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Isolation and Characterization of a 115,000-dalton Matrix-associated Glycoprotein from Chick Aorta*

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Chick aortas were extracted sequentially with phosphate-buffered saline, 6 M guanidine HCl, and 6 M guanidine HCl containing dithioerythritol. The proteins present in the guanidine HCl + dithioerythritol extract were separated by DEAE-cellulose chromatography, and the fractions recovered were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Five major glycoprotein components with apparent M_r = 205,000, 195,000, 150,000, 135,000, and 115,000 (gp 115) were identified. gp 115 was further studied since it was the only noncollagenous protein based on amino acid analysis. The protein was purified to homogeneity by preparative electrophoresis. Its amino acid composition was characterized by a high content of glutamic acid and arginine and a relatively high content of leucine, glycine, and alanine. The concentration of gp 115 in the guanidine HCl + dithioerythritol extract was about 15-fold that in the guanidine and saline extracts. Overall, about 80% of the protein was solubilized with guanidine HCl + dithioerythritol, suggesting that most of it formed large aggregates stabilized by disulfide bonds *in vivo*.

Immunofluorescence studies with specific antibodies showed that gp 115 formed an extracellular fibrillar network in the aorta wall. One-dimensional finger printing with *Staphylococcus aureus* V_8 protease and immunological studies indicated that the protein was unrelated to fibronectin and laminin. The data led us to conclude that gp 115 is a novel extracellular component of chick aorta.

The major extracellular components of blood vessels, including elastin, collagen types I and III, and proteoglycans, have been the subject, in the past, of intense studies, which have resulted in a wealth of information on their structure, biosynthesis, and function (1). More recently, several other extracellular protein components have been identified in blood vessels. Some are collagenous: like collagen type V (2) and the so called "endothelial cell" and "intima type" collagens (3, 4), while others are noncollagenous glycoproteins like fibronectin (5) and laminin (6). It was suggested that these proteins, although present in low amounts, play important roles in maintaining the structure and establishing the normal function of blood vessels. They have been shown to participate in specific interactions between cell surfaces and/or extracellular components and to exhibit distinct distribution patterns in the tissues (7, 8).

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Several authors (9) found that a heterogeneous protein fraction could be extracted from elastic arteries with buffers containing reducing and denaturing agents. This fraction has been repeatedly proposed to contain extracellular glycoproteins, but isolation of discrete protein species and their assignment to the extracellular space have never been reported.

In the search for extracellular protein conponents of blood vessels, we have extracted chick aorta sequentially with phosphate-buffered saline, 6 M guanidine HCl, and 6 M guanidine HCl containing dithioerythritol. The last fraction contained several collagenous proteins and a main glycoprotein of apparent M_r = 115,000, which has been isolated and characterized. Using a specific antibody, we have found that gp 115[1] distributed as an extracellular component of blood vessels wall.

MATERIALS AND METHODS

Preparation of Aorta Extracts—The extraction procedure used was similar to that of Muir *et al.* (10). Aortas and associated blood vessels were excised from 7-day-old chicks and immediately dropped into ice-cold 0.02 M Na phosphate, 0.15 M NaCl buffer, pH 7.4, containing 25 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 5 mM *N*-ethylmaleimide, and 1 mM *p*-aminobenzamidine hydrochloride, (standard homogenizing buffer). All subsequent procedures were carried out at 4 °C unless otherwise indicated. The tissue (about 10 g wet weight) was homogenized in 100 ml of standard homogenizing buffer using a Polytron PCU-2 homogenizer (Kinematica). The pellet obtained after centrifugation at 12,000 × *g* for 45 min was re-extracted twice in 75 ml of standard homogenizing buffer for a total period of 20 h. The supernatants were combined (saline extract). The pellet was resuspended in 0.1 M Tris-HCl, pH 7.5, containing 6 M guanidine HCl, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 5 mM *N*-ethylmaleimide, and 1 mM *p*-aminobenzamidine hydrochloride (10 ml/g wet residue) and extracted for 24 h at room temperature. The precipitate obtained by centrifugation at 12,000 × *g* for 90 min was re-extracted for an additional 24 h in the same buffer, and the homogenate was centrifuged at 12,000 × *g* for 90 min. The supernatant fluids from the two extractions in the presence of guanidine HCl were combined and will be referred to as the guanidine HCl extract. The pellet was extracted two more times as above in 0.1 M Tris-HCl, pH 7.5, containing 6 M guanidine HCl, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 1 mM *p*-aminobenzamidine hydrochloride, and 25 mM dithioerythritol. The last two supernatants were combined and will be identified as the guanidine HCl + dithioerythritol extract. The various extracts were extensively dialyzed against distilled water and lyophilized together with the precipitate which formed during dialysis.

Purification of Collagens and Laminin—Various collagen types were salt-fractionated from pepsin digests of chicken gizzard as described by Mayne and Zettergren (11). Type I and III collagens were further purified from the 0.7 M NaCl precipitate by Cm-cellulose (12) and molecular sieve chromatography on Bio-Gel A-1.5m (13). Collagen type IV and V chains were purified from the 2.2 and 4.4 M NaCl precipitates as described (11, 14). Intima type collagen was purified from the 1.2 M NaCl supernatant following the procedure of Chung *et al.* (4). Laminin was purified from the Engelbreth-Holm-Swarm

[1] The abbreviations used are: gp 115, glycoprotein of apparent M_r = 115,000; SDS, sodium dodecyl sulfate.

mouse tumor as described by Timpl *et al.* (15). Human cold insoluble globulin, purified by affinity chromatography on gelatin-Sepharose, was a gift from Dr. G. Tarone, University of Torino. The protein preparations were found to be more than 90% pure as judged by gel electrophoresis.

Reduction and Alkylation—Reduction and alkylation was performed as described by Gurd (16). The lyophilized guanidine HCl + dithioerythritol extract was dissolved in 1 M Tris-HCl, pH 8.5, buffer containing 6 M guanidine HCl, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, and 15 mM dithioerythritol at a concentration of about 3 mg/ml, and the solution was stirred under nitrogen at room temperature for 6 h. Sodium iodoacetate (30 mM) was added, and the sample was incubated for 1 h at room temperature in the dark. A molar excess of 2-mercaptoethanol over iodoacetate was added, and the sample was dialyzed and lyophilized.

DEAE-cellulose Chromatography—DEAE-cellulose chromatography was performed on a column (2.5 × 25 cm) of DEAE-Sephacel (Pharmacia Fine Chemicals) at 41 °C, equilibrated with 0.05 M Tris-HCl buffer, pH 8.5, containing 1 mM dithioerythritol and 8 M urea freshly deionized by passage through a column of MB-3 (Serva Feinbiochemica). About 100 mg of the carboxymethylated samples, dissolved in the same buffer, were applied to the column. Proteins bound to the DEAE-cellulose were eluted from the column with a linear gradient of 400 ml of 0.05 M Tris-HCl, pH 8.5, containing 8 M urea and 1 mM dithioerythritol in the mixing chamber and 400 ml of the same buffer containing 0.5 M NaCl in the reservoir. The flow rate was 60 ml/h and fractions of 12 ml were collected. The protein elution pattern was determined by measuring the absorbance at 235 nm. Pooled fractions were dialyzed against several changes of distilled water and lyophilized. The recovery of protein varied from 70 to 85%.

Analytical Polyacrylamide Gel Electrophoresis in the Presence of SDS—Polyacrylamide slab gel electrophoresis in the presence of SDS was performed as described by Laemmli (17). Samples were run after reduction with 5% 2-mercaptoethanol. The gels were stained with 0.25% (w/v) Coomassie brilliant blue R-250 (Sigma) in 45% (v/v) methanol, 5% (v/v) acetic acid for 30 min and destained in a mixture of 7.5% (v/v) methanol and 5% (v/v) acetic acid. Some gels were stained with the periodic acid-Schiff method described by Fairbanks *et al.* (18).

Molecular weight protein standards used were: myosin (200,000) from chick pectoral muscle (kindly supplied by Dr. S. Schiaffino, University of Padova), β' (165,000) and β (155,000) subunits of RNA polymerase from *Escherichia coli* (provided by Dr. J. Krakow, New York University), phosphorylase *b* (94,000), bovine serum albumin (68,000), ovalbumin (43,000), and soluble bovine skin collagen (19).

Preparative Polyacrylamide Gel Electrophoresis in the Presence of SDS—A discontinuous gel system consisting of a stacking gel of 3% acrylamide and 0.08% bisacrylamide in 0.1 M Na phosphate, pH 6.4, 0.1% SDS and a separating gel of 5% acrylamide, 0.13% bisacrylamide in 0.1 M Na phosphate, pH 7.4, 0.1% SDS was used. Protein samples (5–10 mg) were dissolved in 2 ml of 0.1 M phosphate buffer, pH 7.4, containing 20% sucrose, 2% SDS, 0.001% bromphenol blue, and 5% 2-mercaptoethanol and heated in a boiling water bath for 3 min. Using a stained gel strip as a guide, the portion of the gel containing the protein of interest was cut out by a microtome blade. Proteins were eluted electrophoretically from the gel pieces into a dialysis bag using a Buchler apparatus for disc gel electrophoresis. Eluted proteins were dialyzed against 0.03% SDS (w/v), lyophilized, and redissolved in water, and SDS was removed by adding 10 volumes of acidified acetone (1 ml of 1 N HCl, 40 ml of acetone). The precipitate formed after a 24-h incubation at −20 °C was collected by centrifugation, dissolved in 0.5 ml of distilled water, and lyophilized.

Amino Acid Analysis—Samples for amino acid analysis were hydrolyzed in closed vials in 6 N HCl containing 0.05% (v/v) 2-mercaptoethanol at 110 °C for 24 h under nitrogen. The hydrolysates were analyzed on a Jeol JLC-5AH amino acid analyzer using a sodium citrate system.

Peptide Mapping—Proteins (fibronectin, laminin, and an aliquot of the DEAE-cellulose fraction containing gp 115) were labeled with ^{125}I by the chloramine-T method (20) and run on 5% (w/v) polyacrylamide gels under reducing conditions. Radioactive bands of interest were detected by autoradiography, cut out from the gel, and used for one-dimensional peptide mapping by limited proteolysis with *Staphylococcus aureus* V_8 protease or α-chymotrypsin (21).

Immunological Studies—Mice were immunized subcutaneously with 30 μg each of a purified preparation of gp 115 in Freund's complete adjuvant. Booster injections in Freund's complete adjuvant were given after 3 and 5 months.

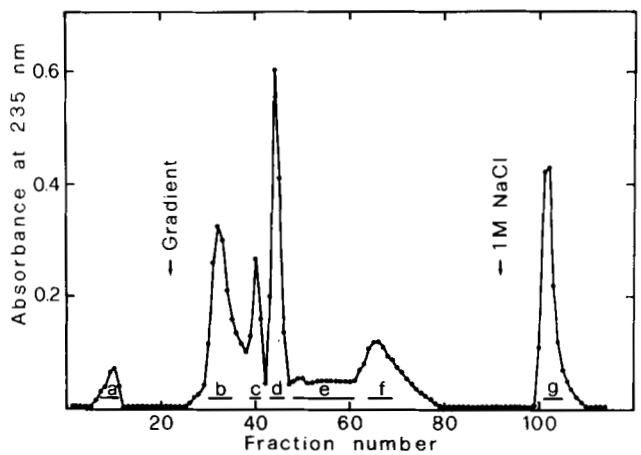


FIG. 1. **DEAE-cellulose chromatography of the 6 M guanidine HCl + dithioerythritol extract.** Proteins were eluted from the column with a linear gradient from 0 to 0.5 M NaCl. *Underlined fractions* were collected.

A solid phase binding assay (22) was used to test reactivity of antiserum and to detect the antigen in different extracts. Briefly, proteins were dissolved in 1% (w/v) SDS, diluted in TEN buffer (20 mM Tris-HCl, pH 7.6, 1 mM EDTA, 100 mM NaCl), plated into microtiter plates, and desiccated at 37 °C. Free binding sites were first saturated by incubation with 1% (w/v) bovine serum albumin at 0 °C for 2 h, and then the absorbed antigens were reacted with diluted antiserum followed by affinity-purified ^{125}I-rabbit IgG anti-mouse IgG (10–15 μCi/μg). The radioactivity specifically bound was solubilized by 2 M NaOH and counted. Background radioactivity, obtained with the preimmune serum, was subtracted to give specific binding values.

For immunofluorescence studies, antibodies were affinity-purified on gp 115 immobilized on nitrocellulose filters following the procedure described.[2] Frozen aorta sections (5 μm) were treated for 2 h with the affinity-purified antibody, washed with saline buffer, and incubated for 1 h with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Miles Laboratories, Inc.). Excess antibody was removed by repeated washing, and the slides were mounted in 50% (v/v) glycerol in saline and examined in a Leitz microscope equipped with epifluorescence optics. Preimmune serum or immune serum pre-absorbed on gp 115 was used as negative first antibody control.

Immunoblotting was performed as described (23). Proteins separated by SDS-polyacrylamide gel electrophoresis were transferred onto nitrocellulose filters which were sequentially incubated with anti-gp 115 antiserum (or preimmune serum) and ^{125}I-rabbit IgG anti-mouse IgG or goat antiserum to chick fibroblast fibronectin (kindly supplied by Dr. H. K. Kleinman, National Institutes of Health, Bethesda, MD) and ^{125}I-protein A. The filters were then exposed to Kodak X-Omat AR films with intensifying screen (DuPont). Evaluation of protein transfer onto nitrocellulose filters was done by Amido black staining.

RESULTS

Analysis of the Proteins Present in 6 M Guanidine HCl + Dithioerythritol—The amount of material solubilized in the presence of guanidine HCl + dithioerythritol represented 6% of the aorta dry weight. After reduction and alkylation, the extract was fractionated by DEAE-cellulose chromatography. The material separated into a small void volume peak and four main peaks that eluted with the gradient (Fig. 1). The fractions corresponding to the various peaks were pooled, extensively dialyzed, and lyophilized. Aliquots of the pooled fractions obtained by DEAE-cellulose chromatography were analyzed by SDS-polyacrylamide gel electrophoresis. Proteins eluted at the void volume (Fig. 2, *lane a*) had mobilities similar to bovine skin acid-soluble collagen and probably represented chick type I collagen chains. The electrophoretic pattern of proteins eluted in the gradient region was very complex (Fig.

[2] A. Colombatti, G. M. Bressan, I. Castellani, and D. Volpin, manuscript in preparation.

2, *lanes b–g*). We therefore limited our analysis to the major glycosylated proteins, identified by staining the gel with the periodic acid-Schiff procedure (18). Major periodic acid-Schiff-positive bands (identified in Fig. 2 by *dots*) included: two bands from *peak b* of Fig. 1 with mobilities similar to myosin (Fig. 2, *lane b*) and three bands from *peak c* with mobilities similar to α_2(I) and α_1(I) chains of bovine skin collagen and the subunits of RNA polymerase (Fig. 2, *lane c*). Based on globular protein standards, the apparent molecular weights of the major glycoproteins were 205,000 and 195,000

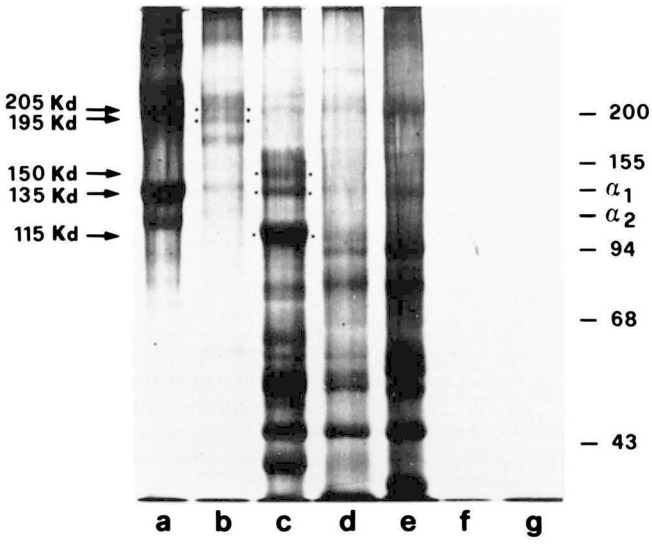


FIG. 2. **SDS-polyacrylamide gel electrophoresis of material from DEAE-cellulose chromatography.** A 6% acrylamide gel was used. Lanes are labeled according to the corresponding peaks of Fig. 1. *Dots* identify the major glycoprotein species revealed by periodic acid-Schiff staining, and their molecular mass is indicated by *numbers* on the *left*. *Numbers* on the *right* correspond to the migration of the following molecular weight standards: myosin (200,000), β subunit of RNA polymerase from *E. coli* (155,000), phosphorylase *b* (94,000), bovine serum albumin (68,000), and ovalbumin (43,000), α_1 and α_2 indicate the mobility of α_1 and α_2 chains of acid-soluble bovine skin type I collagen.

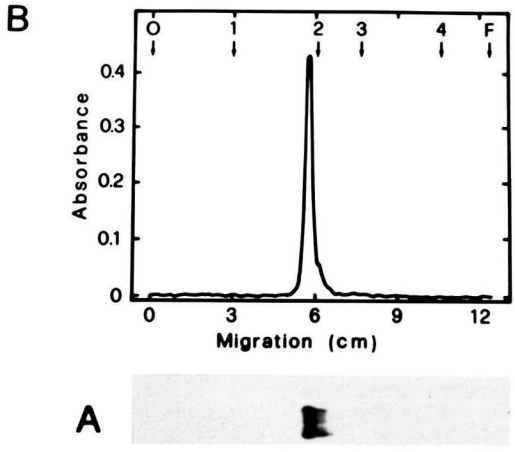


FIG. 3. **SDS-polyacrylamide gel electrophoresis of the 115,000-dalton protein.** A 6% acrylamide gel was used. The protein was purified by preparative SDS-polyacrylamide gel electrophoresis from pooled *peak c* obtained by DEAE-cellulose chromatography of the guanidine HCl + dithioerythritol extract (Fig. 1). *A*, Coomassie blue staining of the gel; *B*, scanning pattern of the gel. *1*, *2*, *3*, *4* indicate mobilities of myosin from chick pectoral muscle, phosphorylase *b*, bovine serum albumin, and ovalbumin. *O*, origin; *F*, dye front.

TABLE I

Amino acid composition of the 115,000-dalton protein from the guanidine HCl + dithioerythritol extract of chick aortas

Analyses were uncorrected for hydrolytic losses or for incomplete hydrolysis. The values represent the mean of three determinations on different preparations in which the coefficient of variation for each amino acid was less than 8%.

Amino acid	Residues/1000 amino acid residues
4-Hyp	0
Asp	70
Thr	36
Ser	69
Glu	142
Pro	67
½-Cys	10[a]
Gly	165
Ala	102
Val	48
Met	6
Ile	20
Leu	93
Tyr	18
Phe	24
Hyl	0
Lys	31
His	17
Arg	82

[a] Determined as *S*-carboxymethylcysteine.

TABLE II

Purification of the 115,000-dalton glycoprotein from chick aorta

Data were obtained from one experiment where 110 chicks were used.

Fraction	Material	Protein as 115,000-dalton band[a]	Purification[b]	Yield
	mg	%	*-fold*	%
Guanidine HCl + dithioerythritol extract	100[c]	2	1	100
DEAE-cellulose gp 115-containing fractions	8[c]	18.8	9.4	75
Preparative electrophoresis	0.38[d]	96	48	19

[a] Calculated from densitometric scans of SDS gels stained with Coomassie blue.

[b] Based on a percentage of total material as 115,000-dalton band in the guanidine HCl + dithioerythritol extract.

[c] Samples were weighed after dialysis and lyophilization.

[d] Calculated by amino acid analysis.

(Fig. 2, *lane b*) and 115,000, 135,000, and 150,000 (Fig. 2, *lane c*).

Characterization of the Major Glycoproteins Present in 6 M Guanidine HCl + Dithioerythritol—The major glycoprotein species of the guanidine HCl + dithioerythritol extract were purified by preparative SDS-polyacrylamide gel electrophoresis, and their amino acid composition was determined. Proteins with apparent M_r = 205,000, 195,000, 150,000, and 135,000 were collagenous since they contained different amounts of hydroxyproline and hydroxylysine and a high amount of glycine (data not shown). These proteins are now being further characterized and will be the subject of a separate report. The 115,000-dalton component (gp 115), which appeared as a single band in Coomassie-stained gels (Fig. 3), had an amino acid composition characterized by a high content of arginine and glutamic acid and a relatively high amount of glycine, alanine, and leucine (Table I). Since hy-

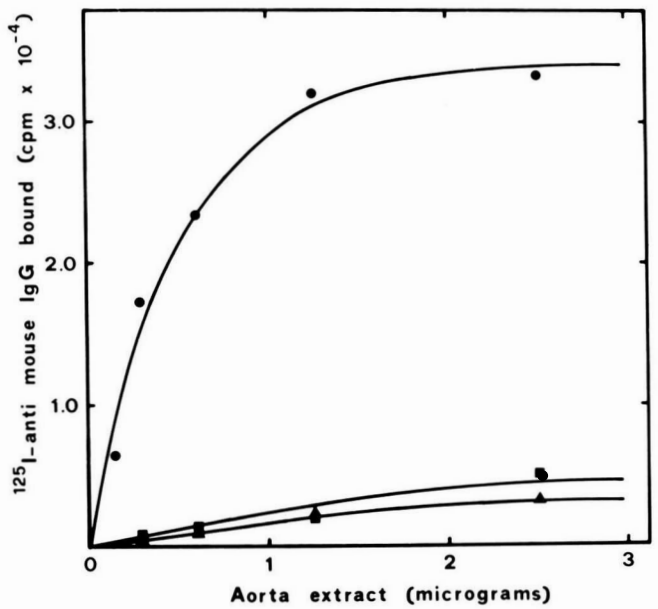


FIG. 4. **Binding of anti-gp 115 antiserum to aorta extracts.** Antibody binding was measured by solid phase radioimmunoassay as described under "Materials and Methods" using 40 μl of diluted antiserum (1:500), 40 μl of ^{125}I-labeled rabbit IgG anti-mouse IgG (0.37 μg/ml, 15 μCi/μg), and different amounts of protein from 7-day-old aortas extracted with saline (■), 6 M guanidine HCl (▲), and 6 M guanidine HCl + dithioerythritol (●).

droxyproline and hydroxylysine were not detected in analyses from different preparations, gp 115 was considered noncollagenous in nature.

The purification steps and yields of gp 115 are outlined in Table II. The protein constituted about 2% of the material solubilized by 6 M guanidine HCl + dithioerythritol. The relative amount of gp 115 in the saline, 6 M guanidine HCl, and 6 M guanidine HCl + dithioerythritol extracts was calculated by a solid phase radioimmunoassay using an antiserum specific for the protein (Fig. 4). The concentration of the protein in the 6 M guanidine + dithioerythritol extract was about 15-fold greater that in the 6 M guanidine HCl and saline extracts. Since the amounts of material solubilized by guanidine HCl and saline were about 2 and 0.75 times that obtained with 6 M guanidine HCl + dithioerythritol, it could be calculated that roughly 80% of gp 115 was recovered in the guanidine HCl + dithioerythritol extract. From Table II and Fig. 4 it was also estimated that gp 115 represented about 0.14% of the aorta dry weight.

Immunological Studies—The specificity of the anti-gp 115 antiserum used was established by immunoblotting. In the guanidine HCl + dithioerythritol extract, which showed a very complex polypeptide composition (Fig. 5, *lane a*), the antiserum bound mainly to a band with the mobility of gp 115 (Fig. 5, *lane b*). Upon prolonged exposure time, additional faster migrating bands were also detected (see also Fig. 9, *lane c*). These probably represent degradation products of gp 115 since they were still apparent when immunoblotting was performed with antibodies affinity-purified on electrophoret-

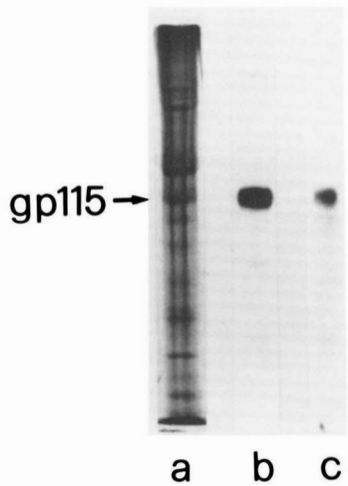


FIG. 5. **Specificity of gp 115 antiserum detected by immunoblotting.** Aliquots of 6 M guanidine HCl + dithioerythritol aorta extract were separated by electrophoresis in a 6% SDS-polyacrylamide gel and stained with Coomassie brilliant blue R-250 (*lane a*) or transferred to nitrocellulose filters, and the strips were developed with whole antiserum (*lane b*) or with affinity-purified anti-gp 115 antibodies (*lane c*).

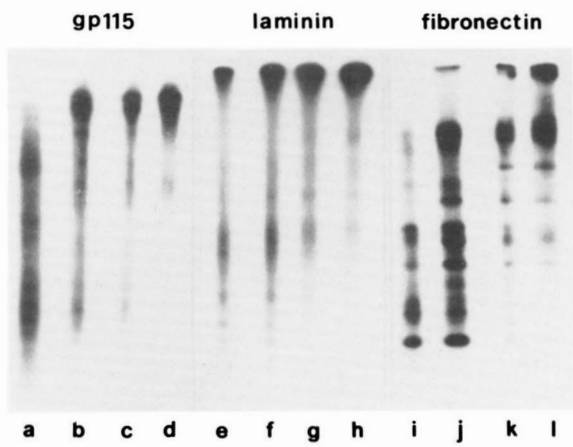
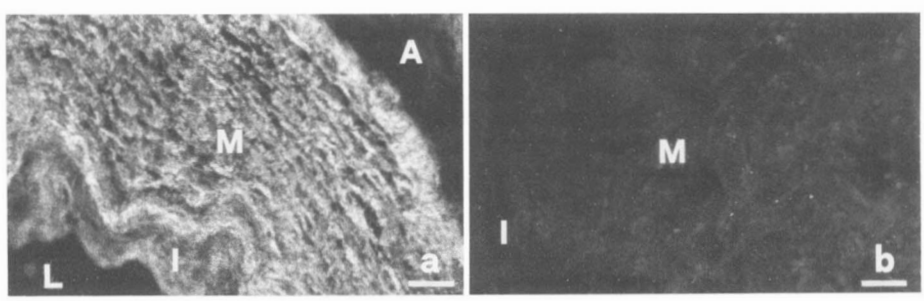


FIG. 7. **Structural analysis by one-dimensional peptide mapping.** Separation was obtained by electrophoresis in a 12.5% SDS-polyacrylamide gel. Peptide maps of ^{125}I-labeled gp 115 (*lanes a–d*), laminin (*lanes e–h*), and fibronectin (*lanes i–l*) were obtained after digestion with 500 (*lanes a, e,* and *i*), 100 (*lanes b, f,* and *j*), 25 (*lanes c, g,* and *k*), and 6 (*lanes d, h,* and *l*) ng/ml of *S. aureus* V_8 protease. In the case of laminin, the data refer to the 200,000-dalton subunit. Peptide maps of the subunit of M_r = 400,000 were also different from those of gp 115 (data not shown).

FIG. 6. **gp 115 localization by indirect immunofluorescence.** Aorta frozen sections from a 2-day-old chick were stained with affinity-purified anti-gp 115 antibody (*a*) and preimmune serum (*b*). Fluorescein isothiocyanate-conjugated goat anti-mouse IgG was used as second antibody. Elastin fibers exhibited a slight yellow autofluorescence. *Magnification bar* is 20 μm. *A*, adventitia; *M*, media; *I*, intima; *L*, lumen.



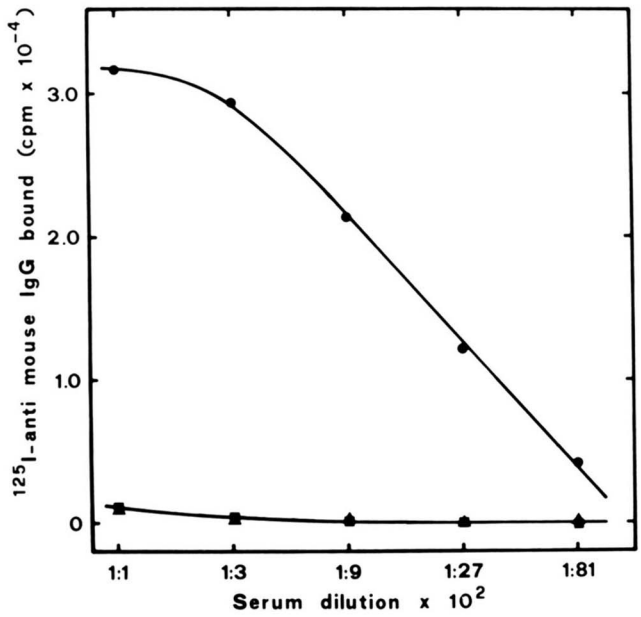


FIG. 8. **Binding of gp 115 antiserum to various extracellular proteins.** Purified proteins were plated at 1 (fibronectin and laminin) or 0.25 (gp 115) μg/well onto 96-well microtiter plates, and the solid phase radioimmunoassay was performed as described under "Materials and Methods". ●, gp 115; ▲, human plasma fibronectin; ■, mouse laminin.

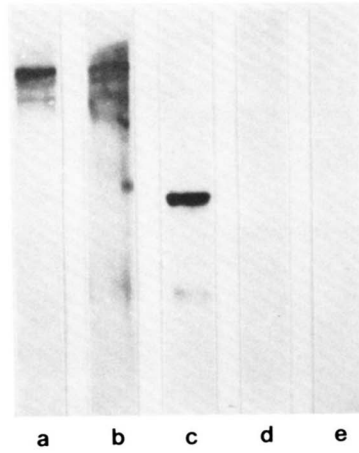


FIG. 9. **Immunological unrelatedness between chick aorta fibronectin and gp 115 revealed by immunoblotting.** Samples of 6 M guanidine HCl + dithioerythritol extract (*lanes a, c, d,* and *e*) and human plasma fibronectin (*lane b*) were separated by electrophoresis in a 6% SDS-polyacrylamide gel and transferred to nitrocellulose filters, and the strips were developed with a goat antiserum to chick fibroblast fibronectin diluted 1:20 (*lanes a* and *b*), a mouse antiserum to gp 115 diluted 1:50 (*lane c*), normal goat serum diluted 1:20 (*lane d*), and normal mouse serum diluted 1:50 (*lane e*). Immunological reactivity was detected either by ^{125}I-labeled protein A (*lanes a, b,* and *d*) or ^{125}I-labeled rabbit IgG anti-mouse IgG (*lanes c* and *e*). Each nitrocellulose strip was incubated with 5×10^5 cpm of ^{125}I-labeled probe.

ically pure gp 115 immobilized on nitrocellulose filters[2] (Fig. 5, *lane c*). Moreover, a monoclonal antibody specific for gp 115 also reacted with these minor bands.[3]

Aorta frozen sections were stained with affinity-purified antibodies, and the distribution of fluorescence confirmed the extracellular localization of gp 115 as suggested by the conditions needed for solubilization (Fig. 6). The entire vessel wall was stained. In the media, which showed the brightest fluorescence, the antigen was distributed in a fibrillar pattern between the circumferential rows of smooth muscle cells. In the intima and adventitia, the staining was less brilliant and more randomly distributed.

Given the extracellular localization of gp 115, we performed a series of experiments to determine whether this glycoprotein beared any structural relation to well characterized extracellular proteins of aorta. As shown in Fig. 7, one-dimensional peptide maps of ^{125}I-labeled human plasma fibronectin, mouse laminin, and gp 115 obtained with *S. aureus* V_8 protease were completely different. α-Chymotryptic peptide maps of the three proteins were also different (data not shown). Moreover, anti-gp 115 antisera did not bind, in a solid phase radioimmunoassay, to human plasma fibronectin and mouse laminin (Fig. 8) and to chick collagen types I, III, IV, and V and intima type collagen (data not shown).

These data indicated that gp 115 and fibronectin were distinct proteins. However, the finding that the tissue distribution of gp 115 and fibronectin was similar[2] prompted us to further investigate the possibility that gp 115 could contain antigenic determinants present only in chick tissue fibronectin and absent from human plasma fibronectin. Samples of 6 M guanidine HCl + dithioerythritol extract were electrophoresed after reduction, transferred to nitrocellulose filters, and reacted either with an antiserum to chick fibroblast fibronectin or an antiserum to gp 115 (Fig. 9). The antiserum to chick fibroblast fibronectin identified two polypeptides in the guanidine HCl + dithioerythritol extract (*lane a*) with mobilities similar to the subunits of human plasma fibronectin (*lane b*) which was included for reference. Slightly below, a third band, probably a degradation product of fibronectin, was also present in both samples. The antiserum to gp 115 bound to gp 115 present in the guanidine HCl + dithioerythritol extract, but failed to recognize proteins in the molecular weight region of the fibronectin subunits (*lane c*). These studies strongly suggested that gp 115 was not related to chick aorta fibronectin and may well represent a novel matrix protein.

[3] A. Colombatti, G. M. Bressan, I. Castellani, and D. Volpin, unpublished results.

DISCUSSION

Glycoproteins have been found to play an important role in organizing the extracellular matrix and in mediating the interactions between cell surface and surrounding environment (24). Several glycoproteins have been detected in blood vessels where they have been localized in the pericellular matrix of smooth muscle cells and fibroblasts and in the basement membrane of endothelial cells (5, 6, 28). A glycoprotein-rich fraction is obtained from elastic arteries by sequential extraction with saline, 6 M guanidine HCl, and 6 M guanidine HCl + dithioerythritol (9). In the search for extracellular glycoprotein components of blood vessels, we have analyzed by ion exchange chromatography and SDS-polyacrylamide gel electrophoresis the glycoprotein-rich fraction solubilized from chick aorta by 6 M guanidine HCl + dithioerythritol. Five discrete glycoprotein species were identified: four were collagenous (M_r = 205,000, 195,000, 150,000, and 135,000) as revealed by amino acid composition and one (M_r = 115,000) was noncollagenous by the same criterion. Of these, only the 150,000- and 135,000-dalton components had molecular masses similar to proteins previously identified in tissue and cell culture extracts obtained with denaturing solvents with and without reducing agents (25, 26). The absence of a glycoprotein similar to gp 115 in extracts obtained by other authors (9, 10, 25, 26) with caotropic and reducing agents could be ascribed either to different experimental

approaches (biosynthetic studies) or systems used (cell cultures, organs different from aorta; and species other than chick).

Two observations support the conclusion that gp 115 might have a structural function. First, it formed a fibrillar network in the extracellular matrix of aorta (Fig. 6). A fibrillar or laminar pattern of distribution has been observed for two other well characterized structural glycoproteins, namely fibronectin and laminin (6, 27). Second, gp 115 was preferentially extracted from the tissue with buffers containing reducing agents, indicating that a large part of it formed disulfide-bonded aggregates tightly associated with the insoluble aorta residue. This suggests that the protein could aggregate *in vivo* into supramolecular structures forming, alone or together with other extracellular components, particular morphological constituents of the media of blood vessels. Immunoelectron microscopic studies should allow the description of the fine distribution of gp 115 in the tissue and contribute to elucidate its function.

Several glycoproteins with structural function have been recently identified in connective tissues from various animal species using purification methods different from ours. The more extensively studied include fibronectin (7), laminin (15), and entactin (28). The molecular weight and amino acid composition, characterized by a high glutamic to aspartic molar ratio and a high arginine content, distinguished gp 115 from the subunit(s) of the above mentioned proteins. Structural (Fig. 7) and immunological studies (Figs. 8 and 9) definitely indicated that gp 115 was not a partial degradation product of either fibronectin or laminin. While the data of Figs. 7 and 8 were obtained with fibronectin and laminin purified from species other than chick, the conclusion that they were not related to gp 115 is very likely, given the fact that fibronectin (29) and possibly laminin (6) are evolutionary conserved proteins and antisera display strong cross-reactivity with molecules isolated from different species. Additional experimental evidence supported the notion that chick fibronectin was immunologically unrelated to gp 115; an antiserum against gp 115 failed to recognize fibronectin subunits which were present in the guanidine HCl + dithioerythritol aorta extract (Fig. 9). Finally, the distribution reported in the literature for laminin and entactin (6, 15, 28, 30) differed from that of gp 115. We have immunohistological evidences that antibodies to gp 115 stain blood vessels throughout the organism as well as extracellular matrix of kidney, gut, lung, and other tissues; on the contrary, no immunoreactivity was detected with skin and cornea basement membranes.[2]

Thrombospondin, a major platelet α-granule glycoprotein, was recently reported to be produced by endothelial cells (31, 32) and fibroblasts (33) in culture. Although, to our knowledge, studies on the occurrence of thrombospondin in intact tissues have not yet been published, the protein should be regarded as a possible constituent of the extracellular matrix of blood vessels. Given their markedly different amino acid compositions, thrombospondin and gp 115 are very likely unrelated proteins. Immunoblotting analysis of proteins extracted from chick embryo aorta and tendon cells using anti-gp 115 antibodies, apart from gp 115 itself, failed to detect any protein in the molecular weight range expected for thrombospondin.[3]

All the data available suggest that gp 115 is a novel matrix glycoprotein associated with blood vessels.

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