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Lysyl hydroxylation in collagens from hyperplastic callus and embryonic bones

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Tissue from two patients with osteogenesis imperfecta suffering from a hyperplastic callus was studied. Although collagen type I from the compact bone and the skin and fibroblast cultures of these patients showed normal lysyl hydroxylation, collagen types I, II, III and V from the callus tissue were markedly overhydroxylated. Furthermore, the overhydroxylation of lysine residues covered almost equally the entire $\alpha 1(I)$ collagen chain, as demonstrated by the analysis of individual CNBr-derived peptides. In addition, collagen type I was isolated from femoral compact bone of 33 individuals who died between the 16th week of gestational age and 22 years. Lysyl hydroxylation rapidly decreased in both collagen $\alpha 1(I)$ and $\alpha 2(I)$ chains during fetal development, and only little in the postnatal period. The transient increase in lysyl hydroxylation and the involvement of various collagen types in callus tissue argue for a regulatory mechanism that may operate in bone repair and during fetal development.

INTRODUCTION

Collagen, the predominant structural component of all connective tissues, provides the fibrillar scaffold in the supramolecular organization of the extracellular matrix. Thus biomechanical properties such as skeletal stability and tensile strength largely depend on a well-co-ordinated expression of collagen genes and the ordered execution of the post-transcriptional and post-translational processing. In particular, there is a growing, although circumstantial, body of observations that post-translational modifications of collagen molecules such as prolyl and lysyl hydroxylation may play a crucial role in tissue development and maturation. Deficiency in lysyl hydroxylation has been shown to be the molecular defect in Ehlers–Danlos syndrome type VI [1,2]. In contrast, overhydroxylation of lysine residues was found to be associated with a group of patients suffering from osteogenesis imperfecta (OI). In the latter condition, there is a general agreement that a perturbation of triple-helix formation due to mutations in genes coding for collagen type I allows the modifying enzymes to act for an extended period on the individual chains. In consequence, OI patients may suffer from highly fragile bones, skeletal deformities and growth disturbance [3–6]. Among the various subtypes of OI, generalized osteoporosis is a common pathophysiological hallmark, whereas hyperplastic callus formation is a rare and unique clinical feature of a well-defined group of OI patients [7–10]. Previous studies have indicated that hyperplastic callus formation and embryonic bone formation may have some molecular features in common: (1) decreasing extents of lysyl hydroxylation in collagen type I with time and tissue maturation; (2) the presence of relevant amounts of collagen types III and V beside the predominance of collagen type I [11]. In order to deepen our understanding of the importance of overhydroxylation of lysine residues in collagen derived from callus tissue we undertook the following studies. (1) We compared the degree of lysyl hydroxylation in collagen types I, II, III, V, all isolated from callus tissue of two OI patients, with collagen from various control tissues derived from early embryonic stages to adulthood. (2) We studied the distribution of hydroxylysine residues along the overhydroxylated $\alpha 1(I)$ collagen chain. (3) We compared the triple-helix stability of overhydroxylated collagen type I with that of normal collagen type I.

METHODS

Patients

Patient 1 was a 15-year-old boy with OI type IV according to the Sillence classification [12]. After removal of a stationary 5-year-old callus and implantation of an intramedullary rod, a rapidly growing hyperplastic callus developed that became available for biochemical studies after an additional operation (later referred to as callus 1). Recently a more detailed report on the patient's history has been published [11]. Patient 2 was a 24-year-old male. OI type III was diagnosed at birth because of bone deformities, fractures and X-ray criteria. The family history was negative. The slightly blue colour of the sclerae at birth turned to white during adolescence. He suffered from more than 80 fractures and a severe kyphoscoliosis. At the age of 24 years he developed a hyperplastic callus of the proximal metaphysis of the right femur over 24 months without any obvious trauma or spontaneous fracture. The excised tumour (callus 2), pieces of skin and compact bone were available for biochemical studies,

Table 1. Summary of information on callus 1 and callus 2

	Callus 1	Callus 2
OI type	IV	III
Age of donor (years)	15	24
Sex	Male	Male
Age of callus (months)	5	24
Localization	Proximal right femur	Right femur metaphysis
Size	15 cm × 10 cm × 5 cm	11 cm × 4 cm × 5 cm
Collagen type composition	I, II, III, V	I, III, V

Abbreviation used: OI, osteogenesis imperfecta.

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and fibroblast cultures could be established. The clinical information of both patients is summarized in Table 1.

Controls

Femoral compact bone was obtained at autopsy from 33 individuals who had died of diseases not related to connective tissue such as infectious diseases or respiratory distress syndrome. Owing to limited amount of bone in some of the fetal controls, three pools were collected, each consisting of five individuals. The first pool comprised femoral bones from fetuses of 16–20 weeks, the second 23–27 weeks and the third 34–40 weeks of gestational age. From the other 18 individuals sufficient material for collagen studies was available. Control skin for biochemical analyses and establishment of fibroblast cultures was obtained at surgery from patients suffering from diseases unrelated to connective tissue.

Chemicals

All chemicals were of analytical grade, unless otherwise stated.

Preparation of tissue

The different tissues were separated carefully from adjacent fat, tendons, vessels and periosteum. They were cut into cubes from which blood and fat were removed by sequential washing with water and 96% ethanol (v/v). After homogenization under liquid N_2, residual fat was removed by additional washing with ethanol. The freeze-dried material was decalcified by dialysis over 3 weeks (0.5 M-EDTA/20 μM-phenylmethanesulphonyl fluoride/10 μM-4-chlormercuribenzoate, pH 7.4, at 4 °C) with repeated changes. After 2 and 3 weeks, a dialysis step against 0.05% (v/v) acetic acid was carried out.

Extraction of collagens

Collagen was extracted by limited pepsin digestion (pig gastric pepsin, EC 3.4.23.1; Boehringer–Mannheim) (0.1 mg/ml in 0.5 M-acetic acid, pH 1.6 adjusted with HCl, for 24 h at 4 °C) five consecutive times. The insoluble remainder was collected by centrifugation at 65000 **g** for 1 h at 4 °C.

SDS/PAGE

SDS/PAGE was performed with or without delayed reduction [13]. For the separation of collagen chains, 4% (w/v) acrylamide stacking gels and 6% (w/v) separation gels were prepared [14]. For the separation of CNBr-derived peptides, 12% (w/v) separation gels were used. Gels were stained with Coomassie Blue and the relative amounts of the different components were measured by whole-band densitometry with the use of a video scanner (Biometra, Göttingen, Germany).

Sequential salt precipitations

Sequential salt precipitations were performed under neutral conditions by sequential dialysis against 0.7 M, 1.8 M- and 2.5 M-NaCl solutions in 0.05 M-Tris, pH 7.4, at 4 °C with four changes over 48 h. To separate collagen types II and V, the supernatant of 2.5 M-NaCl was dialysed against 0.7 M-NaCl in 0.5 M-acetic acid, pH 3.0. After centrifugation at 65000 **g** for 1 h at 4 °C, the pellets were dissolved in 0.05% (v/v) acetic acid and extensively dialysed against the same solvent to remove residual salt [15].

Preparation of α1(I) and α2(I) chains

Collagen type I obtained by salt precipitation was dissolved in 4 M-guanidinium chloride (Enzyme grade; BRL, Gaithersburg, MD, U.S.A.; 1 mg/ml), heat-denatured (at 56 °C for 10 min) and quenched on ice. The γ- and β-components were separated from the monomers on a Superose TM 6 f.p.l.c. molecular-sieve column (Pharmacia, Uppsala, Sweden) as described by Bateman *et al.* [16] with minor modifications (0.05 M-sodium phosphate buffer, pH 6.5, containing 0.15 M-NaCl and 4 M-urea; flow rate 0.1 ml/min). The monomeric fraction was loaded on to a C_{18} reverse-phase column (Vydac 201TP54; Separations Group, Hesperia, CA, U.S.A.) in 200 μl portions at room temperature. In order to resolve the α-chains, the column was developed with a linear gradient from 19.5% (v/v) to 32.5% (v/v) acetonitrile (h.p.l.c. grade; Baker, Deventer, The Netherlands) over 35 min at a flow rate of 1 ml/min [16].

Separation of CNBr peptides of α1(I) and α2(I) chains

Collagen type I was cleaved with CNBr (50 mg/ml in 70% formic acid for 4 h at 30 °C) [17]. The freeze-dried cleavage products were dissolved in 4 M-guanidinium chloride and separated on two tandemly connected TSK G3000SW columns (Pharmacia) under running conditions described for the Superose system with a flow rate of 0.2 ml/min. The two peaks containing either α1-CB7, α1-CB8 and α2CB4 or α1-CB3 and α1-CB6 were rechromatographed individually on a C_{18} column (see above) applying a linear gradient from 16.25 to 32.5% (v/v) acetonitrile over 30 min.

Preparation of α1(III) chains

Collagen type III was concentrated to about 50% in the precipitate of 1.8 M-NaCl. To remove the collagen type I, the precipitate was chromatographed on a molecular-sieve column under the conditions indicated above. The fraction containing trimers was pooled and dialysed against 0.05% acetic acid. The freeze-dried samples were reduced at room temperature (0.1 M-methanethiol/5 M-urea, pH 8 adjusted with Tris, for 4 h) and carboxymethylated for 10 min by adding sodium iodoacetate to a final concentration of 0.2 M [18]. In order to isolate the reduced and carboxymethylated collagen type III chains, rechromatography on the molecular-sieve column was performed. The monomeric fraction contained pure type III collagen as judged by SDS/PAGE (Fig. 1*a*, lane d).

Purification of collagen types II and V

Salt-precipitated collagen types II and V were further purified on the C_{18} reverse-phase column under the conditions described for the separation of collagen type I.

Cell culture studies

Skin fibroblasts were grown in 175 cm^2 flasks (Greiner, Frickenhausen, Germany) under 5% CO_2. They were maintained in Dulbecco's minimal essential medium supplemented with 10% (v/v) fetal-calf serum, ascorbate (50 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml) and glutamine (2 mM). To prevent cross-linking, β-aminoproprionitrile fumarate (100 μg/ml) was added. Medium was harvested every third day and immediately frozen up to 200 ml. After pepsin digestion collagen was precipitated with $(NH_4)_2SO_4$ (243 g/l), sedimented at 65000 **g** for 1 h at 4 °C and dialysed against 0.05% acetic acid. The freeze-dried collagen was fractionated on a molecular-sieve column and separated into α-chains as described above.

Amino acid analysis

Amino acid analysis was performed on an automated amino acid analyser (System 6300; Beckman, Palo Alto, CA, U.S.A.) after hydrolysis for 22 h with 6 M-HCl under N_2 at 110 °C.

C.d. and transition profiles

C.d. spectra were recorded on a Jasco J-500 A spectropolarimeter (Jasco, Tokyo, Japan), equipped with a temperature-controlled Gilford quartz cell of 1 cm path-length. The molar ellipticity expressed in degrees·$cm^2 \cdot mol^{-1}$ was calculated on the

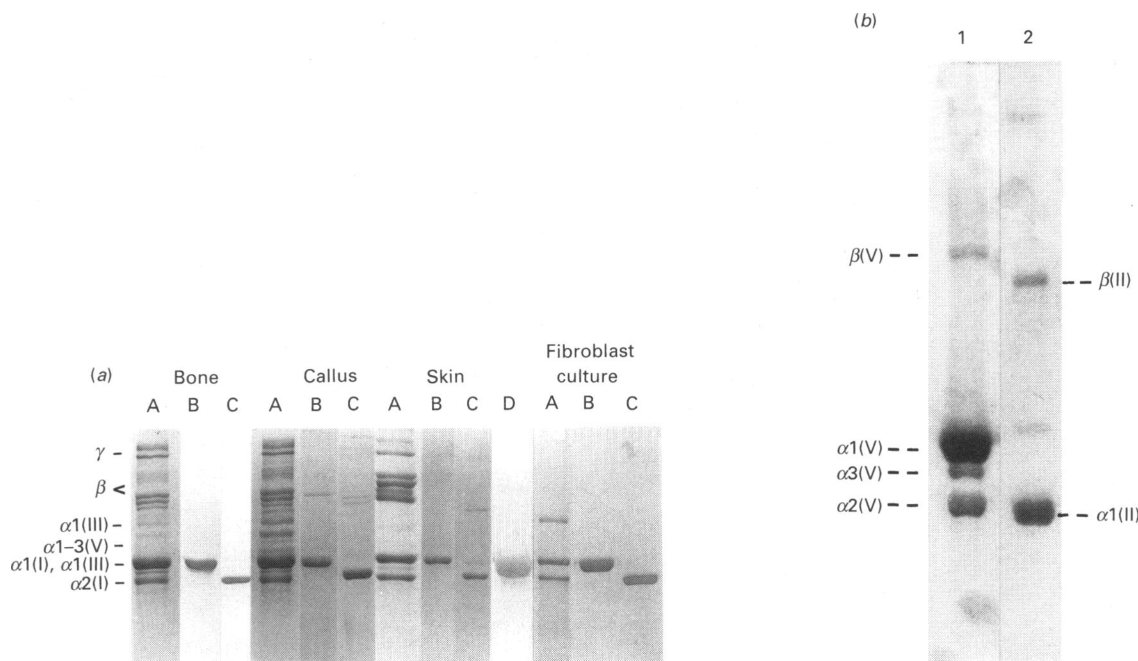

Fig. 1. (a) Purified collagen from tissue and fibroblast cultures of patient 2 and (b) collagen types II and V derived from callus 1 pepsin extracts after acidic salt precipitation

(a) Lanes A, total pepsin extracts; lanes B, α1(I) chains; lanes C, α2(I) chains; lane D, α1(III) chains. (b) Lane 1, collagen type V; lane 2, collagen type II.

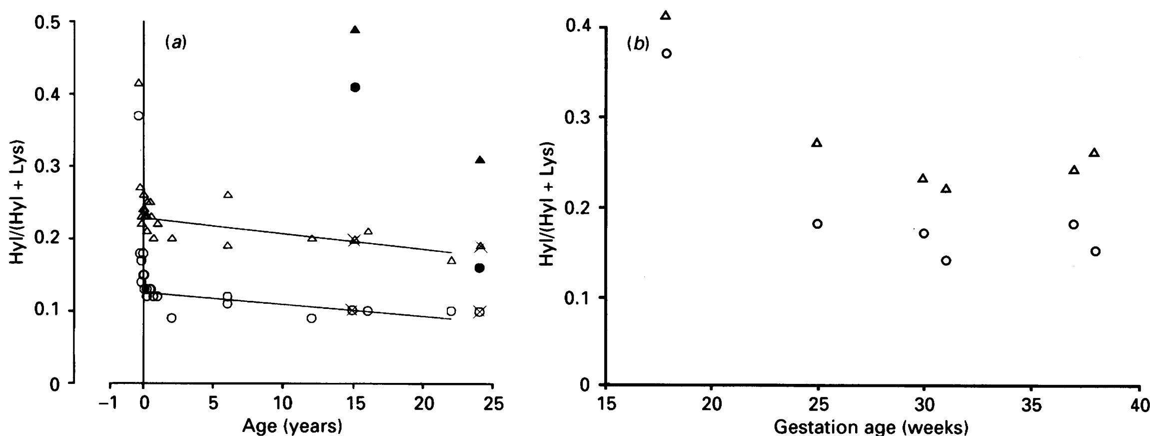

Fig. 2. Degree of lysyl hydroxylation of collagen type I derived from compact bones of various ages and hyperplastic callus tissue

Collagen was pepsin-extracted from decalcified compact bones of individual donors (controls), patients' compact bones and their hyperplastic calluses. Collagen type I monomers obtained by molecular-sieve chromatography were subsequently separated into individual α-chains by reverse-phase h.p.l.c. Hydroxylation of lysine was determined by amino acid analysis. ○, α1(I) of individual donors; △, α2(I) of individual donors; ○, α1(I) of pooled compact bones (five individuals); △, α2(I) of pooled compact bones (five individuals); ●, α1(I) of callus tissue; ▲, α2(I) of callus tissue; ⊗, α1(I) of compact bones of patients with hyperplastic callus; ⩓, α2(I) of compact bones of patients with hyperplastic callus; ——, postnatal fit.

basis of a mean residue molecular mass of 98. The degree of 0% helix–coil conversion corresponds to the molar ellipticity of totally denatured collagen triple helix at 221 nm. Thermal transition curves were recorded at 221 nm by raising the temperature linearly at the rate of 30 °C/h with the use of a Gilford temperature programmer. The sample concentration was 20 μl/ml. Sample buffer was 0.05% acetic acid. Changes in thermal stability due to radiation damage as reported by Hayashi *et al.* [19] could not be detected.

RESULTS

Degree of hydroxylation of proline and lysine residues in collagen type I

Collagen extracted by limited pepsin digestion was further fractionated by differential salt precipitation. Individual α1(I) and α2(I) collagen chains were obtained by reverse-phase chromatography under denaturing conditions and the purity and identity of these chains was shown by SDS/PAGE (Fig. 1*a*, lanes

Table 2. Lysyl and prolyl hydroxylation of different collagen types

α-Chains of pepsin-extracted collagen types I, II, III and V from various sources were purified by h.p.l.c. and the amino acid compositions were determined. The degree of lysyl hydroxylation and prolyl hydroxylation is expressed as Hyl/(Hyl+Lys) ratio and Hyp/(Hyp+Pro) ratio respectively. Abbreviations: N.F., not found; N.D., not determined.

	α1(I)		α2(I)		α1(II)		α1(III)		α1(V)+α2(V)+α3(V)	
	Lys	Pro	Lys	Pro	Lys	Pro	Lys	Pro	Lys	Pro
Mineralized tissue										
Callus 1	0.41	0.47	0.49	0.48	0.52	0.46	0.40	0.48	0.65	0.45
Callus 2	0.16	0.45	0.31	0.44	N.D.		0.27	0.53	N.D.	
Compact bone, patient 1	0.10	0.45	0.20	0.44	N.F.		N.D.		N.D.	
Compact bone, patient 2	0.10	0.44	0.19	0.44	N.F.		N.D.		N.D.	
Control compact bone, ($n = 15$; mean ± 2 s.d.)	0.12 ± 0.03	0.45 ± 0.02	0.22 ± 0.04	0.43 ± 0.02	N.F.		N.D.		N.D.	
Control compact bone, 34–40 weeks	0.18	0.45	0.24	0.41	N.F.		N.D.		0.54	0.45
Control cartilage	N.F.		N.F.		0.40	0.46	N.F.		N.D.	
Soft tissue										
Skin, patient 2	0.12	0.41	0.28	0.41	N.F.		0.15	0.52	N.D.	
Control skin, 25 year old	N.D.		N.D.		N.F.		0.14	0.48	N.D.	
Control skin, 46 year old	N.D.		N.D.		N.F.		0.13	0.48	N.D.	
Control skin, 46 year old	N.D.		N.D.		N.F.		0.12	0.48	N.D.	
Control skin, 48 year old	N.D.		N.D.		N.F.		0.09	0.48	N.D.	
Cell culture										
Fibroblasts, patient 2	0.24	0.46	0.42	0.45	N.F.		N.D.		N.D.	
Control fibroblasts ($n = 9$; mean ± 2 s.d.)	0.27 ± 0.065	0.47 ± 0.02	0.44 ± 0.067	0.43 ± 0.02	N.F.		N.D.		N.D.	

B and C). Whereas the Hyl/(Hyl + Lys) ratios for compact bone from both patients were within control levels, the respective values for collagen from the callus tissue were strikingly higher than those from age-matched controls [0.41 for the α1(I) and 0.49 for the α2(I) collagen chain from callus 1; 0.16 for the α1(I) and 0.31 for the α2(I) collagen chain from callus 2] (Fig. 2*a*). The values for callus 1 exceeded even those of the earliest fetal bones. Interestingly, fibroblasts grown from the skin of patient 2 and maintained in tissue culture produced a collagen type I with a normal degree of lysyl and prolyl hydroxylation (Table 2). The amino acid analysis of both α1(I) and α2(I) collagen chains derived from control femoral compact bones showed a dramatic decrease in lysyl hydroxylation during fetal development whereas prolyl hydroxylation was unchanged (Fig. 2*b*). The bone collagen from the earliest embryos (16–20 weeks of gestation) had Hyl/(Hyl + Lys) ratios that were about twice as high as those found in bones from fetuses who died around birth [0.37 versus 0.15 for the α1(I) and 0.41 versus 0.23 for the α2(I) collagen chain]. In the postnatal period a further slight decrease was observed (Fig. 2*a*).

Lysyl and prolyl hydroxylation in CNBr-derived peptides of collagen type I

The preceding experiments clearly demonstrated that excessive hydroxylation of lysine residues affects α1(I) and α2(I) collagen chains in parallel both during normal development of bones and in callus formation. The following experiments were designed to establish the pattern of lysyl hydroxylation along the collagen type I molecule. Virtually two-thirds of the length of the α1(I) collagen chain comprising peptides α1(I)-CB6, α1(I)-CB7, α1(I)-CB3 and α1(I)-CB8 was overhydroxylated. Also α2(I)-CB4, the peptide close to the *N*-terminus end of the α2(I) chain, was overmodified. The Hyl/(Hyl + Lys) ratio in each CNBr-cleavage peptide obtained from both α-chains was consistently elevated by an increment of 0.23. The CNBr-cleavage peptides from the callus showed a homogeneous migration pattern that was indistinguishable from that of control peptides (Fig. 3). Prolyl hydroxylation expressed as Hyp/(Hyp + Pro) varied from 0.38 [α1(I)-CB6] to 0.5 [α1(I)-CB3] and was identical in control and callus peptides.

Hydroxylation of proline and lysine residues in collagen types II, III and V

To address the question whether or not overhydroxylation of lysine residues also occurs in collagens other than type I, collagen types II, III and V were isolated and purified from callus tissue (Fig. 1*a*, lane D, and Fig. 1*b*, lanes 1 and 2). The data for lysyl hydroxylation of the different collagen types from callus tissue and controls are summarized in Table 2. Collagen type II from callus 1 showed a moderate overhydroxylation of lysine residues compared with the pool of collagen type II extracted from fetal cartilage (articular cartilage from five infants who had died between 34 and 40 weeks of gestation). Collagen type II could not be isolated from callus 2, although it was seen as a faint staining by immunohistochemistry on callus tissue sections with anti-(collagen type II) sera (results not shown). The level of lysyl hydroxylation of collagen type III from callus 1 was 3-fold and that from callus 2 about 2-fold higher than collagen type III from control skin of various donors. Skin collagen type III was used as a control since adolescent/adult bone contains collagen type III only in trace amounts. It is also worth mentioning that collagen type III from callus and collagen type III from the skin of patient 2 differed by a factor of 1.8-fold. The proportion of collagen type V in callus tissue was at the upper limit (14 %) by comparison with adult compact bone (7.4 ± 3.5 %, mean ± s.d., $n = 18$). Hydroxylation of lysine residues in collagen type V from callus 1 was markedly higher than that of controls. Collagen type

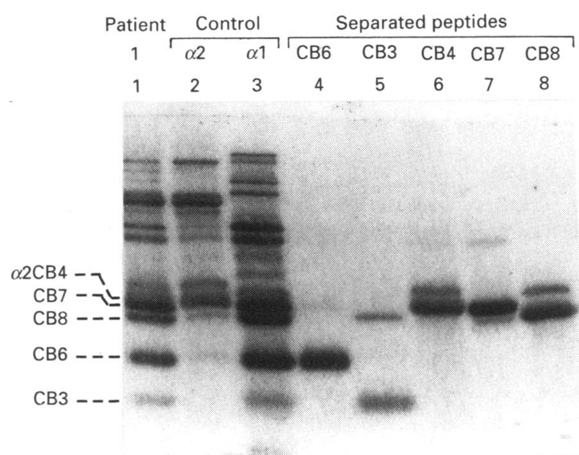

Fig. 3. Electrophoretic separation of CNBr-cleavage peptides

Collagen type I derived from callus 1 was cleaved by CNBr. Both collagen chains of the patient were cleaved together (lane 1), whereas cleavage followed separation into the α1- and α2-chains in the adult control (lanes 2 and 3). The peptides were isolated by fractionation on a molecular-sieve column followed by separation on a C_{18} reverse-phase h.p.l.c. system. From the α2-chain only peptide CB4 could be purified. Lanes 4–8 show peptides purified from callus 1.

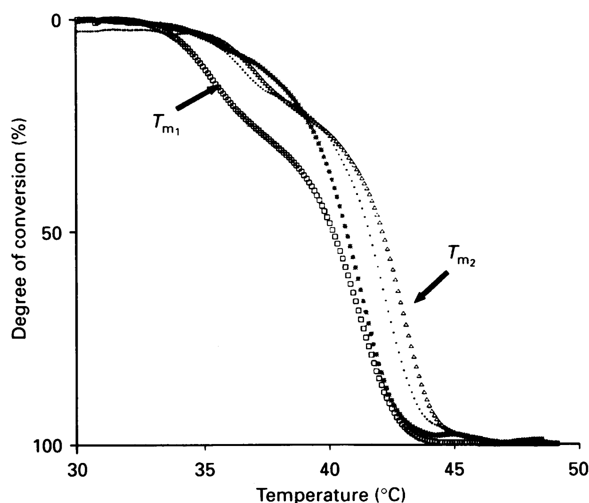

Fig. 4. Thermal transition curves of collagen

The pepsin-extracted and salt-fractionated collagen samples were dissolved in 0.05% acetic acid and the helix-to-coil transition was monitored in a spectropolarimeter at 221 nm. Melting curves were plotted as normalized ellipticities versus temperature of the samples. Collagen type I was derived from callus 1 (■), callus 2 (△), control skin from a 39-year-old (*) and corticalis pool, 36–40 week gestational age (□). T_{m_2} represents the melting temperature of intact collagen type I molecules whereas T_{m_1} is the melting temperature of a shortened collagen type I molecule [20].

V from callus 2, which always co-purified with collagen type I, was not included in our analysis.

Lysyl hydroxylation and melting temperature of collagen type I

Since prolyl hydroxylation was unaltered in all our collagen preparations whereas lysyl hydroxylation varied remarkably, we could study the impact of lysyl overhydroxylation, if any, on thermal stability of the triple helix of collagen type I by c.d. Collagen type I from callus 1 and 2 had a slightly increased melting temperature (42.5 °C for callus collagen and 41.5 °C for control collagen). The biphasic profile of the melting curves is typical for collagen extracted from mineralized tissue (Fig. 4). We recently reported a detailed analysis of the biphasic melting behaviour of pepsin-digested collagen extracts [20]. Collagen type I from skin showed a melting profile with a single T_m value at 41 °C. These melting temperatures correspond to those found by proteolytic degradation experiments by the methods developed by Bruckner *et al.* [21].

DISCUSSION

Physiological functioning of connective tissue largely depends on a co-ordinated expression of genes coding for structural proteins and on their appropriate post-translational processing. One such step in collagen synthesis is the hydroxylation of lysine residues, which is claimed to be of crucial importance since both the lack of hydroxylation as well as overhydroxylation are found to be associated with pathological conditions [1–6].

We have here demonstrated that lysyl hydroxylation rapidly decreases during embryonic development in both α1(I) and α2(I) collagen chains derived from femoral compact bone. In the postnatal years the level of lysyl hydroxylation is fairly constant or continues to fall by very small increments. Similar observations, although not on individual collagen chains, have previously been made on avian bones and on human embryonic bones [22–24]. Accordingly, a decrease in lysyl hydroxylation has been found in rabbit achilles tendon during fetal and postnatal life [22]. Furthermore, Eyre *et al.* [25] found a decrease in the Hyl/Hyp ratio during human life in hydrolysates of femoral compact bone. Since the content of hydroxyproline is fairly constant, this observation might also indicate a further decline in lysyl hydroxylation in collagen type I. Supposedly, the changing levels of lysyl hydroxylation are due to a decrease in lysyl hydroxylase activity, as shown for aging human skin [26].

Callus formation occurs in response to bone fracture and is accompanied by a transient stimulation of lysyl hydroxylation [27]. Hyperplastic callus formation is characterized by an excessive production of poorly structured and mineralized tissue, as is seen in a unique group of patients suffering from OI, a disease resulting in fragile bones and skeletal deformities. Lysine residues are highly overhydroxylated in collagen types I and III, and markedly so in collagen types II and V. In this pathological condition an aberrant regulation of lysyl hydroxylation resulting in overhydroxylation obviously affects all collagen types present and, as previously noted, is a transient event [11]. Here we have shown that the overhydroxylation in callus tissue also depends on the time period for which the hyperplastic callus exists *in vivo*. Callus 1 was surgically removed about 5 months after the first symptoms appeared, whereas callus 2 was removed after 24 months. Clearly collagen from callus 1 has the highest levels of lysyl hydroxylation, which were even higher than those seen in the earliest embryonic bones studied. It is also interesting to note that collagen type I extracted from compact bone, the skin, or synthesized by fibroblasts grown *in vitro* from the patient with callus 2 had normal values of lysyl hydroxylation. In view of all the above observations, we favour the notion that lysyl hydroxylation is under physiological and tissue-specific control, which temporarily fails during excessive callus formation in some patients with OI. Although we have not carried out an analysis to identify an underlying genetic defect in these patients, we consider the observation of overhydroxylation in several collagen types as evidence against a mutation in collagen type I. Accordingly, one would have to assume mutations in all other overhydroxylated collagen types as well.

Since interstitial collagens form rod-like triple-helical molecules, we wanted to see if the additional hydroxylysine residues are concentrated in certain regions or distributed along the entire molecule. CNBr-derived peptides from $\alpha 1(I)$ and $\alpha 2(I)$ collagen chains show that the hydroxylating enzyme seems to target virtually all lysine residues along the entire collagen molecule. Hydroxylysine residues can participate in collagen cross-link formation and are also sites of glycosylation. Although it is known that cross-links contribute to the mechanical strength of the extracellular matrix, the physiological function of the glycosylation of collagen is poorly understood. It seems to interfere with fibril diameter [28]. Triple-helix stability of collagen molecules is closely related to prolyl hydroxylation and may also depend on lysyl hydroxylation and subsequent glycosylation [29]. As shown in Fig. 4 an excess of hydroxylated lysine residues apparently has only a marginal effect on increasing the thermal stability of collagen type I triple helices. This would imply that over-hydroxylated molecules have a more or less normal enzymic susceptibility to degradation *in vivo*. Obviously lysyl hydroxylation has no marked effect on helix stability, since under-hydroxylation of lysine also does not alter T_m values of collagen type I triple helices [30].

Further studies will be needed to show what factors influence collagen lysyl hydroxylation *in vivo* and *in vitro* and what intermediate steps in tissue organization (fibrillogenesis, mineralization) depend on an ordered lysyl hydroxylation in order to maintain physiological function and to avoid clinical symptoms.

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