Preparation of Type III Procollagen and Collagen from Rat Skin†

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ABSTRACT: Neutral salt solutions preferentially extract precursor forms of type I and III collagens from rat skin. These proteins were isolated by chromatography on DEAE-cellulose and identified on the basis of the peptides produced by cleavage with cyanogen bromide. Some type III collagen present in these extracts was isolated and found to contain α chains similar in size and composition to those in type III collagen isolated from skin after limited digestion with pepsin. Two forms of precursor chains, pro α1(III) and pα1(III), were resolved from the type III procollagen fraction after reduction. The smaller form, pα1(III), was isolated and found to contain proportionally less glycine and hydroxyproline and more acidic amino acids and cysteine than α1(III). The thermal stability (T_m) of type III procollagen (and type III procollagen treated with pepsin) was similar to that of type I collagen. However, renaturation was more rapid and complete with the type III preparations, probably due to the presence of intramolecular disulfide bonds.

Biosynthetic precursors (procollagens) of several types of collagen found in vertebrate tissues have been identified. Among these are the precursors of the major collagenous protein of skin, bone, and tendon (type I procollagen) (Layman *et al.* 1971; Bellamy and Bornstein 1971), the cartilage specific collagen (type II procollagen) (Dehm and Prockop 1973), and a collagenous component of basement membranes (type IV procollagen) (Grant *et al.*, 1972).

Miller *et al.* (1971) postulated the existence of another collagen (type III) in skin besides type I to account for two peptides, designated α1(III)-CB3 and α1(III)-CB4,5, which they isolated from cyanogen bromide digests of insoluble human fetal skin. They suggested that these peptides were derived from a chain of the α1 type because of the similarity to the compositions of α1(I)-CB3 and α1(I)-CB4 plus α1(I)-CB5. Recently type III collagen has been solubilized from skin, leiomyomata, and aorta by limited digestion of the tissues with pepsin (Chung and Miller, 1974; Trelstad 1974; Epstein, 1974).

We previously resolved the soluble collagenous proteins synthesized by human skin cells in culture into three fractions, and those extracted from rat skins into two fractions, by chromatography on DEAE-cellulose (Smith *et al.*, 1972; Martin *et al.*, 1973). We identified one fraction from both cells and from skins as type I procollagen. Subsequently, Church *et al.* (1973) who studied collagenous proteins synthesized by dermatosparaxic calf skin cells in culture by the same technique found that a second fraction generated only α1 type chains following incubation with pepsin and suggested that it contained a genetically distinct collagen. We have recently found that this component is a precursor to type III collagen (J. R. Lichtenstein *et al.*, in preparation).

We have modified the extraction and ion-exchange chromatographic techniques that have been previously described (Smith *et al.* 1972; Martin *et al.* 1973), to prepare type III procollagen and type III collagen from rat skins and have partially characterized these proteins.

Materials and Methods

To preferentially extract precursor forms and to limit modification due to proteolysis, skins from fifty 75-g Sprague-Dawley rats were ground with an electric grinder into 2 l. of iced extracting buffer at 0–4° which consisted of 150 mM NaCl–50 mM Tris-HCl–20 mM EDTA–1 mM *p*-mercuribenzoate–10 μM phenylmethanesulfonyl fluoride, all adjusted to pH 7.4 (modified from Monson and Bornstein, 1974). In certain experiments 100 μCi of [3,4-^{3}H]proline or [U-^{14}C] glycine (New England Nuclear) were administered by intracardiac injection 20 min, 2 hr, or 24 hr before the animals were killed. All subsequent procedures were carried out at 4°. The skins were homogenized for 30 sec in a Waring Blendor and then for 30 sec at full power in a Polytron tissue homogenizer. Gross particulate matter was removed by filtration through a laboratory towel and the solution was further clarified by filtration through coarse paper and Celite (Fisher Hi-Flo Super Cel). Solid ammonium sulfate (Schwarz/Mann Ultra Pure grade) was added to the filtrate to 30% of saturation. The precipitate that formed was collected by centrifugation (15,000*g* for 20 min) and washed twice with the extracting buffer modified to contain 20% (w/v) NaCl, and the precipitate was extracted twice with 300 ml of the extracting buffer containing 1 M NaCl.

After clarification by centrifugation (70,000*g* for 15 min), collagenous protein was reprecipitated by the addition of NaCl to 20%, the precipitate that formed was collected by centrifugation (70,000*g* for 15 min), suspended in 200 ml of water, and dialyzed against water, and then the solution was adjusted to 200 mM NaCl–50 mM Tris-HCl (pH 7.6). The collagenous proteins were separated from acidic macromolecules by passage through a DEAE-cellulose column (4 × 25 cm) equilibrated with 200 mM NaCl–50 mM Tris-HCl (pH 7.6) (Miller, 1971). The material which passed directly through the resin was desalted by dialysis and then adjusted to 20 mM NaCl–30 mM Tris-HCl–2 M urea (Schwartz/Mann Ultra Pure grade), and the pH was adjusted to 7.5. The preliminary removal of acidic macromolecules doubled the amount of collagen precursors that dissolved at the subsequent step (approximately 80–90% of these precursors was solubilized as judged by radioactive label). Undissolved matter was removed by centrifugation

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and the sample was applied to a column of DEAE-cellulose (Whatman DE-52, 2.5 × 10 cm) equilibrated with 20 mM NaCl–2 M urea–30 mM Tris-HCl, (pH 7.5) and then eluted with a linear salt gradient from 20 to 200 mM NaCl over a total volume of 600 ml at 8° (Smith *et al.*, 1972; Müller *et al.*, 1973; Martin *et al.*, 1973). The column effluent was monitored continuously with a Beckman DB-GT spectrophotometer at 228 nm and collected in 12-ml fractions and the tubes corresponding to each peak were pooled, desalted by dialysis, lyophilized, and stored at 4°.

To isolate type III collagen, the collagenous proteins that failed to adsorb to DEAE-cellulose in 20 mM NaCl–30 mM Tris-HCl–2 M urea (pH 7.5) were precipitated by the addition to the pooled column front of NaCl to 10% and of acetic acid until the pH was 3.0. Type III collagen was separated from type I by two extractions of the precipitate with 1.5 M NaCl–50 mM Tris-HCl (pH 7.5) which left type III collagen in the precipitate. The residue was dissolved in 10 ml of 0.5% acetic acid and exhaustively dialyzed against 0.1% acetic acid. Solid urea was added to 8 M concentration, an equal volume of 0.06 M NaOAc (pH 4.8) was added, the sample was heated to 50° for 20 min and then applied to a CM-cellulose (CM-52, Whatman) (12 × 1.2 cm) column equilibrated with 30 mM NaOAc–4 M urea (pH 4.8) and eluted with a linear salt gradient of 30–120 mM NaCl over a total volume of 400 ml at 45° (Piez *et al.*, 1963; Bellamy and Bornstein, 1971).

The component polypeptide chains of type III procollagen were separated by chromatography on CM-cellulose. The sample was dissolved in 8 M urea, reduced for 1 hr at room temperature with 1% β-mercaptoethanol, and then dialyzed against 20 volumes of starting buffer for 1 hr prior to application to the column. The conditions of chromatography are as described above.

Electrophoresis in sodium dodecyl sulfate acrylamide was performed as described by Furthmayr and Timpl (1971) in 5% gels for 5 hr at 8.5 mA/tube or in 7.5% gels for 5 hr at 6 mA/tube. Gels were stained for 30 min in Coomassie Blue and destained in 7% acetic acid containing 5% methanol.

Amino acid analysis was done, following hydrolysis in 6 N HCl for 24 hr using an automated amino acid analyzer and the buffer systems described by Miller and Piez (1966).

Collagenase digestion with purified bacterial collagenase (Advanced Biofactors) was performed as described by Peterkofsky (1972) and limited cleavage with pepsin as described by Layman *et al.* (1971).

Digestion of collagenous proteins with cyanogen bromide was performed using 70% formic acid, a 15-fold weight excess of reagent to the 1–2 mg samples, at 30° for 4 hr. Excess cyanogen bromide was removed by lyophilization (Miller *et al.*, 1971).

The denaturation of collagen and procollagen was studied in a Cary Model 60 recording spectropolarimeter. Weighed samples were dissolved in 0.1% acetic acid and then dialyzed into 150 mM NaCl–10 mM phosphate at pH 7.5 and diluted to concentrations of approximately 10 μg/ml. After any suspended particles were sedimented, the dilute solutions were placed in a water-jacketed cell (5-cm light path) and the temperature of the bath was raised at a constant rate of 6.4°/25 min while the rotation was constantly monitored at 313 nm. At 46° the temperature of the coolant bath was quenched quickly to 15° and the rotation monitored at that temperature for 2 hr. The concentration of each sample in solution was determined assuming the specific rotation at 45° to be −825°. The specific rotation was then determined for several points on the curve using the calculated concentration (Piez and Carrillo, 1964).

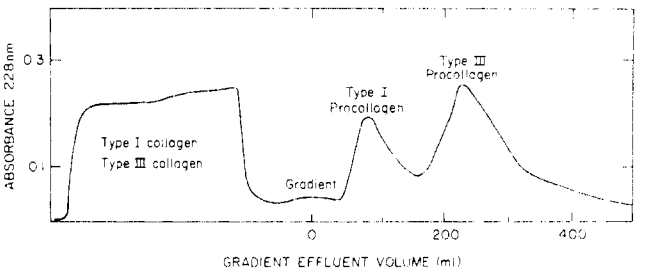

FIGURE 1: DEAE-cellulose chromatography of procollagens from rat skin. The protein not adsorbed to the resin contains both type I and III collagens. The linear salt gradient separates the precursor to type I collagen from the precursor to type III.

Results

Characteristically, collagenous precursor molecules are more soluble in physiological solutions than are collagen molecules (Layman *et al.*, 1971). Because they have large collagenous components, they can be separated from noncollagenous proteins by the traditional methods used to purify collagen and can also be separated from collagen molecules (non-cross-linked) by ion-exchange chromatography on DEAE-cellulose (Figure 1). Although the primary purpose of this report is to describe type III collagen and its precursor chains, we will include some characteristics of type I procollagen for purposes of comparison.

The fractions eluted from DEAE-cellulose were examined by sodium dodecyl sulfate acrylamide gel electrophoresis. After reduction with β-mercaptoethanol, six bands were generated from type I procollagen [which includes small amounts of α1(I) and α2], and three bands from type III procollagen (Figure 2). Studies from several laboratories (reviewed by Martin *et al.*, 1974) indicate that the bands labeled pro α1(I) and pro α2 are the unaltered chains of procollagen type I (Uitto *et al.*, 1972; Goldberg *et al.*, 1972; Fessler *et al.*, 1973; Monson and Bornstein, 1974); they have molecular weights estimated at approximately 140,000. The two more rapidly migrating bands with molecular weights of approximately 120,000 are labeled pα1(I) and pα2 and represent other forms of the pro α chains that arise subsequent to translation. By analogy to the findings with type I procollagen it is likely that the bands, labeled pro α1(III), and pα1(III) in Figure 2, represent similar forms of the precursors of type III collagen chains. None of these bands was observed without prior reduction; rather the material migrated during electrophoresis as a high molecular weight band larger than trimeric components (not shown). Incubation of the sample with bacterial collagenase destroyed these components. Incubation with pepsin under conditions known to produce limited cleavage of procollagen generated a single high molecular weight band which after reduction migrated in the region of α1. Reduction produced small amounts of higher molecular weight components in the regions of dimeric and trimeric components (Figure 3). These studies indicate that these three bands were collagenous, that before reduction they contained disulfide linkages in a helical region or a pepsin resistant nonhelical segment, and that they were converted by pepsin and reduction to α1-like components.

Conclusive identification of the material in this peak as a type III collagenous protein was obtained by comparison of

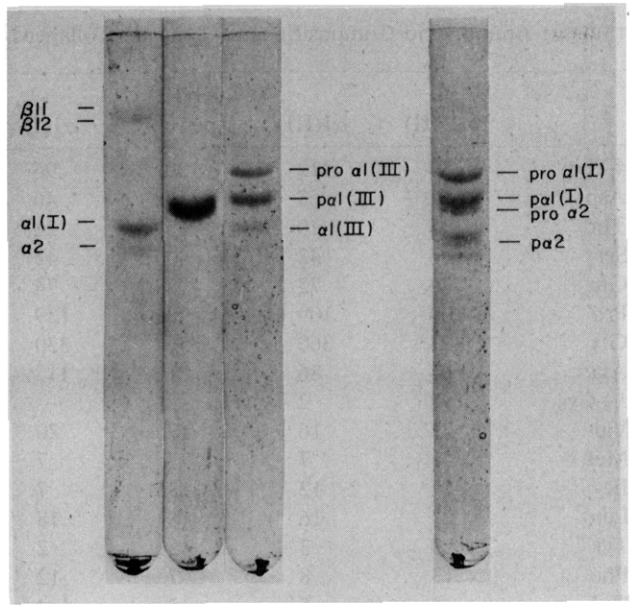

FIGURE 2: Separation of component chains of type III procollagen and type I procollagen by electrophoresis in 5% sodium dodecyl sulfate acrylamide gels. All samples were reduced with 1% β-mercaptoethanol prior to electrophoresis; 50 μl of sample containing 1 mg/ml of protein was loaded onto each gel. Type III procollagen generates those bands labeled pro α1(III), pα1(III), and α1(III). The center gel on the left demonstrates the isolation of pα1(III) by chromatography on CM-cellulose (see Figure 5).

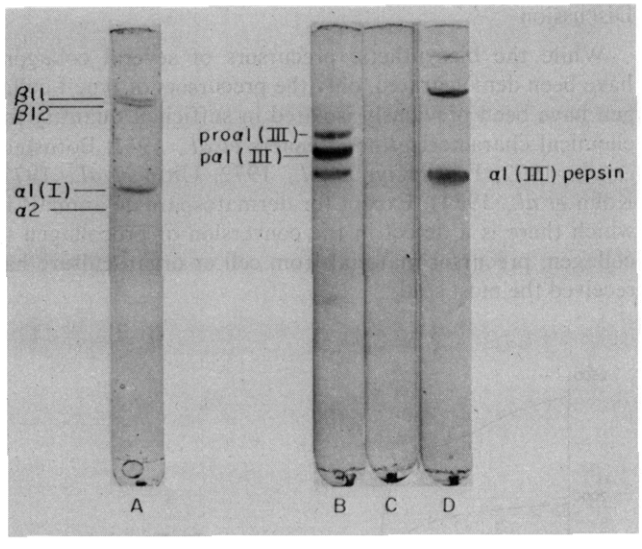

FIGURE 3: Electrophoresis of pepsin-treated and collagenase-treated type III procollagen: (a) type I collagen, (b) type III procollagen preparation (reduced), (c) type III procollagen digested with collagenase (reduced), (d) type III procollagen digested with pepsin (reduced). Each sample (50 μg) was loaded onto each gel.

cyanogen bromide peptides with those from α1(I), α1(II), and with those from α1(III) prepared by pepsin extraction of newborn rat skins by the method of Chung and Miller (1974). The peptides produced from each type of α chain gave a characteristic pattern upon electrophoresis in 7.5% sodium dodecyl sulfate acrylamide gels. The peptide pattern from procollagen type III was similar to that of α1(III) isolated by pepsin digestion of newborn rat skins but different from those of α1(I) and α1(II). There was no visible indication of contamination with α1(I), or α1(II) peptides (Figure 4).

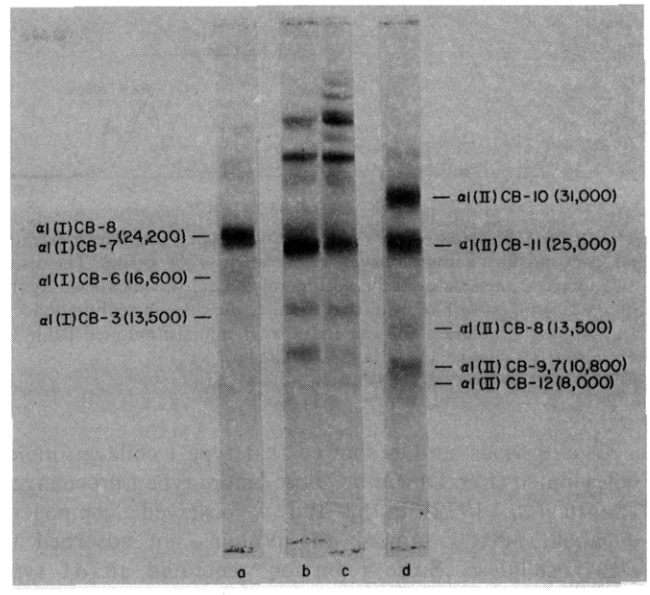

FIGURE 4: Comparison of cyanogen bromide peptides of type III procollagen with peptides from α1(I), α1(II), and α1(III) extracted with pepsin from newborn rat skins: (a) α1(I), (b) α1(III), (c) type III procollagen, (d) α1(II). All chains are from rat tissues; 100 μg of peptides was loaded onto each gel. The α1(II) was extracted from a rat chondrosarcoma described by Smith *et al.* (1974).

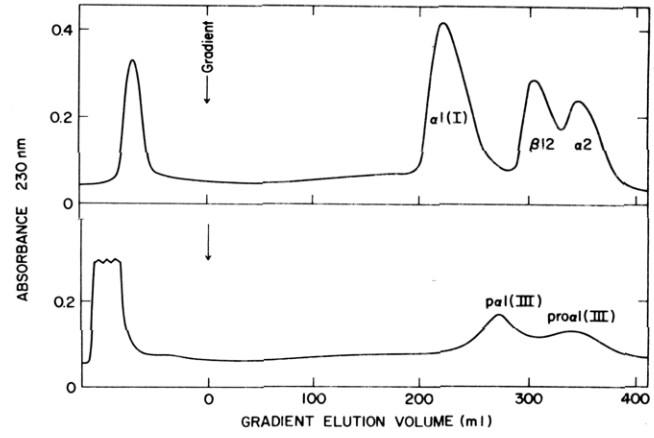

FIGURE 5: Separation of component precursor chains of type III procollagen by CM-cellulose chromatography. Top, type I collagen; bottom, type III procollagen; pα1(III) was resolved from pro α1(III) but α1(III) cochromatographed with pro α1(III).

When rats were killed and skinned 20 min following the intracardiac administration of radioactive glycine or proline, the late eluting peak from DEAE-cellulose was labeled but this label was largely lost in the next 2–24 hr (not shown). The transient labeling and the presence of the components [pro α1(III) and pα1(III)] larger than the α1(III) chain are the characteristics which identify this protein as the biosynthetic precursor of type III collagen.

The major components of type III procollagen were partially resolved by chromatography on CM-cellulose after denaturation and reduction of disulfide bonds with β-mercaptoethanol. Two peaks were eluted with the salt gradient and accounted for 30–40% of the material applied to the column (Figure 5). The first peak contained only the material labeled pα1(III) as judged by subsequent electrophoresis (as in Figure 2) and the second peak, which eluted in the position of α2 contained the bands labeled pro α1(III) and α1(III).

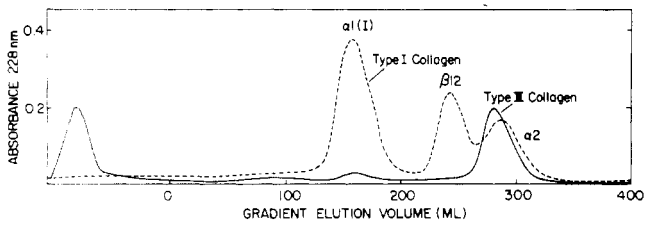

FIGURE 6: CM-cellulose chromatography of type III collagen extracted from rat skins without enzymatic digestion of the tissues. The disulfide linked trimer elutes in the position of $\alpha 2$ and was identified by the characteristic pattern of cyanogen bromide peptides in sodium dodecyl sulfate acrylamide gels and the amino acid composition (see Table I). (——) Type III collagen; (- - -) type I collagen.

Our previous studies showed that type I collagen molecules eluted from DEAE-cellulose before type I procollagen (Smith *et al.*, 1972). In this study we observed a component of about 300,000 among the proteins not adsorbed to DEAE-cellulose. Since reduction generated an $\alpha 1$ type chain from this component, it seemed likely that it was type III collagen. Type III collagen was separated from type I collagen by differential salt precipitation. When this fraction was applied to CM-cellulose under denaturing conditions but without reduction most of the material eluted late in the gradient in a single symmetrical peak in the region of $\alpha 2$ (Figure 6). Type III collagen solubilized from newborn rat skins by limited digestion with pepsin eluted just following $\alpha 2$ (not shown). Electrophoresis of the material in the CM-cellulose peak (labeled type III collagen in Figure 6) gave a trimeric component (molecular weight approximately 300,000) and after reduction a band migrating with $\alpha 1$(III) (not shown); the major cyanogen bromide peptides in 7.5% sodium dodecyl sulfate acrylamide gels were identical to those from $\alpha 1$(III) pepsin or type III procollagen (not shown).

The amino acid compositions of $\alpha 1$(III), $\alpha 1$(III) prepared by pepsin digestion, p$\alpha 1$(III), and $\alpha 1$(I) are presented in Table I. The composition of $\alpha 1$(III) differs markedly from that of $\alpha 1$(I) but is virtually identical with that of the pepsin-solubilized $\alpha 1$(III). Particularly noteworthy are the high levels of glycine and hydroxyproline, the low levels of alanine and the relatively high content of histidine in relation to those of $\alpha 1$(I) and the presence of half-cystine in $\alpha 1$(III). P$\alpha 1$(III) has a lower proportion of glycine, hydroxyproline, and proline and somewhat higher levels of acidic amino acids and cysteine, similar to the findings of other collagen precursor chains in relation to the collagen chains.

Little difference from the pattern obtained with type I collagen was noted in the thermal stability of procollagen type III or procollagen type III treated with pepsin (Figure 7). The measured T_m was 38.6, 38.6, and 38.4° for type III procollagen, the same material treated with pepsin and type I collagen. However, both type III preparations underwent rapid renaturation in contrast to that observed with type I collagen. The curves shown in Figure 7 were constructed using the previously calculated specific rotations for type I collagen. The different base-line levels of specific rotation for procollagen type III and for pepsin-treated type III procollagen may be due to the presence of the noncollagenous portions of the molecule; a different specific rotation for the two collagens; the presence of denatured chains in the preparation or the failure of the type III proteins to lose all helical structure at 45°. The significant aspect of the experiment is the behavior of the type III species which differs so markedly from type I.

TABLE I: Amino Acid Compositions of Type III Collagens.

	pα1(III)	α1(III)	α1(III) pepsin	α1(I)
4-Hyp	113	112	120	96
Asp	61	53	55	46
Thr	18	10	15	20
Ser	46	42	40	42
Glu	80	72	78	74
Pro	100	109	98	129
Gly	323	360	356	330
Ala	65	86	81	112
½-Cys	8	2	2	—
Val	23	16	17	20
Met	6	7	7	7
Ile	17	12	13	7
Leu	23	16	18	18
Tyr	9	3	2	2
Phe	13	8	7	12
Hyl	5	5	5	4
Hist	11	8	12	4
Lys	34	35	34	30
Arg	45	44	42	49

Discussion

While the biosynthetic precursors of several collagens have been demonstrated, only the precursors of type I collagen have been previously isolated in sufficient quantity for chemical characterization (Lenaers *et al.*, 1971; Bornstein *et al.*, 1972; Furthmayr *et al.*, 1972; Uitto *et al.*, 1972; Kohn *et al.*, 1974). Except for dermatosparaxic animals in which there is a defect in the conversion of procollagen to collagen, precursor material from cell or organ culture has received the most study.

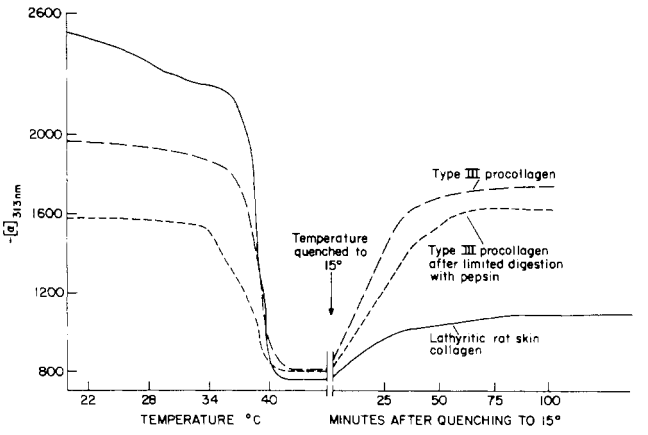

FIGURE 7: Denaturation and renaturation of type III procollagen before and after limited digestion with pepsin. Each sample was heated slowly to 46° as the optical rotation was continuously monitored. The specific rotation was calculated on the basis of the concentration determined by the rotation at 46°. At time zero the temperature was rapidly quenched to 15° and rotation monitored continuously over the following 2 hr. Both type III preparations regained only 15% of initial rotation if the temperature were quenched to 35 or 37°. The measured T_m was 38.6, 38.6, and 38.4° for type III procollagen, pepsin treated type III procollagen, and lathyritic rat skin collagen, respectively.

We have found that it is possible to isolate precursors to type I and III collagens from the skins of normal animals. Approximately 5–10 mg each of type III collagen, type I procollagen, and type III procollagen is obtained from the skins of 50 rats. The recovery of isolated chains by subsequent chromatographic procedures is poor (30–40%). The studies presented here show that chains with molecular weights of approximately 140,000, 120,000, and 95,000 can be isolated from procollagen type III. The fastest migrating polypeptide chain on electrophoresis contained in procollagen type III was identified as α1(III) since it migrated with pepsin treated α1(III) and with the chains of authentic type III collagen.

The polypeptides larger than α1(III) in procollagen type III are probably the precursor chains that correspond to pro α1(I) (140,000) and to pα1(I) (120,000). Pα1(III) was isolated and found to differ in composition from α1(III) in the manner analogous to that described for differences between pα1(I) and α1(I) (Lenaers *et al.*, 1971; Bornstein *et al.*, 1972); that is, by the inclusion of additional half-cystine residues, increased amounts of acidic amino acids and decreased proportions of glycine and hydroxyproline. Although pro α1(III) was not resolved from α1(III) by the chromatographic methods employed here and so its identification must be considered tentative, we think that it is the unmodified precursor chain. There is still insufficient evidence to determine whether the enzymatic conversion of procollagen to collagen occurs by a single step or is complex such that pα chains are necessary intermediates.

The glycine content of the pα1(III) chain isolated by ion-exchange chromatography is considerably higher than that of the analogous precursor to α1(I) (Lenaers *et al.*, 1971; Bornstein *et al.*, 1972; P. H. Byers *et al.*, unpublished). This is, however, in accord with the unusually high glycine content of the intact α1(III) chain. The collagen, in addition to the high glycine content (greater than 33% of the total residues), has, in comparison to type I and II collagens, a greater proportion of hydroxyproline than proline. Recently, several investigators have suggested that hydroxyproline is important for the thermal stability of the collagen helix (Sakakibara *et al.*, 1973; Jimenez *et al.*, 1973; Berg and Prockop, 1973). Since the denaturation temperature of type III procollagen is not significantly different from that of type I collagen (or type I procollagen) the high hydroxyproline content of this collagen may stabilize a helix destabilized by a high glycine content (Traub and Piez, 1971).

Unlike type I collagen, the type III collagen species regained helix rapidly following quenching. Although the initial rotation was different for each species, both type III preparations regained essentially all rotation whereas type I collagen regained only 10–15%. In this manner their behavior is similar to that of collagens containing a predominance of highly cross-linked components such that following denaturation, the chains will rapidly renature. In type III collagen disulfide bonds rather than lysine-derived covalent cross-links hold the chains in register.

The function of cysteine in type III collagen is unclear. There are intramolecular, interchain disulfide bonds since reduction in addition to denaturation is required to generate α1(III) chains during electrophoresis. Trelstad (1974) has suggested that there may be intermolecular disulfide bonds which reduce the solubility of the molecule under most conditions. While it is possible to solubilize the collagen from skin using physiological solvents, the amount dissolved is only a very small proportion of the collagen present and may be the native collagen molecule prior to cross-link formation. However, intermolecular disulfide cross-links need not be the sole source of covalent cross-links; lysine-derived cross-links may also contribute.

Acknowledgments

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References

Bellamy, G., and Bornstein, P. (1971), *Proc. Nat. Acad. Sci. U. S. 68,* 1138.

Berg, R. A., and Prockop, D. J. (1973), *Biochemistry 12,* 3395.

Bornstein, P., von der Mark, K., Wyke, A. W., Ehrlich, H. P., and Monson, J. M. (1972), *J. Biol. Chem. 247,* 2808.

Chung, E., and Miller, E. J. (1974), *Science 183,* 1200.

Church, R. L., Tanzer, M. L., and Lapière, C. M. (1973), *Nature (London), New Biol. 244,* 188.

Dehm, P., and Prockop, D. J. (1973), *Eur. J. Biochem. 35,* 159.

Epstein, E. H., Jr. (1974), *J. Biol. Chem. 249,* 3225.

Fessler, L. E., Burgeson, R. E., Momis, N. P., and Fessler, J. H. (1973), *Proc. Nat. Acad. Sci. U. S. 70,* 2993.

Furthmayr, H., and Timpl, R. (1971), *Anal. Biochem. 41,* 510.

Furthmayr, H., Timpl, R., Stark, M., Lapière, C. M., and Kuhn, K. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 28,* 247.

Goldberg, B., Epstein, E. H., Jr., and Sherr, C. J. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 3655.

Grant, M. E., Kefalides, N. A., and Prockop, D. J. (1972), *J. Biol. Chem. 247,* 3539.

Jimenez, S., Harsch, M., and Rosenbloom, J. (1973), *Biochem. Biophys. Res. Commun. 52,* 106.

Kohn, L. D., Isersky, C., Zupnik, J., Lenaers, A., Lee, G., and Lapière, C. M. (1974), *Proc. Nat. Acad. Sci. U. S. 71,* 40.

Layman, D. L., McGoodwin, E. B., and Martin, G. R. (1971), *Proc. Nat. Acad. Sci. U. S. 68,* 454.

Lenaers, A., Ansay, M., Nusgens, B. V., and Lapière, C. M. (1971), *Eur. J. Biochem. 23,* 533.

Martin, G. R., Byers, P. H., and Piez, K. A. (1974), *Advan. Enzymol.,* (in press).

Martin, G. R., Byers, P. H., and Smith, B. D. (1973), *in* Biology of the Fibroblast, Kulonen, E., and Pikkarainen, J., Ed., London, Academic Press, p 339.

Miller, E. J. (1971), *Biochemistry 10,* 1652.

Miller, E. J., Epstein, E. H., Jr., and Piez, K. A. (1971), *Biochem. Biophys. Res. Commun. 42,* 1024.

Miller, E. J., and Piez, K. A. (1966), *Anal. Biochem. 16,* 320.

Monson, J. M., and Bornstein, P. (1974), *Proc. Nat. Acad. Sci. U. S. 70,* 352.

Müller, P. K., McGoodwin, E. B., and Martin, G. R. (1973), *in* Biology of the Fibroblast, Kulonen, E., and Pikkarainen, J., Ed., London, Academic Press, p 349.

Peterkofsky, B. (1972), *Arch. Biochem. Biophys. 152,* 318.

Piez, K. A., Balian, G., Click, E. M., and Bornstein, P.

(1972), *Biochem. Biophys. Res. Commun. 48,* 990.
Piez, K. A., and Carrillo, A. L. (1964), *Biochemistry 3,* 908.
Piez, K. A., Eigner, E. A., and Lewis, M. S. (1963), *Biochemistry 2,* 58.
Sakakibara, S., Inouye, K., Shudo, K., Kishida, Y., Kobayashi, Y., and Prockop, D. J. (1973), *Biochim. Biophys. Acta 303,* 198.
Smith, B. D., Byers, P. H., and Martin, G. R. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 3260.
Smith, B. D., Martin, G. R., Miller, E. J., Dorfman, A., and Swarm, R. (1974), *Arch. Biochem. Biophys.* (in press).
Traub, W., and Piez, K. A. (1971), *Advan. Protein Chem. 25,* 243.
Trelstad, R. L. (1974), *Biochem. Biophys. Res. Commun. 57,* 717.
Uitto, J., Jimenez, S. A., Dehm, P., and Prockop, D. J. (1972), *Biochim. Biophys. Acta 278,* 198.