Actin Conformation Is Drastically Altered by Direct Interaction with Membrane Lipids: A Differential Scanning Calorimetry Study†

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ABSTRACT: One of the current dogmas in cytoskeleton research holds that actin filaments are attached to the cell membrane through integral membrane actin-binding proteins. We have challenged this concept, using an *in vitro* system composed of pure actin and liposomes, and have found that actin may also interact with membrane lipids. Differential scanning calorimetry (DSC) shows that when the actin molecule is in contact with such lipids, it undergoes a major conformational change which results in the complete disappearance of its phase transition. Conversely, DSC scans reveal that the phase transition of the membrane lipids is only weakly affected by the presence of actin. Indeed, the lipids' main transition shows only slight shifts in T_m, from 56.6 to 57 °C, and ΔH_{cal}, from 10.1 to 8.8 kcal/mol. In the lipids' pretransition, T_p is shifted from 52.7 to 53.7 °C, and ΔH_{cal} is shifted from 0.75 to 0.33 kcal/mol. This interaction between purified actin and membrane lipids is inhibited by high concentrations of KCl, thus indicating that the phenomenon is primarily electrostatic in nature. The ultrastructural consequences of this change in actin conformation were investigated by electron microscopy, which revealed the formation of paracrystalline arrays of actin filaments at the surface of the liposomes. We therefore propose a model in which a limited number of lipid molecules may interact with specific sites on the actin molecule, resulting in the protein's observed conformational change.

Actin is a ubiquitous cytoskeletal protein which is involved in cell motility and morphogenesis. Many studies have documented the attachment of actin filaments to the plasma membrane in a variety of cell types and organisms (Gruenstein *et al.*, 1975; Mescher *et al.*, 1981; Davies, 1984; Luna *et al.*, 1984). Indeed, this actin–membrane interaction is essential to many cellular activities such as motility (Comly, 1973), intracellular organelle movement (Kuznetsov *et al.*, 1992), plasma membrane dynamics (Buss *et al.*, 1992), exocytosis (Aunis & Perrin, 1984), pinocytosis (Klein & Stockem, 1979), phagocytosis (Bailey *et al.*, 1987), ruffling in lymphocytes and fibroblasts (Buckley & Raju, 1976), and cytokinesis (Fukui & Inoue, 1991).

Most of the evidence to date has supported the predominant view that actin filaments are anchored to the membrane through actin-binding proteins. Likely candidates include integral membrane proteins such as ponticulin in *Dictyostelium* (Wuestehube & Luna, 1987), the laminin receptor (Brown *et al.*, 1983), the glycoprotein II/III$_a$ complex in platelets (Parise & Phillips, 1986), hisactophilin (Scheel *et al.*, 1989), myosin I (Korn & Hammer, 1990; Pollard *et al.*, 1991; Zot *et al.*, 1992), and the glycoprotein complex in tumor cell microvilli (Carothers *et al.*, 1991). Alternatively, the attachment has been proposed to occur via a multi-protein network involving spectrin/fodrin (Bennett, 1989), protein 4.1 (Bennett, 1984), α-actinin (Rotman *et al.*, 1982), vinculin (Otto, 1990), and talin (Burridge & Connell, 1983). Although the exact molecular mechanisms controlling actin–membrane interactions are still poorly understood in many systems, the need for membrane proteins acting as linkers of actin filaments remains the predominant concept in this field of research (Niggli & Burger, 1987; Isenberg, 1991). The list of such proteins involved in cytoskeleton–membrane interactions has grown rapidly, and with it the necessity for new reviews (Niggli & Burger, 1987; Carraway & Carraway, 1989; Burridge & Jackman, 1990; Stossel, 1990; Isenberg, 1991; Luna & Hitt, 1992).

The possibility that actin may also interact directly with membrane lipids has rarely been envisaged, having been mentioned in only a few preliminary reports. Okimasu *et al.* (1986) showed that liposomes are able to bind many cytoplasmic proteins, and they identified one of these as F-actin. Also, using the monolayer technique, Llerenas and Meza (1980) found that actin modified the surface isotherm of lipids, suggesting a possible interaction. This was recently supported by the observations of Bärmann *et al.* (1992), who, using DSC, documented similar subtle changes in the thermograms of lipids. However, these studies were preliminary in nature and therefore rather inconclusive. Using an *in vitro* system composed of pure preparations of lipid vesicles and actin, we have previously demonstrated that actin can interact directly with lipids without the need for intermediate linker proteins (St-Onge & Gicquaud, 1989, 1990). Importantly, this was observed under conditions compatible with those prevailing *in vivo* (St-Onge & Gicquaud, 1990).

In the present study, we have used differential scanning calorimetry (DSC) to determine the thermodynamic parameters of this direct actin–lipid interaction, using purified actin and liposomes. Interestingly, we find that this primarily electrostatic interaction induces a major conformational change in the actin but not in the lipid molecules. This appears to result in the formation of a highly ordered array of actin filaments on the liposome membrane surface, as observed by electron microscopy.

MATERIALS AND METHODS

Preparation of Actin. Actin was extracted from rabbit striated muscle acetonic powder by the method of Spudich

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and Watt (1971) as modified by Nonomura *et al.* (1975). Actin was obtained in its monomeric form and dissolved in a low ionic strength buffer composed of 2 mM Tris·HCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, and 0.01% sodium azide, pH 8.0.

Preparation of Liposomes. Liposomes were prepared by the freezing–thawing method of Bally *et al.* (1988) as follows: A solution of 50 mg of distearoylphosphatidylcholine (DSPC), from Sigma Co., in chloroform was evaporated under vaccum in a rotoevaporator to get a film of dry lipids. Lipids were hydrated in 5 mL of low ionic strength buffer and resuspended by vortexing. This lipid suspension was frozen in liquid nitrogen and thawed at 70 °C, above the phase transition of DSPC. This was repeated three times. Each preparation was checked by electron microscopy to confirm the formation of single unilamellar liposomes.

Analysis by Differential Scanning Calorimetry. In a standard preparation, 0.4 mL of 10 mg/mL DSPC liposomes was mixed with 0.4 mL of a G-actin solution of appropriate concentration. After 30 min of incubation, actin was induced to polymerize by addition of $MgCl_2$ solution at a final concentration of 2 mM. Each solution was degassed for 5 min under low pressure before use.

Differential scanning calorimetry was performed with a Hart differential scanning calorimeter equipped with three stainless steel ampules. The first one contained 0.9 mL of actin alone; the second, liposomes alone; and the third, actin + liposomes. The scanning rate was 40 °C/h for all experiments. The baseline was determined by using the buffer alone.

Calorimetric enthalpy, ΔH_{cal}, was calculated from the aera under the curve by using a program provided with the calorimeter. The van't Hoff enthalpy was calculated using the equation $\Delta H_{vH} \simeq 6.9 T_m^2 / \Delta T_{1/2}$)(Mabrey-Gaud, 1981). The cooperative unit, which represents the degree of cooperativity between the molecules during the phase transition, is defined as CU = $\Delta H_{vH}/\Delta H_{cal}$ (Blume, 1991; McElhaney, 1992.)

Electron Microscopy. Samples were diluted to get a final concentration of actin between 0.1 and 0.05 mg/mL. One drop of the solution was deposited on a Formvar–carbon-coated grid treated with a plasma glow discharge. The samples were negatively stained with 1% uranyl acetate in water.

RESULTS

Differential Scanning Calorimetry. Preparations of pure actin alone, pure liposomes alone, and a mixture of both were analyzed in parallel by DSC, yielding the thermograms shown in Figure 1. Actin alone shows a single sharp transition having a T_m = 71 °C, which corresponds to the thermal denaturation of the actin molecule. The pure liposome preparation shows a phase transition typical of DSPC, with two peaks: a small pretransition at 52.7 °C and a main transition at 56.6 °C. The thermogram of the actin + liposome mixture shows important modifications, clearly due to the interactions between the two components. These modifications are summarized in Table I. The most important change is the complete disappearance of the phase transition of actin revealing a drastic change in the conformation of the actin molecule. The phase transition of the lipid is only slightly modified in the presence of actin. The peak of the main transition shows a T_m shift of only 0.4 °C, from 56.6 to 57 °C, and the calorimetric enthalpy decreases only from 10.1 to 8.8 kcal/mol. The peak of the main transition is broadened, indicating a lower cooperativity between the lipid molecules. The cooperative unit decreases from 46 to 38. Changes are more pronounced on the pretransition: Although T_p is only shifted from 52.7 to 53.7 °C, ΔH_{cal} is greatly diminished, from 0.75 to 0.33 kcal/mol, in the presence of actin.

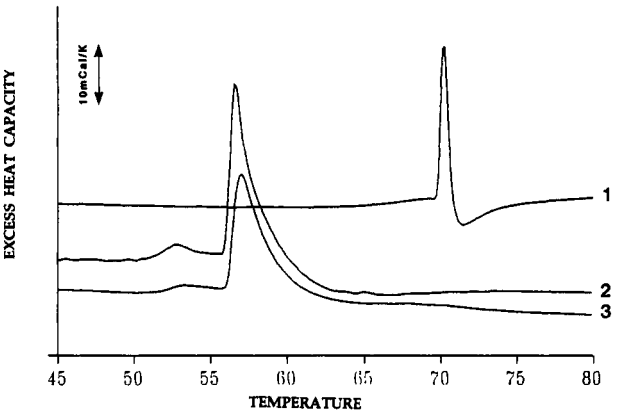

FIGURE 1: DSC scans of actin alone (1), DSPC liposomes alone (2), and actin + liposomes (3) dissolved in the low ionic strength buffer. The concentration of actin was 1.4 mg/mL, and that of DSPC was 4.2 mg/mL. The curves are translated for clarity of presentation.

Table I. Comparison of Calorimetric Parameters of DSPC Liposomes Alone and in the Presence of Actin[a]

	pretransition		main transition			
	T_p (°C)	ΔH_p (kcal/mol)	T_m (°C)	ΔH_{cal} (kcal/mol)	ΔH_{vH} (kcal/mol)	CU
DSPC	52.7	0.75	56.6	10.1	468	46
DSPC + actin	53.1	0.33	57.0	8.8	341	38

[a] T_p and ΔH_p are the temperature and the enthalpy of the pretransition. T_m and ΔH_{cal} are the temperature and the calorimetric enthalpy of the main transition. ΔH_{vH} is the van't Hoff enthalpy; CU = cooperative unit.

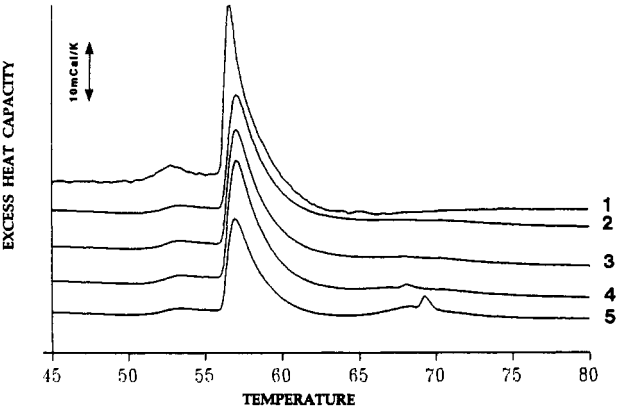

FIGURE 2: DSC scans of DSPC liposomes in the presence of increasing concentrations of actin. The concentration of DSPC liposomes was constant at 4.2 mg/mL. Actin:lipid ratios: (1) 0, (2) 0.28, (3) 0.33, (4) 0.50, and (5) 0.99 mg of actin/mg of lipid.

The results of this calorimetric data show that actin interacts with liposomes composed of DSPC and that this interaction results in a major conformational change in the actin molecule, with only minor modifications to the lipid organization in the membrane bilayer.

We further studied this interaction by examining liposomes in the presence of increasing concentrations of actin (Figure 2). The resulting thermograms reveal that the actin phase transition is undetectable at low concentrations of actin (below 0.5 mg of actin/mg of lipid), but begins to appear as a small peak at higher concentrations. This change is believed to be caused by the saturation of actin-binding sites on the liposome

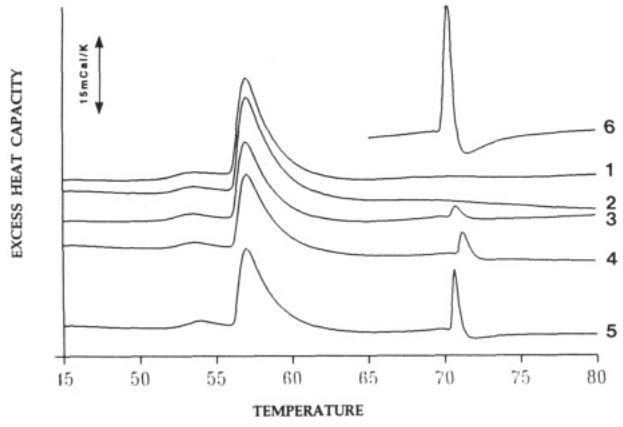

FIGURE 3: DSC scans of DSPC liposomes and actin in the presence of increasing concentrations of KCl. Actin concentration wa 1.4 mg/mL; DSPC liposome concentration was 4.2 mg/mL. KCl concentrations were (1) 0, (2) 10, (3) 50, (4) 100, and (5) 200 mM. Trace 6 shows actin alone, without liposomes.

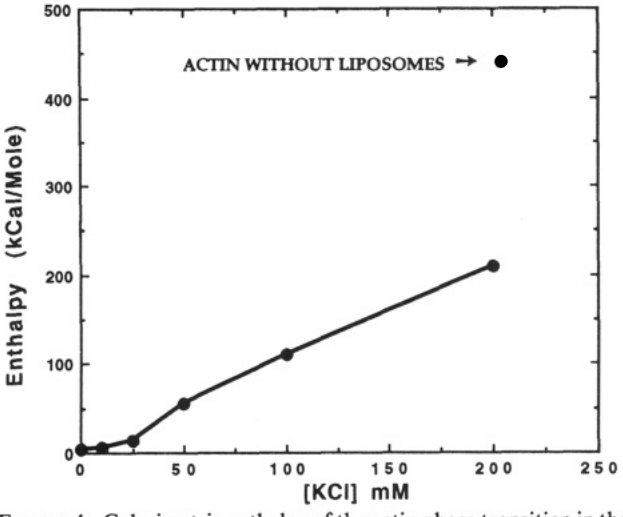

FIGURE 4: Calorimetric enthalpy of the actin phase transition in the presence of liposomes at high KCl concentrations. Actin concentration was 1.4 mg/mL, and DSPC liposome concentration was 4.2 mg/mL.

surfaces. Indeed, only actin molecules which are in direct contact with the liposome surface are expected to undergo the said conformational change, resulting in the disappearance of their phase transition. However, any actin present in excess of the available binding sites on the liposomes cannot interact directly with the lipids and, therefore, is not expected to undergo the same changes. Hence, these excess actin molecules are believed to be responsible for the reappearance of the phase transition at high actin:liposome ratios.

The nature of the direct actin–lipid interaction was investigated by measuring the phase transitions of actin–lipid preparations in the presence of increasing concentrations of KCl (Figure 3). These experiments show that low levels of KCl do not noticeably change the phase transition profile of these preparations (compare profiles 1 and 2 of Figure 3). However, at KCl concentrations above 50 mM, the actin phase transition peak reappears progressively (compare profiles 3–5 with profiles 1 and 6 of Figure 3). It should be noted, however, that for the highest concentration of KCl assayed (200mM) the calorimetric enthalpy of the actin peak remains inferior to that of the peak of actin alone (Figure 4), suggesting that some of the actin remains attached to the liposomes even at such high KCl concentrations

Electron Microscopy. When actin is mixed with a suspension of liposomes, visible aggregates immediately form,

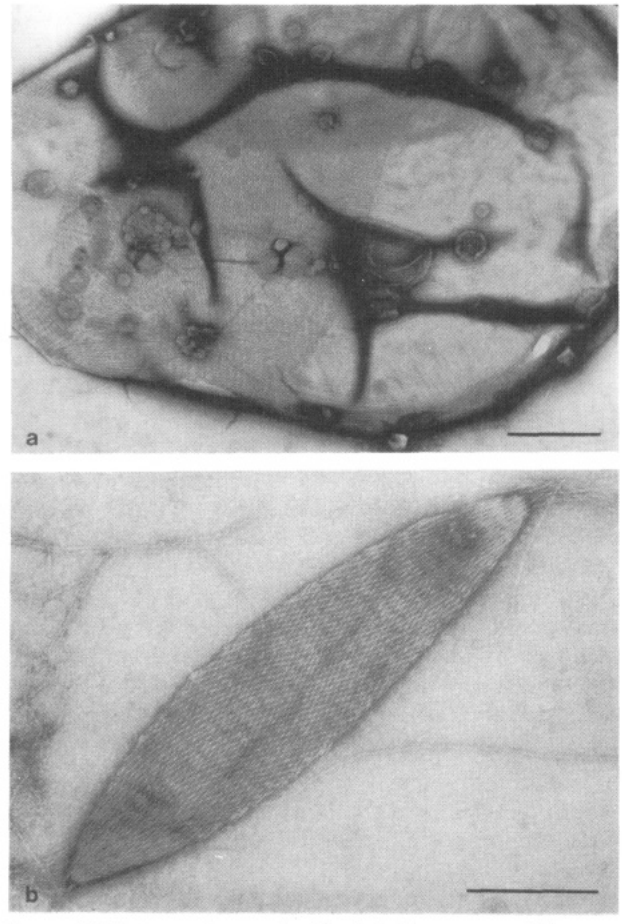

FIGURE 5: Electron micrographs of actin in the presence of liposomes. (a) A large liposome which occupies nearly the entire field of the micrograph is covered with a sheet of parallel actin filaments. In the lower part of the field, the filaments are in register, forming a paracrystalline sheet. (b) The actin filaments on this liposome are organized in a net-like organization and constrain the liposome to an elongate shape. Actin filaments which are not in contact with the liposome do not exhibit ordered patterns and appear as ordinary actin microfilaments. Bar = 0.5 μm.

indicating the occurrence of an interaction between both constituents. This is not produced by precipitation of denatured actin, since electron microscopic examination of the preparation shows the actin filaments covering the entire surface of the liposomes and organized in highly ordered arrays. Two types of organization may be observed: paracrystalline sheet and net-like organization.

Figure 5a shows a large liposome (3 μm diam.) which occupies nearly the entire field of the micrograph. At its surface, actin can be seen organized in multiple arrays of parallel filaments, covering the liposome completely. In several regions (particularly visible in the lower part of the field), the said filaments are in exact register, forming a paracrystalline sheet. In Figure 5b, a single liposome is shown to be covered by two distinct subpopulations of parallel filaments at an angle to one another, in a net-like organization. The constraint imposed by these filaments appears to cause an elongation of the liposome. Both types of organization may exist on the same liposome. In a standard preparation, 90% of filaments are in a paracrystalline sheet and 10% are in a net-like organization. Only the actin molecules at the surface of liposomes are organized in paracrystalline sheets or nets: actin not in contact with liposomes is present as "conventional" actin filaments. This is particularly evident in Figure 5b, on both extremities of the liposome, where filaments organized in regular arrays at the liposome surface become randomly

distributed as they lose contact with the membrane. This observation supports the earlier interpretation of the reappearance of the actin phase transition at high actin:liposome ratios (Figure 2).

DISCUSSION

In the present study, we have demonstrated that actin can interact with liposomes and form well-defined superstructures. Since no other proteins are present in these assays, it is concluded that the change in actin conformation demonstrated by calorimetric data and the formation of highly ordered actin filament arrays observed by electron microscopy both result from direct binding of actin to membrane lipids. Furthermore, the paracrystalline and net-like patterns of actin arrays observed on the surface of liposomes rule out the possibility that this may result from nonspecific adsorption of actin on lipids. This would be expected to yield a random deposit of filaments on the liposome surface, like that seen between liposomes in these preparations.

The most important effect of the observed actin–lipid interaction is the complete loss of the actin unfolding endotherm. This disappearance of the phase transition is not due to a stabilization of the actin, since this would be expected to result in an upward shift of the phase transition temperature, and this has not been detected in scans up to 100 °C.

The alternate possibility that the actin phase transition may be shifted to the 55–60 °C range where it could be masked by the lipid transition peak is also not the case here. Indeed, similar experiments using DLPC, which has a phase transition around 0 °C showed no actin phase transition, even within the 55–60 °C range (not shown).

Hence, disappearance of the actin phase transition may be due to an extreme broadening of the peak, whereby the excess heat capacity would disappear into the baseline. This can result from a change in the conformation of the actin molecule, induced by the contact with lipid, or from the influence of the lipid phase during the proteins' unfolding process. Indeed, a positive ΔC_p for protein unfolding is mostly attributed to the exposure of buried apolar groups to water. In a recent paper, Fu and Freire (1992) showed that the change in heat capacity (ΔC_p) for protein unfolding decreases with increasing solvent hydrophobicity. In our system, actin is present at the surface of liposomes and, hence, in very close proximity to the very hydrophobic phase of the lipids. One might therefore indeed expect a drastic decrease in ΔC_p. If this is the case, it implies that the acyl chains of the lipids play a role in actin unfolding.

Contrary to actin organization in the presence of lipid, the molecular packing of the lipid molecules composing the bilayer is only weakly affected in the presence of actin. The slight decrease in pretransition is consistent with a change of the bilayer from an $L_{\beta'}$ organization, where the lipid molecules are parallel and tilted with respect to the bilayer normal, to a $P_{\beta'}$ phase whereby the bilayer displays periodic ripples (Blume, 1991, 1988). Then, in the presence of actin, it appears that the rippling of the lipid bilayer is impeded or, possibly, that the bilayer is already rippled. A tripartite classification scheme for lipid–protein interactions, proposed by Papahadjopoulos *et al.* (1975), is commonly used. The three categories are defined by the effects of these interactions on the main lipid phase transition. In the present case of direct actin–lipid interaction, however, this parameter is not significantly affected. Thus, our DSC results do not permit the classification of the actin–lipid interaction within this scheme and may signify a fourth type of lipid–protein interaction.

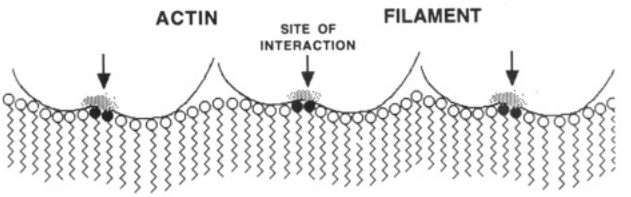

FIGURE 6: Proposed model for the direct interaction of actin with membrane lipids.

The mechanism of actin–lipid binding is proposed to involve a lipid-induced change in actin conformation, permitting periodic lateral associations between actin filaments and the lipid component of biomembranes. This is then believed to give rise to the observed paracrystalline and net-like patterns of filament arrays on the liposome surfaces. We propose the following model, presented in Figure 6, in which actin interacts with lipids through a limited number of binding sites. Occupation of these sites by lipid molecules induces a change in actin conformation and leads to its subsequent paracrystallization. However, since the surface area of the actin molecule is very large compared to that of the lipid molecule, only a small proportion of available membrane lipid molecules is expected to be involved in this binding. The predicted effect on the lipids' molecular packing should then be minimal, which would explain the observed negligible changes in the lipid thermograms in the presence of actin. Finally, this actin–lipid binding is thought to be mainly electrostatic in nature. Indeed, actin is a very hydrophilic protein, and its interaction with lipids is reversed by high salt concentrations. Also, we have shown previously that it is favored by millimolar concentrations of divalent cations, namely, either magnesium or calcium (St-Onge & Gicquaud, 1990). Since the phosphate group of the phospholipids has high affinity for Ca^{2+} and Mg^{2+}, these cations will bind to the lipid bilayer (Herbette *et al.*,1984; Mc Laughlin *et al.*, 1978; Lis *et al.*, 1981.), giving rise to a charged surface of the sort that has been previously shown to polymerize actin and to bind actin filaments (Laliberte & Gicquaud, 1988) and paracrystals (Ward *et al.*,1990; Taylor & Taylor, 1992.)

Certain lines of evidence have tended to discredit the possibility of a direct actin–lipid interaction. Particularly, Bercovici and Gitler (1978) Meyer and Burger (1979), and Sigrist-Nelson *et al.* (1977) have reported that actin is not labeled with the photoaffinity probe INA when it is incorporated into membranes, and they have therefore concluded against a direct actin–lipid interaction. However, it is known that the INA molecule partitions to the hydrophobic part of the membrane. In view of our present model, which predicts an interaction of actin with the polar head groups of a limited subset of lipid molecules, their results are not incompatible with ours. Furthermore, an interesting exception to the INA data was reported in platelets (Rotman *et al.*, 1982).

It should also be noted here that although this mechanism is conceivable for actin and pure lipids *in vitro,* the *in vivo* situation may be more complex, since it is possible that part of the actin may be acylated by palmitic acid (Stadler *et al.*, 1985) or isoprenoid residues (Cutts *et al.*, 1989). The function of this acylation is not known, but it is expected to affect the hydrophobicity of actin and, therefore, its interaction with lipids.

We have found a direct interaction between actin and membrane lipids *in vitro,* and as usual, this provokes the question: does this phenomenon occur *in vivo*? In cells, the concentration of Mg^{2+} is estimated to be between 1 and 8 mM (Fulton, 1985), and that of K^+, around 100 mM. Although

on the basis of our findings these ionic conditions are not optimal for direct actin–lipid binding, they are clearly predicted to offer a suitable environment to promote these interactions. Even if our results do not prove that this phenomenon exists *in vivo*, they suggest that it is possible.

Previous studies have shown that most of the actin which is attached to cell membranes can be readily removed by washing, whereas the rest is tightly bound (Davies, 1984; Gert de Couet *et al.*, 1984; Gruenstein *et al.*, 1975). This tightly bound actin appears to be associated with membrane proteins. It is conceivable that part or all of the easily extractable actin may interact with lipids by the mechanism we have described here. Hence, our results are not incompatible with the studies which have proposed that actin is bound to membranes via membrane proteins. Rather, they lead to the conclusion that two types of actin–membrane attachment may exist in cells, direct interaction with lipids and anchoring via integral proteins.

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