Self-Assembly of Actin Scaffolds at Ponticulin-Containing Supported Phospholipid Bilayers

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ABSTRACT Phospholipid vesicles containing ponticulin have been used to form solid supported and tethered bilayer lipid membranes. The ponticulin serves as both a nucleation site for actin polymerization as well as a binding site for F-actin. Studies of F-actin binding to such bilayers have demonstrated the formation of an in vitro actin scaffold. The dissociation constant for the binding of F-actin filaments to a ponticulin-containing tethered bilayer was found to be 11 ± 5 nM, indicative of high affinity binding.

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The cytoskeleton found in eukaryotic cells is usually anchored to the plasma membrane by protein complexes, an example of which is the dystroglycan/dystrophin complex. However, in *Dictyostelium discoideum*, this role is carried out by a single protein, ponticulin (1). Ponticulin has a molecular weight of ~17000 and is a Type VI membrane protein (2). It spans the plasma membrane, and also has a glycosyl-phosphatidylinositol anchor (3). Its main role within *D. discoideum* is to anchor the actin network to the plasma membrane, although it is also known to act as a nucleation site for the polymerization of G-actin (4). Sackmann et al. have previously studied actin networks on lipid bilayers using membrane associated proteins and charged lipids (5). However, here we show that the transmembrane protein ponticulin can be incorporated into planar supported and tethered phospholipid bilayers and can be used to create an in vitro mimic of the cytoskeletal scaffold. This opens new opportunities to study cytoskeletal function on planar surfaces, making them amenable to the wide battery of surface analytical techniques currently available.

D. discoideum was grown axenically and the ponticulin extracted following the scheme of Chia et al. (4). An F-actin sedimentation assay performed upon the extract showed that the major actin binding protein present was ponticulin. The ponticulin-containing extract was reconstituted into egg-derived phosphatidyl choline (egg-PC) lipid vesicles by detergent solubilization and dilution (6). This technique generates vesicles ranging in diameter (from 80 nm to 2 μm). Incubation of such vesicles with F-actin leads to vesicle decoration of the filaments (Fig. 1). Analyses of such micrographs show that ~85% of the ponticulin-containing vesicles are in contact with the F-actin filaments. In contrast, if ponticulin is not present in the vesicles only ~16% of vesicles are in close proximity to the filaments.

These egg-PC vesicles were used to form solid supported phospholipid bilayers, on clean glass coverslips. Their fluidity was determined from fluorescence recovery after photobleaching experiments, by incorporating 1% of a fluorescently labeled lipid (NBD-PC) (7–9). When 6% (w/w) ponticulin-containing extract is included, the fluidity of the bilayer drops from $2.7 \pm 0.3 \times 10^{-8}$ cm^2/s, for pure egg-PC bilayers, to $1.4 \pm 0.3 \times 10^{-8}$ cm^2/s. Previously reported values for fluid phase egg-PC bilayers are between 2 and 10×10^{-8} cm^2/s (10).

Fig. 2 shows the results of studies of F-actin binding to both solid-supported and tethered lipid bilayer membrane systems (both with and without ponticulin). Comparison of Fig. 2, *b* and *f*, clearly demonstrates that the presence of ponticulin dramatically increases the amount of actin irreversibly bound to the membrane surface.

Tethered lipid bilayer membranes can also be formed from ponticulin-containing vesicles. Here mixed self-assembled monolayers (SAM) containing 40% Eo3-cholesterol and 60% mercaptoethanol (by surface coverage) were formed via adsorption from a 1 mM propanol solution (11,12). This gives rise to ~60% of the surface area being covered by a phospholipid bilayer, whereas the remainder is a hybrid bilayer consisting of a phospholipid monolayer adsorbed onto the hydrophobic cholesterol regions. Our previous surface plasmon resonance studies on egg-PC bilayers leads one to expect a change in thickness, after bilayer formation, of 39 ± 1 Å (12). With ponticulin-containing bilayers we find an average adsorbed layer thickness of 42 ± 1 Å. This close agreement is expected because there is <6% protein present within the bilayer.

After interaction with F-actin we find in the case of pure egg-PC, only a small amount of nonspecific binding, equivalent to an average increase in thickness of 0.6 ± 0.5 Å (Fig. 2 *d*). In the presence of ponticulin, however, the effective thickness increase, associated with the binding of the F-actin, is 9.3 ± 1.3 Å (Fig. 2 *h*). If it assumed that the actin is present

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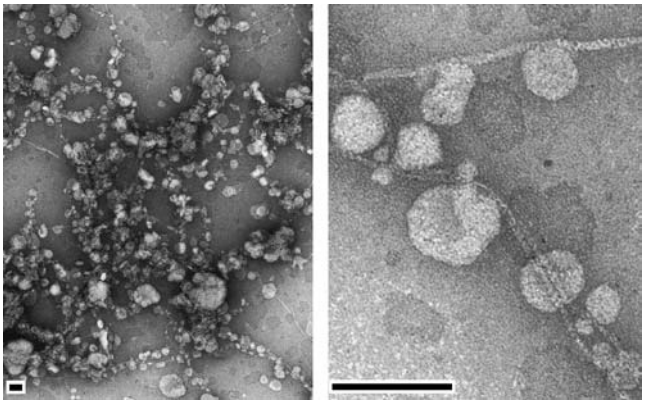

FIGURE 1 Transmission electron micrographs showing the decoration of F-actin filaments with ponticulin-containing egg-PC phospholipid vesicles. Scale bars represent 100 nm.

as a planar network situated on the bilayer, with an average filament diameter of 7 nm, an estimate for the percentage surface coverage can be made. In the absence of ponticulin the actin surface coverage is calculated to be 0.9%, whereas in the presence of ponticulin, it is calculated to be 13%.

By measuring the amount of adsorbed material (change in SPR resonance position) as a function of actin concentration one can determine the dissociation constant (K_d) for the interaction of F-actin filaments with a ponticulin-containing egg-PC lipid bilayer. Fig. 3 shows the equilibrated change in thickness versus actin concentration (where actin concentration refers to the concentration of G-actin before polymerization). The change in thickness was determined from the plot of the adsorbed layer thickness versus time (Fig. 3, *inset*). The Hill equation was used to fit the data using an iterative method from which K_d was calculated to be 11 ± 5 nM. This suggests relatively high affinity binding between the

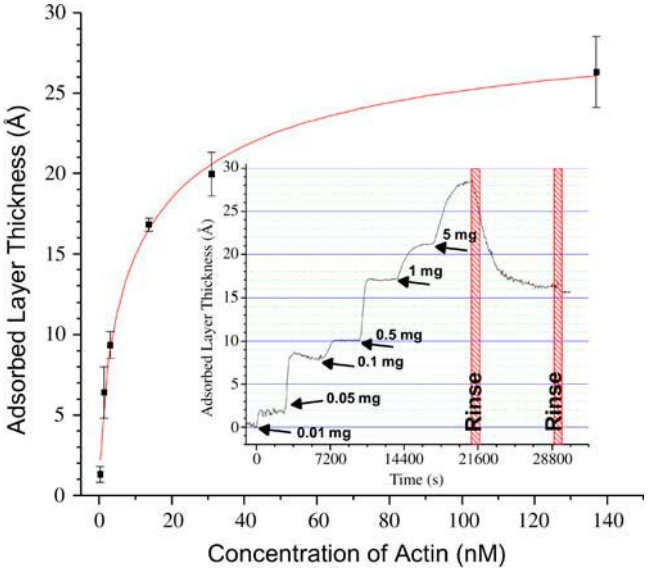

FIGURE 3 A plot showing the thickness of bound actin (measured by surface plasmon resonance spectroscopy) versus actin concentration, for a ponticulin-containing lipid bilayer. The data were fitted (*red line*) using the Hill equation. The inset shows kinetic data after the sequential addition of increasing quantities of F-actin. Arrows mark times of actin addition.

ponticulin-containing lipid bilayer and the prepolymerized, and stabilized, F-actin filaments. This is the first estimate of the ponticulin-actin dissociation constant and places ponticulin toward the high affinity end in the range of actin binding proteins (13–17).

In conclusion, we have shown that the transmembrane protein, ponticulin, acts as an anchor for the actin scaffold both on glass and SAM modified gold supports. The ability to create mimics of the actin cytoskeletal scaffold on

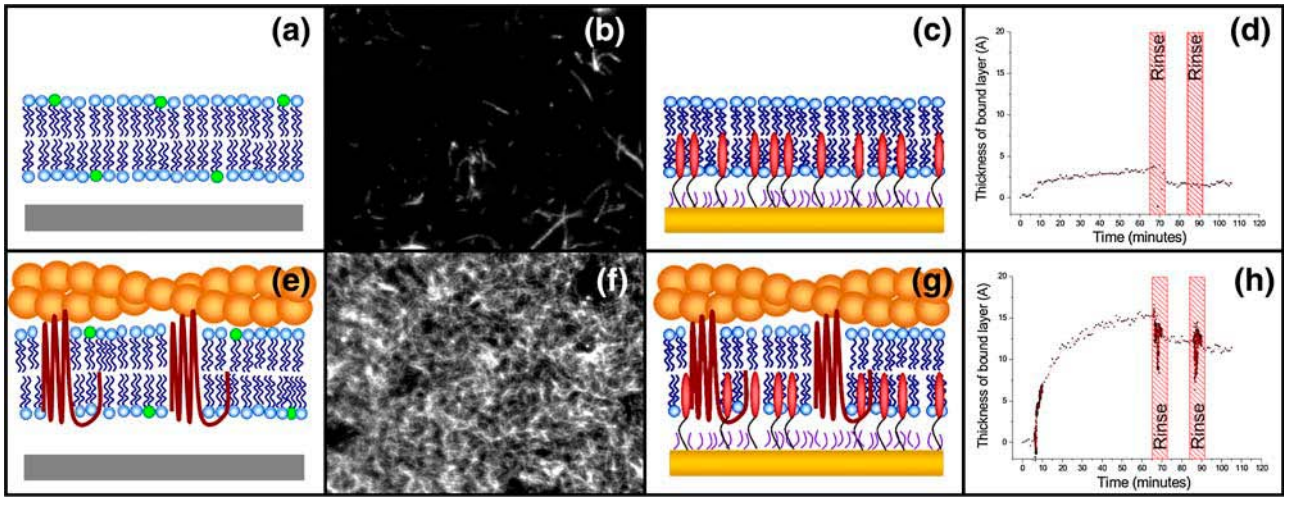

FIGURE 2 The interaction of F-actin with solid supported and tethered egg-PC lipid bilayers in the absence (*a–d*) and presence (*e–h*) of ponticulin. Panels *b* and *f* are fluorescence micrographs (post rinse) showing rhodamine-labeled, bound F-actin filaments. Panels *d* and *h* show typical plots for the change in adsorbed material as determined by surface plasmon resonance studies.

supported and tethered bilayers provides a model platform for studying the in vivo behavior of the cytoskeleton.

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