Biochem. J. (1983) **211**, 295–302
Printed in Great Britain

Structural diversity and domain composition of a unique collagenous fragment (intima collagen) obtained from human placenta

Erich ODERMATT,* Juha RISTELI,† Vera VAN DELDEN† and Rupert TIMPL†
**Abteilung für Biophysikalische Chemie, Biozentrum der Universität Basel, Basel CH-4056, Switzerland, and †Max-Planck-Institut für Biochemie, D-8033 Martinsried, Federal Republic of Germany*

(*Received 11 October 1982/Accepted 15 December 1982*)

Intima collagen was obtained from pepsin digests of human placenta in two forms, which differ to some extent in the size of their constituent polypeptide chains (M_r 50000–70000). These chains are connected by disulphide bonds to large aggregates. The aggregates are arranged in a triple-helical conformation with a remarkably high thermal stability (T_m 41–62°C) and are resistant to further proteolytic digestion. Reduction of as little as 5% of the disulphide bonds produces mainly monomeric triple helices (M_r about 160000) with T_m 32°C. Partially reduced material can be separated into triple-helical and non-collagenous domains by proteolysis. Pepsin releases a collagenous component with chains of M_r 38000. Bacterial collagenase liberates two non-collagenous segments (M_r 15000–30000) rich in cystine. Treatment with collagenase before reduction separates intima collagen into a large fragment composed of collagenous (T_m 41°C) and non-collagenous structures and a single non-collagenous segment. The data support the electron-microscopical model of intima collagen [Furthmayr, Wiedemann, Timpl, Odermatt & Engel (1983) *Biochem. J.* **211**, 303–311], indicating that the basic unit of the fragment consists of a continuous triple helix joining two globular domains.

Collagens type I to type V represent a family of related proteins found in the extracellular space (Bornstein & Sage, 1980). A further, unique, collagenous component was originally identified in a pepsin digest of aortic intima (Chung *et al.*, 1976) and was apparently not related to any of the known collagenous proteins. Similar fragments were also obtained from human placenta and kidney (Furuto & Miller, 1980; Risteli *et al.*, 1980), bovine placenta (Jander *et al.*, 1981) and bovine skin (Laurain *et al.*, 1980). As a matter of convenience we will refer to these materials as intima collagen. Common features of these fragments are their high solubility in NaCl solutions when compared with other collagenous proteins. They constitute large disulphide-bonded aggregates of several polypeptide chains with M_r about 50000. Two or three distinct chain constituents have been characterized (Furuto & Miller, 1980; Jander *et al.*, 1981). These polypeptide chains may be assembled to form a triple-helical structure or exist as non-triple-helical components associated through disulphide bonds (Furuto & Miller, 1981).

Structural models have not yet been established for intima collagen. Certain differences in electrophoretic mobility and composition (Furuto & Miller, 1980; Jander *et al.*, 1981) have suggested that structural diversity exists among these components. In the present study we have isolated and compared two different forms of intima collagen derived from human placenta. Our results indicate a complex arrangement of triple-helical strands and of globular segments within these fragments. This was confirmed by electron microscopy, and together with the chemical studies it has been possible to propose a structural model for intima collagen (Furthmayr *et al.*, 1983).

Materials and methods

Isolation of intima collagen

Prewashed human placenta was kindly supplied by Behringwerke A.G., Marburg, Germany. It was digested with pepsin (Boehringer, Mannheim, Germany) by two different procedures, in each case with 100mg of enzyme per kg wet wt. of tissue. In the first procedure the placenta was homogenized in 0.5M-formic acid, pH2.5 (1.5 litres/kg), and incubated with pepsin for 24h at room temperature. In the second method the homogenization was in

0.5M-acetic acid, pH2.9, and the incubation with pepsin was for 24h at 6–8°C. Interstitial and basement-membrane collagens were removed from both digests by precipitation with 1.2M-NaCl for 24h at 6–8°C. The NaCl concentration of the supernatants was then raised to 1.7M (Furuto & Miller, 1980), which precipitated intima collagen together with some non-collagenous proteins. The precipitate was dissolved in 0.1M-acetic acid and dialysed at 6–8°C against a large volume of 20mM-sodium phosphate buffer, pH7.2. The precipitated protein obtained was once more subjected to fractional NaCl precipitation and precipitation by dialysis. The final precipitate was then dissolved in 0.1M-acetic acid (about 5mg/ml) and dialysed against 1M-$CaCl_2$/50mM-Tris/HCl buffer, pH7.4, and a volume of the solution containing about 100mg of protein was then passed over an agarose A5m column (3cm × 130cm), equilibrated in the same buffer. Intima collagen emerged as a major fraction close to the void volume of the column, and this fraction was dialysed against dilute acetic acid and freeze-dried.

Proteolytic modification

All digestions of collagen were for 24h at an enzyme/substrate ratio of 1:100 (w/w). For digestion with pepsin at 20°C intima collagen was dissolved in 0.5M-formic acid (2mg/ml). Acid solutions of the protein were dialysed against 0.2M-sodium phosphate buffer, pH8.0, before digestion with trypsin, elastase (both from Worthington Biochemical Corp., Freehold, NJ, U.S.A.), staphylococcal proteinase (Miles Laboratories, Elkhart, IN, U.S.A.) and thermolysin (Merck, Darmstadt, Germany) at 20°C. For digestion with bacterial collagenase (form CLSPA; Worthington Biochemical Corp.) the material was dialysed against 0.2M-NaCl/2mM-$CaCl_2$/50mM-Tris/HCl buffer, pH7.4. Digestions were then done at 37°C before or after heat denaturation of intima collagen at 55°C for 15min. The extent of degradation was evaluated by electrophoresis under reducing conditions (see below) and molecular-sieve chromatography on a Sephadex G-100 column (2.6cm × 100cm) equilibrated in 50mM-formic acid. Collagenase digests (2h, 37°C) were also prepared from partially reduced material and were separated on a Sephacryl S-200 column (1.5cm × 110cm) equilibrated in 0.2M-ammonium bicarbonate buffer, pH7.9.

Reduction and separation of chain constituents

For reduction under non-denaturing conditions samples were dissolved in 0.1M-acetic acid (2mg/ml) and dialysed at 4°C against 0.4M-NaCl/50mM-Tris/HCl buffer, pH7.4. Reduction was then performed with various concentrations of dithioerythritol, 2-mercaptoethanol or cysteine at 15°C for periods of 30min to 24h and stopped by addition of a 4-fold excess of ethyleneimine followed by 1h incubation at 15°C. Reagents were removed by dialysis against 0.1M-acetic acid. Proteins were then precipitated by dialysis against 20mM-sodium phosphate buffer, pH7.2, and against water at 4°C, which removed some smaller peptides remaining in the supernatant. The precipitated material was finally dissolved in 0.1M-acetic acid. For chromatography on CM-cellulose (Furuto & Miller, 1981) the material was dialysed at 4°C against 1M-urea/20mM-sodium acetate buffer, pH4.8. Elution of protein from the column was accomplished with a linear gradient of 0–0.4M-NaCl.

For the separation of the chain constituents the samples were completely reduced in 8M-urea and were *S*-aminoethylated (Gollwitzer *et al.*, 1974). Purification steps included chromatography on an agarose A1.5m column (3cm × 120cm) equilibrated in 1M-$CaCl_2$/50mM-Tris/HCl buffer, pH7.4, and on a CM-cellulose column as described above.

Analytical methods

Peptide samples were hydrolysed under N_2 with 6M-HCl (24h, 110°C) and the amino acid composition was determined on a Durrum D-500 analyser. To account for hydrolytic losses, values for serine and threonine were corrected by the factors 1.21 and 1.08 respectively. Cysteine was determined as cystine or after alkylation as *S*-aminoethylcysteine. Polyacrylamide-gel electrophoresis in sodium dodecyl sulphate followed a previously described procedure (Furthmayr & Timpl, 1971). Runs were calibrated with collagen I and CNBr-cleavage peptides of collagen I or with globular proteins (serum albumin, ovalbumin, cytochrome *c*) in order to estimate molecular weights.

Physical measurements

Molecular weights were determined by sedimentation-equilibrium runs at 5000–8000rev./min in a Spinco model E ultracentrifuge (Beckman Instruments) equipped with a scanner and double-sector cells. A partial specific volume of 0.73ml/g was assumed in the calculations. Molecular weights measured in 0.1M-acetic acid were determined by extrapolation to $c = 0$. C.d. spectra were measured in a Cary 61 spectropolarimeter equipped with quartz cells and thermostatically controlled with a water jacket. Melting profiles were determined by raising the temperature (12°C/h) with an automatic temperature programmer (Lauda P 120).

Results

Isolation and chemical characterization of two forms of intima collagen

We have isolated from pepsin digests of human placenta two forms of a collagenous fragment with

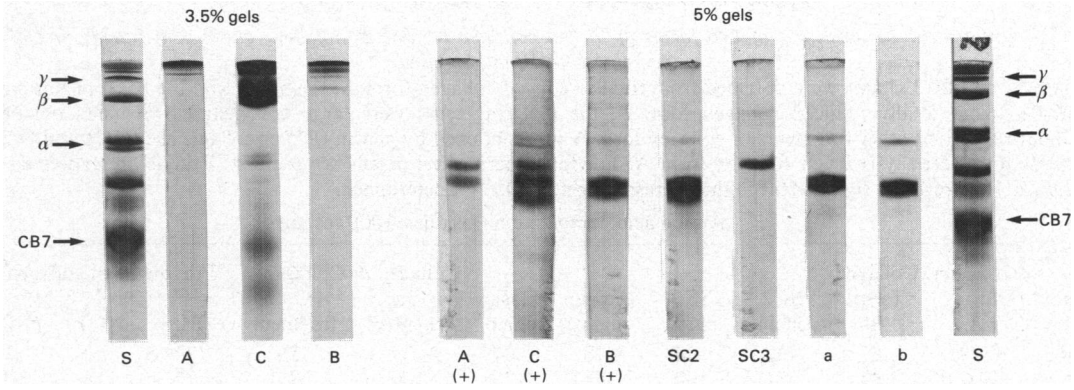

Fig. 1. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of intima collagens, of their purified constituent chains and of their fragments*

The samples were intima collagen form A (A) or B (B), fragment Col (C) of form A, the SC2 and SC3 chains of form A and the acidic (a) and basic (b) chains of form B. (+) denotes samples that were reduced with 2-mercaptoethanol before electrophoresis. For full experimental details see the text. The runs were calibrated with a mixture of collagen I and its CNBr-cleavage peptides (S). The arrows indicate the positions of γ- and β-components, α-chains and peptide α1-(I)CB7.

unique solubility properties. Form B was consistently found in digests prepared in acetic acid, and contains the basic and acidic chains described for similar material isolated from human aortic intima and placenta (Chung *et al.*, 1976; Furuto & Miller, 1980). About half of the digests prepared in formic acid contained another form, A, which is composed of slightly larger chain constituents resembling the chains SC2 and SC3 found in bovine placenta (Jander *et al.*, 1981). The other digests prepared in formic acid contain, however, form B or a mixture of both forms. We also noted a considerable variability in the yield of purified fragment in the range 10–60 mg per kg wet wt. of placental tissue.

The structure and relationships of both forms of the fragment were studied by a variety of methods. Polyacrylamide-gel electrophoresis of non-reduced material showed for both forms mainly large aggregates that did not penetrate the gels. After reduction, however, two bands were observed, which differ distinctly in mobility (Fig. 1). Both forms of intima collagen show a similar amino acid composition (Table 1), which is characterized by less than one-third of glycine but distinct amounts of hydroxyproline and hydroxylysine, This indicates the presence of both collagenous and non-collagenous segments in the fragments.

Both forms of intima collagens show a c.d. spectrum typical of collagenous proteins (Brown *et al.*, 1972), with a negative amplitude of 43000 deg.·cm^2·dmol^{-1} at around 200 nm and a positive amplitude of 3700 deg.·cm^2·dmol^{-1} at 222 nm. After heating of intima collagen the c.d. signal at 222 nm changes from a positive to a negative value, which is characteristic for the triple-helix–coil transition of collagens. The thermal transition profile of intima collagen A is clearly biphasic, showing sharp thermal transitions (T_m) at about 41°C and 62°C (Fig. 2). Thermal unfolding is partially reversible, as indicated by the recovery of about 30% of the c.d. signal after cooling the sample immediately to 20°C. Intima collagen B exhibits a similar profile with lower T_m values. However, several batches showed a more shallow, irregular, transition profile in the range 38–55°C similar to that profile found in a previous study (Furuto & Miller, 1981).

Characterization of the constituent polypeptide chains

Complete reduction of disulphide bonds could be achieved in 8 M-urea, as judged by amino acid analysis. The chain constituents SC2 and SC3 (Jander *et al.*, 1981) of the form A could easily be separated from each other on agarose, since the latter emerged in the void volume of the column, indicating that they were aggregated. In addition, we observed a third peak of small, mainly non-collagenous, peptides accounting for about 5–10% of the total material (Fig. 3). The chains were further purified on CM-cellulose. Chain SC3 appears to be more acidic than chain SC2, and both polypeptides are homogeneous by electrophoresis (Fig. 1). Completely reduced intima collagen form B was directly passed through CM-cellulose, which allowed the separation into the acidic and the basic chain constituents (Fig. 4). They showed a small but distinct difference in electrophoretic mobility, and were

Table 1. *Amino acid compositions of two forms of intima collagen (A and B), of their chain constituents and of their proteinase-resistant domains*

The SC2 and SC3 chains were obtained from totally reduced collagen form A; the acidic and the basic chains were obtained from totally reduced collagen form B; the Col fragments were large collagenase fragments obtained without reduction; other fragments of collagen form A were obtained by pepsin (P1) or collagenase (NC1 and NC2) treatment of partially reduced collagen form A. For full experimental details see the text. Results are expressed as residues/1000 residues rounded off to the nearest integer. N.D., Not determined.

	Amino acid composition (residues/1000 residues)										
							Collagen A	Collagen B	Fragments of collagen A		
Amino acid	Collagen form A	Collagen form B	SC2 chain	SC3 chain	Acidic chain	Basic chain	Col fragment	Col fragment	P1	NC1	NC2
Hyp	39	62	68	24	78	67	37	58	77	2	32
Asp	102	88	81	100	84	77	100	95	72	118	107
Thr	28	21	16	46	12	14	36	26	19	60	26
Ser	47	35	35	53	22	25	46	35	33	57	47
Glu	106	103	107	94	120	108	105	99	106	115	124
Pro	76	107	90	73	99	101	77	92	86	67	71
Gly	243	257	312	230	296	306	219	262	318	96	224
Ala	51	48	46	56	46	38	51	46	41	54	48
Cys	23	21	12	6	9	10	25	25	15	59	40
Val	37	25	22	43	20	19	31	25	21	59	26
Met	5	7	5	3	10	5	6	N.D.	3	13	12
Ile	29	27	22	40	21	22	34	27	22	45	29
Leu	43	34	29	64	24	24	51	32	22	63	38
Tyr	15	14	13	9	15	13	17	16	15	20	14
Phe	22	14	16	31	16	14	21	15	14	27	14
His	6	6	4	6	5	3	8	4	3	19	11
Hyl	37	42	42	22	52	57	37	51	53	9	49
Lys	28	24	16	27	15	22	35	25	15	65	45
Arg	63	65	64	73	56	75	64	67	65	52	43

contaminated with small amounts of larger, presumably dimeric, material (Fig. 1). Usually, the basic chains appeared as a closely spaced doublet of bands, in agreement with a previous study (Furuto & Miller, 1980).

The acidic and the basic chains show distinct differences in amino acid composition, as expected from their behaviour after ion-exchange chromatography. Even larger differences were noted between the SC2 and SC3 chains (Table 1), in agreement with data for bovine placenta (Jander *et al.*, 1981). Ultracentrifugation demonstrated M_r 50000 for the SC2 chain, M_r 60000–70000 for the SC3 chain and M_r 50000–53000 for the acidic and the basic chains isolated from intima collagen B. These data, as well as the yields of individual polypeptides (Figs. 3 and 4), indicate a molar ratio slightly above 2:1 for the SC2/SC3 chains and for the basic/acidic chains.

Partial reduction under non-denaturing conditions

Intima collagen was reduced in the absence of denaturing agents under a variety of conditions that dissociated between 3 and 90% of the disulphide bonds (Table 2). These treatments did not destroy the triple helix, as judged by c.d. spectroscopy and electron microscopy (Furthmayr *et al.*, 1983). Almost complete reduction was achieved with 20mM-dithioerythritol for 24h. This material migrated in sodium dodecyl sulphate/polyacrylamide-gel electrophoresis with the mobility of the constituent chains but occasionally contained dimeric and trimeric chains (Fig. 5*a*). Reduced intima collagen shows the c.d. spectrum of an intact triple helix, but its thermal stability is decreased to a T_m of 31–33°C (Fig. 2). Chromatography of reduced samples under non-denaturing conditions on CM-cellulose demonstrated complete binding of the protein, and after elution from the column the molecules are still in a triple-helical conformation. We failed to observe a lack of binding of significant amounts of acidic chains to the column as described by Furuto & Miller (1981).

Reduction of 5–30% of the disulphide bonds (e.g. by 1mM-mercaptoethanol or 10mM-cysteine for 1h) produces a different electrophoresis pattern (Fig. 5*a*). The major component migrates with a mobility corresponding to M_r 160000, indicating that it consisted of three disulphide-bonded chains. Since this material has the shape of a single triple-helical molecule, it apparently constitutes the basic subunit of the aggregated form of intima collagen

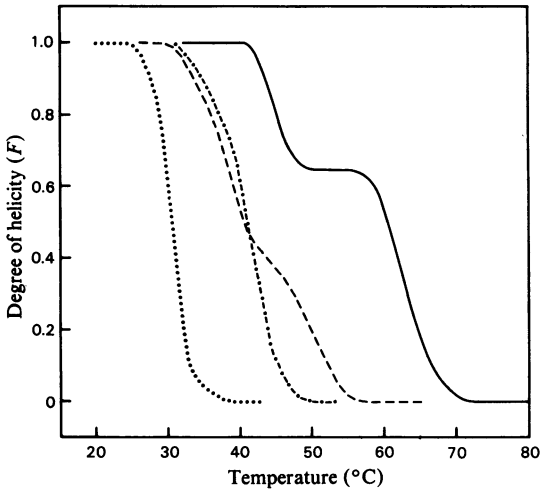

Fig. 2. *'Melting' profiles of the triple helix in intima collagen and its fragments monitored by c.d. spectroscopy at 222 nm*

The samples used were form A (——), form B (– – –), fragment Col of form A (–·–·–) and monomers of form A (·····) obtained by non-denaturing reduction with 20 M-dithioerythritol (Table 2). Proteins were examined in 0.1 M-acetic acid at a concentration of about 0.3 mg/ml. For full experimental details see the text. The degree of helicity was calculated as previously described (Risteli *et al.*, 1980).

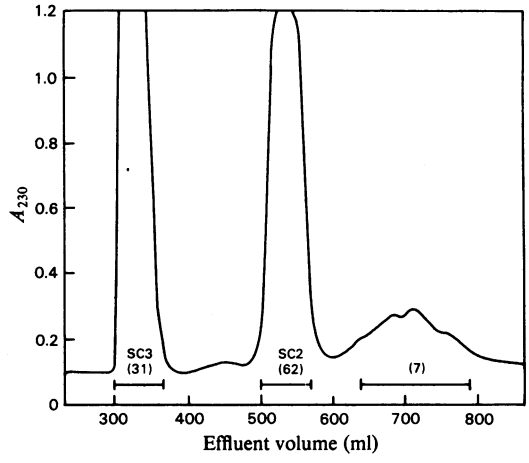

Fig. 3. *Separation of the constituent chains SC2 and SC3 of intima collagen form A on agarose A 1.5m*

The column (3 cm × 120 cm) was equilibrated in 1 M-$CaCl_2$/50 mM-Tris/HCl buffer, pH 7.4, and loaded with 100 mg of protein. For full experimental details see the text. Horizontal bars indicate the pools used for further studies. Numbers in parentheses refer to the relative amounts of peptide material (in %) contained in each pool.

(Furthmayr *et al.*, 1983). Intermediate forms of these aggregates, e.g. a dimer with M_r 300000, can also be observed after reduction of less than one disulphide bond in the monomeric subunit (Table 2 and Fig. 5*a*).

Proteolytic modifications of disulphide-bonded intima collagen

Intima collagen A was completely resistant to further digestion with pepsin and a variety of neutral proteinases, as judged by electrophoresis and molecular-sieve chromatography. Only minor effects were produced by bacterial collagenase when it was used at 37°C. After thermal denaturation at 55°C the protein is degraded by collagenase into several fragments. The major fragment, Col, enters the polyacrylamide gel with a mobility intermediate between those of collagen β- and γ-components (Fig. 1). After reduction both constituent chains are apparently converted into slightly smaller variants.

Dialysis of the digest against water precipitates most of the fragment Col, which accounts for about 70% of the mass of the original material. The amino acid composition of this fragment is similar to that of the starting material (Table 1), suggesting that

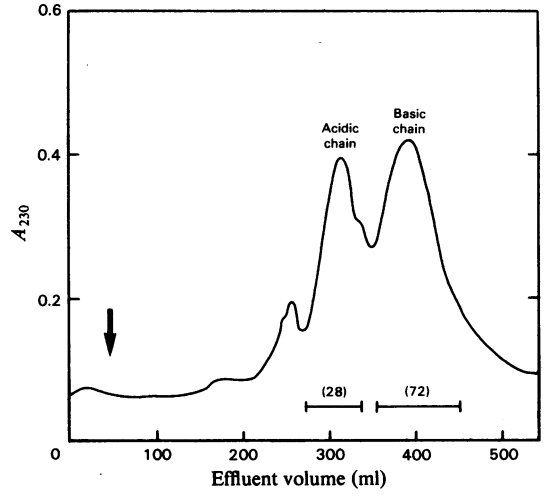

Fig. 4. *Separation of the acidic and the basic chain constituents of intima collagen form B on CM-cellulose*

The column (2 cm × 10 cm) was equilibrated in 2 M-urea/20 mM-sodium acetate buffer, pH 4.8, and loaded with 40 mg of protein. Bound material was eluted by a linear gradient (start indicated by arrow) of 0–0.4 M-NaCl (400 ml/400 ml). For full experimental details see the text. Numbers in parentheses refer to the relative amount of peptide material (in %) contained in each pool (horizontal bars).

Table 2. *Effect of partial reduction under non-denaturing conditions on the size of intima collagen form A*
Reduction was performed in 0.4 M-NaCl/50 mM-Tris/HCl buffer, pH 7.4, at 15°C with dithioerythritol, 2-mercaptoethanol or cysteine, followed by *S*-aminoethylation. *S*-Aminoethylcysteine content is expressed as residues/monomer with M_r 160000; the total content is 34 half-cysteine residues/monomer. Apparent molecular weights were determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 5*a*). For full experimental details see the text. The shapes of the molecules as observed by electron microscopy after rotary shadowing (Furthmayr *et al.*, 1983) are also indicated in the Table.

Treatment	*S*-Aminoethylcysteine content (residues/monomer)	M_r of major polypeptide chains	Shape of major particle
20 mM-Dithioerythritol, 24 h	30	50000	Single strand without globules
0.4 mM-Dithioerythritol, 0.5 h	10	160000 + 50000	Single strand with globules
1 mM-2-Mercaptoethanol, 1 h	2.5	160000	Single strand with globules
10 mM-Cysteine, 1 h	1.6	160000	Single strand with globules
1 mM-Cysteine, 1 h	0.8	300000 plus aggregates	Dimeric strands and larger aggregates

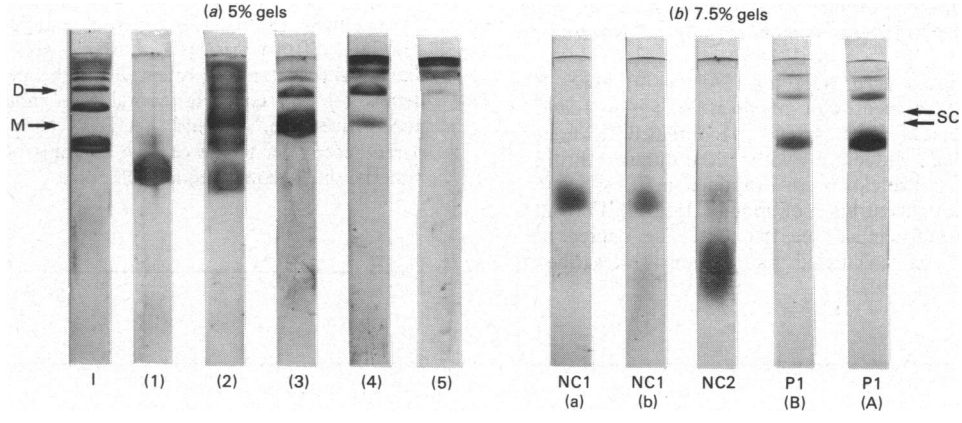

Fig. 5. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of partially reduced intima collagen (a) and of its proteinase-resistant domains (b)*
Samples in (*a*) were a collagen I standard (I) and intima collagen B with 30 (1), 10 (2), 2.5 (3), 0.8 (4) and no (5) reduced half-cystine residues/monomer (see Table 2). For full experimental details see the text. The positions of the monomeric (M) and dimeric (D) bands are indicated by arrows. Samples in (*b*) were fragments NC1 obtained after (a) or before (b) reduction, fragment NC2 and fragments P1 obtained from intima collagen form A or B. For full experimental details see the text. The arrows (SC) indicate the migration positions of the SC2 and SC3 chains.

collagenous and non-collagenous structures are retained after the treatment. This fragment still showed a c.d. spectrum typical of that for collagen, but the 'melting' profile now became homogeneous, with T_m 41°C (Fig. 2). Another fragment, NC1, could be purified from the supernatant prepared after removal of fragment Col by chromatography on Sephacryl S-200. This step removes small amounts of contaminating fragment Col and small peptides (results not shown). Fragment NC1 clearly shows a non-collagenous composition and is particularly rich in half-cystine (Table 1). It appears in electrophoresis as a single band (Fig. 5*b*), with M_r 30000 as estimated from calibration with globular proteins.

Intima collagen B could not be further degraded by pepsin at 20°C. Treatment with bacterial collagenase at 37°C produces similar changes to those found for intima collagen A after thermal denaturation. It was possible to isolate a large fragment Col with an amino acid composition similar to that of intima collagen B (Table 1). The digest lacked fragment NC1 but contained a smaller peptide (M_r about 15000) with a non-collagenous composition.

Proteolytic separation of collagenous and non-collagenous segments after partial reduction

Reduction of 90% of the disulphide bonds in intima collagen A or B under non-denaturing

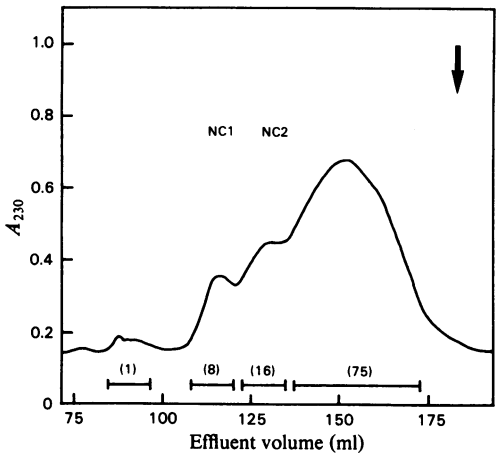

Fig. 6. *Molecular-sieve chromatography on Sephacryl S-200 of a collagenase digest prepared from partially reduced intima collagen form A*

The column (1.5cm × 110cm) was equilibrated in 0.2M-ammonium bicarbonate buffer, pH 7.9, and loaded with 15mg of digest. For full experimental details see the text. The relative recoveries in the individual pools (in %) are indicated by the numbers in parentheses. The arrow denotes the total volume of the column.

conditions (Table 2) allows their further degradation by pepsin at 15°C. Dialysis against water precipitates a large fragment, P1, which has an amino acid composition consistent with that of a collagenous protein (Table 1). No difference could be observed by polyacrylamide-gel electrophoresis between the large fragments obtained from both forms of the protein (Fig. 5*b*). The major component was now a single band (M_r 38000) with a slightly faster mobility than those of the original chain constituents.

A lower degree of reduction (about 30%) was used to achieve degradation of intima collagen A with bacterial collagenase at 37°C. The digest can be separated by molecular-sieve chromatography (Fig. 6) into two peaks containing non-collagenous material and a broad peak of smaller peptides. The latter have a composition similar to that of the collagenous fragment P1. The non-collagenous fragments were further purified by rechromatography on the same column. The material in the first peak is indistinguishable from fragment NC1 (see above) in composition and electrophoretic mobility (Fig. 5*b*). The second peak apparently contains a mixture of fragments, NC2, producing a broad band on polyacrylamide gels after electrophoresis (M_r 9000–17000). They also have an amino acid composition that is different from that of fragment NC1 and is suggestive of another non-collagenous domain (Table 1). Substantial changes in electrophoretic mobility could not be observed after a second reduction of fragments NC1 and NC2 in the presence of sodium dodecyl sulphate.

Discussion

Intima collagen, as isolated by pepsin solubilization from human placenta, appears to consist of aggregates that are composed of basic subunits (monomers) with M_r 160000. The monomers can be released from the larger complexes by reduction of two or three disulphide bridges under conditions that still maintain the triple-helical conformation of the protein. The size of the individual monomers is consistent with M_r 50000–70000 for the constituent polypeptide chains of intima collagen obtained by total reduction. The two polypeptide chains observed are structurally distinct, as shown previously by CNBr cleavage (Jander *et al.*, 1981; Furuto & Miller, 1980). Our study suggests that the two polypeptide constituents are present at a ratio of about 2:1 (SC2/SC3 chain, basic/acidic chain) in single native molecules. The data, however, do not exclude the possibility that different monomers exist that are composed of the two different types of chains.

Compositional data and the proteolytic fragmentation of partially reduced intima collagen demonstrated that the monomers consist of structurally different segments. The major portion of the monomer consists of a triple-helical domain with M_r 115000 that resists pepsin at 15°C and contains about one-third of glycine. A similar structure was previously obtained from bovine placenta (Jander *et al.*, 1981). This glycine content suggests a regular repetitive sequence Gly-Xaa-Yaa characteristic for a triple helix with no or only few interruptions. Assuming a mean residue weight of 105 (see Table 1) and a residue length of 0.286nm (Rich & Crick, 1961), the length of this triple helix should be 100–105nm. This is consistent with the dimensions determined by electron microscopy (Furthmayr *et al.*, 1983).

Treatment with bacterial collagenase of the monomer released two non-collagenous segments NC1 and NC2, which together accounted for about one-quarter of the mass of intima collagen. Fragment NC2 particularly was heterogeneous. This heterogeneity is presumably generated by pepsin and/or partial reduction and prevented further characterization of the peptide material. Segment NC1 was also obtained by collagenase treatment before reduction. In addition, this treatment liberated a large fragment Col consisting of collagenous and non-collagenous structures. As shown by electron microscopy (Furthmayr *et al.*, 1983), the latter fragment represents a dimer of intima collagen that has lost a portion of the triple helix and globular domains located in the terminal positions.

The structure of intima collagen is stabilized by disulphide bonds between the monomers as well as between the constituent chains. The bonds between the monomers are very labile to reduction, since a brief treatment with cysteine (1–10mM) destroyed most of the higher aggregates. As expected, stronger conditions are required to cleave the interchain disulphide bonds, which are presumably located in the globular as well as within the triple-helical domains. As found for pN-collagen III (Nowack *et al.*, 1976), these bonds apparently are responsible for the considerable stability against proteolysis.

The triple helix of the reduced monomer has a sharp 'melting' profile with T_m 32°C. This is comparable with the thermal stability of triple-helical fragments of similar size obtained for example from collagen IV (Timpl *et al.*, 1979). An increase in thermal stability by about 10°C was observed for fragment Col. This indicates stabilization by disulphide bonds and/or by the association of two triple helices in a twisted form (Furthmayr *et al.*, 1983). A more complex 'melting' profile was observed for non-reduced intima collagen, which seems to contain material with higher stability. This is very probably due to the association of the protein to dimers, tetramers and larger aggregates.

Intima collagen was obtained in the present study in two slightly different forms A and B, which are similar to those described by Jander *et al.* (1981) and Furuto & Miller (1980) respectively. We did not find for intima collagen A the low resistance against bacterial collagenase or a third chain constituent, SC1, as described for the material isolated from bovine placenta (Jander *et al.*, 1981). Both forms of intima collagen share a presumably identical triple helix, but seem to differ in their non-collagenous segments, as suggested by compositional data and the size of fragment NC1. Immunochemical studies (H. von der Mark & R. Timpl, unpublished work) show a strong cross-reaction between both variants, indicating that they do not originate from different proteins but rather that they are derived from a common precursor protein that has been degraded to different extents. Yet, it has not been possible to convert form A into form B by a second treatment with pepsin or several neutral proteinases. This suggests that during solubilization from human placenta at low pH endogenous proteinases contribute to degradation, and this also could explain the unpredictable yields of these variants.

Electron microscopy (Furthmayr *et al.*, 1983) demonstrated that both forms of intima collagen are identical in their basic structure, although they could differ in the relative amounts of associated material. The basic monomer consists of a triple-helical rod containing at each end globular domains. This model is compatible with the chemical data, which also suggest that one of the globular regions corresponds to fragment NC1. A possible correlation between fragment NC2 and the second type of globular region is still tentative. The intact form of intima collagen, perhaps obtained by extraction with 6M-guanidine (Gibson & Cleary, 1982) and thus avoiding proteolytic conditions, could provide the starting material to further studies to substantiate our findings.

This study was supported by a grant of the Deutsche Forschungsgemeinschaft (project Ti 95/5).

References

Bornstein, P. & Sage, H. (1980) *Annu. Rev. Biochem.* **49**, 957–1003

Brown, F. R., DiCorato, A., Lorenzi, G. P. & Blout, E. R. (1972) *J. Mol. Biol.* **63**, 85–99

Chung, E., Rhodes, R. K. & Miller, E. J. (1976) *Biochem. Biophys. Res. Commun.* **71**, 1167–1174

Furthmayr, H. & Timpl, R. (1971) *Anal. Biochem.* **41**, 510–516

Furthmayr, H., Wiedemann, H., Timpl, R., Odermatt, E. & Engel, J. (1983) *Biochem. J.* **211**, 303–311

Furuto, D. K. & Miller, E. J. (1980) *J. Biol. Chem.* **255**, 290–295

Furuto, D. K. & Miller, E. J. (1981) *Biochemistry* **20**, 1635–1640

Gibson, A. M. & Cleary, E. G. (1982) *Biochem. Biophys. Res. Commun.* **105**, 1288–1295

Gollwitzer, R., Becker, U. & Timpl, R. (1974) *FEBS Lett.* **47**, 177–180

Jander, R., Rauterberg, J., Voss, B. & von Bassewitz, D. B. (1981) *Eur. J. Biochem.* **114**, 17–25

Laurain, G., Delvincourt, T. & Szymanovicz, A. G. (1980) *FEBS Lett.* **120**, 44–48

Nowack, H., Olsen, B. R. & Timpl, R. (1976) *Eur. J. Biochem.* **70**, 205–216

Rich, A. & Crick, F. M. C. (1961) *J. Mol. Biol.* **3**, 483–506

Risteli, J., Bächinger, H. P., Engel, J., Furthmayr, H. & Timpl, R. (1980) *Eur. J. Biochem.* **108**, 239–250

Timpl, R., Bruckner, P. & Fietzek, P. (1979) *Eur. J. Biochem.* **95**, 255–263