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Research Article

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# Abnormal α2-Chain in Type I Collagen from a Patient with a Form of Osteogenesis Imperfecta

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ABSTRACT Dermal fibroblasts in culture from a woman with a mild to moderate form of osteogenesis imperfecta synthesize two species of the proα2-chain of type I procollagen. One chain is normal. The abnormal chain has a slightly faster mobility than normal during electrophoresis in sodium dodecyl sulfate polyacrylamide gels. Analysis of cyanogen bromide peptides of the proα-chain, the α-chain, and of the mammalian collagenase cleavage products of the proα- and α-chains indicates that the abnormality is confined to the α2(I)CB4 fragment and is consistent with loss of a short triple-helical segment. Type I collagen production was decreased, perhaps because the molecules that contained the abnormal chain were unstable, with a resultant alteration in the ratio of type III to type I collagen secreted into culture medium. Collagen fibrils in bone and skin had a normal periodicity but their diameters were 50% of control; the bone matrix was undermineralized. The structural abnormality in the α2(I)-chain in this patient may affect molecular stability, intermolecular interactions, and collagen-mineral relationships that act to decrease the collagen content of tissues and affect the mineralization of bone.

## INTRODUCTION

Osteogenesis imperfecta (OI)[1] is a heterogeneous group of inherited disorders characterized by bone fragility and other generalized connective tissue abnormalities (1, 2). These disorders can be classified into at least six groups on the basis of clinical findings, mode of inheritance, and underlying biochemical abnormality (2–4). The mildest of these, type I OI, is characterized by osseous fragility that usually decreases at the time of puberty, lack of bone deformity, blue sclerae, and presenile hearing loss. Inheritance is autosomal-dominant and two subtypes can be distinguished on the basis of the presence or absence of dentinogenesis imperfecta. The other varieties are considerably more severe, one leading to death in the newborn period. An additional subtype of intermediate severity, in which there is mild to moderate bone deformity and short stature has been distinguished by some investigators (3, 5–7). We have studied the collagens synthesized in culture by dermal fibroblasts from one such patient and have found that some type I procollagen molecules contain an abnormal proα2(I)-chain; this chain appears to interfere with molecular stability, collagen fibril assembly and/or stabilization, and bone mineralization, thus providing a molecular basis for one form of OI.

*Clinical summary.* The patient is a 56-yr-old caucasian woman. At birth, deformity of her left ankle was noted after a breech delivery. She was said to have had an unusually large head as an infant but was otherwise well until age 11 mo when a fracture of the right tibia occurred. Scleral discoloration was apparently not noticed at an early age. She had a skull fracture at 18 mo and many fractures of her extremities occurred during childhood. A fracture of the right hip at 11 yr required hospitalization. Several postfracture deformities were corrected surgically and she was employed as a textile manufacturing supervisor for over 30 yr. The frequency of fractures decreased at puberty and remained low during her adult years.

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[1] *Abbreviations used in this paper*: bp, base pairs, DMEM, Dulbecco/Vogt modified Eagle's medium; OI, osteogenesis imperfecta.

A partial thyroidectomy was performed for "goiter" (symptoms suggestive of hyperthyroidism) at age 25. Uterine fibroids led to hysterectomy and oophorectomy at 45 and she received estrogen replacement for several months in the postoperative period.

She was the fifth of six children. The parental ages at birth were 42 (father) and 26 (mother). She has never been pregnant. No other family members have or had bone fractures, bone deformities, blue sclerae, or clinically apparent hearing loss. Physical examinations of available relatives (mother, brother, and sister) were normal.

On physical examination the patient was a middle-aged caucasian woman with obvious skeletal deformities who walked with a cane. Her height was 158.6 cm (below the third percentile); weight, 52.2 kg; blood pressure, 136/80; and pulse, 90. Her skin was normal. She had a triangular face with frontal bossing and a prominent occipital overhang. Her sclerae were deep blue in color and she had bilateral annulis senilis. The funduscopic examination was normal. The tympanic membranes were of normal color and architecture. Her teeth were in good repair, were not opalescent, and there was no evidence of unusual wear or exposure of dentin. She had a pectus carinatum and mild kyphoscoliosis. Her lungs were clear to percussion and auscultation; axillary hair was absent, and the heart had no murmurs or clicks. No abdominal organomegaly was palpated. She had mild hyperextensibility of the small joints of the hands, pes planus on the right, mild muscle wasting in both the lower extremities, the right more than the left. Surgical scars were present just above the left knee. In her hands and feet the phalanges appeared to be abnormally thin giving prominence to the distal interphalangeal joints. She had a valgus deformity of the toes on the right foot. The left ankle was abnormal in that the distal ends of the tibia and fibula were subluxed over the talus, which was medially and anteriorly displaced. Reflexes were equal bilaterally.

Summary of laboratory studies included: hematocrit, 35.7; serum calcium, 4.8 meg/liter; phosphorus, 2.6 mg/dl; alkaline phosphatase, 57 U/liter (normal = 36–124 U/liter); thyroxine by radioimmunoassay (RIA), 10.2 (normal = 5.0–12.0); and triiodothyronine by RIA = 212 ng/dl (normal = 122–213 ng/dl). The 24-h urinary excretion of calcium was 4.2 meq and of hydroxyproline was 17 mg (normal = 22–77 mg). Serum parathyroid hormone was 340 ng/ml (normal = 230–630 ng/ml). Radiologic examination of the spine demonstrated reduction in bony density with partial loss of height of multiple dorsal and lumbar vertebrae. The upper lumbar vertebrae were biconcave and several thoracic vertebral bodies were compressed. There were extensive degenerative changes at the posterial intervertebral joints in the mid- and lower lumbar spine. The right tibia and fibula were slightly bowed and had a coarse texture and porosity. There was depression of both the medial and lateral tibial plateaus. Marked calcification of the right achilles tendon was present. The femurs showed marked cortical thinning with slight bowing on the right. There was an abnormal bony texture and osteopenia apparent on x rays of the hands and degenerative changes that affected several of the small joints.

Audiometric examination disclosed a sharply sloping bilateral hearing loss >900 cpm. Otoadmittance tympanometry revealed a characteristically notched curve at 660 Hz suggestive of increased compliance of the middle ear (8).

## METHODS

Dermal fibroblast cultures were obtained from outgrowths of an explant of skin taken from the inner aspect of the upper arm. The cells were grown for five passages after the initial outgrowth and frozen in liquid nitrogen. Biochemical studies were performed on cells between the sixth and twelfth passage. The cultures were maintained in Dulbecco/Vogt modified Eagle's medium (DMEM) containing either 10% fetal calf serum or 10% newborn calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin, 2.5 mM glutamine, and 15 mM Hepes, pH 7.4 in a humidified atmosphere of 9% $CO_2$/air at 37°C. DNA content of cells on a culture dish was measured by using a diaminobenzoic acid assay (9). Control cell strains were obtained from individuals aged newborn to 50 yr who had no evidence of connective tissue disorders.

Collagen production by cells in culture was measured by a modification of the procedure described by Peterkofsky and Diegelmann (10). Cells were preincubated in DMEM that contained 5% dialyzed fetal calf serum and 50 μg/ml of ascorbic acid and then transferred to the same medium that contained 10 μCi/ml of [2,3-$^3$H]proline (New England Nuclear, Boston, MA; 25 Ci/mmol). The cells were incubated for 6 h (total collagen synthesis) or 24 h (typing of collagen or procollagen). In either case the medium and cells were separated, placed on ice, and protease inhibitors (phenymethanesulfonyl fluoride, *N*-ethylmaleimide, and ethylenediamine tetraacetic acid) were added to both to prevent proteolysis (11). The medium and cell layer were recombined to measure total collagen production and to quantitate production of different collagen types.

To measure the relative ratios of types I and III collagens produced by these cells, combined medium and cell layer proteins were precipitated with 15% (wt/vol) trichloroacetic acid and then redissolved in 0.5 N acetic acid adjusted to pH 2 with HCl. The solution was incubated with 1 mg/ml of pepsin at 15°C for 2 h after which the reaction was terminated by addition of 50 μg/ml of lathyritic rat skin collagen. Solid NaCl was added to a concentration of 5% (wt/vol) NaCl and the resulting precipitate was collected, washed once with 20% (wt/vol) NaCl, 50 mM Tris pH 7.5, and then with 18% ethanol. The air-dried residue was dissolved in electrophoresis sample buffer without added reducing agent and heated 5 min in boiling water. The α1(III)-, α1(I)-, and α2(I)-chains were separated on a 7% polyacrylamide slab gel (12) by the technique of interrupted reduction (13). The

radioactive proteins were detected by radioautofluorography (14) and their relative intensities measured by a Transidyne scanning densitometer (model 2955; Transidyne General Corp., Ann Arbor, MI).

The medium procollagens were analyzed on 5% sodium dodecyl sulfate (SDS) acrylamide slab gels as described by Laemmli (12) except that the sample and chamber buffers contained 2 M urea to enhance the separation of pro$\alpha$1(I)- from pro$\alpha$1(III)-chains. The samples were prepared either by precipitation of the medium proteins with 30% saturated ammonium sulfate followed by two washes with 18% ethanol and air drying or by dialysis of the medium against 1 mM ammonium bicarbonate, 0.1 mM phenylmethanesulfonyl fluoride, and 0.5 mM *N*-ethylmaleimide followed by lyophilization.

To label proteins for peptide mapping, $2.5 \times 10^5$ cells were plated in 35-mm culture dishes, allowed to attach and spread overnight, and then incubated with [$^3$H]proline at a concentration of 200 $\mu$Ci/ml in a volume of 0.4 ml of DMEM lacking fetal calf serum but supplemented with 50 $\mu$g/ml of ascorbic acid. Medium and cell layer were harvested separately as described (11) to inhibit proteolysis. Dry samples were dissolved in 50 $\mu$l of electrophoresis sample buffer, which contained dithiothreitol, and denatured in boiling water for 3 min. Some radiolabeled collagenous proteins from culture medium were dialyzed into 0.5 N acetic acid and subjected to limited proteolysis with pepsin to produce collagen-size molecules. Aliquots of medium taken before and after pepsin digestion were analyzed by slab gel electrophoresis; additional aliquots of both were cleaved with purified fibroblast collagenase (15, 16), a generous gift from Dr. Eugene Bauer. The pro$\alpha$-chains, $\alpha$-chains, and fibroblast collagenase cleavage products were separated by electrophoresis in (SDS)-polyacrylamide gels and then digested, in the gels, with cyanogen bromide and the resultant peptides were separated in a second-dimension gel (11, 17). Collagen $\alpha$-chains were isolated by preparative disc-gel electrophoresis and cleaved with cyanogen bromides. The peptides were separated by electrophoresis in 12.5% SDS-polyacrylamide gels.

mRNA from confluent cells in four 100-mm dishes was prepared and translated (18). An aliquot of the translation mixture that contained [$^3$H]proline-labeled prepro$\alpha$-chains was mixed with concentrated sample buffer and applied to a 5% SDS-polyacrylamide slab gel for electrophoresis.

Bone biopsy specimens were obtained either at open biopsy or with a Craig needle (closed biopsy). Specimens were fixed in glutaraldehyde/cacodylate buffer, pH 7.4 for 5 h. Bone was decalcified over 4–5 d at 4°C in 10% EGTA, 0.1 M Tris, pH 7.4. The specimens were then cut into 1-mm pieces, and postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.4 for 1 h. The specimens were dehydrated in ethanol and embedded in a mixture of Depont and Spurr's epoxy media. Thin (7,090-nM) sections were cut, stained with 2% phosphotungistic acid, pH 4.5, for 1 h, and viewed in a Philips 201 transmission electron microscope (Philips Electronic, Mahwah, NJ). Skin for electron microscopy was prepared and examined as previously described (19).

## RESULTS

The dermal fibroblasts from this patient had normal growth characteristics in culture. About 4.2% of the protein produced by the OI cells was collagenous, which is lower than normal (7.2%, $n = 25$, $P < 0.05$). After pepsin digestion of the combined cells and medium proteins, the ratio of $\alpha$1(III) to $\alpha$1(I) was 0.42 (control was 0.19, $P < 0.05$). Because the production of type III procollagen appeared normal (see below and Fig. 1) the decrease in collagen production is accounted for by a decrease in production of type I procollagen.

In the course of measuring the ratio of type I to type III collagen, it was noted that the $\alpha$2(I)-chains from the OI cells migrated as a broad band or doublet during electrophoresis (Fig. 1A). The additional material always migrated more rapidly than normal. The pro$\alpha$2(I)-chain migrated as a broad band, wider than the control (Fig. 1B). The ratio of pro$\alpha$1(III) to pro$\alpha$1(I) was 0.36 (normal 0.20), whereas the ratio of pro$\alpha$1(I) to pro$\alpha$2(I) was 1.8 (within the normal range). mRNA from four confluent 100-mm culture dishes was extracted and the 28 S RNA was translated in a reticulocyte lysate. The collagen mRNA activity for this OI cell strain, measured by translation, was the same as that of control cells. The migration of the prepro$\alpha$2(I)-chain from the patient's cells was normal and the amount of translatable mRNA for prepro$\alpha$1(I) and prepro$\alpha$2(I) was comparable to control (Fig. 1C). These results suggested that the OI cells produced a population of abnormal pro$\alpha$2(I)-chains that were assembled into molecules normally but which, once in a trimer, produced an unstable type I procollagen. The procollagens and collagens synthesized by cells from the patient's mother,

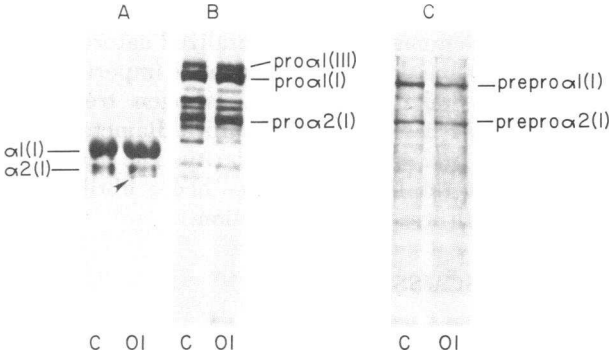


FIGURE 1 Autoradiofluorogram of radiolabeled collagenous proteins synthesized by control (C) and OI cell strains. A. Pepsin-treated procollagens, $\alpha$-chains. B. Pro$\alpha$-chains from whole medium. C. Prepro$\alpha$-chains synthesized by cell-free translation of partially purified mRNA. There is a doublet in the $\alpha$2(I)-chain region of the collagen chains from the patient and the pro$\alpha$2(I)-chain band from the patient is broad. There is no difference in the mobility of the prepro$\alpha$2(I)-chains when the control and affected samples are compared. The amount of translatable mRNA for prepro$\alpha$2(I)-chain is the same in both cell strains as measured by the cell-free synthesis of the chains.

brother, and one sister were normal and there was no evidence of the abnormal α2(I)-chain.

The altered mobility of the proα2(I)- and the α2(I)-chains suggested that they may be missing peptidyl material. To locate the abnormality within the chain, pepsin-treated medium procollagen was cleaved asymmetrically with fibroblast collagenase. Fibroblast collagenase cleaves each chain of type I collagen at a single site three quarters of the length from the $NH_2$-terminal end of the triple helical domain to produce a $TC^A$ (tropocollagen) fragment (large) and $TC^B$ fragment (small) from each chain. Only the $TC^A$ fragment of the affected α2-chain was abnormal (Fig. 2); the $TC^B$ fragments had normal mobility (Fig. 2). When the separated proα2(I)-chains, α2(I)-chains and $TC^A$ fragments of proα2(I)- and α2(I)-chains were digested with cyanogen bromide, the α2(I)CB4 peptide from each abnormal chain had an altered mobility (Fig. 3). The difference in mobility was consistent with a change in apparent molecular weight of ~2,000, or 15–20 amino acids. The change in mobility of the whole α2(I)-chain thus appeared to be a consequence of an alteration that was limited to the domain of α2(I)CB4 (Fig. 4).

The alterations in collagen production and structure affected the nature of the extracellular matrix in bone and skin. Bone from the iliac crest was relatively acellular but had a well-preserved lamellar structure and there was less mineral than normal. Collagen in skin was less dense than normal (Fig. 5). Collagen fibrils in bone and skin (Fig. 5) were smaller in diameter than normal; those in bone were disorganized. These abnormalities of collagen fibril structure and organization in skin demonstrate the generalized nature of the connective tissue defect in osteogenesis imperfecta. A bone biopsy taken 1 yr after estrogen treatment showed an increase in the mean fibril diameter from 58 to 76 nm and enhanced cellularity. The new bone formation on the lamellar surface had a fibrillar appearance with irregular calcification.

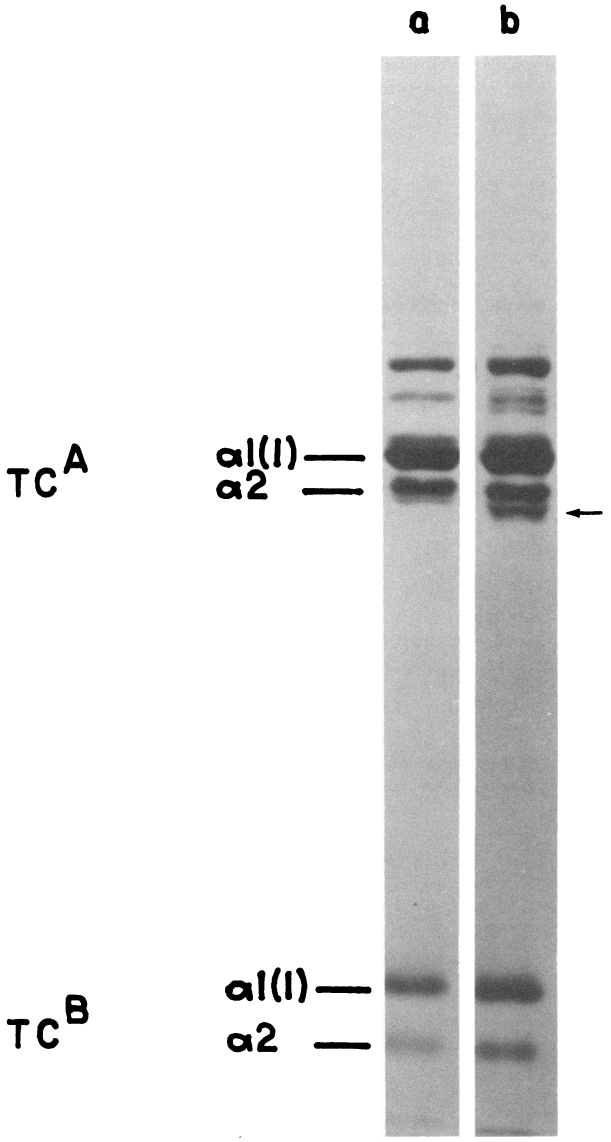


FIGURE 2 Autoradiofluorogram of radiolabeled medium procollagens digested first with pepsin and then with fibroblast collagenase. The $TC^A$ fragment from the patient's α2(I)-chains is represented by a doublet (b); the arrow indicates the altered α2(I) product that has a faster mobility than normal (a), consistent with a decreased molecular weight. The $TC^B$ fragments are normal. The uncleaved α1(I)- and α2(I)-chains are above the $TC^A$ fragments.

## DISCUSSION

The collagens are a family of structurally similar proteins with tissue-specific distributions (20). Type I collagen, the major protein of skin and bone matrix, is synthesized by fibroblasts and osteoblasts (and other cells) as a heteropolymer that contains two genetically distinct chains, α1(I) and α2(I) in a 2:1 ratio: $[\alpha 1(I)]_2\alpha 2(I)$. The genes for these proteins are among the largest and most complex yet isolated (21). The 5,000 base pairs (bp) coding sequences (exons) are distributed over ~40,000 bp of DNA (22–25). The exons that code for sequences in the triple helical domain are small, containing 54–108 bp so that the majority of the gene is made up of intervening sequences (introns). The genes are transcribed in the nucleus to precursor mRNA, colinear with the genes, which are processed to mature mRNA by precise scission of introns (26). The mRNA is transported to the membranes of the rough endoplasmic reticulum where it is translated by membrane-bond ribosomes to produce preproα-chains. The preproα-

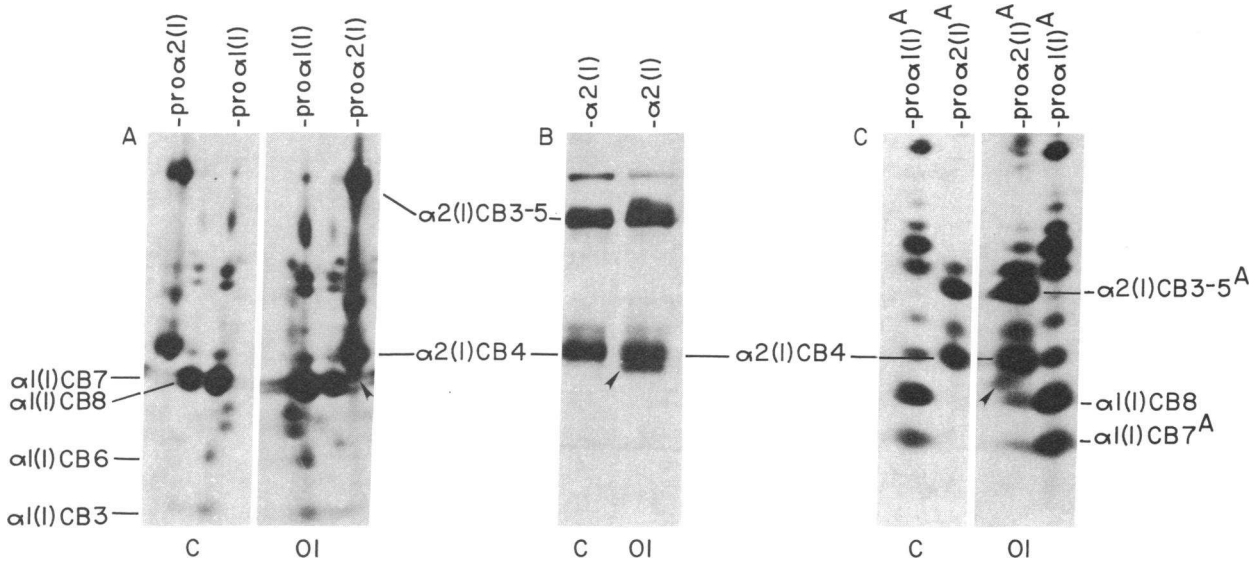


FIGURE 3 Autoradiofluorogram of cyanogen bromide cleavage products of proα-chains, α-chains, and TC^A fragments of proα-chains from control and OI samples. A, proα-chains; B, α-chains, and C, TC^A fragments of proα-chains. The radiolabeled proα-chains and TC^A fragments were first separated in 5% and 7.5% SDS-polyacrylamide gels, respectively, and then cleaved in the gel with cyanogen bromide as described in Methods. The peptides were then separated in the second dimension on a 12.5% gel. The α-chains, prepared by pepsin digestion of whole medium, were isolated by preparative gel electrophoresis. This technique does not separate the normal and abnormal α2(I)-chains so cleavage products of both are represented in the OI α2(I) slot. The methionine between α2(I)CB3 and α2(I)CB5 does not cleave efficiently with cyanogen bromide and the majority of the protein in these peptides is represented in α2(I)CB3-5. The arrows indicate the altered α2(I)CB4 peptide in each sample. Because the mobility of the α2(I)CB3-5 or its fibroblast collagenase product, α2(I)CB3-5^A is normal, neither α2(I)CB3 nor α2(I)CB5 is abnormal in the patient's collagen. The molecular weights of the CNBr peptides are: α2(I)CB3, α2(I)CB4, and α2(I)CB5 all 28,000; α2(I)CB3-5, 56,000; α1(I)CB7 and α1(I)CB8 both 24,000; α1(I)CB6, 16,000; and α1(I)CB3, 13,000.

chains are converted to proα-chains by the loss of the "signal" sequence during transit through the membrane, they are then hydroxylated, glycosylated, and assembled into procollagen molecules which are packaged in the Golgi apparatus, secreted, converted to collagen in the extracellular space, assembled into fibrils, and stabilized by intermolecular crosslinks (see 27, 28 for reviews).

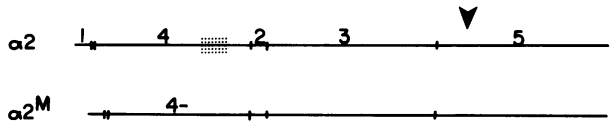


FIGURE 4 Diagrammatic representation of the helical portion of the α2(I)-chain. The vertical bars indicate the location of methionine residues and the cyanogen bromide peptides are numbered. The site of mammalian collagenase cleavage is indicated by the arrowhead. The stippled box is located in the α2(I)CB4 to indicate the altered peptide. The precise location of the abnormality in the peptide is not known and the box is not drawn to scale.

Dermal fibroblasts in culture from this patient with a variety of osteogenesis imperfecta synthesize two species of α2(I)-chains. One is normal but the other has an alteration in the structure of the α2(I)CB4 cyanogen bromide peptide. Although it initially appeared that the abnormality in α2(I) migration could be induced by pepsin, analysis of α2(I)CB4 from the proα2(I)-chain, the α2(I)-chain, and the fibroblast collagenase products of these chains indicate that the faster migrating form of the peptide is present in all these molecular species. A short deletion of peptidyl material from within the helical domain of the α2(I)-chain in the α2(I)CB4 sequence is one explanation for the altered electrophoretic mobilities (3, 11), but we cannot yet exclude the possibility that a single amino acid substitution could alter migration (29). The difference in molecular weight between the normal and abnormal (1,500–2,000 D) α2(I)CB4 could be accounted for by loss of expression of a single exon (54 bp) that codes for 18 amino acids. The genetic material could be lost or an abnormality in splice junctions

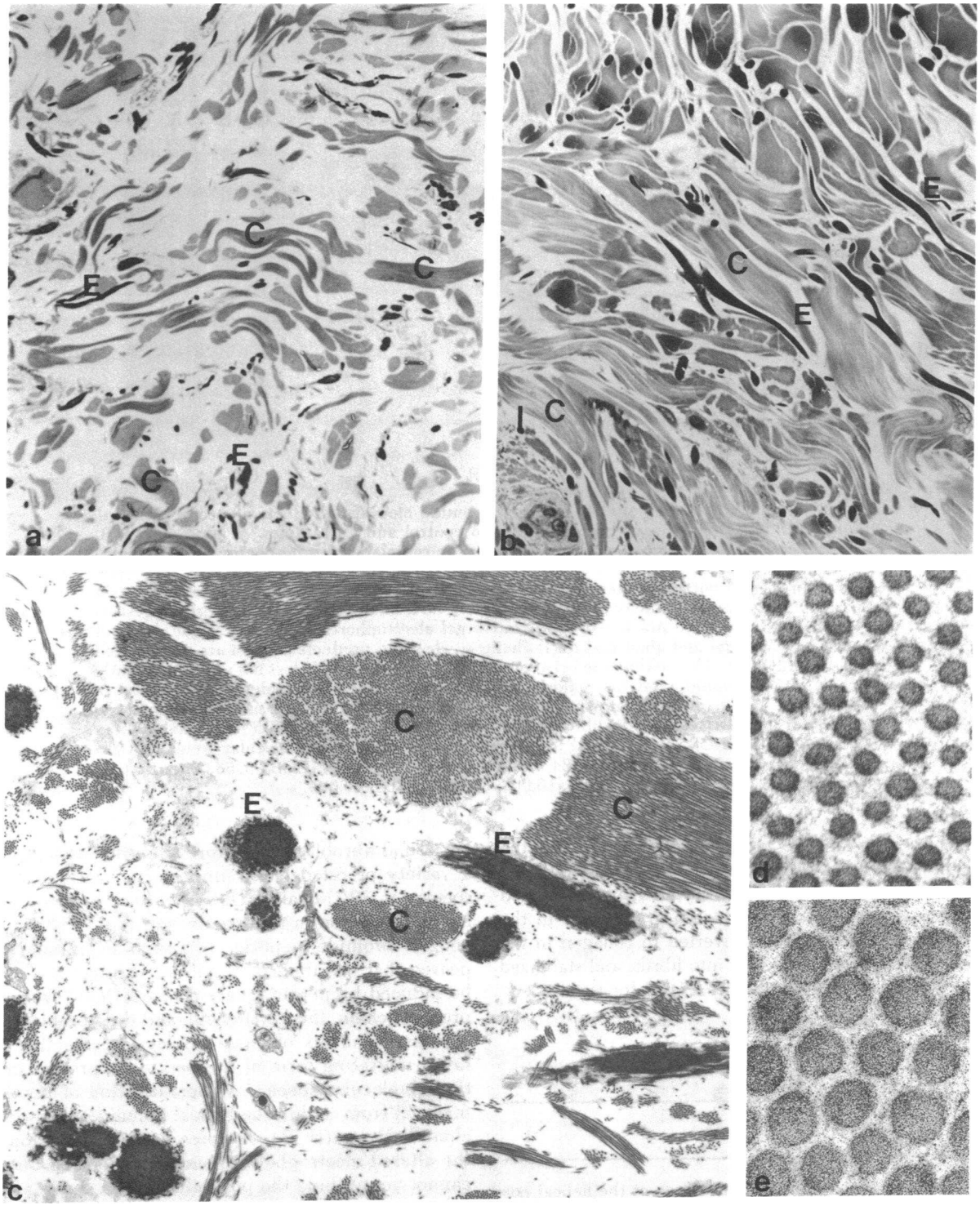


FIGURE 5 Composite light and electron micrographs of dermis from the patient and a normal control. a and b are light micrographs (×400) of collagen in the reticular dermis. The fiber bundles from the OI (a) skin are smaller and more widely separated than the control (b). This feature of the reticular dermis is demonstrated in the transmission electron micrograph (×10,000) of the OI skin (c). Collagen fibril in the OI reticular dermis (d) are about half the diameter of controls (e); magnification in d and e is 24,000.

may lead to loss of a single exon unit in the final mRNA (30).

The ratio of the normal to abnormal α2(I)-chains is greater than one, which suggests that some of the type I procollagen molecules that include the abnormal proα2(I)-chain may be unstable and thus be degraded during processing. This would account for the decreased ratio of type I to III collagen produced by these cells. Because assays of proα1(I) and proα2(I) message levels by translation (see Fig. 1) and by hybridization with specific probes (unpublished observations) indicate that normal amounts of these messages are present, it is unlikely that this woman has inherited an additional abnormal allele that alters the rate of synthesis of proα1(I) such as is seen in some patients with type I OI (31). Further characterization of the defect at the level of gene structure will be required to fully understand the precise mechanisms by which α2(I)-chain structure and the production of type I procollagen have been altered in this patient's cells.

The osseous abnormalities in type I OI are most commonly associated with mild osteoporosis, that is normal mineralization of a decreased amount of organic matrix. In this patient, the histological and ultrastructural studies of bone and skin suggest that there is decreased collagen in the matrix and that there may be defective mineralization. The affected region of the α2(I)-chain, the α2(I)CB4 peptide, appears to have at least two functions. Intermolecular interactions are stabilized in part through crosslinks that involve a lysine or hydroxylysine in α2(I)CB4 (32). Lee and Veis (33) recently suggested that this peptide may be one domain in which phosphoproteins interact with collagen molecules during tissue mineralization. Although the precise mechanisms by which fibril structure and mineralization are affected are not yet clear they most likely result from the alterations in the α2(I)CB4 peptide of some of the α2(I) chains.

Recent investigations of the biochemical basis of OI have demonstrated considerable heterogeneity in the molecular mechanisms. Most patients with type I OI (2) have abnormalities in production of type I procollagen that are reflected in altered ratios of type I to type III collagen in skin (13) and decreased accumulation of type I procollagen in medium of cultured dermal fibroblasts (34). Recently, Barsh et al. (31) have shown that decreased production of type I procollagen by cells from three individuals with type I OI is a consequence of a nonfunctional allele for proα1(I). In these cells half of the normal amount of proα1(I) is synthesized. Because the only stable molecular configurations are $[\alpha 1(I)]_2\alpha 2(I)$ and $[\alpha 1(I)]_3$, decreased synthesis of proα1(I) results in production of half the normal amount of type I procollagen. These patients all had very mild disease with normal stature, no bone deformation, and decreased bone mineral density (31).

There are a number of other patients who have more severe bone disease, one of whom is represented by this patient. These individuals have mild to moderate short stature, moderate bone deformity, and decreased bone mineral density. Bauze et al. (5) and Smith et al. (6) distinguished these patients from those with the mild, "classic" form of type I OI on the basis of clinical findings and by analysis of some physical properties of skin collagen. More detailed studies of collagen synthesized in culture by dermal fibroblasts from other such patients suggests that some individuals in this group may have abnormalities in the production or structure of the proα2(I)-chain (35). One patient, a child with moderate bone deformity, was recently investigated (36, 37) and cells in culture were found to produce $[\alpha 1(I)]_3$ but no normal type I procollagen. Although mRNA for proα2(I) was detectable by a translation assay and proα2(I) was identified intracellularly, there appeared to be little incorporation of the chain into normal type I procollagen molecules (38; Byers, Rowe, Pope, and Nicholls, unpublished observations). Because the parents were consanguineous, the most likely explanation for these findings is that an unstable proα2(I)-chain is produced that does not assemble into type I procollagen molecules. Müller et al. (39) studied a child with moderate deformity and fractures (40) whose cells in culture produced a decreased amount of α2(I) when measured after pepsin digestion of medium procollagens. The molecular details of this disorder were not further explored. We have recently studied an infant whose cells in culture secrete about equal amounts of normal type I procollagen and type I trimer. Whether this is the result of decreased synthesis of proα2(I) or production of a proα2(I) that does not assemble into type I procollagen molecules is not yet clear (Byers, David, and Hunter, unpublished observation). The biochemical, histologic, and clinical findings in these patients suggest that the α2(I)-chain is important for normal bone structure and that some, but not all (41), alterations in its synthesis and structure may result in abnormal bone mineralization.

The clinical and genetic classification of osteogenesis imperfecta (2) provides a useful guideline for genetic counselling. However, as biochemical studies proceed it is becoming clear that even within clinically defined categories there is considerable biochemical heterogeneity (3). As expected, the initial clinical classification is being revised to accommodate new clinical observations and the results of biochemical studies (3, 42). The patient we have described in this report has bone involvement that is intermediate in severity between the mild type I OI and the severe progressive deforming type III OI. Although the mode of inheritance is uncertain, autosomal-dominant inheritance seems most likely, given the single chain defect. In-

creased paternal age is suggestive of a new dominant mutation in this sporadic case (43). We suggest that patients with this intermediate type of severity be classified in a distinct subtype of OI that is distinguished by natural history and basic biochemical defect from mild type I OI and the severe, progressive deforming type III OI. Because these patients have blue sclerae and appear to have dominantly inherited disease, a subgroup of type I OI seems appropriate (3).

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