# Cross-linking of fibronectin to collagen by blood coagulation Factor XIIIa.

D F Mosher, P E Schad

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**Research Article**

Soluble fibronectin is found in body fluids and media of adherent cultured cells and binds to fibrin and collagen. Insoluble fibronectin is found in tissue stroma and in extracellular matrices of cultured cells. Fibronectin is a substrate for Factor XIIIa (plasma transglutaminase) and can be cross-linked by Factor XIIIa to itself and the the alpha-chain of fibrin. We used sodium dodecyl sulfate-polyacrylamide gel electrophoresis to investigate Factor XIIIa-mediated crosslinking of fibronectin to collagen. At O degrees or 37 degrees C, fibronectin could be cross-linked to iodinated cyanogen bromide fragment 7 of the alpha 1(I) chain. At 22 degrees or 37 degrees C, fibronectin could be cross-linked to isolated alpha 1(I) chains of type I collagen. Fibronectin could also be crosslinked to types I and III collagen, but only at 37 degrees C. alpha 1(I)-CB7, alpha 1(I) collagen chains, type I collagen, type III collagen, and fibrin all blocked cross-linking between 125I-alpha 1 (I)-CB7 and fibronectin. alpha 1(I)-CB7 blocked cross-linking between fibronectin and fibrin. These results indicate that the determinants of fibronectin-fibrin and fibronectin-collagen binding and cross-linking are similar. Cross-linking of fibronectin to collagen likely occurs in vivo and may be important for normal wound healing, collagen fibrillogenesis, and embryogenesis.



# Cross-Linking of Fibronectin to Collagen by Blood Coagulation Factor $XIII_a$

DEANE F. MOSHER and PETER E. SCHAD, *Department of Medicine, University of Wisconsin, Madison, Wisconsin 53706*
HYNDA K. KLEINMAN, *Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, Bethesda, Maryland 20014*

ABSTRACT Soluble fibronectin is found in body fluids and media of adherent cultured cells and binds to fibrin and collagen. Insoluble fibronectin is found in tissue stroma and in extracellular matrices of cultured cells. Fibronectin is a substrate for Factor $XIII_a$ (plasma transglutaminase) and can be cross-linked by Factor $XIII_a$ to itself and to the $\alpha$-chain of fibrin. We used sodium dodecyl sulfate-polyacrylamide gel electrophoresis to investigate Factor $XIII_a$-mediated cross-linking of fibronectin to collagen. At 0° or 37°C, fibronectin could be cross-linked to iodinated cyanogen bromide fragment 7 of the $\alpha$1(I) chain. At 22° or 37°C, fibronectin could be cross-linked to isolated $\alpha$1(I) chains of type I collagen. Fibronectin could also be cross-linked to types I and III collagen, but only at 37°C. $\alpha$1(I)-CB7, $\alpha$1(I) collagen chains, type I collagen, type III collagen, and fibrin all blocked cross-linking between $^{125}$I-$\alpha$1(I)-CB7 and fibronectin. $\alpha$1(I)-CB7 blocked cross-linking between fibronectin and fibrin. These results indicate that the determinants of fibronectin-fibrin and fibronectin-collagen binding and cross-linking are similar. Cross-linking of fibronectin to collagen likely occurs in vivo and may be important for normal wound healing, collagen fibrillogenesis, and embryogenesis.

## INTRODUCTION

Fibronectin is high molecular weight glycoprotein found in extracellular fluids, connective tissues, and associated with basement membranes (1, 2). In cell culture, fibronectin is present in cell layers of substrate-attached cells and is organized into an extracellular fibrillar matrix (3–6). Collagen, a second component of the matrix (7, 8), binds to fibronectin (9–12). A specific region of the type I collagen $\alpha$1(I) chain, which is generated by cyanogen bromide cleavage and designated $\alpha$1(I)-CB7, comprises residues 552–822 and contains the principal binding site for fibronectin (11, 12). Ultrastructural localization of fibronectin and collagen at various times after subculture of human skin fibroblasts suggests that fibronectin serves as a template or scaffolding for collagen fiber formation.[1]

Transglutaminases are a class of calcium ion-dependent enzymes that catalyze an acyl transfer reaction which involves glutaminyl residues in proteins and peptides as acyl donors and a variety of primary amines, including lysyl residues, as acyl acceptors. Monosubstituted $\gamma$-amide bonds are thus formed. By this reaction, transglutaminases can catalyze the incorporation of $\epsilon$-($\gamma$-glutamyl)-lysine linkages between proteins. Inasmuch as specific glutaminyl residues are susceptible to the action of transglutaminases and accepting lysyl residues must be in favorable stereochemical juxtaposition, this cross-linking is a highly specific reaction. As recently reviewed by Folk and Finlayson (13), transglutaminases are widespread and are thought to catalyze many biologically important reactions: the cross-linking of vertebrate fibrin by plasma transglutaminase (blood coagulation Factor $XIII_a$), clotting of seminal plasma by transglutaminase from the anterior prostate gland of rodents, cross-linking of hair proteins by hair follicle transglutaminase, cross-linking of keratin by epidermal transglutaminase, and clotting of lobster fibrinogen.

In this paper, we demonstrate that plasma transglutaminase (Factor $XIII_a$) catalyzes the cross-linking of fibronectin and collagen. We speculate that this reaction may be important for wound healing and tissue repair.

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[1] Furcht, L. T., D. F. Mosher, G. Wendelschafer-Crabb, and J-M. Foidart. Fibronectin and type I procollagen fibrillogenesis in cultures of human skin fibroblasts detected by ultrastructural immunocytochemistry. Manuscript submitted for publication.

## METHODS

*Materials.* The following were purchased: $^{125}$I-Bolton-Hunter reagent (1,500 Ci/mmol), New England Nuclear, Boston, Mass.; reagents for polyacrylamide gel electrophoresis and Coomassie brilliant blue R-250, Bio-Rad Laboratories, Richmond, Calif.; trypsin, L-1-tosylamide-2-phenylethylchloromethyl ketone-treated, Worthington Biochemical Corp., Freehold, N. J.; and Form III bacterial collagenase, Advanced Biofactures, Lynbrook, N. Y. Human thrombin (14) was a generous gift from Dr. John Fenton, II, New York State Department of Health, Albany, N. Y.

*Purification of proteins.* Human fibrinogen, Factor XIII, and fibronectin were purified as described previously (15). Fibronectin was also purified from whole plasma by affinity chromatography on immobilized gelatin (16, 17). Adsorbed fibronectin was eluted from the column with 1 M sodium bromide, pH 5.3 (18), dialyzed against Tris-buffered saline, pH 7.4, and concentrated by precipitation with 40% ammonium sulfate. Fibrinogen, Factor XIII, and fibronectin were >95% pure as assayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. However, the fibrinogen and fibronectin preparations contained residual Factor XIII activity (as assayed by ability to cross-link fibrin) unless treated with 3.3 M urea (19).

Rat (from lathyritic animals) and fetal calf skin type I collagen, calf skin type III collagen, $\alpha$1(I) chains, and cyanogen bromide peptides of $\alpha$1(I) chains were prepared as described previously (11, 12). *Ascaris* collagen (20) was a gift of Ms. C. Sullivan, National Institute of Dental Research, Bethesda, Md.

*Radiolabeling of proteins.* $\alpha$1(I)-CB7 was iodinated by the Bolton-Hunter reagent (21). $\alpha$1(I)-CB7 was dissolved in 0.1 M sodium borate, pH 8.5, at a concentration of 2 mg/ml. Ice-cold solution, 400 $\mu$l, was added to 1 mCi of Bolton-Hunter reagent, previously dried from benzene in the reaction vial as recommended by the manufacturer. After 15 min at 0°C, the reaction was terminated by adding 1 ml of 0.2 M glycine in borate buffer, pH 8.5. $^{125}$I-$\alpha$1(I)-CB7 was separated from unbound reagent by chromatography on Sephadex G-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) previously equilibrated with Tris-buffered saline. $^{125}$I-$\alpha$1(I)-CB7 was stored in portions at −70°C in Tris-buffered saline which contained 1 mg/ml bovine albumin. Approximately 0.01 mol of $^{125}$I-reagent was incorporated per mole of $\alpha$1(I)-CB7.

*Cross-linking reactions.* Factor XIII$_a$-mediated cross-linking was performed in 10 mM Tris-chloride, 140 mM sodium chloride, pH 7.4 (Tris-buffered saline), containing the indicated amount of calcium chloride. Proteins to be added were in Tris-buffered saline, with the exception of types I and III collagen. In some experiments, the collagens were dissolved in 100 mM acetic acid, the solutions were dialyzed against 5 mM acetic acid, and insoluble protein was removed by centrifugation at 110,000 *g* for 90 min. In other experiments, the collagens were dissolved in 100 mM acetic acid and dialyzed at 4°C against 0.14 M potassium phosphate, pH 7.4, and then against 0.4 M sodium chloride, pH 7.4. Visible gel formation did not occur when the various collagen preparations were cross-linked to fibronectin. The cross-linking reactions were stopped by addition of an equal volume of 2% sodium dodecyl sulfate and 2% $\beta$-mercaptoethanol followed by heating for 3 min at 95°C.

*Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.* Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed in cylindrical gels (22) or on discontinuous slab gels (23). Proteins were stained with Coomassie brilliant blue (24) and photographed with Polaroid 665 positive-negative film (Polaroid Corp., Cambridge, Mass.). Bands corresponding to the chains of collagen stained redder than the bands corresponding to fibronectin or marker proteins. The metachromasia was corroborated by spectroscopy of gel slices (25). Slabs containing $^{125}$I-proteins were dried and analyzed by autoradiography with Kodak Royal X-Omat R x-ray film (Eastman Kodak Co., Rochester, N. Y.). Densitometry was performed on a Joyce-Loebl densitometer (Joyce, Loebl and Co., Ltd., Gateshead-on-Tyne, England).

## RESULTS

Incubation of fibronectin, the $\alpha$1(I) chain of type I collagen, and Factor XIII$_a$ at 20°C resulted in the formation of high molecular complexes (Fig. 1). The apparent molecular weights of the complexes, ascertained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis after reduction, were $3.1 \times 10^5$, $\cong 5 \times 10^5$, and $>1 \times 10^6$. The complexes were not formed if the incubation mixture lacked fibronectin, $\alpha$1(I), thrombin (needed to activate Factor XIII), calcium ion, or Factor XIII. The complexes were destroyed by trypsin [which cleaved both $\alpha$1(I) chains and fibronectin] and bacterial

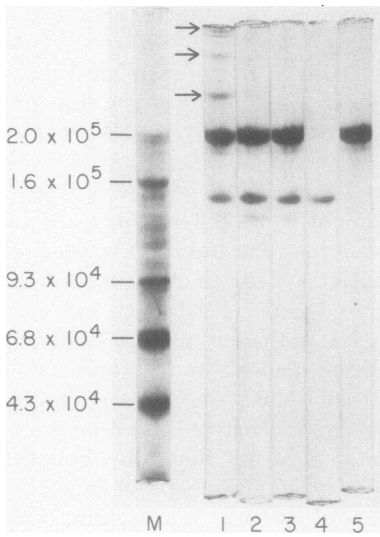


FIGURE 1 Factor XIII$_a$-mediated cross-linking between fibronectin and $\alpha$1(I) collagen chains. The reduced products of five different incubations were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 4% cylindrical gels. Each incubation was for 2 h at 20°C. Incubation 1 contained 490 $\mu$g/ml fibronectin, 270 $\mu$g/ml $\alpha$1(I), 1 U/ml thrombin, 21 $\mu$g/ml Factor XIII, and 4 mM calcium chloride. Incubation 2 lacked thrombin, incubation 3 contained 40 mM EDTA instead of calcium chloride, incubation 4 lacked fibronectin, and incubation 5 lacked $\alpha$1(I) chains. At the end of the incubation, equal volumes of 2% sodium dodecyl sulfate, 2% $\beta$-mercaptoethanol, and 8 M urea were added, the mixture was heated at 95°C for 3 min, and 25 $\mu$l was analyzed by electrophoresis. On gel M were analyzed the following molecular weight markers: fibronectin, $2.0 \times 10^5$; $\alpha$2-macroglobulin, $1.6 \times 10^5$; phosphorylase, $9.3 \times 10^4$; bovine albumin, $6.8 \times 10^4$; and ovalbumin, $4.3 \times 10^4$. The major component of $\alpha$1(I) collagen chains had an apparent molecular weight of $1.3 \times 10^5$. Arrows point to the cross-linked complexes of fibronectin and $\alpha$1(I) chains. In addition, the 3.1, $5 \times 10^5$, and $>10^6$ molecular weight bands were not found if the incubation mixture lacked Factor XIII (not shown).

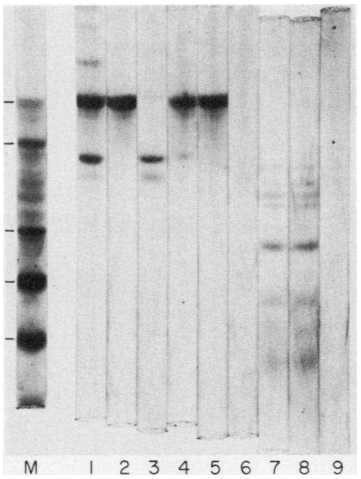


FIGURE 2 Proteolytic digestion of fibronectin, α1(I) chains, and fibronectin-α1(I) chain complex. Cross-linked fibronectin-α1(I) collagen chain complex (formed as described in Fig. 1) (gels 1, 4, and 7), fibronectin alone (gels 2, 5, and 8), and α1(I) chains alone (gels 3, 6, and 9) were analyzed after no further treatment (gels 1–3), digestion with 25 U/ml of collagenase (gels 4–6), or digestion with 10 μg/ml of trypsin (gels 7–9). Protease treatments were performed for 20 min at 37°C in Tris-buffered saline which contained 4 mM calcium chloride. Molecular weight markers (Fig. 1) were analyzed on gel M. Gels 1–6 were cropped just at the top with no loss of bands. Gels 7–9 were from a second electrophoresis and have been aligned to most accurately represent to molecular weights of the cleavage products.

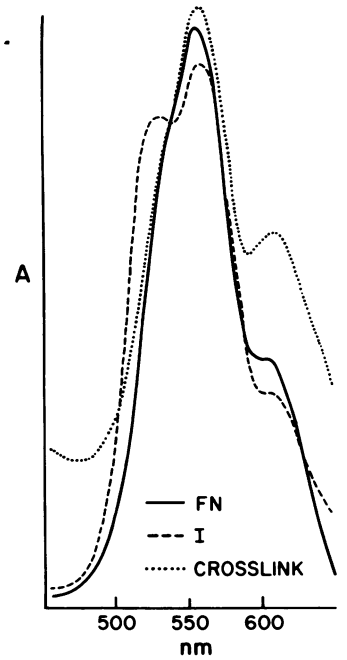


FIGURE 3 Absorbance (A) spectra of fibronectin (FN, solid line), the α1(I) chain of type I collagen (I, broken line), and the 3.1 × $10^5$ molecular weight complex (crosslink, dotted line) after gel electrophoresis and staining. Slices from a duplicate of gel 1, (Fig. 1) stained with Coomassie brilliant blue R-250, were analyzed.

collagenase [which cleaved only α1(I) chains] (Fig. 2). Although the complexes were sensitive to collagenase, the color after staining with Coomassie brilliant blue R-250 was blue and typical of fibronectin rather than collagen (Fig. 3). These results strongly suggest that Factor $XIII_a$ catalyzes the cross-linking of fibronectin to α1(I).

Cross-linking at 37°C could also be demonstrated between fibronectin and types I and III collagen (Figs. 4 and 5). Very high molecular weight complexes formed within 90 min (Fig. 4). At intermediate time points, complexes with apparent molecular weights of 3.1 and 5 × $10^5$ were present. In contrast to cross-linking between fibronectin and α1(I) chains, which occurred at 22° or 37°C, cross-linking between fibronectin and types I or III collagen could only be demonstrated at 37°C (Fig. 5). There were no cross-linking between fibronectin and *Ascaris* collagen (figure not shown).

A preparation of α1(I)-CB7, the cyanogen bromide fragment of α1(I) which is the most effective in compet-

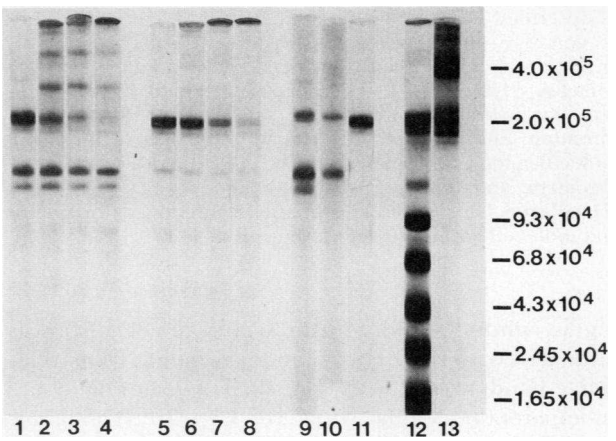


FIGURE 4 Time-course of cross-linking between fibronectin and types I or III collagen. 300 μg/ml fibronectin, 25 μg/ml Factor XIII, 1 U/ml thrombin, and 10 mM calcium ion were incubated at 37°C with either 200 μg/ml type I collagen (gels 1–4), or 200 μg/ml type III collagen (gels 5–8). The collagens were prepared in 5 mM acetic acid as described in Methods. Incubations were sampled after 0 (gels 1 and 5), 5 (gels 2 and 6), 15 (gels 3 and 7), and 90 (gels 4 and 8) min of incubation, and the proteins were analyzed as described for Fig. 1. Gels 9, 10, and 11 contain type I collagen, type III collagen, and fibronectin, respectively; each protein was incubated for 90 min at 37°C with Factor XIII, thrombin, and calcium ion before electrophoresis. The minor bands of the same molecular weight as fibronectin in gels 9 and 10 were metachromatic and represent the β-components of types I and III collagen, respectively. The same amounts of β-components were present before types I and III collagen were incubated with thrombin-activated Factor XIII. Gel 12 contained molecular weight markers: fibronectin, 2.0 × $10^5$; phosphorylase, 9.3 × $10^4$; bovine albumin, 6.8 × $10^4$; ovalbumin, 4.3 × $10^4$; chymotrypsinogen, 2.45 × $10^4$; and hemoglobin, 1.65 × $10^4$. Gel 13 contained nonreduced fibronectin. The bands represent the fibronectin dimer which has a nominal molecular weight of 4.0 × $10^5$ and lesser amounts of fibronectin monomer.

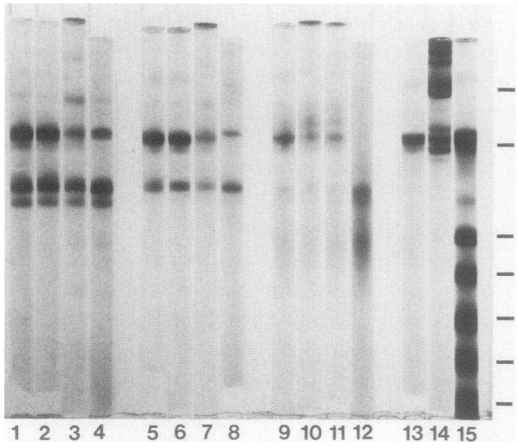


FIGURE 5 Cross-linking of fibronectin to type I, collagen, type III collagen, and α1(I) chains at 0°, 22°, and 37°C. 280 μg/ml fibronectin, 25 μg/ml Factor XIII, 1 U/ml thrombin, and 10 mM calcium ion were incubated for 2 h with 500 μg/ml type I collagen (gels 1–3), 500 μg/ml type III collagen (gels 5–7), or 500 μg/ml α1(I) chains (gels 9–11). The collagens and α1(I) chains were prepared by sequential dialysis in the cold against acetic acid, phosphate, and Tris-buffered saline as described in Methods. Temperatures of incubations were 0° (gels, 1, 5, and 9), 22° (gels, 2, 6, and 10), or 37°C (gels 3, 7, and 11). Gels 4, 8, 12, and 13 contain type I collagen, type III collagen, α1(I) chains, and fibronectin, respectively; each protein was incubated for 120 min at 37°C with Factor XIII, thrombin, and calcium ion. The minor bands of the same molecular weight as fibronectin in gels 4 and 8 were metachromatic and represent the β-components of types I and III collagen. Gels 14 and 15 contained nonreduced fibronectin and molecular weight markers as in Fig. 4.

ing for binding of fibronectin to collagen (11, 12), was iodinated with the Bolton-Hunter reagent. Two radioactive bands were found (Fig. 6). The major band had an apparent molecular weight of $3.3 \times 10^4$. The minor band had an apparent molecular weight of $2.9 \times 10^4$ and did not comigrate with a preparation of α1(I)-CB8, which is also effective in competing for binding of fibronectin to collagen (11, 26). We believe that the minor band is a breakdown product of α1(I)-CB7. Both radioactive bands were diminished when the iodinated preparation was incubated with fibronectin in the presence of Factor $XIII_a$. This was accompanied by the appearance of radioactive bands of higher molecular weight (Fig. 6, lower). The major higher molecular weight radioactive band had an apparent molecular weight of $2.2 \times 10^5$, and the molecular weights of the minor higher molecular weight bands were $>4.0 \times 10^5$. Protein-staining bands (Fig. 6, upper) of molecular weights $4.0 \times 10^5$ and greater also appeared. The cross-linking reaction was complete within 10 min at 37°C (Fig. 6) and within 30 min at 0°C (figure not shown).

The cross-linking of $^{125}$I-α1(I)-CB7 to fibronectin was inhibited if fibrinogen, α1(I) chains, type I collagen, type III collagen, or α1(I)-CB7 was present. Inhibition was quantitated by analysis of densitometry tracings of autoradiograms:

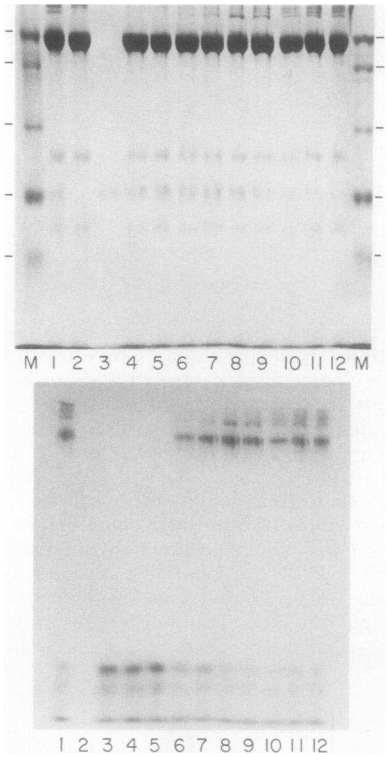


FIGURE 6 Factor $XIII_a$-mediated cross-linking between fibronectin and $^{125}$I-α1(I)-CB7 of α1(I) collagen chains. The reduced products of six different incubations were analyzed by sodium dodecyl sulfate-polyacrylamide electrophoresis through a 3%/8% discontinuous slab gel. The slab was analyzed for stainable protein (above) and by autoradiography (below). The 3% stacking gel was lost during processing. The front is at the bottom of the gel and can be seen by both protein staining (above) and autoradiography (below). Incubations 1–6 were for 2 h at 37°C. Incubation 1 contained 1,500 μg/ml fibronectin, ≅0.6 μg/ml $^{125}$I-α1(I)-CB7 ($2.6 \times 10^5$ cpm/ml), 26 μg/ml Factor XIII, 1 U/ml thrombin, and 5 mM calcium chloride. Incubation 2 lacked $^{125}$I-α1(I)-CB7, incubation 3 lacked fibronectin, incubation 4 lacked Factor XIII, incubation 5 contained 20 mM EDTA instead of calcium chloride, and incubation 6 lacked thrombin. Samples of incubation 1 were taken at 5, 10, 30, 85, 100, and 120 min and were analyzed on slots 7, 8, 9, 10, 11, and 12, respectively. Molecular weight markers (Fig. 1) were run on slots M. The $6.8 \times 10^4$ molecular weight band in slots 1 and 3–12 represent carrier albumin in the $^{125}$I-α1(I)-CB7 preparation. To better establish the size of $^{125}$I-α1(I)-CB7, samples also were analyzed on a 3%/12% polyacrylamide slab gel and chymotrypsinogen ($2.45 \times 10^4$) was used as a molecular weight marker. There were traces of active Factor XIII in the XIII preparation used, because some cross-linking took place in the absence of thrombin (incubation 6).

$$\% \text{ Inhibition} = 100 - 100 \times \frac{\text{peak height of } 2.2 \times 10^5 \text{ molecular weight band in presence of inhibitor/ peak heights of } 3.3 \times 10^4 \text{ and } 2.2 \times 10^5 \text{ molecular weight bands in presence of inhibitor}}{\text{peak height of } 2.2 \times 10^5 \text{ molecular weight band in absence of inhibitor/ peak heights of } 3.3 \times 10^4 \text{ and } 2.2 \times 10^5 \text{ molecular weight bands in absence of inhibitor}}$$

The following values were obtained: fibrinogen, in twofold molar excess of fibronectin, 78%; α1(I) chains, in 1.4-fold molar excess of fibronectin, 87%; type I collagen, in twofold molar excess of fibronectin, 94%; type III collagen, in 0.6 the molar concentration of fibronectin, 86%; and α1(I)-CB7, in the same molar concentration as fibronectin, 55%. In contrast, *Ascaris* collagen did not inhibit $^{125}$I-α1(I)-CB7-fibronectin cross-linking. Because the density of protein staining at the position of fibronectin was less when types I and III collagens were present (Figs. 4 and 5), the percent inhibition for the collagens is not a true estimate.

Lastly, we investigated the effect of adding various amounts of α1(I)-CB7 peptides on the cross-linking of fibronectin to the α-chain of fibrin. α1(I)-CB7, in two- to sixfold excess of fibronectin, inhibited the cross-linking of fibronectin to the α-chain of fibrin at 4°C (Fig. 7). Thus, this collagen peptide can interfere with the fibrin-fibronectin cross-linking just as fibrin interferes with α1(I)-CB7-fibronectin cross-linking.

## DISCUSSION

Fibronectin is one of a limited number of plasma proteins which contain glutaminyl residues susceptible to the action of Factor XIII$_a$ (19, 27, 28). Factor XIII$_a$, in the presence of reagents containing sulfhydryl groups (19), and guinea pig liver transglutaminase (29) catalyze the cross-linking of fibronectin to itself. Factor XIII$_a$ also catalyzes the cross-linking of fibronectin to the α-chain of fibrin (19, 30) and causes the serum concentration of fibronectin to be less than the plasma concentration (27, 31). When denatured collagen is present during clotting, the loss of fibronectin into the fibrin clot is less (9). Conversely, fibrinogen inhibits the binding of fibronectin to denatured collagen (9).

Factor XIII$_a$ also acts upon fibronectin associated with cell surfaces of cultured fibroblasts (32) and readily cross-links labeled cell surface fibronectin to form a very high molecular weight complex (15). Such cross-linking may take place in culture in the absence of exogenous Factor XIII (33). As discussed in the Introduction, the bulk of external fibronectin of cultured fibroblasts is localized, along with collagen, in an extracellular fibrillar matrix.

Our experiments demonstrate that two forms of collagen which are known to bind to fibronectin, the α1(I) chain of type I collagen and cyanogen bromide fragment 7 of the α1(I) chain (11, 12, 26), can be cross-linked to fibronectin by Factor XIII$_a$. A second component of the iodinated α1(I)-CB7 preparation, tentatively identified as a breakdown product of α1(I)-CB7, also was cross-linked to fibronectin. *Ascaris* collagen, which is different from vertebrate collagen (20) and is inactive in cell adhesion assays of fibronectin activity (34), could not be cross-linked to fibronectin and did not block $^{125}$I-α1(I)-CB7-fibronectin cross-linking. Types I and III collagen inhibited Factor XIII$_a$-catalyzed cross-linking between α1(I)-CB7 and fibronectin. Both types of collagen were cross-linked to fibronectin, but only at 37°C.

Fibrin inhibited the cross-linking of α1(I)-CB7 to fibronectin, and α1(I)-CB7 inhibited the cross-linking of fibrin to fibronectin. These results indicate that the

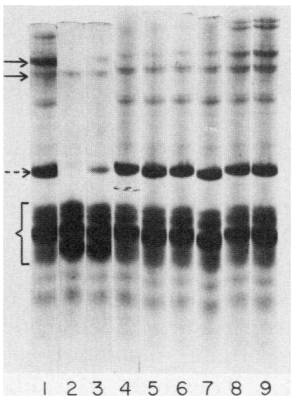


FIGURE 7 Inhibition of Factor XIII$_a$-mediated fibronectin-fibrin cross-linking by α1(I)-CB7. Clotting mixtures contained 1.6 mg/ml fibrinogen, 0.37 mg/ml fibronectin, 10 μg/ml Factor XIII, 1 U/ml thrombin, and 4 mM calcium chloride. After 2 h at 4°C, mixtures were made 20 mM in EDTA and incubated for an additional 30 min at 37°C to free noncovalently bound fibronectin from the clots. The clots were then removed, washed once with Tris-buffered saline that contained EDTA, and analyzed by electrophoresis on 5% cylindrical sodium dodecyl sulfate-polyacrylamide gels. Gels contained the following samples: 1, complete clotting mixture; 2, calcium chloride omitted; 3, Factor XIII omitted; and 4–9, complete mixtures plus 10.6 μM, 8.5 μM, 6.4 μM, 4.3 μM, 2.1 μM, and 1.0 μM α1(I)-CB7, respectively. The concentration of the $2.0 \times 10^5$ molecular weight fibronectin subunit was 1.85 μM. The upper arrow points to the $2.6 \times 10^5$ molecular weight complex between fibronectin and the α-chain of fibrin. The lower arrow points to the noncross-linked fibronectin subunit. The broken arrow points to the γ-chain dimer of fibrin. The brackets show the position of the α-, β-, and γ-chains of fibrin. The fibronectin used in this experiment was not pretreated with 3.3 M urea and therefore had the traces of Factor XIII activity (gel 3).

determinants in fibronectin for fibronectin-fibrin and fibronectin-collagen binding and cross-linking are similar. A major determinant of fibronectin binding to α1(I)-CB7 is located at the site of digestion of α1(I) chains by vertebrate collagenase and has the sequence Gly-Ile-Ala-Gly-Gln-Arg (12). α1(I)-CB8 (35) and the penultimate cyanogen bromide fragment of the α-chain of fibrin (36, 37) also contain a Gly-Ile-Ala sequence. Further experiments are required to investigate whether the Gly-Ile-Ala sequence is important for binding to fibronectin and which proteins in the pairs fibronectin-fibrin and fibronectin-collagen contribute glutaminyl and lysyl residues to the ε-(γ-glutamyl)-lysine cross-link. It should be noted that we were unable to demonstrate Factor $XIII_a$-mediated cross-linking of collagen to itself as shown in Fig. 5, although such cross-linking has been reported (38). To date, we also have been unable to demonstrate Factor $XIII_a$-catalyzed cross-linking between fibrin and collagen (unpublished results), supporting one report (39) but contradicting a second report (40) in the literature. We are continuing our attempts to demonstrate that Factor $XIII_a$ can catalyze collagen-collagen and collagen-fibrin cross-linking and do not want to make a definitive statement here about whether such cross-linking can occur.

Binding experiments have demonstrated better complex formation between fibronectin and nontriple-helical collagen than between fibronectin and native collagen (9, 10, 16, 17). In as much as the binding experiments were performed at 22°C or lower, our finding that fibronectin cross-links to types I and III at 37°C is not inconsistent with these published results. Collagens are partially denatured at physiological temperature (41). Therefore, it is likely that there is local denaturation of types I and III collagens at 37°C, especially at the collagenase-sensitive site within α1(I)-CB7, which lacks proline and hydroxyproline over a span of 12 residues (12).

Complex formation and cross-linking between fibronectin and collagen may be of considerable physiological significance. Ultrastructural localization of fibronectin and collagen at various times after subculture of human skin fibroblasts indicates that the early matrix of 2- to 10-d-old cells consists of polymerized fibronectin to which nonhelical collagen is bound and that the matrix of 4- to 8-wk-old cells consists of periodic collagen fibrils to which fibronectin is bound.[1,2] Thrombin and other growth factors are known to induce the synthesis of fibronectin (42) and collagen (43, 44). Fibronectin has been noted around new fibroblasts of skin scar tissue (45). Failure of Factor $XIII_a$ to cross-link fibronectin to collagen during wound healing and tissue remodeling may explain the observations that patients deficient in Factor XIII often suffer from poor wound healing and have difficulty carrying pregnancies to term (46).

[2] Furcht, L. T., D. F. Mosher, G. Wendelschafer-Crabb, and J-M. Foidart. Manuscript in preparation.

## ACKNOWLEDGMENTS

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