In Vitro Formation of Disulfide-bonded Fibronectin Multimers*

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Fibronectin purified from a plasma protein side fraction in the absence of denaturant contained 1.5 to 1.9 cryptic free sulfhydryl groups per 200- to 250-kDa subunit. Exposure of sulfhydryl groups in physiologic salt solutions required at least 1 M guanidine, and 3 M guanidine was required for optimal exposure. The sulfhydryl groups were not exposed by collagen, a fibronectin-binding collagen fragment, fibrinogen, heparin, hyaluronic acid, calcium ion, EDTA, deoxycholate, or methylamine.

One- and two-dimensional gel electrophoresis indicated that a molecule of 40-60 kDa was disulfide-bonded to a minor portion of the fibronectin in whole human plasma and in preparations of purified fibronectin. In addition, traces of disulfide-bonded multimers were present in preparations of purified fibronectin. The proportion of fibronectin in disulfide-bonded multimers increased in guanidine-containing solutions. Compared to dimeric fibronectin, these multimers had limited solubility in physiologic buffers, could be readily cross-linked by Factor XIII, and exhibited altered tryptic susceptibility. In free sulfhydryl groups were blocked by prior alkylation with N-ethylmaleimide or iodoacetamide, fibronectin did not form disulfide-bonded multimers in guanidine-containing solutions. The patterns of altered tryptic susceptibility and cysteine cleavage suggested that multimer formation is mediated by both sulfhydryls of fibronectin. The transition from dimeric to multimeric fibronectin can serve as a model for the formation of disulfide-bonded fibronectin multimers in the extracellular matrix.

In an alternate purification, the supernatant after heat precipitation was applied to a 2-liter gradient of 0.07 to 0.30 M sodium chloride in 0.01 M sodium phosphate buffer, pH 7.4, at constant stirring in 600 ml of 0.01 M Tris, 0.4 M sodium chloride, pH 7.4. Undissolved material was removed by centrifugation. Fibronectin was precipitated by heating at 56°C for 3 min and removed by centrifugation. The supernatant was dialyzed against 4 liters of 0.01 M Tris, 0.01 M sodium chloride, pH 7.4, and applied to a column (6.5 X 3.5 cm) of DEAE-cellulose (DE-52). The protein was eluted as a single peak at 0.1 M sodium chloride, pH 7.4. The protein was dialyzed against 0.01 M Tris, pH 7.4, and applied to a column (6.5 X 3.5 cm) of DEAE-cellulose which had been equilibrated with 0.01 M Tris, 0.07 M sodium chloride, pH 7.4. The protein was eluted as a single peak at 0.1 M sodium chloride, pH 7.4, and applied to a column (6.5 X 3.5 cm) of DEAE-cellulose which had been equilibrated with 0.01 M Tris, 0.07 M sodium chloride, pH 7.4. The protein was eluted as a single peak at 0.1 M sodium chloride, pH 7.4, and applied to a column (6.5 X 3.5 cm) of DEAE-cellulose which had been equilibrated with 0.01 M Tris, 0.07 M sodium chloride, pH 7.4. The protein was eluted as a single peak at 0.1 M sodium chloride, pH 7.4, and applied to a column (6.5 X 3.5 cm) of DEAE-cellulose which had been equilibrated with 0.01 M Tris, 0.07 M sodium chloride, pH 7.4. The protein was eluted as a single peak at 0.1 M sodium chloride, pH 7.4, and applied to a column (6.5 X 3.5 cm) of DEAE-cellulose which had been equilibrated with 0.01 M Tris, 0.07 M sodium chloride, pH 7.4. The protein was eluted as a single peak at 0.1 M sodium chloride, pH 7.4.
tion was passed over a column of gelatin agarose, and bound fibronectin was eluted with 1 M sodium bromide at pH 5.0 (20).

**Polyacrylamide Slab Gel Electrophoresis—**Electrophoresis in one dimension was performed using the discontinuous slab system of Ames (23). In some experiments, a gradient separating gel was formed by rapidly pouring gels whose bottom third was 12%, middle third was 8%, and top third was 4% acrylamide. Nonreduced-reduced two-dimensional electrophoresis was performed as described elsewhere (24). Unless indicated otherwise, slab gels were stained with Coomassie brilliant blue to visualize the protein bands.

Electroblotting (25) was done in an apparatus purchased from Bio-Rad Laboratories. After the protein was transferred to nitrocellulose paper by electroblotting, replicate sections were analyzed by protein immunostaining. One section of paper was stained with 0.1% naphthol blue black in 45% methanol and 10% acetic acid and destained in 45% methanol and 10% acetic acid. A replicate section of paper was soaked in Tris-buffered saline containing 3% bovine albumin for 1 h at 37 °C, rinsed in Tris-buffered saline, and soaked overnight in Tris-buffered saline containing 3% albumin, 10% fetal calf serum, and either 2% rabbit anti-human fibronectin antiserum or mouse monoclonal anti-fibronectin, 50 μg/ml. The section was washed in Tris-buffered saline and soaked for 1 h in Tris-buffered saline containing 3% albumin, 10% fetal calf serum, and 1% fluorescein isothiocyanate-conjugated goat anti-rabbit IgG or rabbit anti-mouse IgG. The section was washed with Tris-buffered saline, and fluorescent bands were photographed.

Silver staining of polyacrylamide slab gels were done by the procedure of Oakley (26) with modifications described by Giulian et al. (27).

The molecular size markers included: fibronectin dimer, 400 kDa; fibronectin monomer, 200 kDa; phosphorylase, 90 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; chymotrypsinogen, 24.5 kDa; and hemoglobin, 16.5 kDa. The sizes of fibronectin are based on previous experiments in which the apparent size of reduced plasma fibronectin was determined by comparison to the heavy chain of myosin and the subunit of Factor VIII-related antigen/von Willebrand factor (28). In this analysis, the sizes assigned to myosin and Factor VIII-related antigen/von Willebrand factor were literature values for the subunit sizes of these proteins as determined by sedimentation equilibrium in 6 M guanidine.

Other Methods—**Sulfhydryl groups were quantified with 5,5'-di thio bis-(2-nitrobenzoic acid) or 2,2'-dipyridyl disulfide as described elsewhere (7) except that titrating reagent was added prior to addition of guanidine and the molar extinction coefficient for 2-thiopyridone was assumed to be 7060 M⁻¹ cm⁻¹ (29).** Fibronectin with blocked sulfhydryl groups was prepared by incubating the protein, 5 mg/ml, with 10 mM N-ethylmaleimide or iodoacetamide and 3 M guanidine for 2.5 h at 22 °C. Excess alkylating agent and guanidine were removed by dialysis. For cleavage of fibronectin at free sulfhydryl groups (30) fibronectin was incubated with 0.25 mM iodoacetamide and 3 M guanidine in Tris-buffered saline, pH 9.0, for 15 min at 22 °C and then treated with 20 mM potassium cyanide. The reaction was stopped after 30 min by adding 1 M acetic acid, and the samples were dialyzed into 0.1 M Tris, 0.15 M sodium chloride, pH 6.8.

Before most manipulations, proteins were dissolved in or dialyzed extensively against Tris-buffered saline (0.01 M Tris, 0.15 M sodium chloride, pH 7.4). Fibronectin multimers were dissolved in or dialyzed

**TABLE I**

| Fraction | Volume | Protein concentration | Total Protein concentration | Total Fibronectin concentration | Purine | Yield |
|----------|--------|----------------------|-----------------------------|---------------------------------|-------|-------|
|          | ml     | mg/ml                | mg                            | mg/ml                           | %     | %     |
| Before heat precipitation (1)* | 740 | 22.4 | 16,380 | 2.65 | 1,960 | 12b | 100 |
| Before DEAE-cellulose (3)* | 1,080 | 2.1 | 2,270 | 1.50 | 1,620 | 71b | 83 |
| Passthrough from DEAE-cellulose (4)* | 1,300 | 0.6 | 780 | 0.15 | 190 | 24b | 83 |
| Peak from DEAE-cellulose (5)* | 682 | 1.8 | 1,230 | 1.53 | 1,045 | 85' | 53 |
| Product (6)* | 51.45 | 20.1 | 1,030 | 19.2 | 990 | 99' | 51 |

* Numbers refer to the gel slots in the left half of Fig. 1 on which the samples were analyzed.

**Fig. 1.** DEAE-cellulose chromatographic purification of fibronectin from the fibronectin-rich super natant after heat precipitation. The chromatographic profile and results of polyacrylamide gel electrophoresis in sodium dodecyl sulfate (10% gel) are shown. Samples shown include the initially dissolved material before heat precipitation (lane 1), after heat precipitation (lane 2), after dialysis and before the DEAE-cellulose column (lane 3), the passthrough from the DEAE-cellulose column (lane 4), the peak from the DEAE-cellulose column (lane 5), and the final product after concentration with ammonium sulfate, shown both reduced (lane 6R) and nonreduced (lane 6N). All other samples are shown after reduction with 2% 2-mercaptoethanol. Molecular size markers are indicated on the left.
against CAPS\(^1\)-buffered saline (0.01 M CAPS, 0.15 M sodium chloride, pH 11.0).

Fibronectin antigen was measured by the immunoturbidimetric method (31) with a kit supplied by Boehringer-Mannheim Biochemicals (Indianapolis, IN).

RESULTS

Large quantities of human plasma fibronectin were purified from a fibronectin-rich side fraction of commercial Factor VIII production (Table I and Fig. 1). Aside from the step in which fibrinogen was precipitated by heating at 56 °C for 3 min, fibronectin was kept at near physiologic conditions throughout the purification. Preparations with the same qualities were obtained when chromatography on gelatin-agarose with elution with 1 M sodium bromide, pH 5.0 (20), was done in place of chromatography on DEAE-cellulose.

When purified fibronectin was analyzed by polyacrylamide gel electrophoresis after reduction, 99% of the protein was in a band of 200 kDa (Table I and Fig. 1). The 200-kDa band could be resolved into 2 closely spaced of equal intensity if only 1-2 µg of protein were analyzed (22, 33). When purified fibronectin was analyzed by electrophoresis without reduction, minor bands of 180, 200, 240, 460, approximately 800, and greater than 1000 kDa were observed in addition to the major band of 400 kDa (Figs. 1 and 2). The heterogeneity of the unreduced sample was best appreciated by indirect immunofluorescent staining of electroblotted protein (Fig. 2). When whole human plasma was analyzed by the same technique, fluorescent bands of 180, 200, 240, 400, and 460 kDa were detected whereas the bands of approximately 800 and greater than 1000 kDa were not (Fig. 2).

Gel electrophoresis of purified fibronectin without reduction followed by gel electrophoresis after reduction (Fig. 5) indicated that the 460-, 400-, and 240-kDa bands in the nonreduced dimension were composed of 200-kDa subunits. The 200- and 180-kDa bands, in contrast, fell on the diagonal. On the basis of this analysis, we hypothesize that a molecule of 40–60 kDa is disulfide-linked to a small proportion of fibronectin molecules (see under "Discussion").

Fibronectin in Tris-buffered saline had no reactive free sulfhydryl groups whereas fibronectin in 3 M guanidine had 1.5 sulfhydryl groups per 200-kDa subunit or 1.9 sulfhydryl groups if a subunit size of 250 kDa is assumed (7). Greater than 1 M guanidine was required to expose sulfhydryl groups, and additional reactive sulfhydryl groups were not exposed at guanidine concentrations greater than 3 M (Fig. 4). Greater than 2 M urea also caused exposure of sulfhydryl groups, and 4 M urea caused optimal exposure (data not shown). A variety of substances which are known to interact with fibronectin did not cause exposure of sulfhydryl groups (Table II). In addition, incubation with calcium ion, EDTA, deoxycholate, or methylamine did not cause exposure of sulfhydryl groups (Table II).

When fibronectin, 7 mg/ml, was incubated in 3 M guanidine, disulfide-bonded multimers were formed in a time- and temperature-dependent manner (Fig. 5). Upon removal of guanidine by dialysis against Tris-buffered saline, a granular precipitate formed on the sides of the dialysis bag. With time, the granules coalesced to form a thick stringy precipitate (Fig. 6). After dialysis at 22 °C, the precipitate contained approximately 80% of the fibronectin when the protein concentration in the bag was greater than 1.5 mg/ml (Table III) and was enriched in the larger multimers (Fig. 5). After dialysis at 4 °C, the precipitate contained approximately 75% of the fibronectin (Table IV). The precipitate readily dissolved in CAPS-buffered saline, pH 11, and fibronectin dialyzed directly from guanidine into CAPS-buffered saline did not precipitate.

Multimeric fibronectin in CAPS-buffered saline precipitated when diluted in or dialyzed against Tris-buffered saline, pH 7.4. In contrast, dimeric fibronectin in CAPS-buffered saline did not precipitate when diluted in or dialyzed against Tris-buffered saline.

Fibronectin with blocked free sulfhydryl groups did not form a precipitate after incubation in 3 M guanidine and dialysis against Tris-buffered saline (Table IV) and did not form disulfide-bonded multimers in 3 M guanidine (data not shown). Fibronectin multimers had 45% fewer free sulfhydryl groups than fibronectin dimers (Table V).

Multimeric fibronectin in Tris-buffered saline was readily cross-linked by Factor XIII, under conditions in which little cross-linking of dimeric fibronectin occurred (Fig. 7).
Multimers were tested for ability to bind to gelatin-agarose after dilution in Tris-buffered saline, pH 7.4, to a final concentration (500 μg/ml) at which precipitation did not occur. Under standardized conditions, 79% bound to gelatin-agarose as compared to 77% for dimeric fibronectin which had been previously dialyzed into CAPS-buffered saline, pH 11.

Multimers and dimers were digested differently by trypsin (Figs. 8 and 9). The differences were of fragments recognized by a monoclonal antibody to an epitope close to the more carboxyl-terminal of the two free sulfhydryls (7). Thus, the intensities of previously described 180-, 135-, 71-, and 31-kDa fragments (7, 22) were much decreased both by protein staining and by immunofluorescent staining (Figs. 8 and 9). In contrast, fragments of 165, 150, and 39 kDa, which were not recognized by the monoclonal antibody, were present in digests of both multimers and dimers (Fig. 8). Fragments of 80 kDa, which have previously been shown to contain a free sulfhydryl group (7), were present in digests of both dimers and multimers (Fig. 8). We were not able to identify disulfide-linked pairs in comparisons of protein-stained gels run without and with reduction, i.e. we could not identify fragments which

**Table II**

| Potential modifier   | Concentration | ΔA235/A200 | Free-SH exposed/2 x 10^4 g |
|----------------------|---------------|------------|--------------------------|
| Collagen type III    | 0.2 mg/ml     | 0.004      | 0.08                     |
| Heparin              | 0.2 mg/ml     | 0.003      | 0.06                     |
| α1(I)-CB7            | 0.4 mg/ml     | 0.004      | 0.08                     |
| Hyaluronic acid      | 0.1 mg/ml     | 0.001      | 0.02                     |
| Fibrinogen           | 2.4 mg/ml     | 0.002      | 0.04                     |
| EDTA                 | 1 mM          | 0.001      | 0.02                     |
| Ca^{2+}              | 2 mM          | <0.001     | <0.02                    |
| Deoxycholate         | 0.8%          | 0.001      | 0.02                     |
| Methylamine          | 400 mM        | 0.001      | 0.02                     |

*Fig. 4.* Exposure of free sulfhydryl groups in fibronectin by denaturation in guanidine. Fibronectin, 1 mg/ml, was incubated with various guanidine concentrations in 0.1 M Tris, 0.15 M NaCl, pH 7.4, for 1 h at 22 °C. 2,2'-dipyridyl disulfide, 0.1 mM, was added and the change in absorbance at 343 nm was noted after 2 min.

*Fig. 5.* Formation of disulfide-bonded fibronectin multimers after exposure to guanidine. Fibronectin, 7 mg/ml, was incubated in Tris-buffered saline containing 3 M guanidine, pH 7.4, for 0, 5, 30, 60, 120, and 240 min at 22 °C (lanes 1–6), for 120 min at 37 °C (lane 7), and for 120 min at 0 °C (lane 8). A control sample which did not contain guanidine was incubated for 240 min at 22 °C (lane 9). At the end of the incubation, the sample was diluted with 2 parts of Tris-buffered saline and shortly thereafter was dialyzed against Tris-buffered saline at 22 °C. Hence, the sample in lane 1 was incubated in Tris-buffered saline containing 1 M guanidine for about 15 min at 22 °C prior to dialysis. Digests of the 120- and 240-min samples from the incubation at 22 °C and the 120-min sample from the incubation at 37 °C produced precipitates (lanes 10, 11, and 12). The precipitates were removed with a stirring rod, rinsed with water, and suspended in the original volume of Tris-buffered saline. Samples were analyzed by electrophoresis on 6% polyacrylamide gels after dialysis in an equal volume of 2% sodium dodecyl sulfate. Reduced samples analyzed by electrophoresis all had a single band of 200 kDa (not shown).

In other experiments, incubation of fibronectin in Tris-buffered saline containing 1 M guanidine for 120 min at 22 °C did not result in multimer formation. The lines on the right indicate the tops of the stacking (upper) and resolving (lower) gels.

*Fig. 6.* Precipitate formed by multimeric fibronectin. Fibronectin was incubated for 2 h at 22 °C in Tris-buffered saline containing 4 M guanidine, pH 8.2, and then dialyzed against Tris-buffered saline. After dialysis, the bag was photographed in indirect light. Bar represents 1 cm.
split into smaller fragments with reduction. This finding was confirmed by nonreduced/reduced two-dimensional gel electrophoresis, in which all of the spots of <200 kDa fell on the diagonal (data not shown). Several new fragments were recognized in digests of multimers: fragments with apparent sizes nonreduced/reduced of 54/57 and 36/37 kDa, detected by protein staining (Fig. 8) and nonreduced of 70 and 32 kDa, detected by immunofluorescence (Fig. 9). In addition, there were increased amounts of small antigenic fragments detected by immunofluorescence at the front (<20 kDa) of the gel of nonreduced digests (Fig. 9).

Cyanide cleavage of fibronectin dimer and multimers resulted in patterns in sodium dodecyl sulfate-polyacrylamide gels similar to those described by Wagner and Hynes (30). However, the yield of the principal 156-kDa cleavage component (determined by densitometry) was 5.4% in the digestion of multimers as compared to 12.7% in the preparation of dimer. Cleavage products were not detected in the 20- to 50-kDa region of the gels, even after silver staining.

### Table III

| Starting fibronectin concentration | Amount of precipitate (0 to ++++) | mg/ml | % lost in precipitate |
|----------------------------------|----------------------------------|-------|----------------------|
| mg/ml                            | mg/ml                            | mg/ml | % lost in precipitate |
| 6.6                              | +++                              | 4.8   | 30                   |
| 3.3                              | ++                               | 2.3   | 30                   |
| 1.8                              | +                                | 1.4   | 22                   |
| 0.82                             | 0                                | 0.72  | 22                   |
| 0.39                             | 0                                | 0.35  | 22                   |

### Table IV

| Protein        | Starting protein concentration | Amount of precipitate (0 to ++++) | mg/ml | mg/ml | % lost in precipitate |
|----------------|--------------------------------|----------------------------------|-------|-------|----------------------|
| Fibronectin    | 7.4 mg/ml                      | +++                              | 1.8   | 76    |                      |
| CAM-fibronectin| 5.7 mg/ml                      | +                                | 4.9   | 14    |                      |
| NEM-fibronectin| 6.2 mg/ml                      | +                                | 5.2   | 16    |                      |

### Table V

| Sample         | Assay/Flx | Free SH | % \(A_{208/280}\) |
|----------------|-----------|---------|-----------------|
| Dimer, no GnHCl| 0.007     | 0.1     | 0.1             |
| Dimer in 4 mg GnHCl | 0.075 | 1.4     | 0.1             |
| Multimer, no GnHCl | 0.008 | 0.1     | 0.1             |
| Multimer in 4 mg GnHCl | 0.043 | 0.8     | 0.1             |

**Fig. 7. Cross-linking of multimeric fibronectin by Factor XIII.** Dimeric fibronectin, 0.4 mg/ml (lanes 1-3), or multimeric fibronectin, 0.4 mg/ml (lanes 4-6), in Tris-buffered saline were treated with nothing (lanes 1 and 4) or with thrombin, 1 unit/ml, and Factor XIII, 20 μg/ml, in the presence of 10 mM EDTA (lanes 2 and 5) or 5 mM calcium ion (lanes 3 and 6). Prior to the experiments, both proteins had been dialyzed against CAPS-buffered saline, pH 11. Therefore, the proteins were diluted 8-fold in Tris-buffered saline prior to the experiment. The samples were incubated at 37 °C for 2.5 h and then treated with electrophoresis buffer containing 2% 2-mercaptoethanol and analyzed on 10% polyacrylamide slab gels. No visible precipitates formed during the incubations. Size markers are shown on the left. The lines on the right indicate the tops of the stacking (upper) and separating (lower) gels.

**Fig. 8. Sequential trypsinization of multimeric fibronectin.** Multimeric or dimeric fibronectin in CAPS-buffered saline, pH 11, was diluted 8-fold in Tris-buffered saline, pH 7.4. The final concentration of each was 1 mg/ml. The proteins were digested at 37 °C with trypsin, 1 μg/ml. At 0, 1, 5, 10, 30, 60, and 120 min, samples were removed, denatured in 2% sodium dodecyl sulfate without (NR) or with (R) 2% 2-mercaptoethanol, and analyzed by electrophoresis on a 10% polyacrylamide gel (lane 1, 0 min; lane 2, 1 min; etc.). Proteins were stained with Coomassie brilliant blue. In other gels, not shown, we found that dimeric fibronectin that was not cycled through CAPS-buffered saline had the same bands as dimeric fibronectin that was cycled through CAPS-buffered saline. The arrows point to fragments which were found in one preparation and not in the other. The fragments are designated by their size (in kilodaltons) nonreduced/reduced. Brackets indicate fragments of approximately 80 kDa. Size markers are on the left.
immunofluorescent staining was with a mouse monoclonal antibody as described in Fig. 8. Separated proteins were transferred to nitrocellulose which has been shown previously to react with the 31-kDa fragment of early tryptic digests of fibronectin (7). Shown are the results of immunofluorescent staining. Molecular sizes are not marked, but all of the bands in Fig. 8 were transferred to the nitrocellulose paper.

40- to 60-kDa molecule is HC protein (HC protein has a size of approximately 30-kDa and is found disulfide-linked to many plasma proteins, including IgA and albumin (34)). The fact that fibronectin fragments apparently contain more of the 40- to 60-kDa molecule (i.e., the ratio of 240 to 200 kDa molecules is greater than the ratio of 460 to 400 kDa molecules) suggests that the circulating fibronectin which contains the 40- to 60-kDa molecule originates from tissues with active proteolysis. Fibronectins of approximately 800 and greater than 1000 kDa, which were present in purified preparations but not in plasma (Fig. 2), probably represent protein which was denatured during purification.

Plasma fibronectin (35), as well as fibronectin synthesized in cell culture (13), forms disulfide-bonded multimers upon incorporation into the extracellular matrix. A variety of substances known to bind to fibronectin did not expose free sulfhydryl groups (Table II). Thus, we can offer little insight into if or how free sulfhydryl groups of fibronectin are exposed in the cell layer and participate in disulfide-bonded multimer formation. Multimer formation is a slow process (12, 13). If a small fraction of free sulfhydryl groups are exposed at any one time and the fibronectin molecules in the cell layer are aligned optimally, multimer formation could occur without a conformational change to further expose free sulfhydryl groups. Exposure could be facilitated by binding of fibronectin to two substances simultaneously (e.g., collagen and a glycosaminoglycan) or to a substance not tested in our experiments. The free sulfhydryl groups of fibronectin synthesized by cells may be exposed in a manner different from plasma fibronectin. For instance, the difference in antigenicity of the sulfhydryl-containing COOH-terminal region of hamster cellular fibronectin (36) may be accompanied by a conformational change which makes the sulfhydryl group more accessible. Alternatively, modification at a potential site of N-glycosylation which has been identified adjacent to a free sulfhydryl group in bovine plasma fibronectin (37) might make the free sulfhydryl group more accessible. Finally, disulfide multimer formation in the cell layer may not involve free sulfhydryl groups at all, but disulfide exchange of existing sulfides.

The failure of methyla mine to expose free sulfhydryl groups suggests that fibronectin does not contain cysteinyl residues in thiolester linkages (as is the case with α2-macroglobulin and CS (98, 99)).

Disulfide-bonded fibronectin multimers could be produced in solution by exposing concentrated fibronectin to 3 M guanidine at 22 or 37 °C (Fig. 5). These multimers had limited solubility in physiologic buffers, bound to gelatin agarose, and exhibited altered tryptic susceptibility in the 31-kDa COOH-terminal region. Formation of such multimers may explain the limited solubility that is sometimes observed with fibronectin prepared by elution of gelatin-agarose with 4 M urea.

Previous studies have demonstrated that a 31-kDa early tryp tic fragment and an 80-kDa late tryp tic fragment of human plasma fibronectin contain free sulfhydryl groups (7). The 31-kDa fragment and precursors of the 31-kDa fragment were largely missing from trypsinates of multimers whereas the 80-kDa fragment and precursors of the 80-kDa fragment were present and apparently not disulfide-bonded into a larger aggregate (Figs. 8 and 9). The decreased yield of the 156-kDa fragment upon cyanide cleavage, however, suggests that there was also some oxidation of the sulfhydryl group in the 80-kDa region. Disulfide bond formation potentially could involve free sulfhydryl groups in both the 31- and 80-kDa regions, and disulfide bonds could form between the two halves of the dimers as well as between two dimers. Thus, six different disulfide arrangements are possible.
dryl groups were blocked in the multimers (Table V), and the trypsinization study suggests that the majority of the arrangements involve the sulfhydryl group in the 31-kDa fragment. The altered tryptic susceptibility suggests that exposure and oxidation of the cysteinyl residue in the 31-kDa region causes a local conformation change.

The multimers, like fibronectin in the cell layer (40), were readily cross-linked by Factor XIII. As described in the introduction, multimeric cell surface fibronectin has several biologic properties which are not shared by dimeric plasma fibronectin (17-19). We have found that multimeric plasma fibronectin is more potent than dimeric fibronectin in the promotion of growth by primary guinea pig glomerular cells in defined medium (41). Multimeric plasma fibronectin, but not dimeric fibronectin, caused aggregation of platelets in platelet-rich plasma.3 Experiments are currently underway to study whether multimeric plasma fibronectin, like cell surface fibronectin, causes transformed cells in culture to assume a more normal shape, agglutinates fixed red blood cells, and binds hyaluronic acid. It will also be interesting to learn whether multimeric fibronectin can bypass the initial binding pool (35) and become incorporated directly into the detergent-insoluble extracellular matrix when added to cultures of non-transformed fibroblasts. Thus, we believe that the multimers will be a valuable derivative for studies of the polymerization and insolubilization of fibronectin and in structure-function studies of the interactions of fibronectin with cells.

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