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Interaction of iodinated vinculin, metavinculin and α-actinin with cytoskeletal proteins

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Iodinated vinculin, metavinculin and α-actinin were used to probe the interaction of these proteins with electrophoretically separated cytoskeletal proteins. Using the gel overlay technique, we detected strong binding of ^{125}I-vinculin and ^{125}I-metavinculin to α-actinin, 175 kDa polypeptide, talin, vinculin and metavinculin themselves, and moderate binding to actin. ^{125}I-α-actinin was capable of interacting with vinculin and metavinculin. The specific binding of ^{125}-I-α-actinin to vinculin and metavinculin immobilized on a polysterene surface was also demonstrated. We suggest that the ability of vinculin and α-actinin to form a complex may be realized in microfilament-membrane linkages.

Vinculin; Metavinculin; α-Actinin; Microfilament-membrane interaction

1. INTRODUCTION

The interactions between microfilaments and the cell membrane play an important role in cell functioning. The proteins talin and vinculin have been localized in specialized regions of plasma membrane-microfilament association and have been established as cytoskeletal markers of microfilament-membrane attachment sites of cultivated cells and cell-cell contact regions of smooth and striated muscle [1–11]. Vinculin and talin are able to form a tight complex [12]. Talin has been found to interact with an integral plasma membrane protein – fibronectin receptor [13,14]. Recently, a 175 kDa membrane protein was identified in different muscles which was able to interact specifically with vinculin [15]. One vinculin isoform, metavinculin, displays solubility properties of an integral membrane protein [7]. At present, at least 3 proteins are candidates for serving as linkers for the talin-vinculin complex and plasma membrane. However, despite the intracellular localization of vinculin and talin at the microfilament-membrane attachment sites and their interactions with integral membrane proteins and each other, talin and vinculin have not been shown to be actin-binding proteins [4,12,16]. Therefore, the role of these proteins in F-actin-membrane linkages is poorly understood. We suggest that intracellular components must exist which link the vinculin-talin complex to F-actin. To support this suggestion, we demonstrate here that both vinculin and metavinculin have the ability to interact specifically with the major intracellular actin-binding protein, α-actinin.

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2. MATERIALS AND METHODS

Human uterus smooth muscle vinculin, metavinculin, α-actinin, filamin, myosin and actin were isolated according to [17]. Skeletal muscle tropomyosin was a gift from Dr S. Potekhin, Institute of Protein Research, Poustchino. A tubulin preparation was kindly provided by Dr V. Gelfand, Moscow State University. 175 kDa vinculin-binding protein was prepared from chicken gizzards [15]. Fibronectin was obtained from human

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plasma [18]. All proteins used were at least 95% pure as judged by SDS-polyacrylamide gel electrophoresis. α-Actinin, vinculin and metavinculin were iodinated (spec. act. 300–500 μCi/mg) using a water-soluble oxidizing agent (1,3,4,6-tetrachloro-3,6-diphenylglycouril, Iodo-Gen).

For the overlay 7–12 μg of various proteins were separated by SDS-polyacrylamide gel electrophoresis using 6–15% gradient gels [19]. Preparation of gels for the overlay procedure and all incubations were performed essentially as described by Otto [20].

To investigate the binding of ^{125}I-α-actinin to vinculin and metavinculin these proteins were

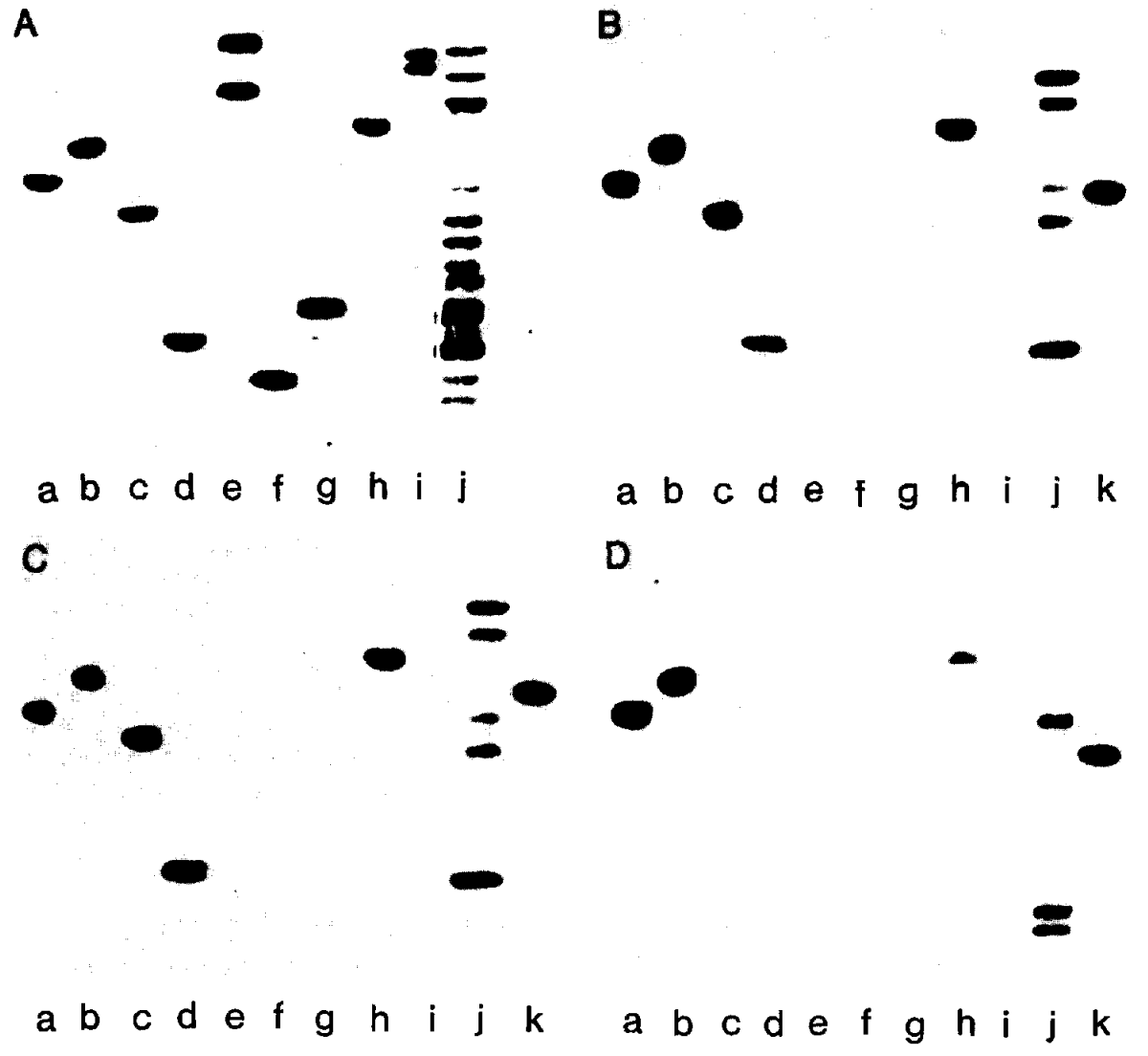

Fig.1. (A) SDS-polyacrylamide gel analysis of different cytoskeletal protein preparations. Proteins were visualized by Coomassie blue staining. Lanes: a, vinculin (130 kDa); b, metavinculin (150 kDa); c, α-actinin (100 kDa); d, actin (43 kDa); e, filamin (250 kDa) and myosin (200 kDa); f, tropomyosin (36 kDa); g, tubulin (58 kDa); h, 175 kDa vinculin-binding protein; i, human plasma fibronectin (240/230 kDa); j, human platelet extract. (B–D) Autoradiographs of gels overlaid with ^{125}I-vinculin (B), ^{125}I-metavinculin (C), ^{125}I-α-actinin (D). Lane k: in (B) ^{125}I-vinculin, in (C) ^{125}I-metavinculin, in (D) ^{125}I-α-actinin.

NaCl, 0.02 M Hepes, 1% NP-40, 0.5% NaN_3 and 0.5% BSA (pH 7.6). Unbound ^{125}I-α-actinin was extensively washed out; polysterene wells were excised and counted in a gamma counter.

3. RESULTS AND DISCUSSION

To study the interaction of ^{125}I-vinculin and ^{125}I-metavinculin with cytoskeletal proteins the gel overlay procedure was used [20]. Under our experimental conditions ^{125}I-vinculin and ^{125}I-metavinculin displayed equal ability to interact with individual cytoskeletal proteins (fig.1A–C). ^{125}I-vinculin and ^{125}I-metavinculin were both found to bind to α-actinin, 175 kDa protein, actin, to themselves and to each other, but failed to interact with filamin, myosin, tropomyosin, tubulin and fibronectin. The autoradiographs of platelet protein samples after incubation of gels with ^{125}I-vinculin or ^{125}I-metavinculin revealed polypeptides of 235 (talin), 200, 130, 100 and 43kDa. Obviously, the last three polypeptides correspond to vinculin, α-actinin and actin. We could not identify the 200 kDa polypeptide which was able to interact with vinculin and metavinculin. It does not appear to be myosin, since fig.1B and C shows that vinculin and metavinculin did not bind to myosin. The 200 kDa polypeptide may be a proteolytic fragment of the 235 kDa vinculin-binding protein, talin. Using preheated (90°C, 5 min) iodinated vinculin and metavinculin we could not detect any significant binding of these proteins (not shown). This observation also suggests that binding of vinculin and metavinculin was specific. In the second series of experiments, binding of ^{125}I-α-actinin to cytoskeletal proteins was studied. As shown in fig.1D, ^{125}I-α-actinin was able to bind to vinculin and metavinculin but could not interact with any other cytoskeletal proteins tested. We also detected binding of α-actinin to polypeptides with apparent molecular masses of 130 and 30–35 kDa in an extract from human platelets. The 130 kDa polypeptide corresponds to vinculin recently identified in platelets [22]. Therefore, the gel overlay experiments demonstrate that vinculin and metavinculin are the most prominent α-actinin-binding proteins. To support this conclusion the interaction of ^{125}I-α-actinin with vinculin and metavinculin immobilized on a polysterene surface was studied. Under our experimental conditions, α-actinin appeared to complex with vinculin and metavinculin, rather than with such sticky proteins as fibronectin (fig.2). Preheated (90°C, 10 min) ^{125}I-α-actinin did not bind to vinculin and metavinculin. Semiquantitative analysis has demonstrated that the K_{diss} values for α-actinin complexes with vinculin and metavinculin are approx. 10^7 M^{-1}. The affinity of α-actinin for vinculin (metavinculin) in solution is probably very low, as we were not able to detect complexes by ultracentrifugation. Previously, it was shown that iodinated vinculin was able to interact strongly with talin and vinculin, moderately with actin and weakly with α-actinin [20,21]. Two new facts have been established in the present study: (i) vinculin and metavinculin have the same ability to interact with cytoskeletal proteins – talin, 175 kDa protein, α-actinin and G-actin; (ii) vinculin and metavinculin are the major α-actinin-binding proteins. The latter finding seems to us very important, since α-actinin, being a classical F-actin-binding protein and interacting with vinculin or metavinculin,

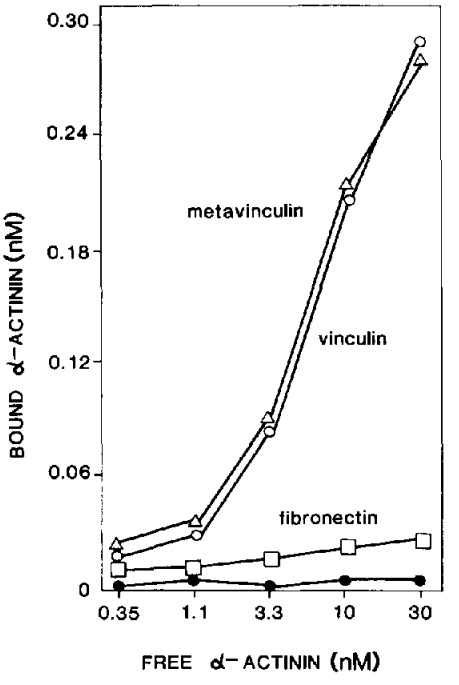

Fig.2. ^{125}I-α-actinin binding to proteins immobilized on a polysterene surface. The wells were coated with vinculin (○), metavinculin (△), fibronectin (□) and vinculin (●), but ^{125}I-α-actinin was preheated (90°C, 10 min).

might be directly involved in F-actin-membrane linkages. α-Actinin was found at the ends of microfilament bundles in non-muscle cells and was suggested as a possible linker between microfilaments and membranes [23]. However, the apparent distance between α-actinin and the cell membrane is too large to suggest that α-actinin is a linker between F-actin and the plasma membrane [24]. The present data allow us to suggest that vinculin (metavinculin), talin, some membrane components (integrin) and α-actinin can complex, forming a special subcellular domain. This domain at the same time can interact directly with the plasma membrane and with the ends of microfilaments.

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