OSTEOGENESIS IMPERFECTA: ALTERED CONTENT OF TYPE III COLLAGEN AND PROPORTION OF THE CROSSLINKS IN SKIN

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1. Introduction

Osteogenesis imperfecta (OI), one of the hereditary diseases of connective tissues, is characterized by a fragile skeleton, poor teeth, thin skin, blue sclera, deafness and hypermobility of joints. Although the pathogenesis of this syndrome has not been fully elucidated, recent studies on connective tissues from patients with osteogenesis imperfecta congenita or tarda have generally indicated that collagen metabolism is primarly altered in this disease. The findings of increased solubility of the collagen [1], decreased stability of polymeric collagen [2,3] and altered lysine and hydroxylysine content [4–6] implied a defect of crosslinking or a decreased number of crosslinks in OI collagen. In addition, a study of collagen synthesis by cell cultures derived from OI skin has shown delayed replacement of Type III collagen by Type I collagen [7], indicating abnormal collagen synthesis in this disease.

In this paper we wish to report that the skin from patients with OI contains more Type III collagen and has a different proportion of reducible crosslinks in its collagen fibers, compared to normal skin.

Abbreviations: OI, osteogenesis imperfecta; DTT, dithiothreitol

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2. Materials and methods

This study is based on two patients who were diagnosed as having osteogenesis imperfecta tarda (Case 1: 12 years of age, male; Case 2: 14 years of age, female). The sample skin was biopsied from the pelvic region. Age and sex matched normal skin was dissected from the corresponding area of normal individuals within 48 h after accidental death.

Skin was cleaned and cut into small pieces at 4°C and powdered in a liquid nitrogen-cooled freezer/mill. The powdered skin was sequentialy extracted with 1.0 M NaCl in 0.05 M Tris buffer, pH 7.4, and 0.5 M acetic acid. The residue obtained after these extractions, which is primarily insoluble collagen, was washed with distilled water and then lyophilized.

Following hydrolysis of insoluble collagen in 6 N HCl at 110°C for 24 h, amino acid analysis was carried out by using a Beckman Model 116 amino acid analyzer.

Insoluble collagen was digested with pepsin (enzyme/substrate ratio 1/10) in 0.5 M acetic acid (substrate concentration, 10 mg/ml) at 5°C for 48 h [8]. The pepsin-released collagen in the supernatant fluid was precipitated twice by dialysis against large volumes of 0.02 M Na_2HPO_4. The precipitate was collected by low-speed centrifugation, dissolved in 0.1 M acetic acid, then lyophilized.

Disc electrophoresis of pepsin-released collagen was carried out in a 6.3% polyacrylamide gel (pH 5.8), with or without prior DTT reduction [9]. A mixture of pure Type I and Type III collagens, originally obtained from fetal calf skin, was run on parallel gels. Standard migration positions were noted, with or without prior DTT treatment, as described [10].

The samples of insoluble collagen were reduced with NaB^3H_4 in sodium phosphate buffer at 37°C as described [11]. The tritiated proteins were hydrolyzed in 3 N HCl at 110°C for 48 h then dried by rotary evaporation. Crosslink analysis of each hydrolysate was performed by using an amino acid analyzer as described [12]. Radioactivity was monitored in a toluene-based scintillation fluid employing Beckman Biosolve-3.

3. Results and discussion

The amino acid compositions of insoluble collagen from OI and normal skin are shown in table 1. Noteworthy was the increased cysteine content of the insoluble collagen from both OI skin samples.

Although normal skin collagen was slightly more digestible by pepsin, about 64% of the original insoluble collagen was released from both the normal and OI samples. Prior to electrophoretic analyses of these pepsin-released collagens in polyacrylamide gels, the migration positions of purified Type I and Type III reference collagens were evaluated. α1 (III) and proα1 (III) chains migrated into the α-chain region of the gel only in the presence of DTT and migrated in positions different from α1(I) and α2 [10]. We found that pepsin treatment of such Type I and Type III collagens did not alter their gel patterns except that proα1 (III) now moved to the α1 (III) position. Figure 1 shows the densitometric tracings of stained polyacrylamide gels following electrophoresis, in DTT, of pepsin-released collagens from normal and OI skin (case 2). Note that the proportion of the α1 (III) chain in OI skin is higher than in normal skin; the same result was observed for case 1 and its control. The increased cysteine content

Table 1
Amino acid composition of insoluble collagen from OI and normal skin

Amino acid	Case 1	Normal 1	Case 2	Normal 2
	(12 year, male)		(14 year, female)	
4-Hydroxyproline	70.5	76.3	72.3	80.5
Aspartic acid	48.8	45.9	43.9	46.9
Threonine	19.3	17.2	17.5	17.1
Serine	42.0	34.7	33.5	33.9
Glutamic acid	75.4	73.1	69.6	74.0
Proline	110.8	122.7	117.9	122.0
Glycine	324.1	334.4	328.9	333.2
Alanine	109.2	112.3	118.9	111.1
Valine	39.6	31.3	42.2	31.7
Methionine	7.9	7.0	6.8	6.9
Cysteine	3.6	1.2	2.0	1.0
Isoleucine	7.5	13.3	15.8	12.2
Leucine	34.8	28.1	31.6	26.5
Tyrosine	8.7	5.4	7.4	4.9
Phenylalanine	15.6	13.7	14.6	13.5
Hydoxylysine	3.4	4.9	3.7	4.4
Lysine	29.1	26.8	25.9	27.6
Histidine	6.1	5.4	5.1	5.5
Arginine	43.6	46.3	42.4	47.1

Values are expressed as residues per 1000 total residues and are the mean of duplicate analyses

No corrections have been made for destruction or incomplete release during hydrolysis

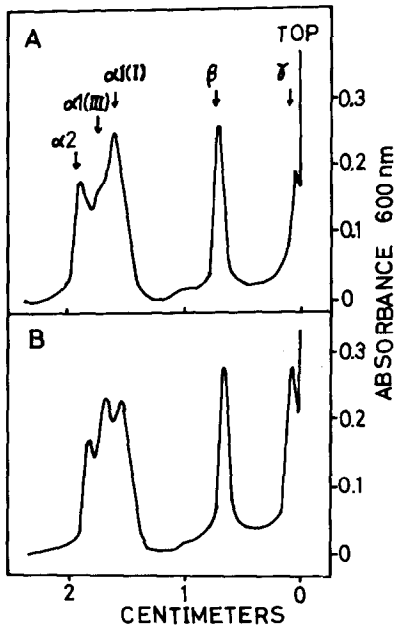

Fig.1. Densitometric tracings of stained polyacrylamide gels following electrophoresis, with DTT reduction, of pepsin-released collagens. (A) Normal, 14 years of age, female; (B) OI, 14 years of age, female.

in the insoluble collagen from OI skin may reflect its increased content of Type III collagen which contains cysteine [10]. Thus, these data from direct analyses of OI skin support the study of Penttinen et al. which reported delayed replacement of Type III collagen by Type I collagen in cultured cell strains from OI skin [7].

The specific radioactivities of the NaB^3H_4-reduced insoluble collagen from normal and OI skins are compared in table 2. The results show that the insoluble collagen from both OI skins incorporated slightly less tritium than the controls.

The chromatographic patterns of the radioactive components in acid hydrolysates of NaB^3H_4-reduced insoluble collagen from normal and OI skin are shown in fig.2. The proportion of radioactivity in each of the major components, compared to the total radioactivity eluted from the column, was determined. Although previous investigators implied a defect of crosslinking or a decreased number of crosslinks in OI collagen [1–6], the present study showed significant formation of the reducible crosslinks in OI skin collagen. Comparison of normal and OI skin collagens showed that a significantly lower proportion of hydroxylysinonorleucine (peak H) was observed in both OI skin collagens. Previously, we found that reconstituted fibrils of Type III collagen had a lower specific radioactivity than Type I collagen and that lysinonorleucine was the major reducible crosslink rather than hydroxylysinonorleucine [10]. Thus, the diminished specific radioactivity and diminished proportion of hydroxylysinonorleucine in OI skin collagen may reflect its higher content of Type III collagen.

A recent study of Epstein has shown the progressive decrease in the content of Type III collagen in skin with age [13]. Accordingly, a higher proportion of Type III collagen in OI skin may indicate delayed maturation of collagen fibers. Our previous studies of OI bone collagen reported that the insoluble collagen from OI bone show a higer proportion of dihydroxylysinonorleucine, the most abundunt reducible crosslink in bone collagen, and higher specific radioactivity, compared to controls [14]. These findings also indicate immaturity of the collagen matrix in OI bone, since previous aging studies of human tissues showed a significant decrease in the proportion of reducible

Table 2
Specific radioactivity of the NaB^3H_4-reduced insoluble collagen from OI and normal skin

	Case 1 (12 year, male)	Normal 1	Case 2 (14 year, female)	Normal 2
^3H (cpm × 10^{-2}/mg)[a]	1070	1344	857	1003
^3H (cpm/μg)[b]	1200	1380	876	1022

Results are expressed as counts per minute (cpm) per milligram (dry weight) of reduced insoluble collagen[a] and per microgram of hydroxyproline[b] in the acid hydrolysates

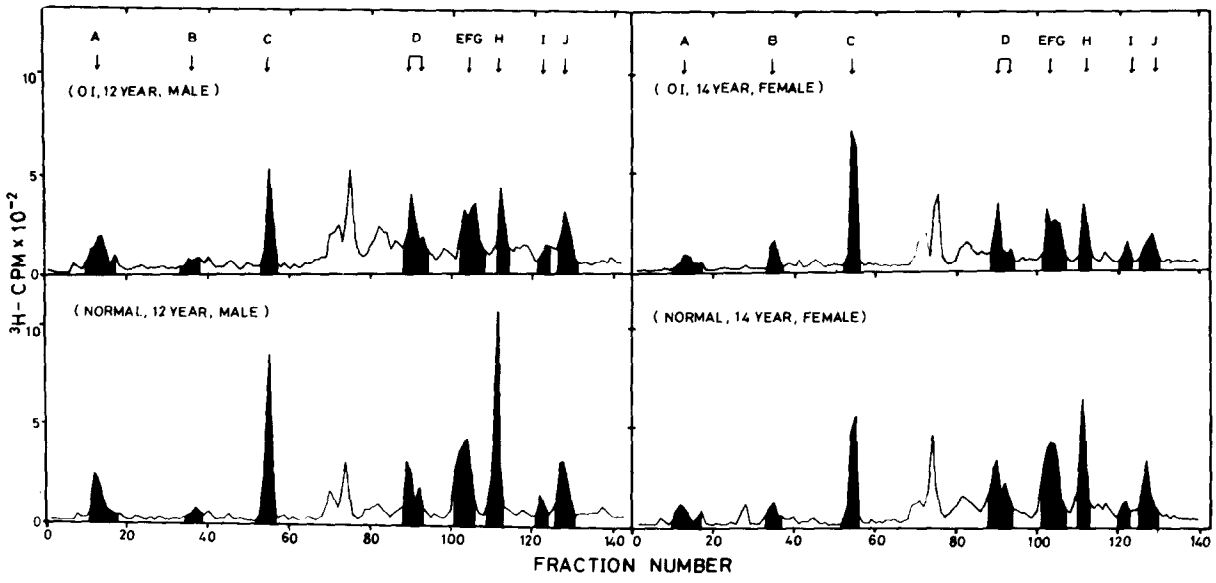

Fig. 2. Chromatographic patterns of the radioactive components in acid hydrolysates of NaB^3H_4-reduced insoluble collagens from normal and OI skin fractionated on a Beckman UR-30 column (0.9 × 69 cm). The peaks are: (A) unknown (fall through); (B) dihydroxynorleucine; (C) hydroxynorleucine; (D) N^{ϵ}-hexosylhydroxylysine; (E) N^{ϵ}-hexosyllysine; (F) aldol histidine; (G) dihydroxylysinonorleucine; (H) hydroxylysinonorleucine (I) lysinonorleucine; (J) histidinohydroxymerodesmosine.

crosslinks and in the total content of such reducible compounds of collagen with age [15,16]. Thus, the composite data suggest that the clinical symptoms of osteogenesis imperfecta may be related to abnormal regulation of collagen synthesis in which immature collagen fibers form labile intermolecular crosslinks.

References

[1] Brown, D. M. (1972) Pediat. Res. 6, 394 abstract.
[2] Francis, M. J. O., Smith, R. and Macmillan, D. C. (1973) Clin. Sci. 44, 429–438.
[3] Bauze, R. J., Smith, R. and Francis, M. J. O. (1975) J. Bone Joint Surg. 57-B, 2–12.
[4] Niemann, K. M. W. (1969) J. Bone Joint Surg. 51-A, 804 abstract.
[5] Bleckmann, H., Wollensak, K. J. and Buddecke, E. (1971) Z. Kinderheilk 110, 74–84.
[6] Trelstad, R. L., Rubin, D. and Gross, J. (1977) Lab. Invest. 36, 501–508.
[7] Penttinen, R. P., Lichtenstein, J. R., Martin, G. R. and McKusick, V. A. (1975) Proc. Natl. Acad. Sci. USA 72, 586–589.
[8] Rubin, A. L., Drake, M. P., Davison, P. E., Pfahl, D., Speakman, P. T. and Schmitt, F. O. (1965) Biochemistry 4, 181–190.
[9] Lenaers, A., Ansey, M., Nusgens, B. V. and Lapiere, C. M. (1971) Eur. J. Biochem. 23, 533–543.
[10] Fujii, K., Tanzer, M. L., Nusgens, B. V. and Lapiere, C. M. (1975) Biochem. Biophys. Res. Commun. 69, 128–134.
[11] Tanzer, M. L. (1968) J. Biol. Chem. 243, 4045–4054.
[12] Tanzer, M. L., Housley, T., Berube, L., Fairweather, R., Franzblau, C. and Gallop, P. M. (1973) J. Biol. Chem. 248, 393–402.
[13] Epstein, E. H., jr. (1974) J. Biol. Chem. 249, 3225–3231.
[14] Fujii, K. and Tanzer, M. L. (1977) Clin. Orthop. 124, 271–277.
[15] Fujii, K. and Tanzer, M. L. (1974) FEBS Lett. 43, 300–302.
[16] Fujii, K., Kuboki, Y. and Sasaki, S. (1976) Gerontology 22, 363–370.