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Interaction of NBD-talin with lipid monolayers

A film balance study

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Fluorescently labelled smooth muscle talin like native talin interacts with negatively or partly negatively charged lipid monolayers This was measured in time/area diagrams using the film balance technique combined with fluorescence imaging after double photolabelling of talin and phospholipids.

NBD-talin; Lipid monolayer; Protein/lipid double labelling

1. INTRODUCTION

In previous studies we have presented evidence that talin is a lipid [1] and an actin binding protein [2,3]. Moreover, we have investigated its nucleation promoting activity for actin filament formation in solution [4] and after reconstitution into lipid vesicles [5].

Here, we monitored the insertion behaviour of talin into negatively or partly negatively charged lipid monolayers by the film balance method measuring time/area diagrams. For direct observation we added *N*-(Texas red-sulfonyl)dipalmitoyl-L-α-phosphatidylethanolamine) to the lipids and labelled talin with 7-chloro-4-nitrobenzeno-2-oxa-1,3-diazole (NBD) using the fluorescence imaging technique.

2. MATERIALS AND METHODS

2.1. *Talin preparation*

Talin was isolated from chicken gizzard by the O'Halloran et al. method [6]. Talin was further purified by passing it through a gel filtration column as described in [4] and labelled when required with 7-chloro-4-nitrobenzeno-2-oxa-1,3-diazole (NBD) following the protocol of Detmers et al. [7]. Using this method approx. 5% of total proteins were labelled.

2.2. *Lipid preparation*

Mixtures of dimyristoyl-L-α-phosphatidylcholine (DMPC), dipalmitoyl-L-α-phosphatidylcholine (DPPC) and dimyristoyl-L-α-phosphatidyl-glycerol (DMPG) supplied from Avanti Polar (Birmingham, USA) were dissolved in chloroform/methanol solution of 9:1 (v/v). If not otherwise stated, these lipids were then spread onto a solution (subphase), containing 10 mM HEPES, 10 mM NaCl at pH 7 and 20°C, rendering a homogeneous lipid monolayer. *N*-(Texas red-sulfonyl)dipalmitoyl-L-α-phosphatidylethanolamine was purchased from Molecular Probes (Eugene, OR, USA). Water purified by Millipore Milli-Q-System was used for all buffers.

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2.3. *Film balance technique*

All experiments were performed on a film balance apparatus developed by Heyn et al. [8]. This unit consists essentially of a fluorescence microscope which is placed above a Langmuir trough. The microscope is mounted on a motorized *x–y* translation stage which allows one to observe most of the fluid surface. The base of the 30 ml trough is brass-plated and Peltier elements are installed below for temperature regulation (accuracy ~ 0.2°C) Most parts of the trough are covered by a glass slide to protect a spread lipid monolayer from impurities, air convection and fluid condensation. Surface pressure of a solution in the trough is measured by a Wilhelmy system. Pressure area diagrams of lipid monolayers are obtained by isothermal compression and expansion. Further, time scans at constant pressure recording area or at constant area recording pressure can be obtained. Scanning speed of the film balance apparatus and the process of recorded lateral pressure measurements are controlled by an IBM-compatible PC-AT. Images detected by fluorescence microscope could be directly observed by means of a SIT-camera (Hamamatsu, C 2400) on a video screen.

3. RESULTS

3.1. *Surface pressure of talin solutions*

For control, the surface pressure of talin was determined in various aqueous solutions. An increase in surface pressure over time was measured for native and fluorescently labelled talin with increasing salt concentrations (Fig. 1). To exclude buffer effects and an influence of protein labelling, subsequent experiments were carried out at a surface pressure of 25 mN/m, which is above the recorded surface pressures under all conditions. In analogy with other proteins [9], further addition of talin at a given saturation condition did not increase the surface pressure (data not shown).

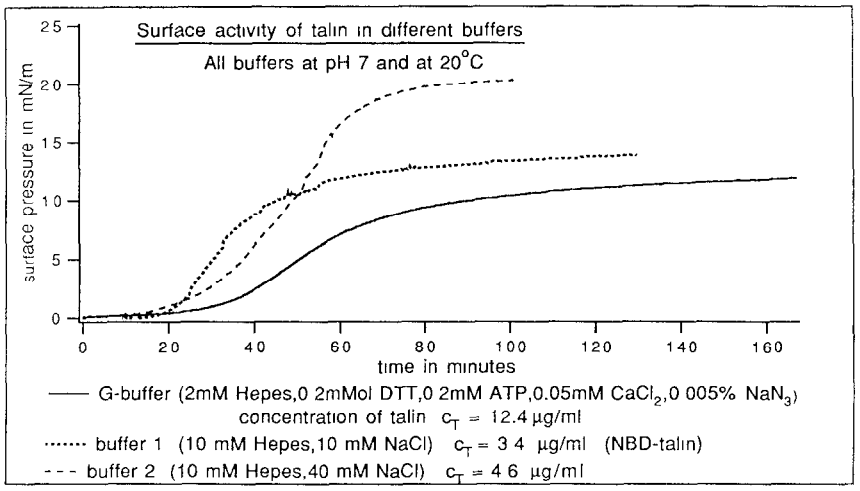

Fig. 1. Surface activity of talin in different buffers.

3.2. *Time/area diagrams*

The film balance apparatus allows the recording of time/area diagrams of lipid monolayers at constant pressure. All lipid monolayers at thermodynamic equilibrium normally show a constant area with time. Fig. 2 shows the effect of talin when injected (marked by arrow) into the subphase for a variety of lipids at different ratios. In these experiments, the pressure for all lipids was kept constant at 25 mN/m which was above the surface saturation pressure (cf. Fig. 1). Uncharged lipid monolayers (DPPC/DMPC) show no significant change in relative area with time; however, negatively (DMPG) or partly negatively (DPPC/DMPG) charged monolayers exhibit pronounced increases in relative area over time with saturation behaviour. For control, gelsolin known to interact at least with phosphatidylinositol-4,5-bisphosphate (PIP2) (cf. [10]), under identical experimental conditions did not exhibit any appreciable effect on the surface pressure of negatively charged lipid monolayers (Dietrich, unpublished observation).

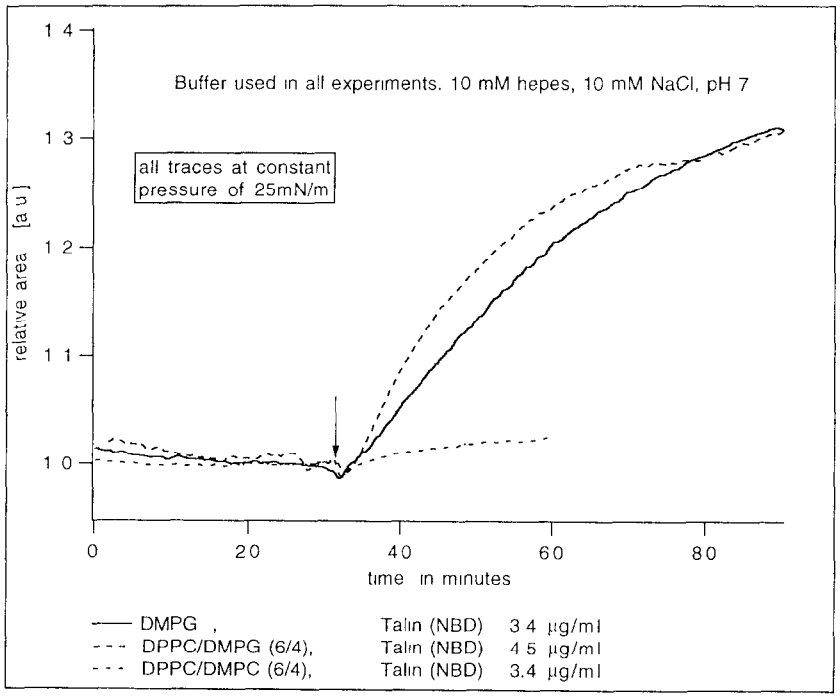

Fig. 2.

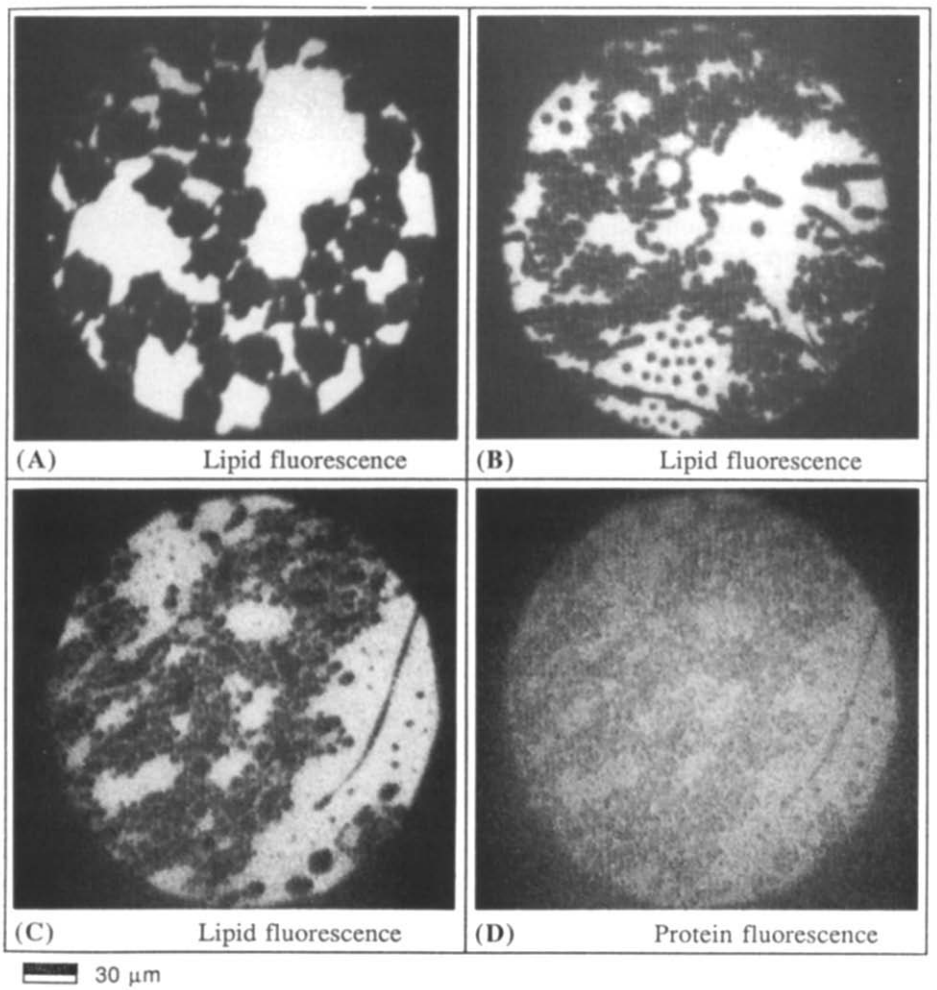

Fig. 3. DPPC/DMPG at a molar ratio of 6:4. (A) In the absence of talin (B) In the presence of talin (C) In the presence of talin 50 min after injection. (D) In the presence of talin 50 min after injection. Note: Fig. 3A–C display *Lipid fluorescence*; Fig. 3D shows *Protein fluorescence*. Experimental conditions: 0.2 nM talin; lipids compressed to 25 mN/m; Buffer: 10 mM HEPES, 10 mM NaCl, pH 7, 20°C.

3.3. *Fluorescence imaging of talin–lipid monolayer interaction*

For direct observation of talin–lipid monolayer interaction two different fluorophors were applied. For lipid labelling, small amounts (~ 0.1 mol%) of *N*-(Texas red-sulfonyl)dipalmitoyl-L-α-phosphatidyl-ethanolamine were added to the lipid solution. Excitation and emission wavelengths of 580 nm and 610 nm were used, respectively. Talin was labelled with NBD, and the fluorescence was measured at 530 nm after excitation at 480 nm. Analysis of the fluorescences was accomplished by using two different sets of filters which were easily interchangeable. This procedure allowed the observation of the two fluorophors at the same location.

In the experiment described here, we used DPPC/DMPG at a ratio of 6:4. The monolayer was compressed to a surface pressure of 25 mN/m for 30 min. At this pressure the monolayer is within the two-phase region [11]. Fig. 3A and B show the lipid fluorescence in the absence (Fig. 3A) and presence of 0.2 nM (NBD)-talin (Fig. 3B) 5 min after injection. The dark domains of these images represent the *crystalline* phase of the monolayer from which the Texas-red marked lipids are excluded; the light regions show the *fluid* phase. After injection of talin, significant changes of the *crystalline* domains – shown by lipid fluorescence (Texas-red) – are observed. Fig. 3C and 3D demonstrate lipid (Texas-red) and talin (NBD) fluorescence 50 min after injection, respectively. Since the fluorescence signal is multiply enhanced, it is not readily possible to correlate its efficiency with protein concentration.

4. DISCUSSION

Deduced from time/area diagrams (Fig. 2) the following features are of importance. (1) The curvature of all these traces show saturation behaviour with different area increase. (2) The increase of area for pure DMPG of about 40% can only be explained by the fact that talin inserts into the hydrophobic region of monolayers since the adsorption of protein at the lipid head group region can hardly explain the size of area increase. (3) The

protein incorporation cannot be due to the interaction of the protein with the water/air interface since then a continuous flow of protein from the subphase into the water/air interface should be observed. This behaviour was only seen for talin in the absence of lipids (data not shown).

In our experiments we used binary lipid mixtures which normally show a phase transition inducing partial separation of the two components [11]. It is, therefore, expected that dark domains are rich of components of lower phase transition pressure (here: DPPC) and the *fluid* regions are enriched with components of higher transition pressure (here: DMPG or DMPC). For DPPC/DMPG monolayers, it is hence assumed that these are composed of less charged, *crystalline* regions and negatively charged, *fluid* regions. As demonstrated in Fig. 3C and D talin predominantly appears in light, negatively charged *fluid* regions. (Note: lipid and protein fluorescence display the same images.)

In control experiments we replaced negatively charged DMPG with uncharged DMPC. DPPC/DMPC (6:4) monolayer exhibited similar domain structure. After talin injection, neither a contrast in protein images nor a change in lipid fluorescence of the *crystalline* domains could be detected.

In summary, using the film balance technique, we show that fluorescently labelled (NBD) talin as well as native talin are interacting with negatively charged lipid monolayers. This alternative technique agrees with previous observations by differential scanning calorimetry and Fourier transform infrared spectroscopy [1], selective lipid labelling with [^{3}H]PTPC/11 [3] as well as protein–lipid double labelling and light microscopic imaging [5] of talin–lipid interaction.

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