Fibronectin has an affinity to vinculin, α-actinin, tropomyosin and myosin

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1. INTRODUCTION

There has been a considerable amount of research on the problem of cell–cell and cell–extracellular substance contacts [1–4]. Fibronectin being a major component of the extracellular matrix is associated with the cell surface and is known to play an important role in the cell–substrate interaction [5–10]. A lot of data show that fibronectin is involved in controlling cell shape, cell motility, adhesion and some other important cell physiological functions [5–10]. However, actin and actin-associated proteins (myosin, tropomyosin, filamin, vinculin, α-actinin) as well as tubulin form the structural basis of cytoskeleton and cell motility [11–13]. A variety of experimental data demonstrate the relationship between extracellular matrix and cytoskeleton. For instance, the addition of fibronectin to transformed fibroblasts induces microfilament-bundle formation and, dissociation of stress fibers caused by cytochalasin B is followed by the release of fibronectin [14–16]. Microfilaments and fibronectin were shown to be closely associated (maximum separation was of 8–22 nm). These results were obtained by immunoelectron microscopy and goniometry [17,18]. Fibronectin can bind actin; actin-binding fragment of fibronectin molecule was isolated [19–21]. In the cell, actin is associated with various proteins which can influence the arrangement of actin filaments. However, it is not known whether fibronectin has an affinity to actin-binding proteins–α-actinin, tropomyosin, filamin, vinculin, myosin and tubulin.

We demonstrate here the ability of fibronectin to interact with α-actinin, myosin, tropomyosin and vinculin. Complexes with filamin and tubulin were not revealed.

2. MATERIALS AND METHODS

Fibronectin was isolated from human plasma by affinity chromatography on gelatin–Sepharose, followed by ion-exchange chromatography on DEAE–cellulose [22]. Filamin from chicken gizzard was purified as in [23]. Vinculin and α-actinin were isolated from chicken gizzard according to [24]. Tropomyosin was isolated from rabbit skeletal muscle (a gift of Dr S. Potekhin, Institute of Protein Research, Poustchino), heavy meromyosin was prepared from rabbit skeletal muscle myosin (a gift of Dr L. Zheleznaya, Institute of Biophysics, Poustchino), tubulin was isolated from bovine brain (a gift of Dr S. Kuznetsov, Moscow State University). Protein preparations were at least 98% pure.

Interaction of fibronectin with different proteins was tested by enzyme-linked immunoassay (ELISA) [25]. Microtitration plates (Lindbro, Flow Labs, Inglewood CA) were coated with proteins (10 μg/ml) overnight at 4°C. Unbound protein was washed out and fibronectin in 0.14 M NaCl, 0.02 M sodium phosphate buffer (pH 7.4) containing 0.05% Tween 20 was added. Fibronectin was allowed to interact with protein coating the plate for 60 min at 20°C. Affinity-purified rabbit (anti-human plasma fibronectin) IgG followed by horseradish peroxidase-conjugated goat (anti-rabbit IgG) IgG were used to detect fibronectin bound to immobilized protein. *O*-Phenylenediamine was a substrate for peroxidase. The absorbance of reaction products was recorded at 492 nm.

3. RESULTS AND DISCUSSION

Fibronectin is known to associate with actin in

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vitro [19,21] – it decorates F-actin and binds to actin immobilized on the polysterene surface [21,26]. However, a lot of proteins in the cell which may participate in the regulation of actin functional state are associated with microfilaments [13]. Therefore, it is worth knowing whether fibronectin is able to interact with the most important actin-binding proteins, such as myosin (heavy meromyosin), tropomyosin, α-actinin, filamin, vinculin and tubulin. The experiments were carried out according to the following scheme: proteins were immobilized on polysterene surface and then fibronectin was added. The amount of fibronectin bound to actin-binding proteins was determined by enzyme-linked immunoassay. Results of the experiment are shown in fig.1. Fibronectin appeared to complex with tropomyosin, α-actinin, vinculin, heavy meromyosin, rather than with filamin and tubulin. It should be emphasized that the amount of fibronectin bound depends on the concentration of fibronectin added to the immobilized protein. We assume that fibronectin interaction with α-actinin, tropomyosin, heavy meromyosin and vinculin is specific and does not reflect the stickiness intrinsic to fibronectin molecule, as the same time fibronectin did not interact with filamin and tubulin, while it exposed high affinity to gelatin which is considered to be a natural substrate for fibronectin. We also tested the ability of proteins under study to stick to the plastic. All the proteins stuck to polysterene surface under experimental conditions (not shown). The dissociation constant for fibronectin–gelatin complex is 10^{-9} M [8]. Fig.1 shows that complexes of fibronectin with different actin-binding proteins are not so stable, however, in these cases the affinity seems also to be rather high and dissociation constants are at least 10^{-6} M.

Fibronectin affinity to vinculin and α-actinin is of special interest, as these proteins are localized in focal contacts (vinculin), in the brush border of epithelial cells, at fascia adherence of intercalated disc membrane in the cardiac muscle and in dense plaques of smooth muscle cells. These structures are known to be actin microfilament attachment sites [27,28]. However, fibronectin was found in the regions of ventral surface of the cell close to focal contacts [29]. Furthermore, fibronectin being localized between the neighbouring cells stimulates cell–cell interaction [5–10]. Recently, it was

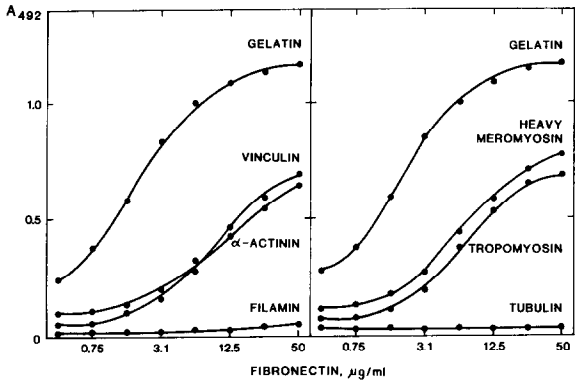

Fig.1. Fibronectin binding to cytoskeletal proteins immobilized on polysterene surface. Fibronectin bound was detected by ELISA procedure.

demonstrated that the distribution of vinculin (intracellular actin-binding protein) and fibronectin is highly coincident in normal fibroblasts [29]. We suggest that fibronectin affinity to actin, myosin, tropomyosin and especially to vinculin and α-actinin plays an important role in the relationship between intracellular actin-containing microfilament bundels and fibronectin, the main protein component of extracellular matrix. However, we cannot exclude the possibility that affinity of fibronectin to cytoskeletal proteins reflects non-specific opsonic functions of this molecule.

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