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Limited cleavage of cellular fibronectin by plasminogen activator purified from transformed cells

(proteases/extracellular matrix/cell migration)

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ABSTRACT **The substrate specificity and direct catalytic activity of plasminogen activator (PA) was examined under conditions where its natural substrate, plasminogen, was missing or inhibited. PA, purified from cultures of transformed chicken fibroblasts, was incubated with purified preparations of potential substrates. The adhesive glycoprotein fibronectin, isolated from normal chicken fibroblast extracellular matrix, underwent limited but specific cleavage by PA in the absence of plasminogen. Analysis of the cleavage products by polyacrylamide gels under both reducing and nonreducing conditions indicated that PA-mediated cleavage occurred near the carboxyl terminus of fibronectin but on the amino-terminal side of the interchain disulfide bridge, thus disrupting the native dimeric fibronectin molecule. Under the identical conditions, chicken ovalbumin was not cleaved while the established substrate, chicken plasminogen, was extensively converted to plasmin. A monoclonal antibody, directed against avian PA and shown to inhibit plasminogen-free, cell-mediated matrix degradation, specifically inhibited the fibronectin cleavage. A human PA, urokinase, also cleaved fibronectin under plasminogen-free conditions yielding a limited number of high molecular weight cleavage products.**

Eukaryotic cell–substratum interactions dictate a wide variety of tissue and cell behaviors including cell migration, cell invasion, embryonic tissue formation, and cell responsiveness to growth regulatory molecules (1–4). The encounter of eukaryotic cells with their underlying substratum involves complex macromolecular interactions between cell surface recognition molecules and a variety of substratum structural components including collagen (5–7) and proteoglycans (8–11) as well as the adhesive glycoproteins fibronectin (12–15) and laminin (16, 17). Although the specific adhesion of cells to substratum is important in many of the above processes, the degradation of the substratum through cell surface proteolytic events also can dictate the ultimate fate of cells and can influence their pathology. The combination of specific cell–substratum adhesion followed by selective and localized substratum degradation might be involved in the basic mechanisms by which cells attach and then migrate across and through basement membranes and extracellular matrix (18).

Selective degradation of the individual proteins of the extracellular matrix and basement membrane has been shown *in vitro* with a wide variety of proteolytic enzymes including collagenase, elastase, trypsin, chymotrypsin, cathepsin, thrombin, and plasmin (19–27). However, the molecular relationship between the degradation of substratum proteins *in vivo* and specific cellular proteolytic enzymes remains, for the most part, undefined. In cell culture the local generation of plasmin through the activity of plasminogen activator (PA), produced by a variety of cells, has been shown to contribute to the degradation of substratum glycoproteins in a number of cellular migration and invasion systems (20, 28, 29). In the absence of plasminogen, PA itself is thought not to be directly involved in substratum degradation since it is a highly specific protease assumed to have a single natural substrate, plasminogen. Moreover, it has been reported (30, 31) that PA in the absence of plasminogen cannot degrade purified fibronectin or matrix fibronectin. Studies in our laboratory, however, indicated that a fibronectin-enriched extracellular matrix was degraded specifically by transformed cells in the absence of plasminogen and that the pattern of inhibition using a variety of specific protease inhibitors indicated that cellular PA was directly involved in this degradation (32). A monoclonal antibody selected for its ability to inhibit the catalytic activity of PA was shown to inhibit plasminogen-free, cell-mediated matrix degradation, further suggesting a direct catalytic role for PA (33). To investigate these apparent contradictory results, plasminogen activator was purified from transformed cells, fibronectin was purified from the extracellular matrix of corresponding normal cells, and direct degradation assays were performed. This report presents evidence that fibronectin undergoes limited but distinct cleavage by purified PA and that the cleavage appears to occur near the carboxyl terminal region in fibronectin, disrupting the native dimeric structure of the fibronectin molecule.

MATERIALS AND METHODS

Cell Cultures. Primary chicken embryo fibroblasts (CEF) were prepared from 10- to 12-day embryos (34). CEF secondary cultures were infected and transformed with Rous sarcoma virus as described (35). All cultures were grown in Eagle's minimal essential medium (MEM) containing high glucose, penicillin, and streptomycin and supplemented with 10% (vol/vol) heat-inactivated fetal calf serum or plasminogen-free fetal calf serum prepared as described (35).

Preparation of Cellular Fibronectin. Cellular fibronectin was prepared from CEF cultures exactly as described by Yamada (36). The CEF cultures were labeled with [^{35}S]methionine (20 μCi/ml; 1 Ci = 37 GBq) in MEM containing 10% of the normal concentration of methionine for 2 days prior to the preparation of fibronectin. The final preparations of fibronectin (8000–18,000 cpm/μg, 0.6–1.2 mg/ml) were dialyzed versus 0.05 M 3-(cyclohexylamino)-1-propanesulfonic acid (Sigma) (pH 11), stored frozen at −70°C in small aliquots, and thawed separately for each individual experiment.

Preparation of Plasminogen Activator. Avian plasminogen activator was purified from cultures of Rous sarcoma virus-

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Abbreviations: PA, plasminogen activator; CEF, chicken embryo fibroblasts.

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infected CEF by a combination of benzamidine-Sepharose chromatography, Sephadex G-100 chromatography, and immunoaffinity chromatography as described (33). The preparation was judged to be a homogeneous protein of 45–46 kDa by $NaDodSO_4$/PAGE. A preparation of the human PA urokinase was obtained from Sterling-Winthrop Laboratories (Rensselaer, NY) and further purified by benzamidine-Sepharose affinity chromatography. The final preparation was >95% high molecular weight urokinase and yielded a single protein band of 50–52 kDa on $NaDodSO_4$/PAGE. A small amount (0–5%) of low molecular weight urokinase (30–33 kDa) was detected in some preparations.

Fibronectin Cleavage Reaction. Samples of fibronectin (2–15 μg) were incubated in Eppendorf plastic centrifuge tubes in a total volume of 0.1 ml containing 0.02 M Tris·HCl (pH 8.1), the different enzymes, antibody, and inhibitor preparation to be tested. When indicated the plasmin inhibitor Trasylol (aprotinin) was added at a final concentration of 200 units/ml. Incubation was carried out at 37°C in an incubator for 2–18 hr with the cap closed on the Eppendorf centrifuge tube. At the end of the incubation period the reaction was terminated by the addition of 25 μl of 5× gel electrophoresis sample buffer (37) with or without 5% (vol/vol) 2-mercaptoethanol. The samples were boiled for 3 min and loaded onto $NaDodSO_4$/polyacrylamide gels.

Gel Electrophoresis. $NaDodSO_4$/PAGE was carried out with slab gels using the system of Laemmli (37). Electrophoresis was conducted at 30 mA for 3–4 hr. Following electrophoresis the gels were stained with either Coomassie blue or silver (38) and prepared for autoradiography by treatment with Amplify according to the manufacturer's directions (Amersham). A series of protein standards were run in parallel lanes; the standards were human plasma fibronectin dimer (440 kDa), fibronectin monomer (220 kDa), myosin (205 kDa), gamma-globulin (150 kDa), β-galactosidase (117 kDa), plasminogen (95 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), and chymotrypsinogen (25 kDa).

Materials. Antibody to avian PA was obtained from a mouse hybridoma culture selected for its ability to produce an IgG with anticatalytic activity (33). Purified IgG was isolated from the hybridoma culture supernatant by Protein A-Sepharose chromatography according to the manufacturer's directions (Bio-Rad). Control IgG was obtained from a mouse hybridoma culture producing antibody to human urokinase. Chicken plasminogen was purified from chicken plasma by two passages over lysine-Sepharose as described (39). Plasmin was prepared by incubating chicken plasminogen (1 μg) with 0.01 μg of purified avian PA for 20 hr in the presence of 20% (vol/vol) glycerol. Highly purified human thrombin was a gift of John Fenton (Division of Laboratories and Research, Dept. of Health, Albany, NY). Trasylol (aprotinin) was obtained from Sigma as a solution containing 29,000 Kallikrein units/ml. Purified ovalbumin was obtained from Sigma.

RESULTS

Cellular fibronectin isolated from [^{35}S]methionine-labeled CEF cultures undergoes limited cleavage when incubated with purified PA isolated from cultures of transformed Rous sarcoma virus-infected CEF (Fig. 1). Cellular fibronectin, as isolated and analyzed under nonreducing conditions, is composed mainly of a 440-kDa dimer with lesser but variable amounts of both high molecular weight aggregate forms and lower molecular weight forms (lane 1). Upon incubation with purified PA (Fig. 1 *Inset*), there was a shift from the 440-kDa protein to 200- to 220-kDa lower molecular weight forms (lane 2). If the polyacrylamide gel analysis was carried out under standard reducing conditions, the alteration in fibronectin was not apparent since the untreated, reduced fibronectin monomer (220 kDa) was barely distinguishable from the PA-generated 200- to 220-kDa polypeptides (lanes 3 and 4).

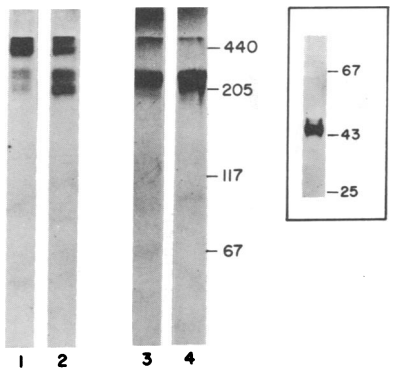

FIG. 1. Cleavage of chicken cellular fibronectin by purified avian PA analyzed under nonreducing and reducing conditions. Samples of [^{35}S]methionine-labeled cellular fibronectin (6 μg, 53,000 cpm) were incubated for 18 hr at 37°C in 0.02 M Tris·HCl (pH 8.1) in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 0.2 μg of purified PA. At the end of the incubation period, the samples were prepared for $NaDodSO_4$/PAGE in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of 5% (vol/vol) 2-mercaptoethanol and analyzed in 6% polyacrylamide gels. An autoradiograph of the gel is shown with standard protein markers in parallel, and their apparent molecular sizes are indicated on the right in kDa. (*Inset*) A sample of the purified chicken PA used in the incubation was analyzed by 10% $NaDodSO_4$/PAGE under nonreducing conditions and stained with silver reagent.

The extent of cleavage of fibronectin by PA was time dependent occurring linearly over a 2- to 18-hr incubation period (Fig. 2). The 440-kDa fibronectin band was progressively lost upon incubation with a concomitant increase in 200- to 220-kDa bands. The appearance of a minor 100-kDa band was also detected during the incubation but this apparent cleavage product represented only 2–4% of the total protein. The appearance of the 200- to 220-kDa and 100-kDa bands was reduced by 80–95% when the incubation was carried out in the presence of 10 μg of antibody to PA.

The cleavage of fibronectin was also dependent on the concentration of PA (Fig. 3*A*). Cleavage was detectable as loss of the 440-kDa band and concomitant increase of 200- to 220-kDa bands at an enzyme/substrate ratio of 1:40 (wt/wt) (lane 2). At higher enzyme/substrate ratios, 1:20 and 1:10 (wt/wt), the 100-kDa cleavage product was clearly observed (lanes 3 and 4). Densitometer tracings of lanes 1–4 indicated that the broad 440-kDa fibronectin band represented 92%, 55%, 40%, and 29%, respectively, of the total protein in each lane (data not shown). That the appearance of the fibronectin cleavage products was due to PA activity was shown by inhibition of cleavage with the monoclonal antibody specific for avian PA and selected (33) for its anticatalytic activity (Fig. 3*A*, lane 5). As little as 2 μg of monoclonal anti-PA IgG significantly reduced the breakdown of 440-kDa fibronectin and prevented the generation of the 100-kDa fragment. Addition of irrelevant monoclonal IgG had no effect on the PA-mediated cleavage of fibronectin (lane 6).

To rule out the possibility that the cleavage was due to plasmin, generated by the action of PA on potential traces of plasminogen that might have contaminated the fibronectin preparation, we analyzed the effect of Trasylol, a polypeptide that is a potent inhibitor of plasmin but does not inhibit PA (40). Fig. 3*B* shows that Trasylol had little effect on the PA-initiated generation of the 200- to 220-kDa and 100-kDa bands (lanes 2 and 3). As a positive control, we added a trace amount of plasmin to the fibronectin incubation mixture (lane 4). This indeed generated a breakdown of fibronectin, but the cleavage products differed from those in the absence of plasmin and, furthermore, the plasmin-mediated cleavage was completely inhibited by Trasylol (lane 5). Thus if

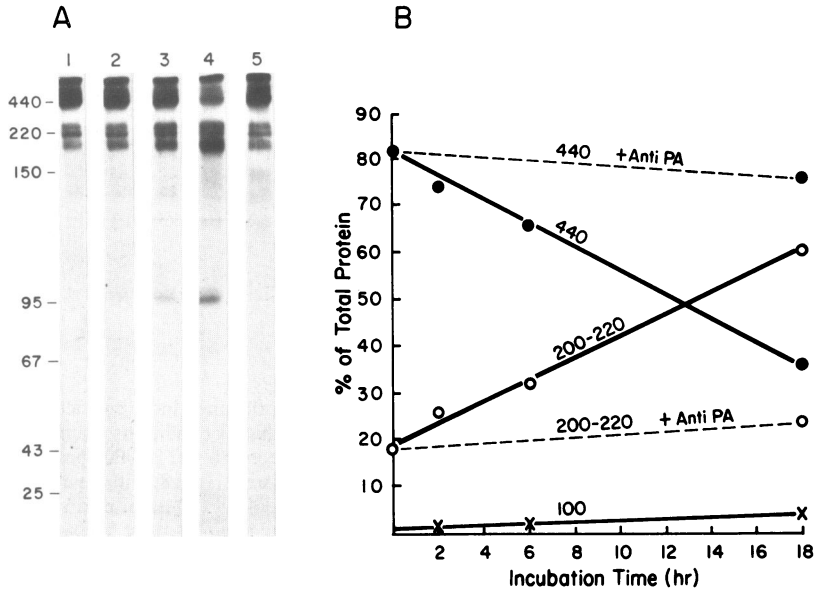

FIG. 2. Time course of PA-mediated fibronectin cleavage. (*A*) PAGE. Samples of [^{35}S]methionine-labeled cellular fibronectin (15 μg, 120,000 cpm) were incubated for the indicated time periods with 0.8 μg of purified PA at 37°C in 0.02 M Tris·HCl (pH 8.1) and analyzed by 6% NaDodSO$_4$/PAGE under nonreducing conditions. Lanes: 1, fibronectin alone (no incubation); 2, fibronectin and PA (2-hr incubation); 3, fibronectin and PA (6-hr incubation); 4, fibronectin and PA (18-hr incubation); 5, fibronectin, PA, and 10 μg of anti-PA IgG (18-hr incubation). Molecular sizes are in kDa. (*B*) Densitometric analysis of the polyacrylamide gels in *A*. Densitometer tracings of lanes 1–5 in *A* were analyzed by integrating the areas under the broad 440-kDa fibronectin band, the protein bands in the 200- to 220-kDa area, and the 100-kDa band. The total area from all three regions was assigned a value of 100%. The area under each protein region was calculated as a percent of the total protein for each time period.

plasminogen or plasmin were present, the Trasylol present in the conditions described for lane 3 would have completely inhibited its activity. As a further test to rule out the involvement of contaminating plasminogen, CEF cultures were grown and maintained in medium containing plasminogen-free fetal calf serum for 5 days prior to the preparation of cellular fibronectin. The resulting fibronectin preparation was cleaved by PA in an identical manner to that shown in Fig. 3 (data not shown).

Although fibronectin was cleaved by PA, it is not cleaved as efficiently as plasminogen. A comparison of PA-mediated fibronectin cleavage with PA-mediated plasminogen cleavage under the same conditions is shown in Fig. 4. The loss of 440-kDa chicken fibronectin is not as extensive as the loss of the 95-kDa chicken plasminogen under conditions where PA is limiting (0.02–0.10 μg). Chicken ovalbumin is not cleaved by PA under identical conditions even at relatively high levels of PA (0.5 μg), indicating that specificity does exist in regard to PA substrates. Gamma-globulin also was not cleaved following incubation with 1.0 μg of PA (data not shown). Graphic illustration of the cleavage reactions (Fig. 4*B*) indicates that at low levels of PA (0.02 μg), fibronectin cleavage is barely detectable while plasminogen is extensively cleaved (>50%) and converted to the 25-kDa and 70-kDa two-chain plasmin molecule. Significant cleavage of fibronectin (10–35%) does not occur until 0.1–0.5 μg of PA is used.

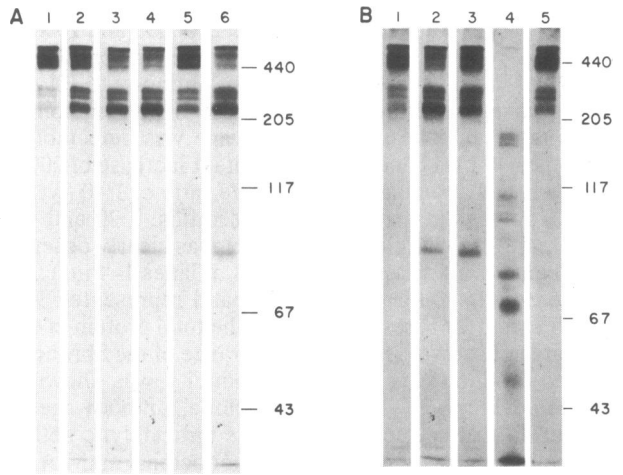

FIG. 3. Analysis of PA-mediated fibronectin cleavage. (*A*) PA dose dependence and inhibition of cleavage by specific antibody. Samples of [^{35}S]methionine-labeled cellular fibronectin (8 μg, 66,000 cpm) were incubated for 18 hr at 37°C in 0.02 M Tris·HCl (pH 8.1) and analyzed by 6% NaDodSO$_4$/PAGE under nonreducing conditions. Lanes: 1, fibronectin alone; 2, fibronectin and 0.2 μg of PA; 3, fibronectin and 0.4 μg of PA; 4, fibronectin and 0.8 μg of PA; 5, fibronectin, 0.8 μg of PA, and 2 μg of anti-avian PA monoclonal IgG; 6, fibronectin, 0.8 μg of PA, and 10 μg of control IgG (anti-human urokinase monoclonal IgG). The autoradiograph was exposed for 1 day. (*B*) Lack of effect on cleavage by the plasmin inhibitor Trasylol. Incubation conditions and gel analysis was the same as in *A*. Lanes: 1, fibronectin alone; 2, fibronectin and 0.8 μg of PA; 3, fibronectin, 0.8 μg of PA, and 20 units of Trasylol; 4, fibronectin and 0.1 μg of chicken plasmin; 5, fibronectin, 0.1 μg of plasmin, and 20 units of Trasylol. The autoradiograph was exposed for 2 days and, therefore, the polypeptide bands are more intense than in *A*. Molecular sizes are in kDa.

A limited cleavage of cellular fibronectin also can be observed when urokinase is incubated with ^{35}S-labeled chicken fibronectin (Fig. 5). Urokinase, purified by affinity chromatography on benzamidine-Sepharose (Fig. 5 *Inset*), caused a dose-dependent cleavage of fibronectin in the presence of the plasmin inhibitor Trasylol. As little as 0.15 μg of urokinase [enzyme/substrate ratio, 1:100 (wt/wt)] generated a 220-kDa polypeptide product and diminished the 440-kDa fibronectin band (lane 2). Incubation with 0.5 μg of urokinase led to nearly complete loss of the 440-kDa fibronectin band and the appearance of a major 200-kDa fragment along with the 220-kDa fragment (lane 3). Incubation with 1.5 μg of urokinase caused little additional change in the polypeptide pattern over that with 0.5 μg of urokinase, indicating that proteolytic cleavage was limited (lane 4). Thrombin, like urokinase, also is an arginine-specific serine protease, but its cleavage of fibronectin was not limited. When 0.5 μg of thrombin was incubated with fibronectin under identical conditions to that of urokinase, more extensive cleavage of fibronectin occurred (lane 5), and peptides of 210 kDa, 140 kDa, 70 kDa, 45 kDa, and 25–27 kDa became the predominant cleavage products. Other serine proteases, including trypsin, elastase, plasmin, and chymotrypsin, also caused extensive cleavage of fibronectin (data not shown). Of the proteases examined only avian PA and urokinase appear to be limited in their extent and rate of fibronectin cleavage. The 100-kDa minor fragment observed when chicken cellular fibronectin was incubated with chicken PA (Figs. 2–4) was not observed with urokinase-mediated cleavage of cellular fibronectin (Fig. 5) or with cleavage of cellular fibronectin by plasmin (Fig. 3*B*), thrombin (Fig. 5), or other serine proteases.

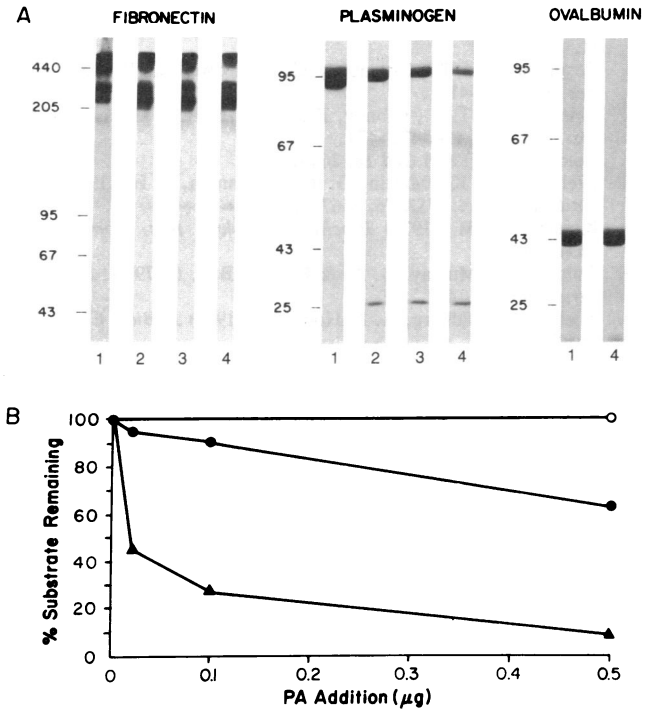

FIG. 4. Cleavage of chicken proteins by purified avian PA. (*A*) Polyacrylamide gel analysis. Fifteen-microgram samples of chicken fibronectin, plasminogen, and ovalbumin were incubated in 0.02 M Tris·HCl (pH 8.1) for 18 hr at 37°C in the absence (lanes 1) or presence of 0.02 μg (lanes 2), 0.10 μg (lanes 3), and 0.50 μg (lanes 4) of purified chicken PA as indicated. Trasylol (20 units) was included in each incubation mixture. At the end of the incubation period the samples were electrophoresed on 6–15% $NaDodSO_4$/polyacrylamide gradient gels and stained with Coomassie blue. The fibronectin samples were electrophoresed under nonreducing conditions, and the plasminogen and ovalbumin samples were under reducing conditions [5% (vol/vol) 2-mercaptoethanol]. Molecular sizes are in kDa. (*B*) Densitometric analysis of the polyacrylamide gels. Densitometer tracings of the gels in *A* were analyzed by integration of the area under each protein peak. The areas under the 440-kDa fibronectin peak (●), the 95-kDa plasminogen peak (▲), and the 43-kDa ovalbumin peak (○) in the absence of added enzyme were assigned a value of 100%, representing the uncleaved substrates. The areas under the respective substrate peaks that remained following progressive enzymatic digestion were calculated and compared to the 100% value.

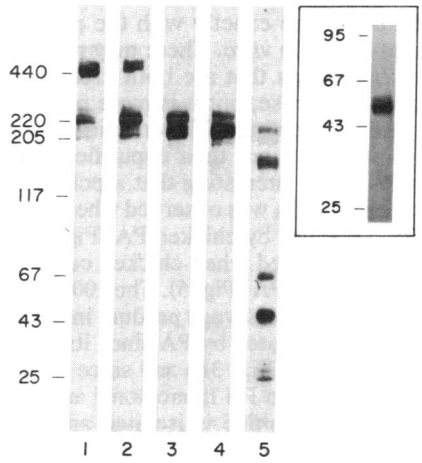

FIG. 5. Cleavage of cellular fibronectin by human PA (urokinase) and compared to thrombin-mediated cleavage. Samples of [^{35}S]methionine-labeled chicken cellular fibronectin (15 μg, 280,000 cpm) were incubated for 18 hr at 37°C in 0.02 M Tris·HCl (pH 8.1) containing 20 units Trasylol in the absence or presence of purified urokinase or thrombin. Samples were electrophoresed on a 4–15% $NaDodSO_4$/polyacrylamide gradient gel under nonreducing conditions and analyzed by autoradiography. Standard proteins were in parallel lanes, and their apparent molecular sizes are on the left in kDa. Lanes: 1, fibronectin alone; 2, fibronectin and 0.15 μg of urokinase; 3, fibronectin and 0.50 μg urokinase; 4, fibronectin and 1.5 μg of urokinase; 5, fibronectin and 0.50 μg of thrombin. (*Inset*) A sample of the purified urokinase preparations used in the incubation was analyzed by 10% $NaDodSO_4$/PAGE under nonreducing conditions and stained with Coomassie blue.

DISCUSSION

Purified plasminogen activator can bring about a limited cleavage of cellular fibronectin in a dose-dependent manner in the absence of plasminogen. That the cleavage is due to the direct catalytic activity of PA was illustrated by the use of highly purified PA in the incubation mixture and by the insensitivity of the cleavage to Trasylol, a potent inhibitor of plasmin. Plasmin cleavage of fibronectin generated a cleavage pattern distinct from that of PA and moreover was completely inhibited by Trasylol, ruling out a possible contamination of plasminogen or plasmin in the enzyme or substrate preparations. Further evidence that fibronectin cleavage was mediated by PA directly is shown by the inhibition of the cleavage reaction with a monoclonal antibody to PA that was selected specifically for its anticatalytic activity against avian PA (33).

Although the focus of this study was chicken PA and chicken cellular fibronectin, the mammalian PA human urokinase was also capable of cleaving cellular fibronectin yielding a similar digestion pattern to that of avian PA (Fig. 4). Chicken ovalbumin and human gamma-globulin were not cleaved by the respective avian and human PAs under identical conditions, indicating that the PAs are selective in terms of their macromolecular substrate specificity. It has been reported (31) that human urokinase is able to directly degrade a 66-kDa protein in the extracellular matrix isolated from human fibroblasts but cleavage of matrix fibronectin was not observed.

The cleavage of fibronectin by PA is clearly limited in that the major products generated from the 440-kDa native fibronectin are large polypeptides of apparent molecular weight 190–220 kDa. Under reducing conditions, these polypeptide products retained their apparent molecular weights and were not distinctly separated by PAGE from reduced fibronectin monomers that have an apparent size of 220 kDa (Fig. 1). Since Furie and Rifkin (25) have shown that the interchain disulfide bridge(s) in the fibronectin dimer is very close to the carboxyl terminus, these results indicate that the PA-mediated cleavage occurs near the carboxyl-terminal region of fibronectin and on the amino-terminal side of the disulfide bridge, yielding large polypeptides (190–220 kDa) not linked by any disulfide bridges. This limited cleavage by PA clearly can be detected only when the PAGE analysis is carried out under nonreducing conditions, possibly explaining why previous workers concluded that fibronectin was not a substrate for purified PA (30, 31).

Although the fibronectin cleavage products generated by PA ranged in apparent molecular mass from 190 kDa to 220 kDa, in dose- and time-dependent incubations the 220-kDa product often appeared initially followed subsequently by the 190- to 220-kDa products (Fig. 5). This might be simply a reflection of sequence heterogeneity in the native fibronectin dimers (41) or could also represent further degradation of fibronectin at either the amino or carboxyl termini of the initially generated 220-kDa product. Fibronectin does possess a highly labile proteolytic site 25–30 kDa from the amino terminus (21, 24, 25, 42); hydrolysis at this site would cause reduction in size from 220 kDa to 190–200 kDa. However, such a fragment has not been detected under the conditions of our analysis.

It is interesting that the cellular fibronectin preparations always contained limited amounts of 190- to 220-kDa poly-

peptides that comigrate exactly with the polypeptide products generated by PA *in vitro*. These may represent contaminating cellular proteins that are both urea extractable and bind to gelatin-Sepharose, but it is more probable that they represent a limited cleavage of fibronectin occurring prior to or during isolation and are then copurified with the intact fibronectin. It is also interesting that a polypeptide with an apparent size of 100 kDa was observed when chicken cellular fibronectin was cleaved by chicken PA (Figs. 2–4). No such polypeptide was detected when chicken cellular fibronectin was cleaved by human PA (Fig. 5). The 100-kDa polypeptide appeared to be a minor cleavage product in the avian system but it was clearly generated by PA since its appearance was insensitive to Trasylol (Fig. 3*B*) and since it was not generated when the anti-avian PA monoclonal antibody was used (Fig. 3*A*). Until the peptide is isolated and sequenced, its position in the fibronectin molecule and the exact proteolytic mechanisms that give rise to its appearance remain unknown.

Although fibronectin is indeed cleaved by PA, in solution it is not the best substrate for PA. The serum zymogen plasminogen is cleaved more efficiently by PA than is fibronectin. A quantitative comparison illustrates that plasminogen cleavage in solution occurs at an enzyme/substrate ratio of <1:100 (wt/wt) (Fig. 4) while under the identical conditions, fibronectin cleavage by PA is just barely detectable. The limited cleavage of fibronectin by PA occurs extensively only at enzyme/substrate ratios of 1:40 to 1:10 (wt/wt), which in terms of enzyme concentration in solution may not be a usual physiological condition. However, such cleavage of fibronectin could take place at close cell–substratum contact points where the local concentration of secreted or membrane-bound PA (35) may be relatively high. In those specific cell types where PA expression can be substantially induced by hormones, growth factors, or oncogenic transformation (43), high PA levels may be attained locally that cause the limited cleavage of matrix fibronectin. If such cleavage disrupted the cell–fibronectin attachment points, which are also located in the carboxyl-terminal region of the fibronectin molecule (44–46), it would allow the cell to detach from the substratum and undergo lateral migration. Reattachment of the migrating cell to native, matrix fibronectin in the immediate environment followed again by PA-mediated cleavage of fibronectin would thus bring about a cycle of specific adhesion and local catalytic detachment resulting in a net movement of cells across or through extracellular matrix. A monoclonal antibody directed against the catalytic activity of avian PA indeed has been shown to inhibit cell detachment and migration of Rous sarcoma virus-transformed avian cells on a fibronectin-enriched matrix in the complete absence of plasminogen (33). The latter results and the results of the present study indicate that PA, bound to cell membranes or secreted locally at cell–substratum contact points, has the catalytic ability to cleave matrix fibronectin near the carboxyl terminus of the fibronectin molecule. Such cleavage within the dynamic milieu that encompasses the cell–substratum attachment points could have pronounced effects on cell migratory behavior.

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