Actin Binding to Lipid-inserted α-Actinin

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ABSTRACT The interaction of α-actinin with lipid films and actin filaments was investigated. First α-actinin was incorporated in lipid films at the air/water interface. Injection of α-actinin into the subphase of a lipid monolayer led to a significant increase of the surface pressure only for lipid films consisting of a mixture of a negatively charged lipid with a high proportion of diacylglycerol. These α-actinin-containing films were transferred onto silanized quartz slides. Photobleaching experiments in the evanescent field allowed quantification of the lateral number density of the lipid-bound α-actinin. In combination with the area increase from the monolayer experiments, the photobleaching measurements suggest that α-actinin is incorporated into the lipid film in such a way that actin binding sites are accessible from the bulk phase. Binding experiments confirmed that the α-actinin selectively binds actin filaments in this configuration. We also showed that, in contrast to actin filaments which are adsorbed directly onto planar surfaces, the α-actinin-bound actin filaments are recognized and cleaved by the actin-severing protein gelsolin. Thus we have constructed an in vitro system which opens new ways for investigations of membrane-associated actin-binding proteins and of the physical behavior of actin filaments in the close neighborhood to membranes.

INTRODUCTION

The mechanical characteristics of eukaryotic cells are determined to a large extent by viscoelastic properties of polymeric networks made up by cytoskeletal proteins (Bereiter-Hahn, 1987; Bray 1992) and by the interaction of elements of the cytoskeleton, in particular by actin filaments, with the plasma membrane (Geiger, 1985; Carraway and Carraway, 1989). The rheological properties of cytoskeletal protein gels have been studied by various biophysical methods (Elson, 1988; Sato et al. 1987; Stossel et al., 1987; Cortese and Frieden, 1988; Schmidt et al. 1989; Janmey et al., 1990; Müller et al., 1991). However, the interaction of membranes with these gels so far has remained the realm of cell biologist approaches adapted to the cellular localization of protein/membrane interaction and the biochemical assignment of functions to the various cytoskeleton-associated proteins (Geiger, 1985; Pollard and Cooper, 1986; Gerisch et al., 1991; Bärmann et al., 1992). Thus, actin filaments have been reported to bind to membranes in two different geometric configurations: (*a*) vertically, with their "barbed ends" pointing to the membrane, mediated by a complex system of actin-binding and/or membrane-binding proteins like integrins, talin, vinculin, α-actinin etc. as in various forms of focal adhesions (Burridge et al., 1988; Critchley et al., 1991; Lu et al., 1992) or (*b*) laterally, as in sarcomeric Z-disks (Takahashi and Hattori, 1989; Goll et al., 1991), by arms or anchors as for instance the calmodulin/110-kDa (Myosin I) complex in brush border microvilli (Mooseker, 1985; Drenckhahn and Dermietzel, 1988; Coluccio and Bretscher, 1989; Mooseker and Coleman, 1989).

The chemical and physical behavior of actin filaments in the close neighborhood of membranes on a submicrometer scale has been hardly investigated at all. Theoretically, hydration conditions and repulsive/attractive forces between a lipid layer surface and macromolecules should vary drastically on the nanometer scale when they are brought close together (Israelachvili, 1992). One of the reasons for the lack of empirical studies on this type of membrane/F-actin interaction may be the absence of appropriate methods. Supported planar lipid films which have already been used as model systems for cell membranes (Thompson and Palmer, 1988; McConnell et al., 1986; Watts et al., 1986) provide an appropriate mean for the employment of surface-sensitive techniques. In our laboratory, especially TIRFRAP (total internal reflection fluorescence recovery after photobleaching) and AFM (atomic force microscopy) methods were used as surface-sensitive methods allowing the quantification of molecular events at planar interfaces (Schmidt et al., 1990; Weisenhorn et al., 1990, Radmacher et al. 1992). Preliminary studies on the binding of actin to surfaces showed that the electrolyte composition of the solution strongly influences the binding behavior of actin to surfaces, and that actin polymerization can be induced by far lower metal ion concentrations close to certain surfaces than in free solution (Schmidt 1988; Zimmermann et al. 1989). But an experimental set-up by the aid of which the interaction of actin filaments oriented in parallel and very close to lipid surfaces could be studied by surface sensitive methods was missing so far. Here, we report on such a set-up based on the properties of α-actinin as a laterally F-actin binding protein.

α-Actinin is a rod-like homodimer of about 4 nm in diameter and 50 nm in length, composed of antiparallel subunits with a molecular mass of about 100 kDa (Suzuki et al., 1976; Blanchard et al., 1989). Each subunit contains one actin-binding domain of 27 kDa in size as a head-peace (Mimura and Asano, 1986), so that the dimer is able to cross-link actin filaments (Jockusch and Isenberg, 1981; Meyer and Aebi, 1990). The actin binding of nonmuscle isoforms of α-actinin is inhibited by micromolar Ca^{2+} concentrations,

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but the smooth muscle form used in our experiments is not regulated by Ca^{2+} (Burridge and Feramisco 1981). Together with spectrin and dystrophin, α-actinin belongs to a family of actin-binding proteins that share a repeated conserved sequence motif coding for a series of three α-helical structural building blocks of the elongated rod part of these molecules (Byers et al., 1989; Matsudaira, 1991; Parry et al., 1992). From α-actinin, this part can be cut out by enzymatic digestion as a 53-kDa fragment and has been shown to directly link actin filaments to the cytoplasmic side of the β_1 integrin subunit in focal adhesions of fibroblasts (Otey et al., 1990; Pavalko and Burridge 1991; Pavalko et al. 1991).

α-Actinin has been demonstrated to be located in the neighborhood of the cytoplasmic surface of membranes (Lazarides and Burridge 1975; Lazarides, 1976; Jokusch et al., 1977). Yet, a direct anchoring function of α-actinin to nonprotein elements of the plasma membrane has not been shown in vivo yet, and it has been questioned whether α-actinin, which is highly soluble in a wide range of electrolyte concentrations, can be looked at as a true membrane binding protein (Chen and Singer, 1982; Small, 1985; Blanchard, 1989). But by in vitro binding experiments with a film-balance technique, Meyer et al. (1982) found that α-actinin specifically bound to lipid monolayers containing diacylglycerol and palmitic acid. That this result hints to a possible physiological function of lipid binding to α-actinin was confirmed by Burn et al. (1985) who isolated diacylglycerol-containing α-actinin/actin complexes from activated platelets. Since diacylglycerol is the membrane-bound fission product of the enzymatic hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), it was speculated that membrane binding of α-actinin might be in some way dependent on PIP_2. This was only recently confirmed by the discovery by Fukami et al. (1992) that PIP_2 is necessary for full gelating activity of muscular α-actinin and is part of the Z-disk of striated muscles.

Motivated by the paper of Meyer et al. (1982), the idea of this study was to construct an experimental model of an α-actinin-containing planar solid-supported lipid layer in such a way that actin filaments could be specifically anchored by laterally binding to α-actinin heads. First, the interaction of α-actinin with Langmuir-Blodgett films of varying composition was to be further characterized. α-Actinin-containing monolayers were then transferred to hydrophobically coated quartz slides, resulting in an unsymmetrical planar lipid layer with fixed α-actinin molecules. In this experimental set-up we tried to answer the questions whether α-actinin incorporated into a lipid layer was still able to bind actin filaments, and whether actin filaments fixed this way at a short distance from a lipid layer were distinguishable from actin directly adsorbed to this layer.

MATERIALS AND METHODS

Chemicals

1,2-Dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylglycerol (DMPG), 1,2-dipalmitoyl-*sn*-glycerophosphatidic acid (DPPA), 1,2-dioleyl-*sn*-glycerol (diacetylglycerol, DAG), palmitic acid (PA), and octadecyltrichlorosilane (OTS) were obtained from Sigma Chemie (Deisenhofen, Germany), and their purity was checked by thin-layer chromatography. Salts, buffer substances, and organic solvents were purchased from Merck (Darmstadt, Germany), ATP-disodium salt and sodium dodecyl sulfate-polyacrylamide gel electrophoresis reagents from Serva (Heidelberg, Germany), and 4-chloro- and 4-fluoro-NBD (7-nitrobenz-2-oxa-1,3-diazole) from Molecular Probes (Eugene, OR). All chemicals were bought at the highest purity available and used without further purification. Trichloromethane was of high-performance liquid chromatographic (HPLC) purity.

Protein preparation and fluorescent labeling

α-Actinin was prepared from smooth muscle exactly as described by Craig et al. (1982) with the exception that fresh turkey gizzard cut into cubes of about 1-cm side-length was used as starting material for the glycerol extraction step. Fluorescent NBD-α-actinin was prepared as follows: NBD-fluoride dissolved in dimethylsulfoxide at a concentration of 5 mg/ml was added dropwise with slow stirring up to a final concentration of 50 μg/ml to a 1 mg/ml solution of α-actinin in PBS (phosphate-buffered saline: 8.2 mM Na_2HPO_4, 1.9 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl 0.02% NaN_3, pH 7.0). The reaction mixture was then incubated at 4°C for 1 h. Unbound dye was removed by gel filtration over Sephadex G-25 (Pharmacia, Freiburg, Germany). Purity and labeling were controlled by HPLC over a Zorbax GF-250 column (Du Pont) on a Kontron HPLC system equipped with a dual-wavelength detector (Fig. 1). The elution buffer was PBS, and the elution time was 1 ml/min.

Actin was prepared according to Pardee and Spudich (1982) with an additional gel filtration step as suggested by MacLean-Fletcher and Pollard (1980) using a Sephacryl S-300 column. Polymerization activity of actin was routinely checked by falling ball viscosimetry. The polymerization buffer (F-buffer) contained: 2 mM Tris-HCl (pH 7.5), 100 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 2 mM dithiothreitol, 5 mM Na_2ATP. As a fluorescent polymerization probe, purified actin was labeled with NBD exactly as described by Detmers et al. (1981). Unlabeled actin and NBD-labeled actin was mixed in a ratio of 10:1. The final concentration of the mixture was 0.7 mg/ml.

Gelsolin purified from pig platelets was a kind gift of R. Gieselmann in our laboratory. The final concentration applied to actin filaments was 0.1 mg/ml in F-buffer. ADP-ribosylated actin prepared with *Clostridium perfringens* iota toxin according to Schering et al. (1988) was the generous gift of K. Aktories (Universität des Saarlandes, Homburg, Germany). The final concentration for the blocking procedure was 1 mg/ml. ADP-ribosylated actin was incubated for 20 min when indicated.

Protein characterization

Protein concentrations were determined by the method of Bradford (1976) with ovalbumin as standard. The concentration of purified actin was measured by UV spectroscopy using an extinction coefficient of 0.63 mg^{-1} cm^{-1} at 290 nm

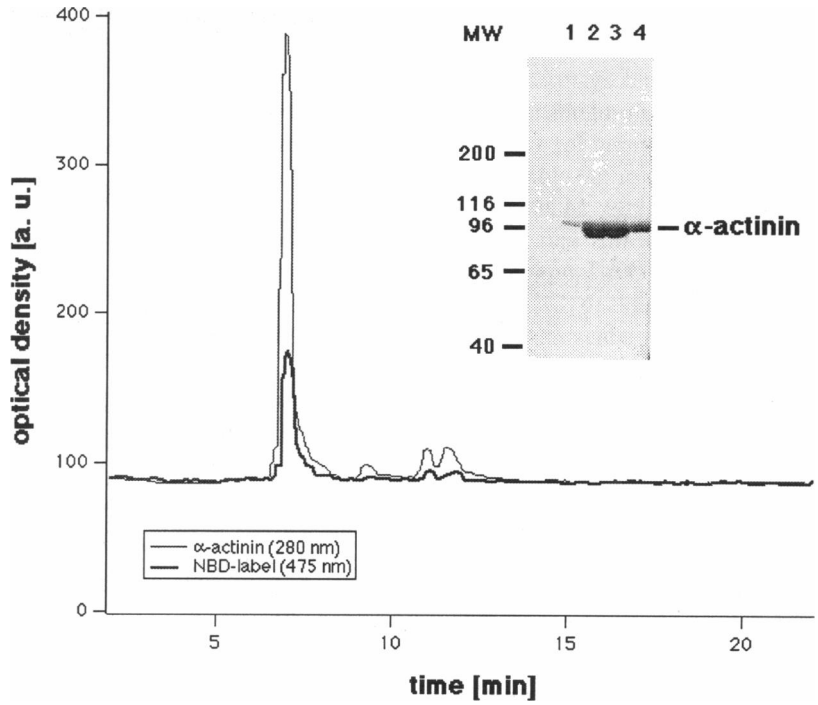

FIGURE 1 HPLC run of NBD-labeled α-actinin measured at 280 and 475 nm. The insert shows a microgel of α-actinin. The elution buffer was PBS, the elution speed was 1 ml/min. Lanes 1–4 show α-actinin after the last purification step.

(Houk and Ue, 1974). The purity of proteins was checked by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to Laemmli (1970). All proteins were sterile-filtered and stored at 4°C in the dark. Polymerization activity of actin, cross-linking activity of α-actinin and capping activity of gelsolin were tested by low shear viscosimetry using a falling ball viscosimeter (Pollard and Cooper, 1982) equipped with a light barrier device for better reproducibility of the measurements.

Lipid monolayers

The monolayer experiments were performed with a small (21.8 cm^2 surface) temperature-controlled Langmuir Blodgett (LB) trough which was built in our lab (Heyn, et al., 1990). 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/KOH, pH 7.0, 100 mM KCl, and 0,02% NaN_3, was used as a subphase. Lipids were dissolved in trichloromethane at 1 mg/ml and spread at the air/water interface. The solvent was allowed to evaporate before the film was slowly compressed to a final pressure of 25 mN/m. Then α-actinin was injected into the subphase providing a final concentration of 50 μg/ml. During an incubation period of 10–15 min at 20°C, the area of the film was kept constant. In the incorporation experiments (see Fig. 2 *b*) the area of the protein/lipid-film was then slowly (100 μm/s) expanded by a certain area. After each expansion step, the film was allowed to establish a new pressure level.

Preparation of supported planar lipid films

For the preparation of supported planar lipid films a protein/lipid film was formed at the air water interface by injection of the α-actinin solution under the lipid monolayer at a pressure of 25 mN/m. The final concentration of α-actinin in the bulk volume was 50 μg/ml. At constant pressure the area increase was monitored. The incorporation of the protein was completed after typically 10 min. In order to separate the lipid/protein film from the α-actinin solution, the film was then pushed at constant pressure via the surface of a wet filter paper bridge onto the surface of another LB-trough. From here, it was transferred by the Langmuir-Schäfer technique (horizontal dipping) (Tamm and McConnell, 1985) onto silanized quartz slides. Silanization of the quartz slides with OTS (octadecyltrichlorosilane) was carried out according to Maoz and Sagiv (1984).

Blocking of these solid supported protein/lipid-layers against nonspecific binding of actin was achieved by incubation with (*a*) 1 mg/ml ADP-ribosylated actin in F-buffer or (*b*) 1 mg/ml thermally denatured actin in F-buffer for 30 min at room temperature and subsequent washing with HEPES buffer (as described above).

For the experiments where α-actinin was just adsorbed to the lipid film the DPPA/DAG monolayer was transferred onto silanized quartz slides and then incubated for 1 h with α-actinin (0.3 mg/ml in PBS). After the blocking procedure described above actin filaments were incubated as demonstrated in Fig. 5, lane B.

Total internal reflection fluorescence microscopy combined with photobleaching (TIRFRAP)

The experimental set-up for the TIRFRAP measurements has been described in detail elsewhere (Zimmermann et al., 1990). Briefly, the interface between the lipid-coated surface and the solution was illuminated by an Argon laser beam

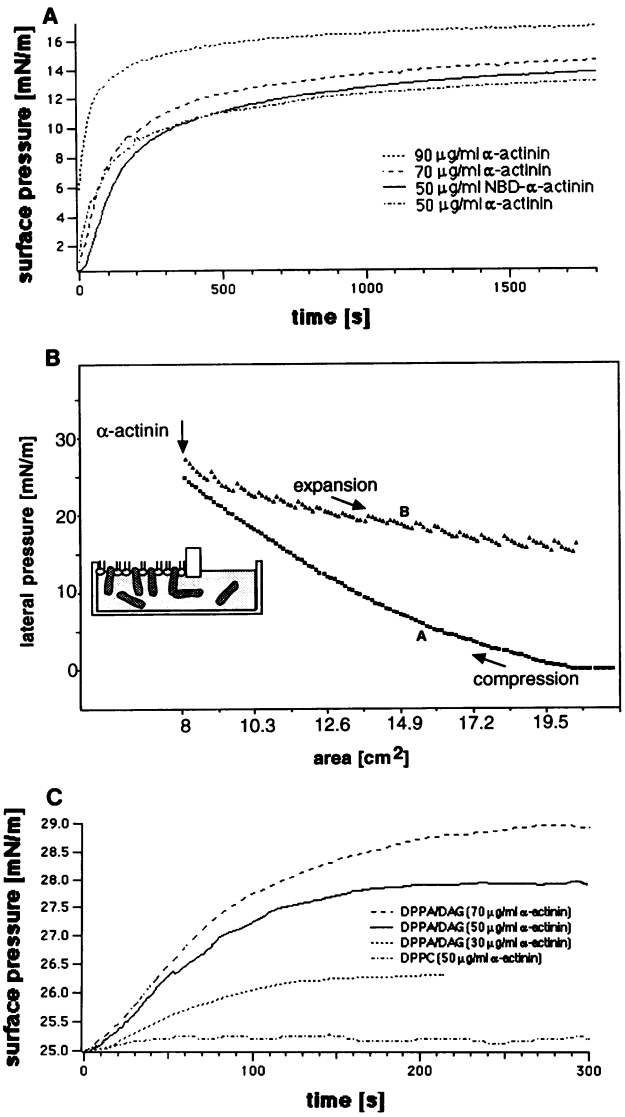

FIGURE 2 (*a*) Time course of the surface pressure increase by adsorption of various concentrations of α-actinin to the free air/water interface. The subphase consisted of 10 mM HEPES/KOH, pH 7.0, 100 mM KCl, and 0.02% NaN_3. (*b*) Pressure area isotherm of the 1/1, DAG/DPPA monolayer on HEPES taken upon compression (*A*). Trace B shows the pressure area isotherm of the same monolayer after injection of α-actinin into the subphase taken by stepwise decompression. (*c*) Pressure change after injection of various concentrations of α-actinin into the presence of a monolayer of 1/1 DPPA/DAG or DPPC, as indicated. The starting pressure of 25 mN/m in all cases.

under an incidence angle larger than the critical angle of total internal reflection, resulting in an evanescent field at the interface. The characteristic penetration depth was in our case 160 nm (Axelrod et al., 1984). The fluorescence emission from within the evanescent field has obviously two contributions: one from bound molecules at the interface (I_s) and one from molecules diffusing freely in the bulk close to the surface (I_b). In cases, where both contributions can be separated experimentally, the known geometry of the evanescent field allows the calibration of the fluorescence from the surface with respect to the known bulk concentration. Under conditions where changes in the quantum yield of the label are negligible, which was shown to be justified for similar systems, the surface concentration c_s is then given by (Zimmermann et al., 1990):

$$c_s = c_b \frac{d}{2} \frac{I_s}{I_b}, \quad (1)$$

where c_b is the concentration of the protein in the volume and d is the penetration depth of the evanescent field.

In our experiments here, the TIRFRAP set-up was used to determine the concentration of bound protein by two different methods: (*a*) With a brief laser pulse, the fluorescence of molecules within the evanescent field was bleached, and the fluorescence recovery was recorded. In contrast to bound molecules, the fluorescence contribution from unbound molecules will recover with a diffusion limited time constant, which is shorter than seconds. (*b*) Freely soluble fluorescent molecules in the volume were replaced by buffer, the fluorescence from the surface was measured and was then bleached with several short laser pulses to the minimal value. The buffer solution was then replaced again by a solution of fluorescently labeled α-actinin, so that the exact contribution of the freely soluble protein could be measured.

RESULTS AND DISCUSSION

Incorporation of α-actinin into lipid films at the air/water interface

To a large extent, the structure of most water-soluble proteins is determined by hydrophobic interactions. In contact with the air/water interface, proteins lose part of their solvation shell and tend to unfold their noncovalent structure. A layer of unfolded and more or less denatured protein molecules is formed, developing a characteristic surface pressure (MacRitchie and Alexander, 1963; Graham and Phillips 1979). This effect can be seen in Fig. 2 *a*. The surface pressure of an α-actinin solution increases with time to approach an equilibrium value. The time course of this process is diffusion-controlled and concentration-dependent, whereas the final equilibrium value does only weakly depend on the concentration. For 50 µg/ml we found a maximum value of 14 mN m^{-1} after 1 h. NBD-labeled α-actinin showed almost identical behavior (see Fig. 2 *a*).

From these findings we concluded that monolayer studies on the interaction of this protein with lipid films at the air/water interface would yield conclusive results on protein/lipid interaction, only if the surface pressure of the lipid film was kept well above the equilibrium spreading pressure of the protein itself. All further experiments were therefore carried out at lipid pressures above 25 mN/m. The experiments were performed as follows (see details in Methods on film balance): a lipid film was spread from organic solution onto the air/water interface of a HEPES buffer subphase. After evaporation of the solvent, the film was slowly compressed to its final pressure of 25 mN/m (see Fig. 2 *b*, *trace A*). Upon expansion the pressure area isotherm followed the same curve without significant hys-

teresis. After the pressure was constant at 25 mN/m for several minutes, the α-actinin solution was injected into the subphase. The change of the pressure for several α-actinin concentrations was recorded and was plotted in Fig. 2 *c*.

In order to distinguish between the different contributions to the surface pressure the film was then expanded by a certain fraction of the area allowing a new equilibrium to form (see Fig. 2 *b*, *trace B*). This expansion was repeated step by step until the surface pressure leveled off at the inherent spreading pressure of the protein (Fig. 2 *a*). We found that this value was insensitive to the lipid composition.

However, the initial pressure change after the injection of α-actinin into the subphase varied drastically for different lipid compositions. This finding is plotted in Fig. 3. The experiments showed clearly that a significant pressure increase was observed (i) only when a high percentage of DAG was in the monolayer and (ii) only when the second lipid was negatively charged. Without DAG, or with a neutral lipid in the presence of DAG, no pressure increase was measured. As controls, bovine serum albumin (BSA), unspecific immunoglobulins, hemoglobin, Dictyostelium-csA (contact site A (Faix et al. 1990)), streptavidin, monomeric actin, and gelsolin were also injected. With the exception of BSA, none of these resulted in a pressure increase. BSA led to a slight pressure increase, which might be due to either fatty acid impurities or the ability of BSA to bind amphiphilic molecules. The pronounced increase of the surface pressure in the case of α-actinin indicates a strong interaction of this protein with the 1:1 mixture of a negatively charged lipid and DAG.

With the known compressibilities of the films, which are obtained directly as the derivatives of the pressure/area isotherms, the pressure changes can be converted into area changes. For a 1/1 DAG/DPPA mixture, the area change at a lateral pressure of $\pi_{lat} = 25$ mN/m was $\Delta A = 4 \pm 1\%$. The work performed by α-actinin molecules inserting into the lipid film and thus increasing the surface area at a constant lateral pressure, $W/A = \Delta A/A \times \pi_{lat}$ is about 1 mJ/m^2. For a better understanding of the insertion mechanism, it would be interesting to measure the temperature dependence of this process. This would give additional insight in the entropic versus enthalpic components of the underlying processes. Unfortunately the thermal stability range of this protein is too small to allow such investigations.

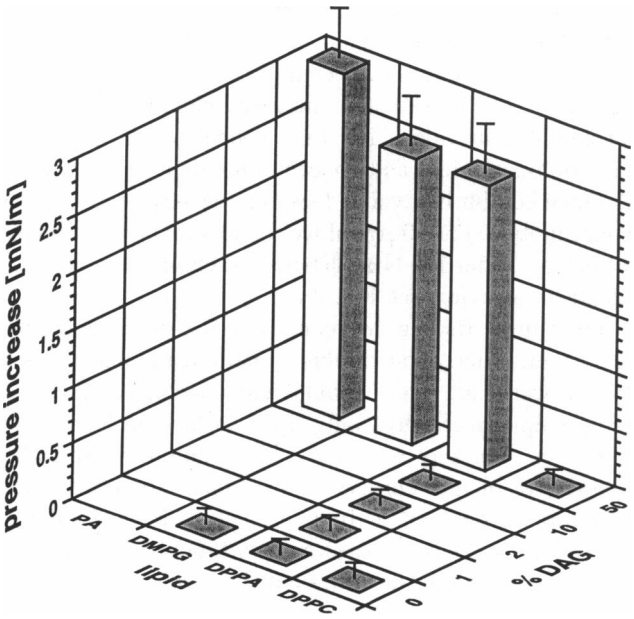

FIGURE 3 Surface pressure increase after injection of α-actinin into the subphase to a final concentration of 50 μg/ml for various compositions of the lipid monolayer.

Formation of supported planar lipid films

The monolayer experiments led us to the assumption that α-actinin incorporates, at least partially, into the lipid film. For further investigation of the functional integrity of α-actinin after its insertion into these lipid monolayers, we transferred the protein/lipid film onto quartz slides. For the formation of supported planar lipid films, lipid films of DAG/DPPA in a 1:1 ratio were compressed to 25 mN/m. Then α-actinin was injected to a final concentration of 50 μg/ml. Earlier studies had revealed (Zimmermann, 1991) that during the transfer of lipid monolayers on solid substrates, defects in the lipid film structure may be created. Free protein molecules from solution may bind to these defects nonspecifically. To prevent this we first removed free protein by transferring the α-actinin/lipid monolayer at a constant pressure of 25 mN/m via a wet bridge (Heyn et al., 1990) onto the surface of a second LB trough filled with buffer. The film was then transferred by Langmuir-Schäfer technique (horizontal dipping) (Tamm and McConnell, 1985) from the air water interface onto a silanized quartz slide (Maoz and Sagiv, 1984). This asymmetric supported lipid film was investigated by TIRFM. The fluorescence intensity from the NBD-labeled α-actinin in the lipid film was measured first in pure buffer and then in a 0.3 mg/ml solution of NBD-labeled α-actinin. Using Eq. 1 the lateral density of the α-actinin in the lipid film was then calculated to be $2 \pm 1 \times 10^{15}$ molecules/m^2. With the measured area increase during incorporation of the α-actinin into the monolayer of 4 ± 1 %, the surface increase upon incorporation of one protein molecule into the lipid film turns out to be 32 ± 15 nm^2.

Using data from Suzuki et al. (1976) for the dimensions of α-actinin, the small cross-section area can be calculated as 12.6 nm^2 and the long cross-section as about 200 nm^2. Therefore our data favor a model for the incorporation into the lipid film in which the molecule is oriented normal rather than tangential to the surface. This orientation is consistent with the idea that at least one actin-binding domain remains accessible from the solution volume after incorporation into the monolayer. Still, the area measured is more than twice the area calculated. Apart from the obvious inaccuracy of our measurements, this difference could be explained by a more complex way of interaction between α-actinin and lipids.

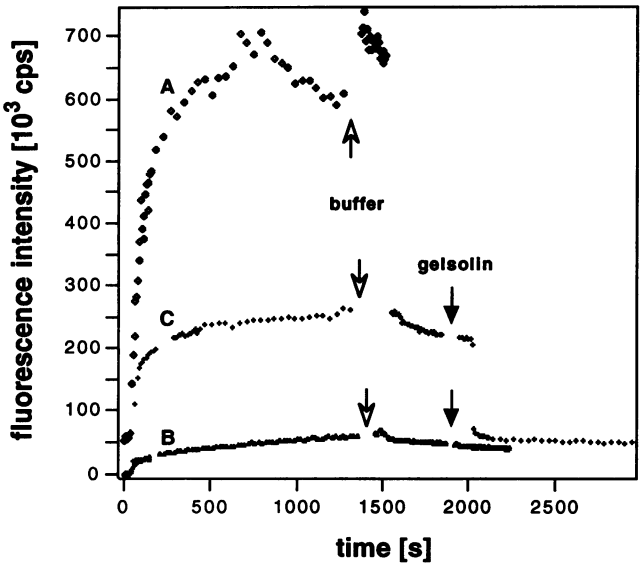

FIGURE 4 Binding of actin filaments to supported planar lipid films: time course of the fluorescence from NBD actin filaments at the supported planar lipid film measured by evanescent excitation. Trace A: 1:1, DAG/DPPA lipid film not blocked against unspecific binding by pretreatment with ADP-ribosylated actin. (*B*) 1:1, DAG/DPPA lipid film but pretreated with ADP-ribosylated actin. (*C*) Lipid film containing α-actinin blocked and incubated with NBD-actin filaments. Flushing with buffer and with gelsolin is indicated.

Actin binding assay

Supported planar lipid films like the ones described above were used to test the ability of lipid-bound α-actinin to bind actin filaments. As mentioned before, these supported planar lipid films may contain defects, leading to nonspecific protein binding. Also, we have to take into account that actin itself, particularly G-actin, may interact with negatively charged surfaces, as has been shown by Schmidt (1988) and that under certain conditions, G-actin spontaneously self-assembles into filaments at negatively charged surfaces (Weisenhorn, et al., 1990) or on phospholipids (Taylor and Taylor, 1992). However, on lipid monolayers, there was no increase of the surface pressure after injection of actin monomers and polymers (data not shown). In contrast, measurements with TIRF showed that NBD-actin filaments adsorbed to supported planar lipid films of the same composition as the monolayers. Fig. 4 shows the time course of the fluorescence of the surface-bound NBD-actin measured in TIRF. In trace A, a solution of NBD-labeled actin filaments was added without blocking. It shows a large increase in surface fluorescence which did not decrease significantly after flushing the chamber with buffer. The surface fluorescence also remained the same even after the actin filaments had been attacked by the actin-severing protein gelsolin. The latter finding indicates a strong interaction of actin with the negatively charged lipids.

This massive interference requires a suitable blocking protocol. In an extended study, we have found that a surface can be protected best against nonspecific adsorption of a protein by blocking with either the same protein or a very similar one (see also Timbs and Thompson, 1990). We have employed actin, ADP-ribosylated by *C. perfringens* iota toxin (Schering et al., 1988), to block against nonspecific binding of the actin filaments to our lipid films. ADP-ribosylated actin does not form filaments but is otherwise structurally very similar to G-actin. Fig. 4, trace B, shows the fluorescence increase at the blocked surface. The fluorescence increase is drastically reduced to less than 6% compared to trace A. Trace C shows the time course of the incubation of the lipid film containing the reconstituted α-actinin, which was blocked with ADP-ribosylated actin. The fluorescence is clearly increased compared to the fluorescence of the lipid film containing no α-actinin. After flushing with buffer, the surface fluorescence remains almost unaltered, indicating that the filaments are bound by the α-actinin inserted into the lipid film. After incubation with gelsolin and subsequent flushing, virtually no surface fluorescence above the background was detectable. This means that in this case the gelsolin was capable of successfully attacking the actin filaments so that the actin could be removed from the surface. Less than 10% remained at the surface. This shows that the α-actinin molecules which we have reconstituted in our supported planar lipid film selectively bind actin filaments. And it shows that the α-actinin molecules bind the actin filaments in such a way, that the filaments are accessible to their physiological regulatory proteins.

In order to address the question of whether or not α-actinin molecules, which are just adsorbed at the surface, would bind actin filaments in the same way as incorporated α-actinin, we performed an experiment where we allowed α-actinin to adsorb to a supported planar lipid film. In a separate experiment, not shown here, we confirmed that under the chosen conditions two- to threefold as many α-actinin molecules do adhere to the lipid film as are incorporated into the monolayer. We then repeated the above actin binding experiments.

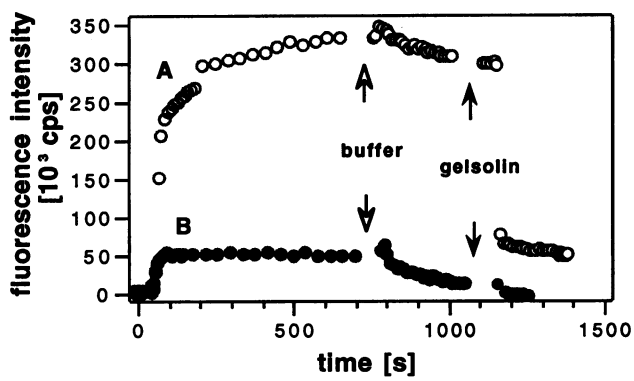

FIGURE 5 Binding of actin filaments to α-actinin adsorbed to or inserted into a 1:1, DAG/DPPA planar supported lipid film: time course of the fluorescence from evanescent excitation. Trace A: 1/1 DAG/DPPA lipid film containing α-actinin blocked and incubated with NBD-actin filaments. Trace B: 1:1, DAG/DPPA lipid film with adsorbed α-actinin blocked and incubated with NBD-actin filaments. Flushing with F-buffer and exchange with gelsolin containing F-buffer (see also Materials and Methods) is indicated. Note that trace A is identical with trace C in Fig. 4.

The result is shown in Fig. 5, trace B. It clearly demonstrates that the binding efficiency for actin filaments to the adsorbed α-actinin is less than 15% compared to the one of the reconstituted α-actinin (*trace A*). This is an additional indication that α-actinin has to be incorporated into the lipid film, not merely adsorbed, in order to remain its function in terms of binding actin filaments.

CONCLUDING REMARKS

We have shown: (*a*) α-actinin selectively interacts with lipid films of a certain composition; (*b*) α-actinin incorporates into these films; (*c*) α-actinin remains in a functional conformation after reconstitution in lipid films on a solid support; (*d*) bound actin filaments to these α-actinin lipid lipid films are accessible to their regulatory proteins. Whether these findings are of physiological relevance is not addressed by this study. Our investigations do show that anchoring of actin filaments to lipid films in vitro is achieved by α-actinin.

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