pC-collagen and 50 units/ml C-proteinase, cleavage of both normal and mutated pC-collagen II was complete in less than 1 h (24, 31, 36). There was no apparent difference in the rate of cleavage of the normal and the mutated proteins (not shown). As the C-propeptides are cleaved from the protein, normal collagen II assembled into fibrils with a lag period of about 1 h, and the assembly was complete in 7.5–10 h (Figs. 5A and 6). Under the same conditions, none of the mutated collagen II assembled into fibrils that remained 160 ng/ml/min under the conditions used (Fig. 6). Although the mutated collagen II increased the lag period for the assembly of normal collagen II, none of the mutated protein was detected in the pellet fractions (Fig. 5C).

Morphology of the Fibrils—As reported previously (24, 31, 36), collagen II assemblies into fibrils that form three-dimensional arcades under the conditions used here (Fig. 8A). Although the mutated collagen II did not assemble into fibrils, the presence of the protein in mixtures with normal collagen II altered the morphology of the fibrils formed by normal collagen II (Fig. 8B). The fibrils were less uniform in diameter. Also, they were shorter, and some showed distorted ends.

Electron microscopy of fibrils formed from mixtures of normal collagen II and mutated collagen II (Fig. 9B) also revealed distortions compared with fibrils of normal collagen II (Fig. 9A). The individual fibrils formed in the presence of mutated collagen II appeared to be thinner and loosely packed into thicker fibrils. Apparently because of loose packing of the structures, no D-period banding was apparent under the same conditions in which a D-period banding pattern was readily demonstrated in the control fibrils.

### Table I
Distances measured between atoms in models of the collagen II triple helix

Distances are between identical residues in the most NH2-terminal and in the middle chain in the model in which each of the three chains was staggered by 1 amino acid residue (chains 1 and 2; see Ref. 3). All the distances were larger between identical residues in the middle chain and the most COOH-terminal chain (chains 2 and 3).

| Collagen II residue | α-Carbon distance | S atom distance |
|--------------------|------------------|----------------|
|                    | Å                | Å              |
| Normal sequence    |                  |                |
| Pro α1–518         | 6.44             |                |
| Arg α1–519         | 5.88             |                |
| Gly α1–520         | 3.33             |                |
| Mutated sequence   |                  |                |
| Cys α1–518         | 6.35             | 8.07           |
| Cys α1–519         | 4.63             | 6.06           |
| Cys α1–520         | 3.81             | 5.97           |

which Cys was placed in the Gly, X, and Y positions, respectively, the α-carbon distances were α-520 < α1–519 < α1–518 when residues in chain 1 (most NH2-terminal) were compared with residues in chain 2 (middle chain). All the α-carbon distances were greater when residues in chain 2 (middle chain) were compared with chain 3 (most COOH-terminal). Therefore, the results were consistent with the conclusion that intramolecular disulfide bonds between two of the chains (chains 1 and 2) can form more readily between Cys residues in the obligate Gly position (α1–520) than Cys residues in the Y position (α1–519). The S atom distances with the Cys-α1–520 and Cys-α1–519 substitutions were essentially the same (Table I), but these distances are more difficult to predict because of the flexibility of the side chains.

**Figs.**

**A**

**B**

**C**

![Fig. 5. Polyacrylamide gel assays of fibril assembly with normal collagen II, collagen II containing the Cys for Arg substitution at α1–519, and mixtures of the two collagens.** Fibril assembly was carried out by incubating normal pC-collagen II (150 μg/ml) with C-proteinase (50 units/ml), 14C-labeled Cys for Arg-substituted pC-collagen II (150 μg/ml) with C-proteinase (50 units/ml), or an equal mixture of the two (total of 300 μg/ml) with C-proteinase (100 units/ml). After incubation at 37 °C in fibril formation buffer for the times indicated, the samples were centrifuged and separated on SDS-polyacrylamide gels. A, samples of normal pC-collagen II incubated for 0.5–24 h. The gel was stained for proteins with colloidal Brilliant Blue G. B, samples of mutated pC-collagen II. Upper part, photograph of the gel stained with colloidal Brilliant Blue G. Lower part, image of the same gel from a phosphor storage plate to detect the 14C-labeled mutated protein. C, mixture of the two p-collagens. Upper part, photograph of the gel stained for protein to detect both normal and mutated collagen II. Lower part, image of the same gel from a phosphor storage plate to detect the 14C-labeled mutant protein only. C, C-propeptide; P, pellet; S, supernatant.
The unique conformation of the collagen triple helix places every third amino acid in the restricted space at the center of the triple helix where only glycine, the smallest amino acid residue, can be accommodated (see Ref. 3). More than 150 naturally occurring mutations that substitute codons for bulkier amino acids for the obligate glycines of fibrillar collagens have now been reported (6–10). Among the first Gly substitutions were single base changes that introduced codons for Cys into collagen I, since monomers containing two Cys for Gly substitutions for Gly residues in the collagen triple helix (Table I). An incidental finding in the computer modeling carried out here is that the distance between Gly residues is closer when measured between chain 1 (the most NH₂-terminal chain) and chain 2 (the middle chain) than between chain 2 and chain 3 (the most NH₂-terminal chain). Since Cys for Gly substitutions in the pro-α1(I) chain of type I procollagen readily generate intramolecular disulfide bonds (8–10), the model-building experiments suggest that the still unresolved staggering order of α chains in type I collagen (see Ref. 3) is α1, α1, and then α2.

The recombinant mutated collagen II did not assemble into fibrils even when incubated at concentrations five times the

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**FIG. 6. Incorporation of collagen II into fibrils.** Fibrils were assembled by enzymic cleavage of pC-collagen II and assayed as in Fig. 6. ( ), normal collagen II (150 μg/ml); (○), mixture of normal collagen II (150 μg/ml) and mutated collagen II (150 μg/ml) containing the Cys for Arg substitution at α1–519. Only the normal collagen II was recovered in the pellet (see Fig. 5C).

**FIG. 7. Lag period as a function of the amount of mutated pC-collagen II added to samples containing 150 μg/ml normal pC-collagen II in the assay for fibril assembly.** M, pC-collagen, mutated pC-collagen containing Cys for Arg at α1–519.

**FIG. 8. Dark-field light microscopy of fibrils.** A, fibrils assembled from normal collagen II. B, fibrils assembled from a mixture of normal collagen II and collagen II containing the Cys for Arg substitution at α1–519. Magnification, × 480.

The results here demonstrate that the Cys at α1–519 did not form intramolecular disulfide bonds in recombinant procollagen II molecules in which all three chains contained the Cys substitution. A small fraction of the protein formed disulfide bonds after processing of the procollagen to collagen II and incubation at 37 °C for 24 h. The disulfide bonds generated under these conditions were probably intermolecular. The failure of the Cys-α1–519 to form intramolecular disulfide bonds is apparently explained by the greater distance between residues in the Y position than with substitutions for obligate Gly residues in the collagen triple helix (Table I). An incidental finding in the computer modeling carried out here is that the distance between Gly residues is closer when measured between chain 1 (the most NH₂-terminal chain) and chain 2 (the middle chain) than between chain 2 and chain 3 (the most NH₂-terminal chain). Since Cys for Gly substitutions in the pro-α1(I) chain of type I procollagen readily generate intramolecular disulfide bonds (8–10), the model-building experiments suggest that the still unresolved staggering order of α chains in type I collagen (see Ref. 3) is α1, α1, and then α2.

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On the basis of the results here, it is unlikely that the disulfide bonds were intramolecular in homotrimERIC monomers containing the mutated α1(II) chains. Instead, they were probably present as intermolecular bonds that formed after monomers containing one or two mutated chains assembled into fibrils.

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FIG. 9. Electron microscopy of fibrils. A, fibrils assembled from normal collagen II. B, fibrils assembled from mixtures of normal collagen II and mutated collagen II containing the Cys for Arg substitution at α1–519. Bars, 100 nm.

critical concentration for the assembly of fibrils for normal collagen II. However, the presence of mutated protein in mixtures with normal collagen II considerably increased the lag period for the assembly of the normal protein into fibrils. Also, the presence of the mutated protein altered the morphology of the fibrils formed by the normal collagen II. Therefore, although the monomers of the mutated collagen II did not have enough affinity to self-assemble into fibrils, their affinity for a nuclei formed by monomers of normal collagen II was apparently great enough to alter fibril assembly by the wild type protein. Although the assays used would have detected as little as 0.1 μg of the mutated monomers in pellet fractions, they probably would not have detected the relatively small numbers of monomers found in nuclei (see Ref. 34).

The five families with the Arg-α1–519 mutations were heterozygous for the mutated allele, and therefore, their tissues probably contained normal collagen II monomers, heterotrimERS comprising one or two mutated chains, and homotrimers of mutated chains such as those examined here. Cartilage from one patient demonstrated that some of the Cys residues at α1–519 formed disulfide bonds linking two α1(II) chains (45).
Collagen II Cys α1–519

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