A STUDY OF ACTIN–FIBRONECTIN INTERACTION

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1. Introduction

Interactions between the proteins exposed on the cell surface and cytoskeletal proteins have been frequently postulated [1–5]. There is a certain correspondence between the topography of the extracellular matrix compounds (fibronectin, collagen), which play an important role in the attachment and spreading of cells, and the intracellular proteins which form cytoskeletal stress fibers (actin, myosin, α-actinin, filamin). A variety of experimental data indicates this:

(1) Transformed cells have a poor extracellular matrix and are deficient in actin-containing microfilament bundles [1,6,7];
(2) Cytochalasin B promotes dissociation of the stress fibers and the release of fibronectin from the cell [8];
(3) Treatment of cells with proteases and chelating agents causes a release of fibronectin into the media and the loss of microfilament bundles [9,10].

Electron microscope studies also suggest the possibility of transmembrane association between fibronectin and actin [4]. However, some authors consider that there is no direct interaction between fibronectin and actin in the focal adhesion sites [11,12]. Thus, the problem of transmembranous interaction of fibronectin and actin has yet to be clarified. Direct in vitro interaction between actin and fibronectin has been demonstrated in [13,14]. Fibronectin from human plasma was retained in an actin–Sepharose column [13], and the protease-digest actin-binding fragment of the fibronectin molecule was isolated [14]. It was concluded that 'fibronectin... can efficiently bind directly to actin' [13]. However, in those studies a commercial lyophilized actin from Sigma was used and the biological activity, i.e., the polymerizability of protein was not tested. It is quite possible that the results obtained do not correspond to the real situation. Thus, the question arises: Can fibronectin interact with biologically active actin in solution or not?

Here, we report studies of the interaction of human plasma fibronectin with purified polymerizable rabbit skeletal muscle actin. Differential spectroscopy, ELISA (enzyme-linked immunoassay) and DNase I inhibition test was used to study the interaction.

2. Materials and methods

Fibronectin from human plasma was prepared by gelatin–Sepharose affinity chromatography with subsequent chromatography on Whatman DE-52 cellulose [15]. The fibronectin preparation was homogeneous and had high biological activity [16,17]. Rabbit skeletal muscle actin was purified according to [18]. Before the experiments G-actin was chromatographed on a Sephadex G-150 column. The actin preparation was ≧98% pure and polymerizable.

The incubation mixture for actin polymerization contained 0.4 mg actin/ml and also fibronectin (when indicated) in 2 mM Tris–HCl buffer (pH 7.5) with 0.2 mM ATP and 0.2 mM $CaCl_2$–buffer A. The polymerization was initiated by addition of $MgCl_2$ to 2 mM and KCl to 8 mM.

The DNase I inhibition test was carried out according to [19]. Aliquots of 8 μl were taken from the actin polymerization mixture and 0.01 ml 0.3 mg DNase/ml solution was immediately added followed by 3 ml calf thymus DNA (0.05 mg/ml). An increase of absorbance at 260 nm was recorded.

The viscosity was measured in the Cannon-Manning semimicroviscosimeter (size 100). Specific viscosities

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(η_{sp}) were calculated as follows: (sample flow time − buffer flow time)/buffer flow time. The buffer flow time was ~60–70 s.

ELISA procedure was carried out on polystyrene microtiter plates according to [20]. For plate-coating, the proteins were diluted to 0.05 mg/ml (collagen) and to 0.2 mg/ml (F-actin, albumin) by 0.02 M carbonate buffer (pH 8.0) containing 0.02% NaN_3 and 2 mM $MgCl_2$ (for coating with collagen, $MgCl_2$ was omitted). Fibronectin was allowed to bind to collagen, F-actin or to albumin-coated plates for 60 min at 20°C in 0.02 M Na-phosphate buffer (pH 7.4) with 0.05% Tween 20. Affinity-purified rabbit anti-human plasma fibronectin antibodies and horseradish peroxidase conjugated to goat IgG against rabbit IgG were used to detect the bound fibronectin. *o*-Phenylenediamine was used as a substrate for peroxidase. The absorbance of the reaction product was measured on a Gilford model 240 spectrophotometer at 492 nm.

Sedimentation analysis was performed at 20°C with a Spinco model E ultracentrifuge using an ultraviolet scanner at 290 nm. The concentration gradient curves are the result of a graphic differentiation of the integral curves obtained in the experiment. The sedimentation coefficients were calculated according to [21]. The concentration was 0.5 A_{290}/ml for both proteins.

For electron microscopy, F-actin samples were diluted to 0.1 mg/ml and negatively stained with 1% uranyl acetate.

3. Results and discussion

To test the binding of fibronectin to native actin, we studied the effect of fibronectin on actin polymerization. Fig.1 shows the time course of actin polymerization in the presence of fibronectin. We recorded the actin polymerization using the DNase I inhibition test (a), and the increase of specific viscosity (b). At the initial point the inhibition of DNase I is complete, as the sample contains only G-actin. But the inhibition decreases with the decrease of the actin monomer concentration. The addition of fibronectin practically does not affect the G-actin/F-actin ratio during polymerization (fig.1a). The specific viscosity measurements also did not reveal the involvement of fibronectin in the actin polymerization process (fig.1b).

Actin polymerization is accompanied by an alteration of the absorbance in the region of 230–350 nm [22]. The difference in the spectral characteristics between F and G-actin has its maximum at 232 nm [22,23]. Fig.2 shows the time course of actin polymerization. The addition of salts ($MgCl_2$ and KCl) leads to an increase of the actin solution absorbance at 232 nm (fig.2a). The addition of a small amount (0.02 mg/ml) of F-actin stimulates polymerization, promoting the nucleation step (fig.2b). If fibronectin was added to G-actin, the increase of absorbance accompanying polymerization was less significant (fig.2c–f). The inhibition was complete if 1 fibronectin molecule was added/10 actin monomer molecules.

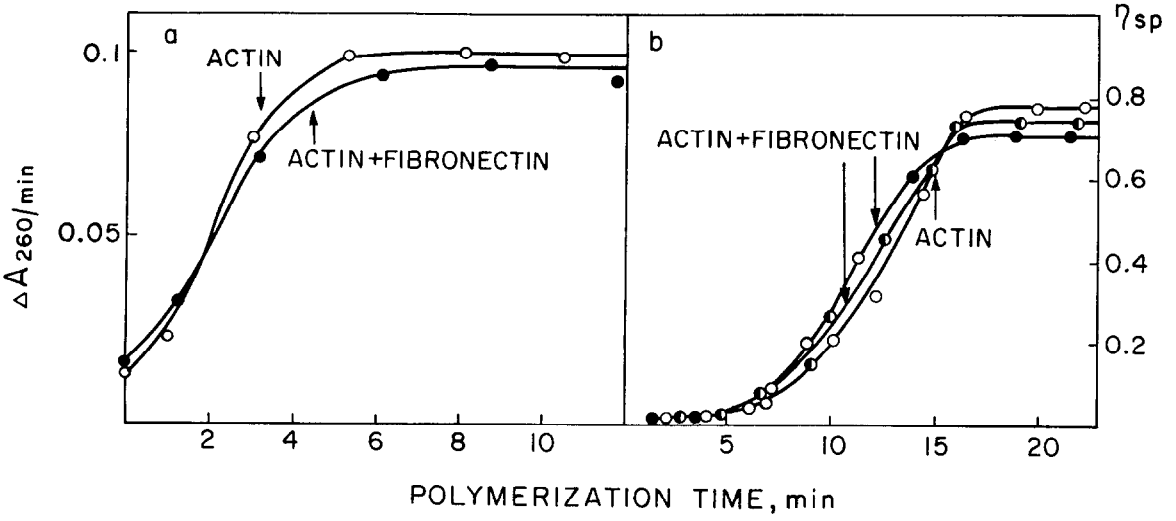

Fig.1. The time course of actin polymerization in the presence of fibronectin: (a) DNase I inhibition test; (b) specific viscosity measurements; (○) actin 0.4 mg/ml; (◐) actin, fibronectin, 0.2 mg/ml; (●) actin, fibronectin, 0.4 mg/ml.

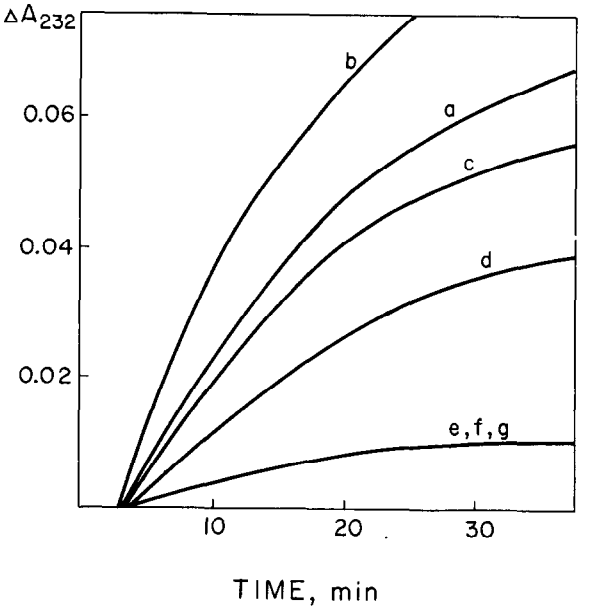

Fig.2. Differential spectroscopy studies of actin polymerization: (a) actin, 0.4 mg/ml; (b) F-actin to 0.04 mg/ml was added at the initial point; (c–g) fibronectin was added, the molar ratio to actin was 1:100 (c), 1:50 (d) and 1:10 (e–g); (f,g) F-actin to 0.04 mg/ml was added at the initial point (f) or at 20 min (g).

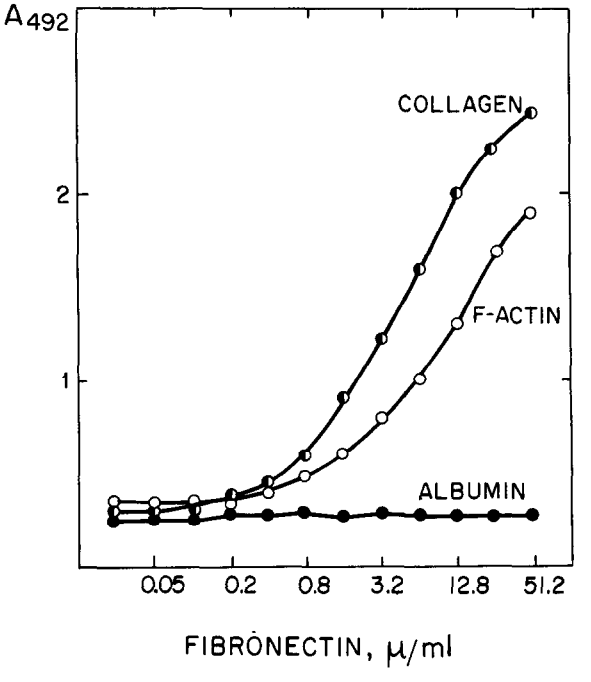

Fig.3. Binding of fibronectin to F-actin immobilized on a polystyrene surface (ELISA procedure). The wells were coated with F-actin (○), collagen (◐) or albumin (●).

Fig.2e,f show that stimulation of actin polymerization with the addition of F-actin was not followed by an increase of absorbance if fibronectin was present.

Viscosimetry and the DNase I test did not reveal a significant effect of fibronectin on actin polymerization, and thus we assume that fibronectin interacts with F-actin and that this association induces alteration of actin spectral characteristics.

To test the binding of fibronectin to F-actin we used an immunochemical method, the microELISA procedure. Fibronectin was added to the microtiter plates coated with collagen type I, F-actin and albumin (fig.3). Fibronectin binds to the collagen or F-actin-coated surface, but does not interact with albumin.

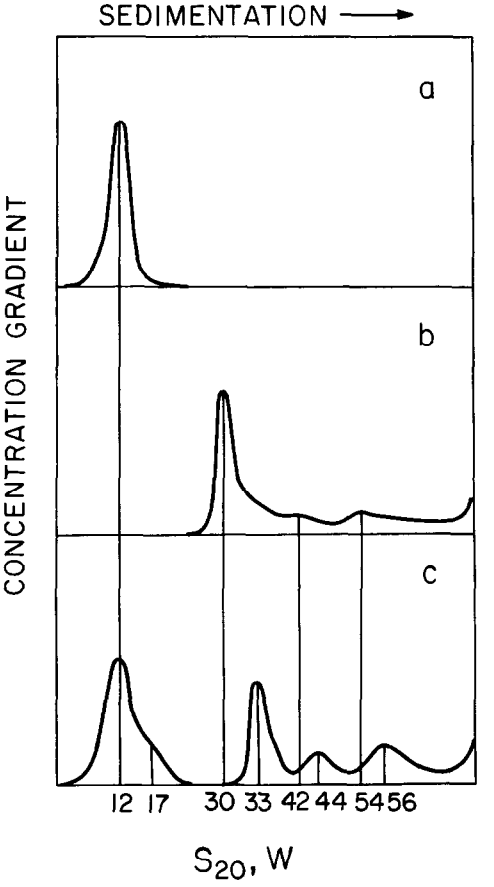

Fig.4. Sedimentation analysis of the F-actin–fibronectin interaction. The cells contained 2 mM Tris–HCl buffer (pH 7.4), 0.2 mM ATP, 0.2 mM $CaCl_2$, 8 mM KCl and 2 mM $MgCl_2$, fibronectin (a), F-actin (b), and actin polymerized in the presence of fibronectin (c). The speed was 10 000 rev./min, the sedimentation time 240 min, the data were corrected to water.

The interaction of fibronectin with collagen is more efficient than with F-actin.

In the next series of experiments we studied the F-actin–fibronectin interaction using analytical centrifugation. Fig.4a shows the results of sedimentation analysis of the fibronectin preparation: one sedimenting boundary is observed and the $s_{20,w}$ was 12 S. The F-actin preparation is heterogeneous (fig.4b), the major component has an $s_{20,w}$ of 30 S and the other components 32–56 S. In the presence of F-actin the boundary of sedimenting fibronectin becomes asymmetric (fig.4c), a distinct shoulder with a sedimentation coefficient of 17 S appears. F-actin sedimentation characteristics also change in the presence of fibronectin, the boundary moves to the bottom faster. These results show the capability of fibronectin to form a complex with F-actin in solution.

Electron microscopy studies also show that fibronectin can interact with actin (fig.5). Fibronectin molecules are located mainly along actin fibers. Some stimulation of the lateral interaction between actin fibrils is observed in the presence of fibronectin. This

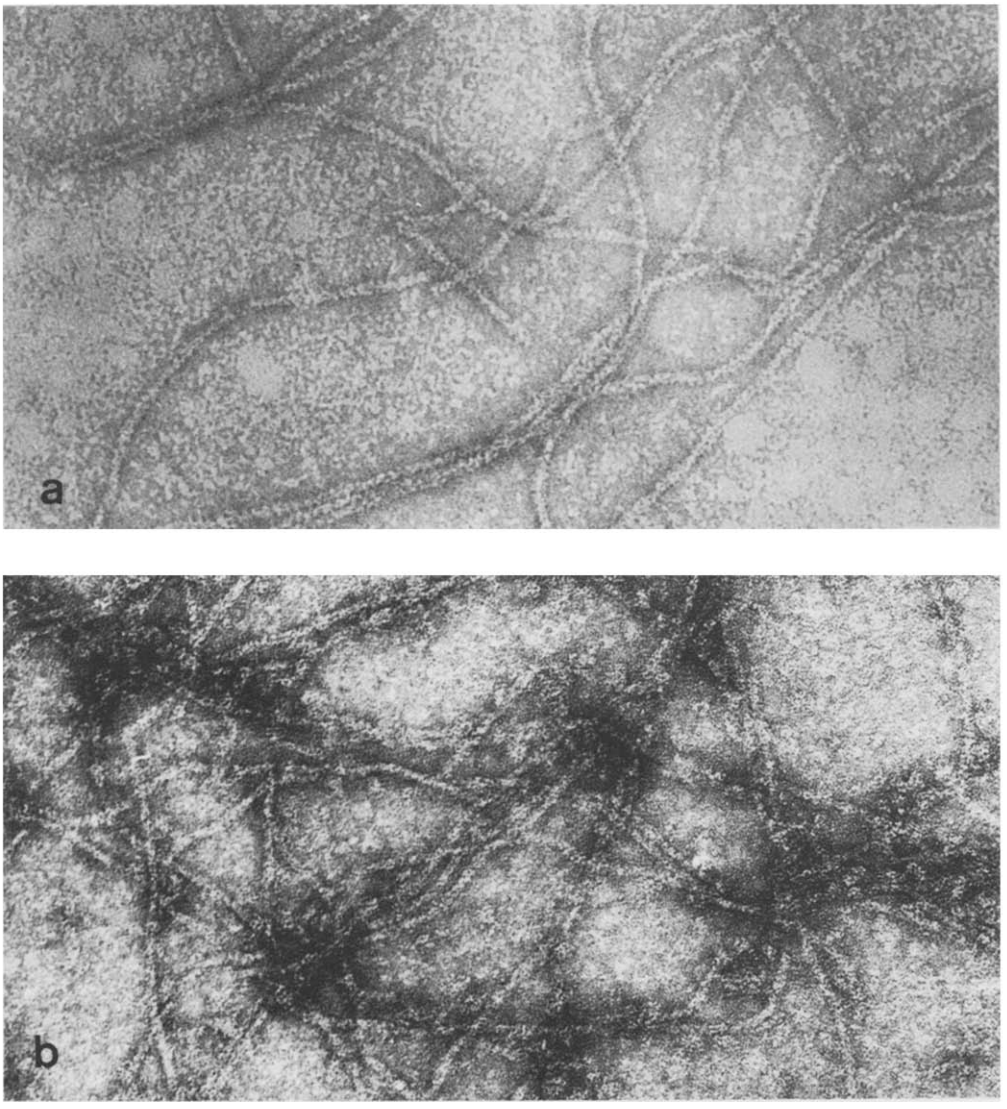

Fig.5. Electron micrographs of F-actin polymerized in the presence and absence of fibronectin. (a) F-actin; (b) F-actin, polymerized in the presence of fibronectin, molar ratio to actin 1:10; magnification 180 000 ×.

seems reasonable, as both polypeptide chains of the fibronectin molecule may contain actinbinding site.

Thus we assume that fibronectin has some affinity to actin. Ultracentrifugation data show that in solution this affinity is rather low. On the other hand, the interaction is stronger if the proteins are allowed to interact on a solid surface (electron microscopy and microELISA data). The plasma membrane and cytoskeletal structures in vivo can provide the necessary surface.

The low affinity of fibronectin to native actin in solution is of special interest. Fibronectin efficiently interacts with denatured actin immobilized on Sepharose [13]. We could not repeat this result with native F- or G-actin, the fibronectin was not retained in the columns containing actin–Sepharose conjugates. Thus, fibronectin probably distinguishes between the native and the denatured forms of the actin molecule. Practically all types of the cells contain a significant amount of actin. Therefore if fibronectin indeed has opsonic functions, its affinity to actin, and especially to denatured protein can help it to recognize and interact with the cellular debris and then to bind to macrophages, thus promoting phagocytosis.

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