ganic phosphate or parathyroid hormone, can effect a change from the 1-hydroxylation system to the 24-hydroxylation system. Because this changeover is slow (*3, 4*), requiring days, it seems possible that new enzyme synthesis and enzyme turnover are involved.

Y. TANAKA
H. F. DELUCA

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison 53706

References and Notes

1. I. T. Boyle, L. Miravet, R. W. Gray, M. F. Holick, H. F. DeLuca, *Endocrinology* **90**, 605 (1972); M. F. Holick, M. Garabedian, H. F. DeLuca, *Science* **176**, 1146 (1972); R. G. Wong, A. W. Norman, C. R. Reddy, J. W. Coburn, *J. Clin. Invest.* **51**, 1287 (1972).
2. I. T. Boyle, R. W. Gray, H. F. DeLuca, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2131 (1971); I. T. Boyle, R. W. Gray, J. L. Omdahl, H. F. DeLuca, in *Endocrinology 1971*, S. Taylor, Ed. (Heinemann Medical Books, Ltd., London, 1972), p. 468; L. Galante, S. J. MacAuley, K. W. Colston, I. MacIntyre, *Lancet* **1972-I**, 985 (1972).
3. M. Garabedian, M. F. Holick, H. F. DeLuca, I. T. Boyle, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1673 (1972); D. R. Fraser and E. Kodicek, *Nat. New Biol.* **241**, 163 (1973); L. F. Hill and E. B. Mawer, *Annu. Gen. Meet. Med. Res. Soc.* (abstr.) (London, 1972); H. Rasmussen, M. Wong, D. Bikle, D. B. P. Goodman, *J. Clin. Invest.* **51**, 2502 (1972).
4. Y. Tanaka and H. F. DeLuca, *Arch. Biochem. Biophys.* **154**, 566 (1973).
5. T. Suda, H. F. DeLuca, Y. Tanaka, *J. Nutr.* **100**, 1049 (1970).
6. The crystalline 25-OH-D_3 was a gift from the Philips-Duphar Company of the Netherlands, the 1,25-$(OH)_2$-D_3 was synthesized enzymatically with use of vitamin D-deficient chickens (*4*), and the 24,25-$(OH)_2$-D_3 was chemically synthesized (*12*). The radioactive 25-OH-D_3 was also chemically synthesized (*13*).
7. P. S. Chen, Jr., T. Y. Toribara, H. Warner, *Anal. Chem.* **28**, 1756 (1956).
8. J. Lund and H. F. DeLuca, *J. Lipid Res.* **7**, 739 (1966).
9. M. F. Holick and H. F. DeLuca, *ibid.* **12**, 460 (1971).
10. P. F. Neville and H. F. DeLuca, *Biochemistry* **5**, 2201 (1966).
11. I. T. Boyle, J. L. Omdahl, R. W. Gray, H. F. DeLuca, *J. Biol. Chem.* **248**, 4174 (1973).
12. H.-Y. Lam, H. K. Schnoes, H. F. DeLuca, T. C. Chen, *Biochemistry*, in press.
13. T. Suda, H. F. DeLuca, R. B. Hallick, *Anal. Biochem.* **43**, 139 (1971).
14. Supported by PHS grants AM-14881 and AM-15512. We gratefully acknowledge the technical assistance of Helen Frank.

26 November 1973

Collagen Polymorphism: Characterization of Molecules with the Chain Composition $[\alpha 1(III)]_3$ in Human Tissues

Abstract. *Collagen molecules with the chain composition* $[\alpha 1(III)]_3$ *have been isolated from pepsin-solubilized collagen of dermis, aorta, and leiomyoma of the uterus by differential salt precipitation. On denaturation, approximately 90 percent of this collagen is recovered as a* γ *component (300,000 daltons). Reduction and alkylation of the high-molecular-weight component yields* $\alpha 1(III)$ *chains (95,000 daltons). In addition to containing cysteine,* $\alpha 1(III)$ *chains exhibit several other compositional differences when compared to* $\alpha 1(I)$, $\alpha 1(II)$, *or* $\alpha 2$ *chains from human tissues.*

The majority of the triple-stranded collagen molecules in several connective tissues such as bone, mature dermis, tendon, and dentin contain two distinct α chains. These chains, $\alpha 1(I)$ and $\alpha 2$, occur in this type of molecule in a ratio of 2 to 1; that is, the chain composition is $[\alpha 1(I)]_2\alpha 2$ (*1*).

In contrast, cartilage collagen is comprised almost entirely of molecules containing three identical α chains, which differ from $\alpha 1(I)$ and $\alpha 2$ chains with respect to amino acid composition and sequence as well as to the content of hydroxylysine-linked carbohydrate. These identical chains have been designated $\alpha 1(II)$ chains (*2*). The predominance of collagen molecules with the chain composition $[\alpha 1(II)]_3$ has been established for several cartilaginous structures in a number of species (*3–5*).

In addition, evidence has been presented for the occurrence of yet another genetically distinct α chain in interstitial collagens. This chain, designated $\alpha 1(III)$, has heretofore been detected only in the form of two cyanogen bromide peptides derived from insoluble collagen of infant dermis (*6*). We report here the isolation of collagen molecules with the chain composition $[\alpha 1(III)]_3$, with the amino acid composition of $\alpha 1(III)$, and indicate some of the tissues in which this type of molecule occurs.

Samples of skin, aortic arch, and thoracic aorta were obtained postmortem from both premature and normal infants who had died from respiratory complications. Uterine leiomyoma was obtained from several cases at surgery. Samples of skin, vascular tissue, and leiomyoma were pooled, cleaned of adhering tissue, and sliced into small pieces approximately 1 mm^3 in size. Each sample was extracted for 4 days at 4°C in 100 volumes of 1.0*M* NaCl containing 0.05*M* tris-HCl, *p*H 7.5, and for an additional 4 days at 4°C in 100 volumes of 0.5*M* acetic acid. As indicated previously, these solvents were capable of solubilizing a portion of the collagen in infant skin (*7*); however, none of the vessel or leiomyoma collagen was brought into solution.

After the extraction procedures, the tissues were lyophilized and portions (1 g) were stirred in 0.5*M* acetic acid containing pepsin as described for cartilage collagen (*8*). Collagen rendered soluble by incubation of the tissues with pepsin was precipitated from the digestion mixture by the addition of NaCl to a concentration of 0.9*M*. The precipitates were redissolved in 1.0*M* NaCl, 0.05*M* tris, *p*H 7.5, and dialyzed exhaustively against 0.5*M* acetic acid at 4°C and lyophilized. The proportion of original tissue collagen recovered as soluble native collagen by this procedure was 65, 55, and 20 percent for skin, leiomyoma, and vessel, respectively.

Collagen samples were redissolved at a concentration of 5 mg/ml in 0.02*M* (Na^+) sodium acetate, *p*H 4.8, containing 1.0*M* urea, denatured by warming to 45°C for 30 minutes, and chromatographed on a column (1.8 by 10 cm) of carboxymethylcellulose as described for cartilage collagen (*4, 8*). A representative elution pattern (Fig. 1A) indicates the presence of three major peaks which were identified as containing $\alpha 1(I)$, $\alpha 1(III)$, and $\alpha 2$ chains by examination of the cyanogen bromide peptide pattern obtained from the material in each peak (*6*). Although Fig. 1A depicts the elution pattern of collagen from leiomyoma, collagen from the vascular tissue and dermis showed the same pattern. Some heterogeneity was observed in each peak, and this may be ascribed to chemical heterogeneity introduced by the activity of pepsin as well as to the presence of a small amount of β_{12} cochromatographing with $\alpha 1(III)$.

The above results suggesting that pepsin-solubilized collagen from these tissues is comprised of a mixture of molecules with the chain compositions $[\alpha 1(I)]_2\alpha 2$ and $[\alpha 1(III)]_3$ were confirmed by the use of differential salt precipitation similar to the technique used to fractionate mixtures of $[\alpha 1(I)]_2$-$\alpha 2$ and $[\alpha 1(II)]_3$ molecules (*9*). For this purpose, the collagen samples were redissolved at 4°C in 1.0*M* NaCl, 0.05*M* tris, *p*H 7.5; and the NaCl concentration was increased in successive steps by 0.1*M* over the range 1.1 to 3.0*M*. Utilizing this procedure we ob-

Table 1. Amino acid composition of the α1(III) chain derived from human tissues.

Amino acid	Residues per 1000 total residues
4-Hydroxyproline	125
Cysteine*	2
Aspartic acid	42
Threonine	13
Serine	39
Glutamic acid	71
Proline	107
Glycine	350
Alanine	96
Valine	14
Methionine	8
Isoleucine	13
Leucine	22
Tyrosine	3
Phenylalanine	8
Hydroxylysine	5
Histidine	6
Lysine	30
Arginine	46

* Analyzed as carboxymethylcysteine; samples in which the reduced protein was oxidized with performic acid (*10*) and cysteine was determined as cysteic acid gave identical results.

tained an initial precipitate at 1.5*M* NaCl, which on denaturation and chromatography on carboxymethylcellulose revealed the presence of a single major component chromatographing in the position of α1(III) (Fig. 1B). An additional precipitate was obtained at 2.4*M* NaCl, which on denaturation was shown to contain α1(I) and α2 chains as well as a small quantity of β_{12}.

To further characterize the α1(III) chain obtained after carboxymethylcellulose chromatography (Fig. 1B), the material was rechromatographed on a standardized column (1.5 by 155 cm) of agarose beads (Bio-Gel A-5m, 200-400 mesh, Bio-Rad Laboratories) with 2.0*M* guanidine-hydrochloride, containing 0.05*M* tris, *p*H 7.5, as the eluant. Approximately 90 percent of the protein applied to the column chromatographed in the position corresponding to γ components, and there was an absence of components of smaller molecular weight. Since amino acid analyses (*8*) showed the presence of half-cystine in the γ component, additional portions of the material eluted from carboxymethylcellulose were dissolved at a concentration of 20 mg/ml in a solution of 5.0*M* urea, 0.1*M* 2-mercaptoethanol, *p*H 8.0 (attained by the addition of tris-free base). The solution was allowed to stand at room temperature for 4 hours at which time the solution was made 0.2*M* in iodoacetic acid (sodium salt) containing sufficient [1-^{14}C]iodoacetic acid (New England Nuclear, NEC-222) to give a final concentration of 200 μc/ml. The reaction mixture was allowed to stand at room temperature for an additional 10 minutes and a 1-ml portion was applied directly to the agarose column. Portions of the column effluent were mixed with Aquasol (New England Nuclear) and counted in a liquid scintillation counter (model LS-233, Beckman Instruments). The agarose elution pattern of the reduced and alkylated preparation showed that approximately 90 percent of the protein originally chromatographing as the γ component or components of higher molecular weight is recovered as radioactive α chains. Rechromatography of the reduced and alkylated α1(III) chains on carboxymethylcellulose gives an elution pattern indistinguishable from that in Fig. 1B for the γ component.

Amino acid analyses of the reduced and alkylated α1(III) chain were obtained on an automatic amino acid analyzer (*8*) and the composition of the chain when derived from dermis, aorta, and uterine leiomyoma is presented in Table 1.

These data indicate that molecules containing the α1(III) chain can be effectively solubilized from human tissues by incubation with pepsin under conditions in which the enzyme activity would be confined to short, nonhelical segments at the extremities of the molecule (*8*). The data further indicate that the α1(III) chain occurs in these tissues in the form of molecules with the chain composition $[\alpha 1(III)]_3$, since all of the collagen containing α1(III) could be separated from $[\alpha 1(I)]_2\alpha 2$ molecules by differential salt precipitation.

The observation that collagen containing the α1(III) chain is comprised entirely of γ^- and higher molecular weight components was somewhat unexpected. Our data demonstrate, however, that these high-molecular-weight complexes are maintained through interchain disulfide linkages. At the moment it is impossible to assess the physiological significance of these bonds in terms of intra- or intermolecular cross-linking since they could have been formed at the time of denaturation. In any event, the data indicate that α1(III) contains cysteine in pepsin-resistant and, presumably, helical portions of the native molecule.

The amino acid composition of α1(III) presented in Table 1 clearly indicates that it is a distinct type of α chain. When compared to human α1(I)

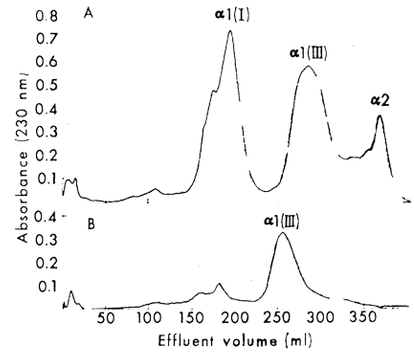

Fig. 1. (A) Carboxymethylcellulose elution pattern of 30 mg of pepsin-solubilized collagen from uterine leiomyoma after denaturation. (B) Carboxymethylcellulose elution pattern of the portion of uterine leiomyoma collagen which precipitates at 1.5*M* NaCl, *p*H 7.5. The amount of denatured protein chromatographed was 10 mg.

and α2 (*7*) as well as α1(II) (*5*), in addition to containing cysteine, the α1(III) chain exhibits significant differences in the content of several amino acids, most notably alanine, valine, isoleucine, phenylalanine, and histidine. In some respects, namely, the relatively high content of hydroxyproline and the presence of cysteine, α1(III) resembles the α1-like chain of basement membrane (*11*). However, the many other compositional differences apparent in α1(III) and the basement membrane chain serve to distinguish these types of chains.

ENDY CHUNG
Department of Pathology,
Institute of Dental Research,
University of Alabama Medical Center,
Birmingham 35294

EDWARD J. MILLER*
Department of Biochemistry,
Institute of Dental Research,
University of Alabama Medical Center

References and Notes

1. E. J. Miller, *Clin. Orthop.* **92**, 260 (1973).
2. ——— and V. J. Matukas, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 1264 (1969).
3. R. L. Trelstad, A. H. Kang, S. Igarashi, J. Gross, *Biochemistry* **9**, 4993 (1970); E. Strawich and M. E. Nimni, *ibid.* **10**, 3905 (1971).
4. E. J. Miller, *ibid.* **10**, 1652 (1971).
5. ——— and L. G. Lunde, *ibid.* **12**, 3153 (1973).
6. E. J. Miller, E. H. Epstein, Jr., K. A. Piez, *Biochem. Biophys. Res. Commun.* **42**, 1024 (1971).
7. P. Bornstein and K. A. Piez, *J. Clin. Invest.* **43**, 1813 (1964).
8. E. J. Miller, *Biochemistry* **11**, 4903 (1972).
9. R. L. Trelstad, A. H. Kang, B. P. Toole, J. Gross, *J Biol. Chem.* **247**, 6469 (1972).
10. S. Moore, *ibid.* **238**, 235 (1963).
11. N. A. Kefalides, *Biochem. Biophys. Res. Commun.* **45**, 226 (1971).
12. Supported by PHS grants HL 11,310-07 and DE-02670.
* Address reprint requests to E.J.M.
23 October 1973; revised 10 December 1973