Essential charged amino acids in the binding of fibronectin to gelatin

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The binding of fibronectin to gelatin–agarose was strictly dependent on pH, having a pH optimum of 7–9. The binding was strongly inhibited by increasing ionic strength. A chemical modification of lysyl and arginyl groups of fibronectin abolished the binding activity. The anionic detergents sodium dodecyl sulphate and sodium deoxycholate in concentrations of 10–100 mM had the same effect. The binding was not affected by the non-ionic detergents Triton X-100, Tween 20 or Lubrol WX. The results demonstrate an important role of ionic interactions in the binding of fibronectin to gelatin. Absence of inhibition by non-ionic detergents suggests that hydrophobic interactions contribute relatively little to the binding of fibronectin to gelatin.

Fibronectin is a high-molecular-weight glycoprotein present in connective tissue and in blood plasma. Fibronectin has affinity for collagen *in vitro* and co-distributes with collagen in the pericellular matrix of fibroblasts. Fibronectin apparently has a function in mediating binding of cells to extracellular collagenous matrix and also in mediating the uptake of denatured collagen by the reticuloendothelial system (for references, see Vaheri & Mosher, 1978; Mosesson & Amrani, 1980; Saba & Jaffe, 1980). The fibronectin–collagen interaction has recently been intensively studied. Collagen-binding peptides have been isolated from proteolytic digests of fibronectin (Ruoslahti *et al.*, 1979; Hahn & Yamada, 1979; Sekiguchi & Hakomori, 1980) and the collagen-binding area has been localized close to the *N*-termini of the polypeptide chains of fibronectin (Balian *et al.*, 1980). Denatured unfolded collagen has generally been found more active in binding fibronectin than native collagen (Engvall & Ruoslahti, 1977; Jilek & Hörmann, 1978). Collagens of different genetic types vary in their affinity for fibronectin (Jilek & Hörmann, 1978; Engvall *et al.*, 1978).

Despite these interesting results, the molecular mechanism of the fibronectin–collagen interaction is not understood. By using the interaction of fibronectin with immobilized gelatin as a model system, it has been shown previously that cationic compounds such as amines and arginine dissociate fibronectin–gelatin complexes (Vuento & Vaheri, 1978). These findings prompted us to study further the role of ionic interactions in the binding of fibronectin to gelatin. In the present paper we show that the

binding is very sensitive to pH, to ionic strength and to chemical modification of charged amino acid side chains of fibronectin. This indicates that ionic interactions are essential for the binding.

Materials and methods

Purification of fibronectin

Fibronectin was purified from human plasma as previously described (Vuento & Vaheri, 1979). The purity of the preparations was assessed by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (Weber & Osborn, 1969). Concentrations of solutions of purified fibronectin were determined by photometry, an $A_{280,1\text{ cm}}^{1\%}$ of 12.8 (Mosesson *et al.*, 1975) being assumed.

Radiolabelling of fibronectin

Fibronectin was labelled with ^{125}I by the chloramine-T method (Greenwood *et al.*, 1963). The labelled protein (10 μg) was isolated from the reaction mixture by affinity chromatography on gelatin–agarose (Ruoslahti *et al.*, 1978). For this purpose, gelatin–agarose (see below) was diluted fivefold with plain agarose gel, and columns (1 ml) were constructed in serological pipettes. The columns were equilibrated with 10 mM-Tris/HCl (pH 7.5)/0.9% NaCl/0.02% NaN_3 /bovine serum albumin (5 mg/ml). After the iodination reaction had been performed, the reaction mixture was diluted to 3 ml with the above buffer and applied on a column. The column was washed with 15 ml of the above buffer, which removed the iodine that had not reacted and other radioactive contaminants. The

labelled protein was eluted with 1 M-arginine hydrochloride (Vuento & Vaheri, 1978) in the above buffer. Arginine was then removed by an extensive dialysis against 10 mM-Tris/HCl (pH 7.5)/0.9% NaCl/0.02% NaN_3 . When rechromatographed on columns of gelatin-agarose, the labelled protein bound completely to the affinity gel.

The solubility of the labelled protein at various pH values was studied by centrifugation in a Spinco Model L preparative ultracentrifuge at 22°C. A rotor speed corresponding to 30000 g was used. After centrifugation for 30 min, the radioactivity present in the supernatant was analysed.

Preparation of gelatin-agarose

Gelatin (Type I; Sigma, St. Louis, MO, U.S.A.) was coupled to CNBr-activated agarose gel (Sephacrose 4B; Pharmacia, Uppsala, Sweden) by using procedures described previously (Vuento & Vaheri, 1979).

Binding of labelled fibronectin to gelatin-agarose

Suspensions (10%, v/v) of gelatin-agarose were prepared in buffers having a varying pH and ionic composition. Portions (250 μl) of the suspension were transferred to plastic tubes (0.5 cm $\times$ 4 cm). Labelled fibronectin (10 ng, 10^5 c.p.m.) was added, and the volume of the reaction mixture was adjusted to 1.0 ml with buffer. In all experiments the reaction mixture contained 1 mg of bovine serum albumin (Type V; Sigma). The presence of albumin was necessary to inhibit the binding of labelled fibronectin to tube walls. The tubes were incubated with end-over-end rotation at room temperature, and then centrifuged (2000 g, 5 min). The supernatants were carefully removed by suction, and the tubes were washed with 1 ml of buffer containing 1 mg of albumin/ml. After centrifugation (2000 g, 5 min) and aspiration of the supernatants, the radioactivity was counted in a Wallac 81000 liquid-scintillation counter using gamma counting vials obtained from Koch-Light Laboratories, Colnbrook, Slough, Berks., U.K.

A time curve of the binding of ^{125}I -labelled fibronectin to gelatin-agarose is shown in Fig. 1. The binding approached a maximum in about 2 h. To ensure complete equilibration under the various assay conditions used, a prolonged incubation time of 16 h was used in all experiments.

The proportion of ^{125}I -labelled fibronectin bound to gelatin-agarose varied according to the concentration of the labelled protein. About 15% were bound at a concentration of 5×10^5 c.p.m./ml, 20% at 1×10^5 c.p.m./ml, 40% at 1×10^4 c.p.m./ml and 50% at 5×10^3 c.p.m./ml. This type of concentration effect is similar to that of a reversible equilibrium reaction. When passed through a column of gelatin-agarose, the labelled protein bound

Fig. 1. Time curve for the binding of ^{125}I -labelled fibronectin to gelatin-agarose

Portions of labelled fibronectin were incubated at room temperature in plastic tubes with 25 μl portions of gelatin-agarose in a total volume of 1 ml. The buffer used in this experiment was 50 mM-Tris/HCl, pH 7.5. The tubes were mixed by end-over-end rotation. After incubation, the gel beads were washed and the tubes were analysed for bound radioactivity. Radioactivity bound to 'plain' agarose has been subtracted from the results.

completely. This complete binding under conditions of high excess of gelatin indicates that the labelled protein was free of non-reactive contaminants and that the relatively low binding in the assay was due to a low affinity (Ruoslahti *et al.*, 1978) between fibronectin and gelatin. For the purpose of detecting even slight changes in the binding, we used a concentration of labelled fibronectin (10^5 c.p.m./ml) giving a binding of 20%. At this concentration of fibronectin, gelatin-agarose was neither saturated with fibronectin nor in a heavy excess. Control experiments were run with plain agarose gel, which only bound 2–3% of total radioactivity. Duplicate measurements were made at each experimental point. Inhibition of binding was calculated as follows. Background binding (the amount of radioactivity in c.p.m. bound to the control agarose gel) was first subtracted from the data, and then the following formula was used:

$$\text{Inhibition (\%)} = \frac{\text{c.p.m.}_{\text{max.}} - \text{c.p.m.}_{\text{sample}}}{\text{c.p.m.}_{\text{max.}}} \times 100$$

where c.p.m._{max.} refers to maximal amount of radioactivity bound to gelatin-agarose when no inhibitory substances were present.

Affinity chromatography

Affinity chromatography of purified intact or chemically modified fibronectin was performed on

columns (2 ml; 1 cm × 2.5 cm) of gelatin–agarose. The columns had a capacity to bind about 1 mg of native fibronectin. The columns were equilibrated with 50 mM-borate buffer (pH 7.5)/0.9% (w/v) NaCl or with 10 mM-Tris/HCl buffer (pH 7.5)/0.9% (w/v) NaCl. Portions of intact or chemically modified fibronectins (0.3–0.4 mg) were applied and the columns were eluted at a flow rate of 30 ml/h. Fractions (1.0 ml) were collected and protein concentration was measured by photometry at 280 nm. Fibronectin bound to the columns was eluted with 8 M-urea in the above buffers. The recovery of protein from the columns was usually complete.

Chemical modification

Modification of amino groups was performed by succinylation (Klotz, 1967). Fibronectin [1–2 mg in 50 mM-borate buffer (pH 7.5)/0.9% (w/v) NaCl] was succinylated with 5 mg of succinic anhydride (Sigma) per mg of protein during an incubation time of 1 h. The pH of the reaction mixture was kept at 7.0–7.5 by adding 0.2 M- Na_2CO_3 . The reagents were removed by dialysis.

Modification of carboxy groups (Carraway & Koshland, 1972) was done with 3-(3-dimethylaminopropyl)-1-ethylcarbodi-imide (Sigma) at pH 4.75 in the presence of 1 M-glycine ethyl ester. Carbodi-imide was used at a concentration of 0.1 M. During the reaction, the pH of the reaction mixture was kept at 4.75 by adding dilute HCl. After an incubation time of 1 h, the reagents were removed by dialysis.

Guanidinium groups (Yankeelov, 1972) were modified in 50 mM-borate (pH 7.5)/0.9% NaCl with 5 mM-butane-2,3-dione (Sigma) or with 5 mM-phenylglyoxal (Sigma). After an incubation time of 1 h, the reagents were removed as described above.

Agarose-gel electrophoresis

Gel electrophoresis was performed in 1% agarose (electrophoresis grade; Merck, Darmstadt, Germany) buffered with sodium barbiturate, pH 8.6, I 0.02. After electrophoresis, the gels were fixed with 10% (w/w) trichloroacetic acid/5% (w/w) sulphosalicylic acid, washed with water, dried, and stained with Amido Black.

C.d. (circular-dichroism) measurements

C.d. spectra of intact and modified fibronectins were recorded with a Cary 61 spectropolarimeter using cuvettes with a 5 mm light path.

Reagents

Triton X-100, Tween 20 and Lubrol WX were obtained from Sigma. Sodium dodecyl sulphate and sodium deoxycholate were obtained from Merck.

Other reagents were obtained from commercial suppliers and were of highest grade available.

Results

Effect of pH and ionic strength

The effect of pH on fibronectin–collagen interaction was tested by using several buffer systems over a pH range of 2.0–10.5. Glycine/HCl buffers were used at pH 2.0–3.0, sodium citrate buffers at pH 3.0–6.0, potassium phosphate buffers at pH 5.5–8.0, Tris/HCl buffers at pH 7.0–9.5 and glycine/NaOH buffers at pH 8.5–10.5. All buffers were used at a concentration of 50 mM. The results from such experiments are shown in Fig. 2. The binding had a distinct pH optimum of 7–9 as measured in Tris/HCl and in glycine/NaOH buffers. Increasing the pH to, and over, 9.5 strongly diminished the binding. Also, lowering the pH from 7 to 6 resulted in strong inhibition of binding. Only a marginal binding was observed in citrate and glycine/HCl buffers with pH lower than 6.

Ultracentrifugation experiments showed that the labelled protein remained fully soluble at pH 2.0–4.5 and at pH 7.0–10.5. However, there was evidence for aggregation in a pH range of 5.0–6.5. The proportion of the labelled protein remaining soluble

Fig. 2. Effect of pH on the binding of ^{125}I -labelled fibronectin to gelatin–agarose

The assay was performed as described in the legend to Fig. 1 and in the Materials and methods section. The buffers used were 50 mM-glycine/HCl, pH 2.0–3.0 (Δ), 50 mM-sodium citrate, pH 3.0–6.0 (□), 50 mM-potassium phosphate, pH 5.5–8.0 (▲), 50 mM-Tris/HCl, pH 7.0–9.5 (○) and 50 mM-glycine/NaOH, pH 8.5–10.5 (●).

after centrifugation (30000 g, 30 min) was, in 50 mM-citrate buffers, 70% at pH 5.0, 71% at pH 5.5 and 80% at pH 6.0. In 50 mM-phosphate buffers, 69% of the labelled protein remained soluble at pH 5.5, 74% at pH 6.0, and 78% at pH 6.5. The values represent means for three experiments.

The effect of ionic strength was studied by adding increasing amounts of NaCl to the assay system in 50 mM-Tris/HCl, pH 7.5. The effects of NaCl are shown in Table 1. Addition of 50 mM-NaCl inhibited the binding by 27%. When the amount of salt was further increased, the degree of inhibition increased slowly, reaching 56% at 1 M-NaCl (Table 1).

Effect of detergents

Several non-ionic and ionic detergents were tested for their ability to affect the binding reaction. The neutral detergents Triton X-100, Tween 20 and Lubrol WX showed no inhibition, even at concentrations of 5% (v/v). However, the binding was

strongly inhibited by the anionic detergents sodium dodecyl sulphate and sodium deoxycholate at concentrations of 10–100 mM (Table 2).

Fig. 3. Agarose-gel electrophoresis of intact and chemically modified fibronectin

The experiments were performed with 1% agarose gel buffered with sodium barbiturate buffer, pH 8.6. Samples (1–2 µg) of the proteins were applied to the wells in the centre of the gels. After completion of the run (100 V, 1.5 h) the gels were fixed and stained as described in the Materials and methods section. The anode is at the left. The upper well in each gel contained control fibronectin. The lower well contained succinylated fibronectin (a), phenylglyoxal-treated fibronectin (b) and carbodi-imide-treated fibronectin (c).

Table 1. Effect of ionic strength on the binding of ¹²⁵I-labelled fibronectin to gelatin-agarose

Portions of labelled fibronectin were equilibrated with gelatin-agarose in 50 mM-Tris/HCl buffer, pH 7.5, to which various amounts of NaCl had been added. After incubation for 16 h at room temperature, the gel was washed and analysed for bound radioactivity. The inhibition of binding was calculated as described in the Materials and methods section.

[NaCl] (M)	Inhibition of binding (%)
None	0
0.05	27
0.10	41
0.50	52
1.00	56

Table 2. Effect of detergents on the binding of ¹²⁵I-labelled fibronectin to gelatin-agarose

The assay was performed as described in the legend to Table 1 and in the Materials and methods section. The concentrations are given: for Triton X-100, Tween 20 and Lubrol WX, in % (v/v); for sodium dodecyl sulphate and sodium deoxycholate, in mM.

Detergent added	Concn.	Inhibition of binding (%)
None		0
Triton X-100		0
	1	0
	5	0
Tween 20	1	0
	5	0
	10	0
Lubrol WX	1	0
	5	0
	10	0
Sodium dodecyl sulphate	0.1	26
	1	38
	10	100
	100	100
Sodium deoxycholate	1	0
	10	28
	100	96

Effect of chemical modification of charged groups

The binding of fibronectin to gelatin–agarose was markedly sensitive to chemical modification of charged groups of fibronectin. Succinylation of

Fig. 4. Affinity chromatography of intact and chemically modified fibronectins on columns of gelatin–agarose. Portions of intact or chemically modified fibronectins (0.3–0.4 mg) were applied on columns (2 ml) of gelatin–agarose equilibrated with 50 mM-sodium borate (pH 7.5)/0.9% NaCl. The columns were eluted with the equilibration buffer. Bound protein was eluted with 8 M-urea, starting after fraction 10. The volume of the fractions was 1.0 ml. (a) Intact fibronectin; (b) succinylated fibronectin; (c) fibronectin treated with phenylglyoxal; (d) fibronectin treated with butane-2,3-dione. In this experiment the modification was performed in borate buffer and the sample was then dialysed against borate buffer (●) or 10 mM-Tris/HCl (pH 7.5)/0.9% NaCl (○).

fibronectin resulted in a considerable increase in negative charge, as indicated by an increased migration rate in agarose electrophoresis (Fig. 3). Succinylated fibronectin was tested for its ability to bind to gelatin–agarose by affinity chromatography. It showed no binding even under conditions where gelatin–agarose was used in an excess (Fig. 4).

When the arginine-specific reagents phenylglyoxal and butane-2,3-dione were included in the binding assay employing radioactive fibronectin, extensive inhibition of binding was observed at concentrations of 1–10 mM (Table 3). The inhibition obtained with butanedione was dependent on the presence of borate (Table 3). Similar results were obtained when fibronectin modified with phenylglyoxal or with butane-2,3-dione was tested by affinity chromatography (Fig. 4). Agarose-gel electrophoresis of phenylglyoxal-treated fibronectin showed a slight increase in the migration rate (Fig. 3), indicating an increase in negative net charge. Fibronectin treated with butanedione had a migration rate identical with that of intact fibronectin (result not shown).

Modification of carboxy groups by carbodi-imide led to an extensive aggregation of fibronectin. Also, the recovery of this modified protein from dialysis bags and from columns was poor. This made a reliable analysis of gelatin-binding activity of the carboxy-group-modified fibronectin difficult. However, in affinity-chromatography experiments with carboxy-group-modified fibronectin, some protein (up to 50% of that applied) consistently eluted with the equilibrating buffer (results not shown), indicating a decreased affinity for the gel. Carbodi-imide-treated fibronectin showed no migration on agarose electrophoresis (Fig. 3).

Table 3. Effect of arginine-specific reagents on the binding of 125 I-labelled fibronectin to gelatin–agarose

The incubations were performed in 50 mM-borate buffer (pH 7.5)/0.9% NaCl or in 10 mM-Tris/HCl buffer (pH 7.5)/0.9% NaCl at room temperature for 16 h. The calculations were performed as described in the Materials and methods section. The concentrations are in mM. Abbreviation used: N.d., not determined.

Reagent added	Concn.	Inhibition of binding (%) in:	
		Borate buffer	Tris buffer
None		0	0
Butane-2,3-dione	0.1	15	0
	1.0	79	0
	10.0	97	14
Phenylglyoxal	0.1	0	N.d.
	1.0	62	N.d.
	10.0	81	N.d.

Table 4. Mean residue ellipticity at 227 nm of intact and chemically modified fibronectins

Measurements of c.d. were performed at 227 nm. The protein concentration was 0.1 mg/ml. Chemical modification was performed as described in the Materials and methods section, and the samples were then dialysed against 10 mM-Tris/HCl (pH 7.5)/0.9% NaCl (¹) or 50 mM-borate (pH 7.5)/0.9% NaCl (²).

Protein	$10^{-3} \times [\theta]_{227}$ (degrees · cm ² · dmol)	(%)
Intact fibronectin ¹	+1.84	100
Intact fibronectin ²	+1.66	90
Succinylated fibronectin ¹	+2.11	115
Phenylglyoxal-treated fibronectin ¹	+1.84	100
Carbodi-imide-treated fibronectin ¹	+0.65	35
Butanedione-treated fibronectin ¹	+1.94	106
Butanedione-treated fibronectin ²	+1.47	80

Circular dichroism

The far-u.v. c.d. spectra of succinylated, phenylglyoxal-treated and butanedione-treated fibronectin showed only slight differences from the spectrum of intact fibronectin. The ellipticities at 227 nm, which in the experience of one of us (K. Österlund, unpublished work) is sensitive to conformational changes occurring, e.g., during thermal denaturation, are given in Table 4. Carbodi-imide-treated fibronectin showed strongly decreased ellipticity at 227 nm (Table 4.).

Discussion

In our hands the binding of fibronectin to collagen showed several characteristics of an ionic interaction. The binding had a distinct pH optimum of 7–9 (Fig. 2).

The aggregation of the labelled protein at pH 5.0–6.5, which indicates a low solubility of this protein at pH values close to its isoelectric point (Vuento *et al.*, 1977), makes the interpretation of the results from the binding experiments in this pH range difficult. However, the inhibition of binding at low pH could be due to protonation of γ -carboxy groups of glutamic acid and aspartic acid residues. These groups have pK values of about 4.7 in proteins.

The observed inhibition at alkaline pH values could be due to an ionization of tyrosine residues (pK 10.0) and/or by a loss of positive charge of ϵ -amino groups of lysine residues (pK 10.2). The guanidinium groups of arginine residues lose protons at very high pH only (pK 12), thus the observed pH effect cannot be directly related to these groups.

In a fairly recent paper (Gold & Pearlstein, 1980), the binding of fibronectin to collagen was reported to be independent of pH over the pH range 2.6–10.6. However, these results were obtained with an assay system employing relatively high concentrations (5 μ g/ml) of fibronectin. Considering the solubility problems encountered even when using highly diluted fibronectin, it seems possible that an aggregation (cf. Vuento *et al.*, 1980a) of fibronectin may have affected the results. It is also possible that

changes in the binding are more sensitively detected at low concentrations of fibronectin and of the affinity matrix.

When tested at a relatively low ionic strength, addition of salt decreased the binding considerably (Table 1). However, a complete inhibition was not achieved even with high concentrations of salt. The present results show that the reaction is sensitive to ionic strength. Once formed, the fibronectin–gelatin complexes are not readily dissociated by NaCl (Ruoslahti & Engvall, 1978; Vuento & Vaheri, 1978). However, some inorganic salts dissociate the complexes more effectively than others (Ruoslahti & Engvall, 1978; Vuento & Vaheri, 1978) and salts of organic cations dissociate the complexes very effectively (Vuento & Vaheri, 1978).

The effect of detergents on the binding varied according to the nature of the detergent tested. Ionic detergents sodium dodecyl sulphate and sodium deoxycholate inhibited strongly. These results agree with those of other investigators (Gold & Pearlstein, 1980). However, in contrast with the results of these workers, we observed no inhibition with non-ionic detergents. Differences in the experimental methods may explain this discrepancy. We used gelatin covalently coupled to agarose gel. Such an affinity matrix may have a reactivity somewhat different from that of collagen non-covalently adsorbed to plastic (Gold & Pearlstein, 1980). Adsorbed collagen might have been dissociated by detergents and such an effect could not be distinguished from inhibition of binding of fibronectin to collagen. Others have found that non-ionic detergents do not elute fibronectin from gelatin covalently coupled to agarose (Ruoslahti & Engvall, 1978).

The inhibition observed with ionic detergents could be due to an interference by these detergents at possibly existing hydrophobic sites involved in the binding of fibronectin to gelatin. On the other hand, it could be related to a denaturing effect these detergents exert on fibronectin (Colonna *et al.*, 1978). That a native conformation of fibronectin is essential for the collagen-binding activity is also supported by findings that reduction and alkylation

of disulphide bridges destroys the activity (Vuento *et al.*, 1980b). Our results do not support the view (Gold & Pearlstein, 1980) that the binding of fibronectin to collagen would be mainly attributable to hydrophobic effects.

Chemical-modification studies showed that at least amino groups and guanidinium groups of fibronectin are essential for the binding. Succinylation of fibronectin inactivated it completely (Fig. 4). This could mean that the free amino groups of fibronectin are directly involved in the binding, or alternatively, that they are necessary for maintaining the native conformation of fibronectin essential for the binding. Succinylation caused only a slight change in the c.d. of fibronectin (Table 4). However, as the ellipticity of fibronectin at the far-u.v. region is exceptionally weak, it is difficult to assess the magnitude of conformational changes by this technique. Thus more information is needed to establish the mode of participation of the amino groups.

The presence of phenylglyoxal and butane-2,3-dione in the incubation mixture caused strong inhibition of binding (Table 3). Similarly, the phenylglyoxal and butanedione-treated fibronectins showed decreased binding on affinity chromatography (Fig. 4). These two reagents are reported to be specific for arginine (Yankeelov, 1972; Lange *et al.*, 1974). Borate is known to stabilize the complex between butane-2,3-dione and arginine (Yankeelov, 1972), and the modification is often reversible on removal of borate. Accordingly, it was found that butane-2,3-dione inhibited the binding of fibronectin to collagen in borate buffer but not in Tris/HCl buffer (Table 3). Also, when fibronectin was incubated with butanedione in borate buffer and then dialysed against Tris buffer, it regained binding activity (Fig. 4). C.d. measurements again revealed only minor changes in ellipticity at 227 nm (Table 4). Considering the reservations made above in context with succinylation experiments, we conclude that arginine residues of fibronectin are essential for the binding, but their mode of participation remains to be clarified.

Carbodi-imide at acid pH and in the presence of a nucleophilic reagent is considered to specifically modify carboxy groups (Carraway & Koshland, 1972). Such treatment caused aggregation of fibronectin, which did not allow quantitative studies on collagen-binding activity. The treatment resulted in marked changes in c.d. (Table 4), indicating conformational changes.

The effect of chemical modification of fibronectin on the cell-adhesion- and spreading-mediating activity of this protein has also been studied (Grinnell & Minter, 1979; Gold & Pearlstein, 1979). A modification of amino groups by succinylation had no effect on the activity, nor did a complete

reduction and alkylation abolish the activity (Grinnell & Minter, 1979; Gold & Pearlstein, 1979). Modification of carboxy groups resulted in a loss of activity in one study (Grinnell & Minter, 1979) but not in another (Gold & Pearlstein, 1979). The cellular-adhesion-mediating activity of fibronectin is probably not directly related to the collagen-binding activity. There is evidence that these activities are located in different parts of the molecule (Ruoslahti & Hayman, 1979). They also have different stability against heat-denaturation (Vuento *et al.*, 1980b).

Immunofluorescence studies have shown that fibronectin and collagen co-distribute in the pericellular matrix of fibroblasts (Vaheri *et al.*, 1978). Our results are a step towards understanding the mechanism of formation of the fibronectin–collagen matrixes. We have established an essential role of ionic interactions in this process and identified lysine and arginine residues of fibronectin as participating in the binding of fibronectin to collagen.

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References

- Balian, G., Click, E. M. & Bornstein, P. (1980) *J. Biol. Chem.* **255**, 3234–3236
- Carraway, K. L. & Koshland, D. E., Jr. (1972) *Methods Enzymol.* **25**, 616–623
- Colonna, G., Alexander, S. S., Yamada, K. M., Pastan, I. & Edelhoch, H. (1978) *J. Biol. Chem.* **253**, 7787–7790
- Engvall, E. & Ruoslahti, E. (1977) *Int. J. Cancer* **20**, 1–5
- Engvall, E., Ruoslahti, E. & Miller, E. J. (1978) *J. Exp. Med.* **147**, 1584–1595
- Gold, L. I. & Pearlstein, E. (1979) *Biochim. Biophys. Acta* **581**, 237–251
- Gold, L. I. & Pearlstein, E. (1980) *Biochem. J.* **186**, 551–559
- Greenwood, F. C., Hunter, W. M. & Glover, J. C. (1963) *Biochem. J.* **89**, 114–123
- Grinnell, F. & Minter, D. (1979) *Biochim. Biophys. Acta* **550**, 92–99
- Hahn, L. S. & Yamada, K. M. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1160–1163
- Jilek, F. & Hörmann, H. (1978) *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 247–250
- Klotz, I. M. (1967) *Methods Enzymol.* **11**, 576–580
- Lange, L. G., Riordan, J. F. & Vallee, B. L. (1974) *Biochemistry* **13**, 4361–4370
- Mosesson, M. W. & Amrani, D. L. (1980) *Blood* **56**, 145–158
- Mosesson, M. W., Chen, A. B. & Huseby, R. M. (1975) *Biochim. Biophys. Acta* **386**, 509–524
- Ruoslahti, E. & Engvall, E. (1978) *Ann. N.Y. Acad. Sci.* **312**, 178–191

- Ruoslahti, E. & Hayman, E. G. (1979) *FEBS Lett.* **97**, 221–224
- Ruoslahti, E., Vuento, M. & Engvall, E. (1978) *Biochim. Biophys. Acta* **534**, 210–218
- Ruoslahti, E., Hayman, E. G., Kuusela, P., Shively, J. E. & Engvall, E. (1979) *J. Biol. Chem.* **254**, 6054–6059
- Saba, T. M. & Jaffe, M. D. (1980) *Am. J. Med.* **68**, 577–594
- Sekiguchi, K. & Hakomori, S.-I. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2661–2665
- Vaheri, A. & Mosher, D. F. (1978) *Biochim. Biophys. Acta* **516**, 1–25
- Vaheri, A., Kurkinen, M., Lehto, V.-P., Linder, E. & Timpl, R. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4944–4948
- Vuento, M. & Vaheri, A. (1978) *Biochem. J.* **175**, 333–336
- Vuento, M. & Vaheri, A. (1979) *Biochem. J.* **183**, 331–337
- Vuento, M., Wrann, M. & Ruoslahti, E. (1977) *FEBS Lett.* **82**, 227–231
- Vuento, M., Vartio, T., Saraste, M., von Bonsdorff, C.-H. & Vaheri, A. (1980a) *Eur. J. Biochem.* **105**, 33–42
- Vuento, M., Salonen, E., Salminen, K., Pasanen, M. & Stenman, U.-H. (1980b) *Biochem. J.* **191**, 719–727
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412
- Yankeelov, J. A., Jr. (1972) *Methods Enzymol.* **25**, 566–579