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Isolation of Type III Collagen from Human Adult Parenchymal Lung Tissue†

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ABSTRACT: The isolation and characterization of type III collagen from adult human lung parenchyma are described. The identity of this molecule as type III collagen has been established on the basis of (a) demonstration of intramolecular disulfide cross-links in the helical portion of the molecule, (b) amino acid analysis characteristic for type III collagen, and (c) composition and size of isolated cyanogen bromide peptides α1(III)-CB3, α1(III)-CB5, and α1(III)-CB8. The molecular weight of lung α1(III) was determined as 93 000 by Agarose chromatography, but its electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gels was slower than that of type I α chains which also have a molecular weight of 93 000.

The lung parenchyma is biochemically and morphologically a complex structure. Approximately 40 different cell types are held together in a complicated supporting structure composed of collagen, elastin, and ground substance (Hance and Crystal, 1975a,b). Collagen is the most abundant structural protein in the lung representing 20-30% of the dry weight of adult human lung (Bradley et al., 1975). It is responsible for the maintenance of airway and vascular stability and contributes significantly to lung recoil. Collagen is fundamental to normal lung structure and function in certain disease states including the fibrotic lung disorders (Hance and Crystal, 1975a,b).

The insolubility of lung collagen has frustrated direct attempts at its characterization (Bradley et al., 1975; Hurst and Baker, 1975). Bradley et al. (1975) were able to extract less than 10% of the total collagen present in lung tissue from 12-17-week-old human fetuses and were unable to extract intact collagen molecules from adult human lung. The latter, it should be noted, has on the average 11 times more collagen per unit lung mass than the fetal tissue (Bradley et al., 1975). The collagen extracted from fetal lung contained α and β chains with chromatographic, molecular weight, and compositional properties similar to those reported for type I collagen extracted from human dermis.

Short-term cultures of lung minces prepared from 3-week-old rabbit lungs have been used to study collagen synthesis by peripheral lung, bronchial tree (first through seventh

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branches), and tracheal tissues. The peripheral lung tissue synthesized $\alpha 1$(I) and $\alpha 2$ chains, while the airway tissue synthesized $\alpha 1$(II) chains characteristic of type II collagen. Hance et al. (1975) have shown that cells washed from newborn rabbit lungs synthesized type I and type III collagen when grown in culture. WI-38 fibroblasts, a well-documented diploid cell line derived from human fetal lung, also synthesized type I and type III collagen molecules in tissue culture.

In vitro biosynthetic data have provided valuable information about the heterogeneity and biosynthesis of lung collagen (Bradley et al., 1974a,b, 1975; Hurst and Baker, 1975; Hance et al., 1975); however, these results must be interpreted with caution when applied to the composition of intact tissue. Chondrocytes synthesize type II collagen as a cell-specific protein as long as they are integrated into the whole organ (Mueller et al., 1975; Layman et al., 1972); however, chondrocytes in culture synthesize type II collagen while in suspension culture, but switch to the synthesis of type I collagen if the cells are grown in monolayer on the surface of a culture flask.

Further evidence for the existence of type III collagen in lung tissue has recently been obtained by Epstein and Munderloh (1975) who isolated a specific peptide from a cyanogen bromide digest of intact adult human lung. The peptide was shown to be identical with the $\alpha 1$(III)-CB8 peptide from human dermal type III collagen.

The present study reports the isolation of the type III collagen molecule from adult human parenchymal lung tissue and describes the identification and characterization of its component $\alpha 1$(III) chain.

Experimental Section

Tissue Preparation. Lung tissue was obtained at autopsy from male patients between 30 and 70 years of age whose death was from nonpulmonary causes. An intact lung was obtained from each patient and studied separately to allow a comparison of data between patients. The lungs were stripped free of pleura, large bronchi, and blood vessels; the remaining parenchymal tissue was minced with scissors, washed with distilled water, and homogenized in a Waring blender. The tissue mince was suspended in 0.5 M acetic acid (1.5 ml/g wet weight) and incubated with porcine pepsin (Sigma, 2 × crystallized) at an enzyme–tissue ratio of 1:50 (wet weight) for 30 h at 11 °C. The pepsin was then inactivated by the addition of NaOH to adjust the pH to 8.0 and the solubilized collagen was dialyzed against 0.02 M Na_2HPO_4 at 4 °C. The resulting precipitate was washed four times with cold 0.02 M Na_2HPO_4, suspended in 0.1 M acetic acid, and dialyzed against 0.1 M acetic acid. Following dialysis this suspension was lyophilized and stored at 4 °C.

Chromatography of Collagen Chains. Pepsin-solubilized lung collagen (200–400 mg) was dissolved for chromatography in 50–60 ml of starting buffer (freshly deionized 8 M urea made 0.02 M in potassium acetate and adjusted to pH 5.5 with NaOH). Chromatography on carboxymethylcellulose (2.5 × 6 cm column) was conducted at room temperature in starting buffer and a 0 to 0.1 M linear LiCl gradient was superimposed over a total volume of 1 l. The flow rate was 150 ml/h and the effluent was continuously monitored for this and all other column chromatography at 226–230 nm in a Gilford Spectrophotometer Model 240. Further purification into component chains was conducted on 6% Agarose (Bio-Gel A-5m, 200–400 mesh) (130 × 2.5 cm column) in 2 M guanidine hydrochloride (Heico), 0.05 M Tris,[1] pH 7.5, at room temperature at a flow rate of 15 ml/h. All column fractions were desalted on Bio-Gel P-2 columns in 0.1 M acetic acid, lyophilized, and stored at 4 °C.

Isolation of Cyanogen Bromide Peptides. Cyanogen bromide peptides were prepared following the method of Epstein (1974). Collagen fractions were dissolved in 70% formic acid (10 mg/ml) and following the addition of 1.5-fold w/w cyanogen bromide (Eastman) were incubated for 4 h under nitrogen at 30 °C. Cyanogen bromide was removed by desalting on Bio-Gel P-2 as described above and the peptides were lyophilized. Cyanogen bromide peptides were initially separated by carboxymethylcellulose chromatography (1.0 × 11 cm column) at 42 °C in 0.02 M sodium formate buffer, pH 3.8, at a flow rate of 50 ml/h. A 0.01 to 0.2 M linear sodium chloride gradient was superimposed over a total volume of 500 ml. Final purification of the cyanogen bromide peptides was achieved by chromatography on 8% Agarose (Bio-Gel A-1.5 m, 200–400 mesh) (1.5 × 130 cm column) at a flow rate of 7.5 ml/h using guanidine-Tris buffer.

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. $NaDodSO_4$–polyacrylamide gel electrophoresis of collagen chains was conducted on 5% polyacrylamide gels (6 h, 6 mA/gel) using the procedure described by Furthmayr and Timpl (1971) except the gels were made 6 M in urea. The samples were prepared for electrophoresis by incubation at 42 °C for 20 min in 0.1 M sodium phosphate buffer, pH 7.40, containing 1% sodium dodecyl sulfate and made 8 M in urea. When reduction of collagen chains was desired, 10 μl of 0.2% dithiothreitol in 8 M urea was added to the top of the gel with the sample. Preliminary studies had revealed that incubation of the sample with dithiothreitol or preparation of the gel in dithiothreitol did not alter the results and was unnecessary. Cyanogen bromide peptides were electrophoresed under similar conditions on 7.5% $NaDodSO_4$–polyacrylamide gels not containing urea.

Molecular Weight Determination. The molecular weights of the collagen chains were determined by chromatography on 6% Agarose (Bio-Gel A-5m, 200–400 mesh) (1.5 × 130 cm column) at a flow rate of 7.5 ml/h using guanidine-Tris buffer with lathyritic rat skin collagen as a standard (Piez, 1968). The cyanogen bromide peptide molecular weights were determined by chromatography on 8% Agarose (Bio-Gel A-1.5m, 200–400 mesh) (1.5 × 130 cm column) at a flow rate of 7.5 ml/h using guanidine-Tris buffer, and electrophoresis on 7.5% $NaDodSO_4$–polyacrylamide gels using authentic cyanogen bromide peptides from human dermis as standards. Standard peptides were a gift from Hans Furthmayr. Cyanogen bromide peptides were also prepared from human skin following the method of Becker et al. (1976). The logarithm of peptide molecular weight was proportional to the elution volume. The reproducibility of the molecular weight was approximately 5%.

Mercaptoethanol Reduction. Reduction and alkylation of isolated type III molecules were performed according to the method described by Daniels and Chu (1975). The reaction was conducted in 0.5 M Tris, pH 8.5, containing 0.2% EDTA and made 8 M in urea.

Borohydride Reduction. α and β chains isolated from reduced and alkylated type III molecules were further reduced with sodium borohydride prior to analysis on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The collagen samples were dissolved in 2 ml of a 2 M guanidine hydrochloride–0.05 M Tris buffer, pH 7.5, containing 1% sodium

[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate.

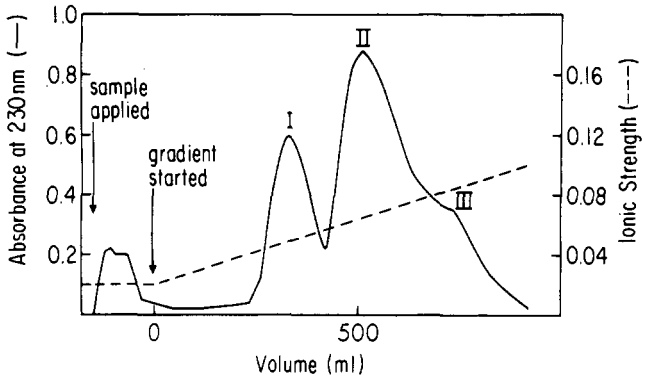

FIGURE 1: Carboxymethylcellulose chromatography of collagen extracted from adult human lung. Collagen was eluted from a 2.5 × 6 cm column (23 °C, 150 ml/h, equilibrated with 8 M urea–0.02 M potassium acetate, pH 5.5) with a LiCl (0–0.1 M) gradient. Fractions (14 ml) were collected; 200 mg of sample was applied.

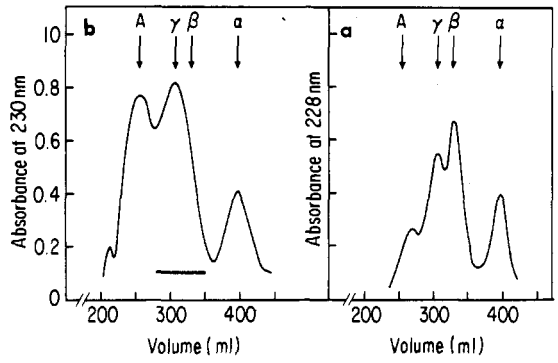

FIGURE 2: (a) Molecular sieve chromatography (Bio-Gel A-5m 130 × 2.5 cm column) of peak II obtained from carboxymethylcellulose chromatography (Figure 1). The column was eluted at a flow rate of 15 ml/h with 2.0 M guanidine hydrochloride–0.05 M Tris, pH 7.5; 100 mg of sample was applied. Horizontal bar denotes γ component subsequently reduced with 2-mercaptoethanol and alkylated with iodoacetic acid (sodium salt). (b) Molecular sieve chromatography of γ component (denoted by horizontal bar in Figure 2a) after reduction and alkylation. Same column and elution conditions; 40 mg of sample was applied.

borohydride; the reaction was terminated after 45 min at 40 °C by the addition of 0.1 ml of glacial acetic acid. The pH was readjusted to 7.4 with NaOH prior to analysis.

Hydroxyproline Analysis. Samples were prepared for hydroxyproline analysis by hydrolysis in 6 M HCl at 108 °C for 24 h and determined by the method of Bergman and Loxley (1963).

Amino Acid Analysis. Samples were prepared for amino acid analysis by hydrolysis under nitrogen in redistilled 6 M HCl at 108 °C for 24 h. One-half-cystine was determined as cysteic acid following performic acid oxidation (Moore, 1963). Prior to amino acid analysis, hydrolysates of cyanogen bromide peptides were treated with pyridine acetate, pH 6.5, at 108 °C for 1 h, to convert homoserine lactone to homoserine. Amino acid analysis was performed on a Beckman Model 119 amino acid analyzer utilizing Durrum DC-6A resin (0.9 × 30 cm column) and Durrum Pico-Buffer System II. Four buffers were used in sequence at a buffer flow rate of 70 ml/h; buffer 1, Pico buffer A adjusted to pH 3.10; buffer 2, Pico buffer A adjusted to pH 3.35; buffer 3, Pico buffer B; buffer 4, Pico buffer C. Buffer change times were respectively 12, 44, and 62 min. A temperature program automatically increased the column temperature from 45 to 55 °C at 35 min. No correction factors were applied for losses during hydrolysis.

Glycosylated Hydroxylysine Analysis. Samples of isolated collagen chains were hydrolyzed in 2 M KOH at 108 °C for 20 h in polypropylene-capped tubes. Following neutralization with 1 M $HClO_4$ the supernatant was analyzed on the amino acid analyzer for glucosylgalactosylhydroxylysine, galactosylhydroxylysine, and free hydroxylysine. Analysis was performed on a Beckman Model 119 amino acid analyzer utilizing Beckman PA-35 resin (0.9 × 11 cm column) and a single buffer, 0.2 N sodium citrate (pH 4.40) at a flow rate of 70 ml/h. A temperature program automatically increased the column temperature from 45 to 55 °C at 35 min. Standards were prepared from commercial sponge (Pinnell et al., 1971).

Results

Initial attempts to solubilize lung collagen utilizing standard solvents, 1 M NaCl at pH 7.5, 0.5 M acetic acid, 8 M urea, and 5 M guanidine, resulted in total extraction of less than 1% of the collagen based on hydroxyproline analysis. The limited proteolytic digestion used here was capable of extracting 20 to 30% of the total parenchymal lung collagen per unit dry weight of lung tissue. The material extracted following pepsin digestion was not soluble in the neutral salt conditions previously described for the isolation of type III collagen derived from other tissues (Chung and Miller, 1974). Attempts to define other conditions for separating type I and type III collagens by neutral salt precipitation were not successful. Therefore, the pepsin-solubilized lung collagen was recovered following precipitation against 0.02 M Na_2HPO_4. The resulting precipitate was only sparingly soluble in 0.5 M acetic acid and was recovered by lyophilization following dialysis against 0.1 M acetic acid. The explanation for the unusual solubility behavior of this collagen is unknown.

Attempts to chromatograph this material directly on carboxymethylcellulose using the conventional solvent systems described for chromatography of collagen (Epstein, 1974; Chung et al., 1974) were not successful, in part, due to the extraordinary insolubility of the precipitate. The 8 M urea–acetate buffer system described here dissolved 95 to 98% of the precipitate and produced the elution pattern seen in Figure 1. The subunit composition of each chromatographic fraction was determined by $NaDodSO_4$–polyacrylamide gel electrophoresis and the relative amounts of the components were calculated from the area of each peak in the carboxymethylcellulose chromatogram. Peak I represented 30.5% of the total sample and consisted of α_1(I) and β_{11}. Peak II consisting of γ and β_{12} components represented 60.0% of the sample and peak III which was α_2 chain represented 9.5% of the sample. There was little variability in subunit composition between different lungs. The identification of type I collagen chains α1(I) and α2 was further confirmed from peaks I and III, respectively, of the carboxymethylcellulose chromatogram by amino acid analysis and cyanogen bromide peptide pattern on 7.5% polyacrylamide gel electrophoresis (data not shown).

When peak II was chromatographed on 6% Agarose (Figure 2a), two major fractions corresponding to aggregated, high-molecular-weight components and γ components were obtained along with a smaller quantity of α and β components. The γ component isolated by Agarose chromatography was reduced, alkylated, and rechromatographed on the same Agarose column. The resulting chromatogram (Figure 2b) showed three distinct peaks corresponding to: (1) α subunits; (2) β subunits; and (3) unreacted γ subunits. This behavior was felt to be consistent with the presence of intramolecular disulfide cross-links in the helical region of the collagen molecule.

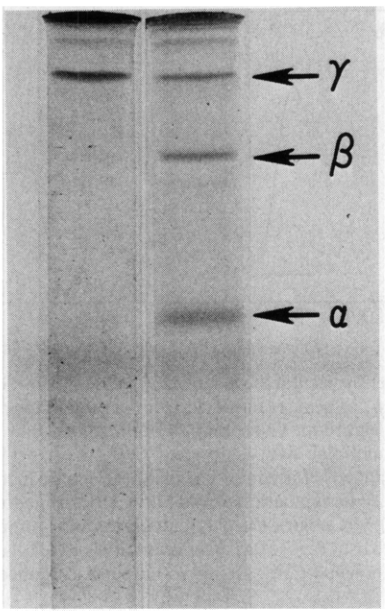

FIGURE 3: $NaDodSO_4$–polyacrylamide gel electrophoresis of γ component in absence (left) and presence (right) of dithiothreitol. Sample on right incubated in 0.2 M dithiothreitol 20 min prior to electrophoresis. Samples were electrophoresed on 6-cm 5% polyacrylamide gels for 6 h at 6 mA/gel.

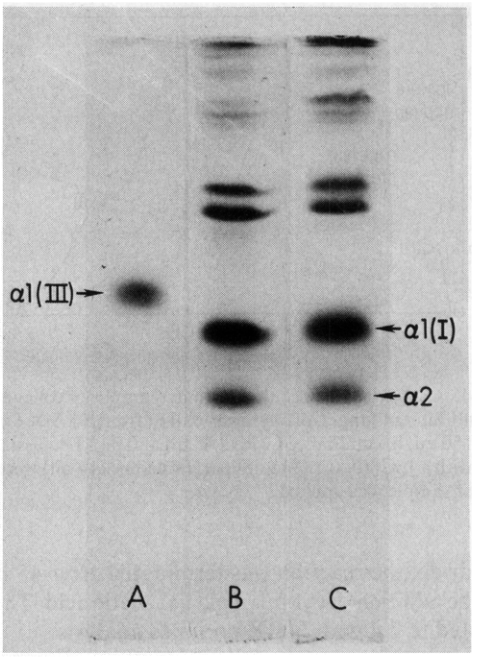

FIGURE 4: $NaDodSO_4$–polyacrylamide gel electrophoresis of (A) α1(III) chain, (B) lathrytic rat skin collaqen, and (C) pepsin extracted human skin collagen. The slower mobility of the α1(III) chain (A) is apparent.

The β and unreacted γ components were isolated and subsequently reduced by sodium borohydride. Analysis by polyacrylamide gel electrophoresis revealed no evidence for further cleavage into α components.

When fractions containing the aggregated and γ components were electrophoresed on $NaDodSO_4$–polyacrylamide gel in the presence of dithiothreitol, two new electrophoretic bands with mobilities corresponding to α and β chains were produced (Figure 3) consistent with the results of Agarose chromatography (Figure 2).

The α component isolated by Agarose chromatography, following reduction and alkylation of the γ component (Figure 2b), moved as a single homogeneous peak on $NaDodSO_4$–polyacrylamide gel electrophoresis (Figure 4). Its amino acid analysis shown in Table I was characteristic of type III collagen. The distinctive features of the amino acid analysis were: (1) a hydroxyproline to proline ratio greater than one; (2) a high content of glycine, histidine, and hydroxyproline; and (3) 2 residues/1000 amino acid residues of ½-cystine.

Only 4.9% of the hydroxylysine residues in the chain were glycosylated and all of these were present as galactosylhydroxylysine. The molecular weight as determined by Agarose chromatography was the same as that reported for the corresponding chain isolated from skin; however, the electrophoretic mobility of the chain from lung on $NaDodSO_4$–polyacrylamide gel was less than that of type I α chains of similar molecular weight.

The isolated α1(III) chain was cleaved by reaction with cyanogen bromide and the resulting peptides electrophoresed on 7.5% $NaDodSO_4$–polyacrylamide gels. The peptide pattern was identical with that obtained for type III collagen from human dermis (data not shown).

To further prove the identity of the chain as α1(III), the material corresponding to peak II (Figure 1) of the carboxymethylcellulose chromatogram was cleaved with cyanogen bromide. The resulting peptides were isolated and purified by a combination of ion exchange and molecular sieve chromatography. The pattern of elution for these peptides from the carboxymethylcellulose column is shown in Figure 5. The chromatogram in Figure 5 is virtually identical with the elution pattern obtained for type III collagen from dermis (Chung et al., 1974). Three peptides corresponding to α1(III)-CB3, α1(III)-CB5, and α1(III)-CB8 were isolated and shown to be closely similar in size and amino acid composition to the corresponding peptides derived from human dermal type III collagen (Table I).

Discussion

The existence of type III collagen was first proposed by Miller et al. (1971) following the isolation and identification of an unique peptide from a cyanogen bromide digest of insoluble skin collagen. Chung and Miller (1974) and Epstein (1974) subsequently purified and isolated the type III molecule from a pepsin digest of human dermis and showed it to be composed of three α1(III) chains which are cross-linked in the helical portion of the molecule by disulfide bonds. Cyanogen bromide peptide data from the α1(III) chain has been reported from human skin (Chung et al., 1974) and placenta (Epstein and Munderloh, 1975) and from bovine peridontium (Butler et al., 1975).

The insolubility of collagen found in adult tissue has been well recognized and generally attributed to the presence of intermolecular cross-linking. Limited proteolytic digestion with pepsin has been widely used to liberate collagen from otherwise insoluble tissue. The collagen molecule is resistant to proteolytic digestion under conditions which preserve the structure of the triple helix. In the case of lung tissue this digestion results in solubilization of both type I and type III collagen molecules.

A differential salt precipitation at neutral pH (Epstein, 1974; Chung et al., 1974) has been used to separate type III from type I collagen following pepsin digestion. This technique was not suitable for use with lung collagen because pepsin-solubilized collagen was only slightly soluble in the neutral buffers used for this procedure. Chung et al. (1975) have postulated the insolubility of type III collagen may result from

TABLE I: Comparison of the Amino Acid Composition of the $\alpha 1$(III) Chain and Component Higher Molecular Weight Peptides of Human Lung and Dermal Collagen.[a]

	α_1(III)		CB-3		CB-5		CB-8	
	Lung	Dermis	Lung	Dermis	Lung	Dermis	Lung	Dermis
4-Hyp	106	125	16	18	28	30	17	15
Asp	53	42	2 (1.8)	2	10	9	7 (7.4)	5
Thr	12	13	1 (0.8)	1	3 (3.2)	3	0 (0.4)	0
Ser	26	39	5 (5.3)	6	8 (8.4)	9	2 (2.1)	1
Glu	83	71	6 (6.2)	6	16	15	10	10
Pro	103	107	13	15	24	28	9 (8.9)	9
Gly	360	350	37	36	86	77	46	45
Ala	99	96	8 (7.5)	8	23	20	18	19
½-Cys[b]	2 (1.9)	2	0	0	0	0	0	0
Val	13	14	1 (0.6)	1	4 (4.2)	4	2 (2.0)	2
Met	8 (8.4)	8						
Ile	13	13	3 (2.9)	3	3 (3.4)	3	2 (1.7)	2
Leu	22	22	2 (2.1)	2	5 (4.8)	5	2 (2.2)	2
Tyr	1 (1.0)	3	1 (1.4)	2	0	0	0	0
Phe	8 (7.8)	8	1 (0.8)	1	2 (1.8)	2	2 (1.8)	1
Hyl	8 (7.8)	5	1 (0.5)	0	1 (1.3)	0.8	1 (0.9)	0.5
Lys	31	30	2 (1.9)	2	8 (8.4)	8	4 (4.4)	5
His	8 (8.0)	6	1 (0.9)	1	0	0	0 (0.2)	0
Arg	48	46	3 (3.1)	4	8	8	8 (7.9)	9
Hse			1 (1.3)	1	1 (1.1)	1	1 (0.9)	1
Total	1000	1000	103	109	231	223	133	126
% Hyl as galactosyl-Hyl	4.9							
% Hyl as glucosylgalactosyl-Hyl	<1.8							
Mol wt by Agarose chromatography	93 000	93 240	9 400	9 500	21 100	20 000	12 100	12 000
Mol wt by $NaDodSO_4$–polyacrylamide gel			10 600		20 000		10 500	

[a] Values for dermis from Chung et al. (1974). Residues rounded off to the nearest whole number. Actual values are in parentheses when less than ten residues were detected. A value of zero indicates less than 0.2 residue. [b] Determined as cysteic acid (see Experimental Section).

the interaction between other macromolecular components of the tissue and the type III molecule. The presence of covalent cross-links in the pepsin-resistant portion of the molecule could also explain the resistance of the molecule to extraction with commonly used solvents. Evidence for the existence of similar cross-links has been found in the collagen extracted from avian, bovine, and human skin following pepsin digestion (Bannister, 1975; Bannister and Burns, 1972; McLain, 1974; Steven, 1966).

The isolation of a collagen molecule from lung which could be converted to α and β components following reduction and alkylation was compatible with the behavior previously demonstrated for type III collagen. The isolation of an α chain with an amino acid composition similar to that of type III collagen from human dermis including cystine and a high hydroxyproline and glycine content further supported the identity of type III collagen in lung.

The isolation and identification of cyanogen bromide peptides $\alpha 1$(III)-CB3, $\alpha 1$(III)-CB5, and $\alpha 1$(III)-CB8 established the identity of this chain as $\alpha 1$(III). The molecular weights and amino acid compositions of the peptides were almost identical with those described for the same peptides from human dermis.

The presence of γ and β components following reduction of type III collagen has been attributed to the incomplete reduction of disulfide cross-links (Chung et al., 1974); however, exposure of these γ and β components to vigorous reducing conditions, including borohydride reduction in the presence of urea, did not result in the formation of α chains. These results are consistent with the presence of covalent cross-links other than disulfide bonds which are resistant to pepsin digestion.

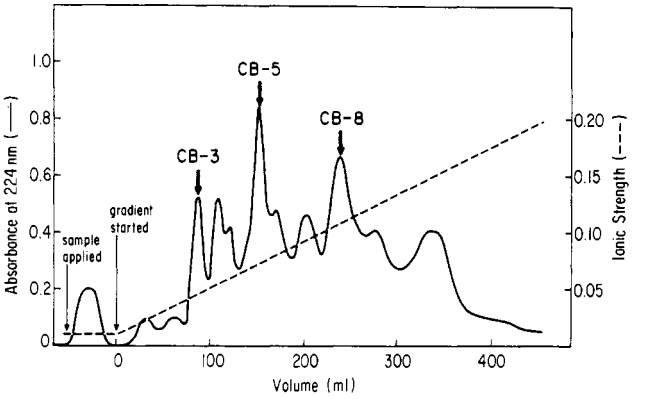

FIGURE 5: Carboxymethylcellulose chromatography of cyanogen bromide digest of peak II (Figure 1). Peptides were eluted from a 1.0 × 11 cm column (42 °C, 50 ml/h, equilibrated with 0.02 M sodium formate buffer, pH 3.8) with a 0 to 0.2 M NaCl gradient. Fractions (8 ml) were collected; 40 mg of sample applied.

The hydroxylysine content of lung $\alpha 1$(III) was elevated over that of $\alpha 1$(III) from dermis. This increased hydroxylysine content may reflect a greater proportion of hydroxylysine-derived cross-links in lung collagen which could, in part, contribute to the insolubility of lung collagen. Type I chains from lung have also been reported with an elevated hydroxylysine content (Bradley et al., 1974a). Although the hydroxylysine content was high, analysis for hydroxylysine glycosides re-

vealed only 5% of the hydroxylysine residues to be glycosylated, and these were entirely galactosylhydroxylysine. No glucosylgalactosylhydroxylysine could be detected. The role for hydroxylysine glycosides is poorly understood, although they may be involved in cross-link regulation (Robins and Bailey, 1974).

The molecular weight of the lung α1(III) chain obtained by Agarose chromatography was 93 000. The α1(III) chain from human dermis has been reported to have a molecular weight of 93 000 (Epstein, 1974; Chung et al., 1974) and that from calf peridontium was reported to have a molecular weight of 95 000 (Butler et al., 1975). Type III procollagen chains have been reported with molecular weights between 115 000 and 120 000 but following pepsin digestion are converted to collagen chains of 95 000 (Byers et al., 1974; Lenaers and Lapiere, 1975; Anesey et al., 1975). The electrophoretic mobility of the lung α1(III) was slower when compared with type I α chains, although the molecular weight is similar. The explanation for this difference is unknown, although it has long been recognized that collagen chains electrophorese aberrantly on NaDodSO$_4$–polyacrylamide gels. Although their molecular weights are identical, α1(I) and α_2 chains also have quite different mobilities on NaDodSO$_4$ gels.

The data shown here do not permit quantification of the amount of type III collagen present in pulmonary tissue, but tissue from a single peripheral adult human lung dissected free of large pulmonary vessels yielded 30 to 60 mg of purified α1(III) isolated as the reduced and alkylated product.

The functional significance of type III collagen in any tissue is unknown, but further characterization of this molecule and its distribution within the lung may provide insight into its importance in pulmonary tissue. Extension of these studies to lung obtained from patients with fibrotic disease may be expected to increase our understanding of the fibrotic process in this pathologic state.

Note Added in Revision

The presence of type III collagen from human lung has been recently reported by Seyer et al. ((1976) *J. Clin. Invest. 57,* 1498).

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