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RESEARCH ON THE MECHANISM OF INTERACTION BETWEEN ACTIN AND MEMBRANE LIPIDS

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SUMMARY. Using an *in vitro* system involving pure actin and liposomes, we have established that actin may interact with membrane lipids without any intermediate proteins, and that the mechanism of interaction depends upon the concentration of divalent cation. In the absence of divalent cation, actin increases membrane permeability. Low concentrations (1 mM) of divalent cation potentialize this interaction. In the presence of high divalent cation concentration, actin deposits on the surface of liposomes in a crystalline organization and reduces the membrane microviscosity as shown by the polarization of fluorescence of the DPH probe. We propose that actin interacts with lipids by hydrophobic association which is facilitated by initial electrostatic binding.

It is well known that actin filaments may attach to the cell membrane. These microfilament-membrane interactions play an important role in governing cell motility, morphology, and organization of components at the cell surface. The current views stress that actin microfilaments are anchored to membranes by integral actin-binding- proteins (1 - 5). Four of these proteins have been identified: laminin receptor (6), a fraction of glycoprotein IIb-III in platelet (7), cytoskeleton-associated glycoprotein from ascites tumor cells (8) and ponticulin from dictyostelium (9). However, in many cells, the linkage is indirect. In erythrocytes, actin filaments are attached to the membrane by a complex of spectrin - band 4.1-glycophorin (10) and in brush border microvilli, lateral attachment of actin is via the 110 kDa- calmodulin complex (11) which has been identified as a myosin I (12).

Direct attachment of actin to phospholipids has rarely been envisioned. This possibility is mentionned in a paper of Weinstein (13) and Okimasu et al (14) but extensive work has not been carried out. Recently, we showed that actin binds to positively charged liposomes, although in unphysiological conditions (15, 16).

In the present communication, we report that actin may interact directly with phospholipids in conditions compatible with those prevailing *in vivo* by a mechanism of hydrophobic association which is facilitated by initial electrostatic binding.

MATERIALS AND METHODS

Preparation of actin. Actin was prepared from acetonic powder extract of rabbit striated muscle by the technique of Spudich and Watt (17) as modified by Nonomura et al (18). Standard preparation of actin was

0006-291X/90 $1.50

dissolved in the low ionic strength buffer having the following composition: Tris HCl 2.0 mM; ATP 0.2 mM; $CaCl_2$ 0.2 mM; b mercaptoethanol 0.5 mM; pH 8.0. Ca^{++} or Mg^{++} concentrations were adjusted using their chloride salts.

Preparation of liposomes. Small unilamellar liposomes (SUV) were prepared by sonication. Briefly , 33 m Mole of lipids dissolved in chloroform were evaporated with a rotoevaporator. The lipids were resuspended in 5 ml of aqueous phase, vortexed, and sonicated 60 min at 30°C with a Megason P-300. The preparation was sedimented 10 min X 110 000 g to eliminate titanium particles. Large unilamellar vesicles (REV) were prepared by the reversed phase technique of Szoka and Papahadjopoulos (19) using 72 m Mole of lipids for each preparation.

Turbidimetry. Interaction of actin with liposomes may produce aggregates. This phenomenon was quantified by measuring the absorbance at 350 nm of mixtures of actin and liposomes. The results are expressed in differential turbidity = (turbidity of the mixture - turbidity of the sum of each constituent alone). In all experiments final concentrations were 0.3 mg/ml for actin and 0.21 mM for lipids.

Electron microscopy. Samples were diluted to get a final concentration of actin between 0.1 and 0.05 mg/ml. One drop was deposited on a formvar-carbon coated grid previously treated with a plasma glow discharge and negatively stained with 1% uranyl acetate solution.

Measure of liposome permeability. The permeability of liposomes membrane was determined by measuring the exit rate of liposome-encapsulated 6 carboxyfluorescein following a protocole described by Weinstein et al (20). 6 carboxyfluorescein was encapsulated into liposomes during the preparation procedure by sonication using 100 mM of carboxyfluorescein in aqueous phase. Unencapsulated carboxyfluorescein was removed from the preparation by chromatography on Sepharose 6 B, 15 x 30 mm column. Carboxyfluorescein encapsulated in liposomes at final concentration 6.5 mM of lipids/ml were mixed with actin at concentrations indicated in the Results. After 30 min of incubation at room temperature the concentration of carboxyfluorescein outside the liposome was measured by fluorescence using 496 nm excitation length and 514 nm emission.

Fluorescence polarization. The effect of actin on the fluidity of the liposome membrane was measured by fluorescence polarization of the DPH probe incorporated into the lipid bilayer. Liposomes were prepared by sonication, and the DPH probe incorporated at a ratio lipids/probe = 750 as recommended by Shinitsky et al (21). These liposomes at a concentration of 0.15 mM lipids/ml in various ionic conditions were mixed with actin. After 30 min of incubation at room temperature, fluorescence was measured using a Shimatzu RF 540 spectrofluorimeter equipped with fluorescence polarization equipment. Excitation wavelength was set at 360 nm and emission at 430 nm. Fluorescence polarization was calculated as Shinitsky et al (21) with correction for light diffusion produced by liposomes alone.

RESULTS

When actin is added to a suspension of liposomes in favorable ionic conditions, the solution become immediately turbid due to the formation of aggregates of actin and liposomes.This reaction shows that a direct interaction may exist between actin and membranar lipids.

The aggregates may be observed directly by electron microscopy. They consist of liposomes linked by actin filaments. Several figures are obtained depending on the size of the liposomes. With large liposomes (1 µm) (Fig. 1A) actin filaments deposit on the surface with a periodicity of 37.5 nm corresponding to the half pitch of the filament, or they cover the surface of the liposome in a net like organization (Fig. 1B). With small liposomes prepared by sonication (100 nm) (Fig. 1C), bundles of parallel actin filaments confine liposomes which adopt a rectangular shape under the constraint imposed by the filaments. These aggregates are formed only in the presence of more than 3 mM of divalent cations which may be either Ca^{++} or Mg^{++} but not in their absence (Fig. 1D).

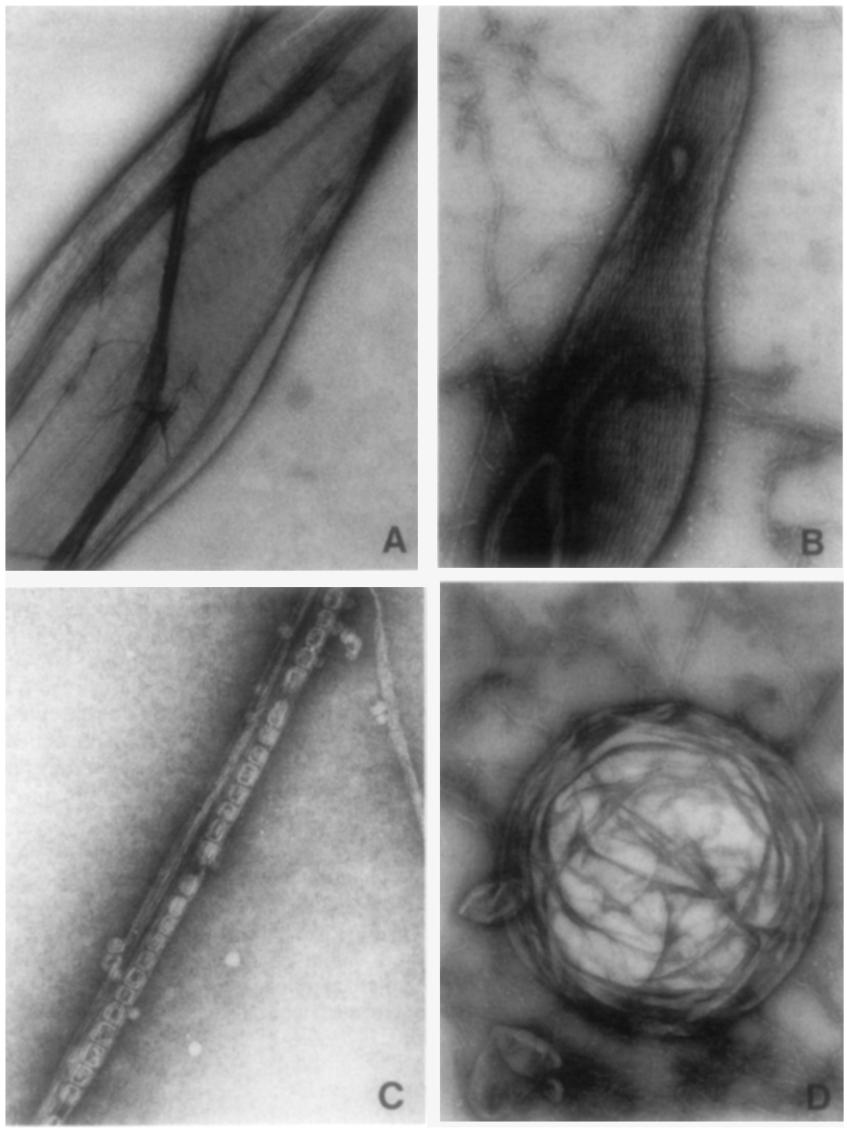

Figure 1
Electron micrographs of negatively stained preparation of liposomes and actin.
A) Reverse phase liposome and actin in the presence of 10 mM of $MgCl_2$. A paracrystalline sheet made by parallel filaments in register cover the surface of the liposome 65000X. B) A liposome in the same preparation. In this case, actin filaments form a net like organization 65000X. C) Small liposomes (sonicated vesicles) and actin in the presence of 10 mM of $MgCl_2$. A bundle of actin filaments confines the liposomes which adopt a rectangular shape 95000X. D) Reverse phase liposome and actin in the presence of 1 mM $MgCl_2$. No clear evidence of interaction may be seen in this preparation 40000X.

This reaction may be quantified by turbidimetry. Fig. 2 represents the differential turbidity (turbidity of the mixture less the turbidity of the sum of each constituent) as a function of Mg^{++} concentration. Maximum turbidity is obtained in the presence of 10 mM of Mg^{++}. The same result is obtained if Mg^{++} is replaced by Ca^{++} but not with monovalent cations such as K^{+}.

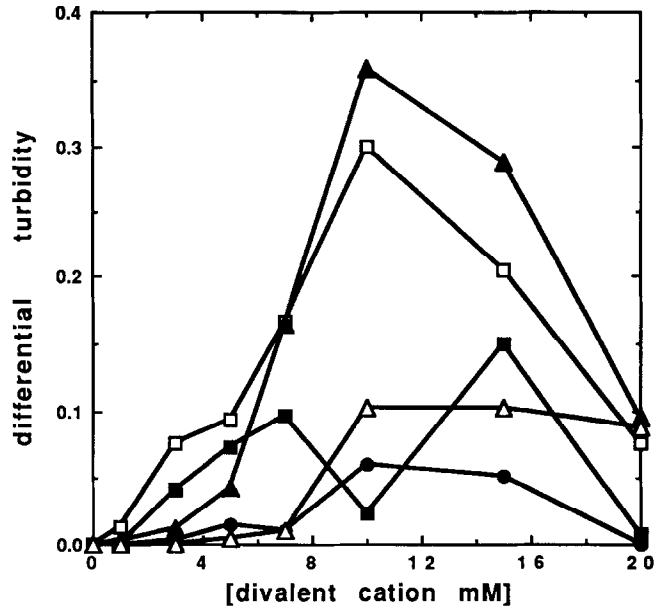

Figure 2
Differential turbidity of mixtures of actin and liposomes as a function of divalent cation concentration.
□ PC; ● PG; ■ CO; ▲ AO; △ PS.

Actin increases the permeability of the liposome membrane to carboxyfluorescein in a dose dependent manner and with a saturation effect (Fig. 3). This action of actin occurs in the absence of a divalent cation. However small concentration of divalent cations (1 mM) potentialize the effect of actin (Fig. 4) but higher

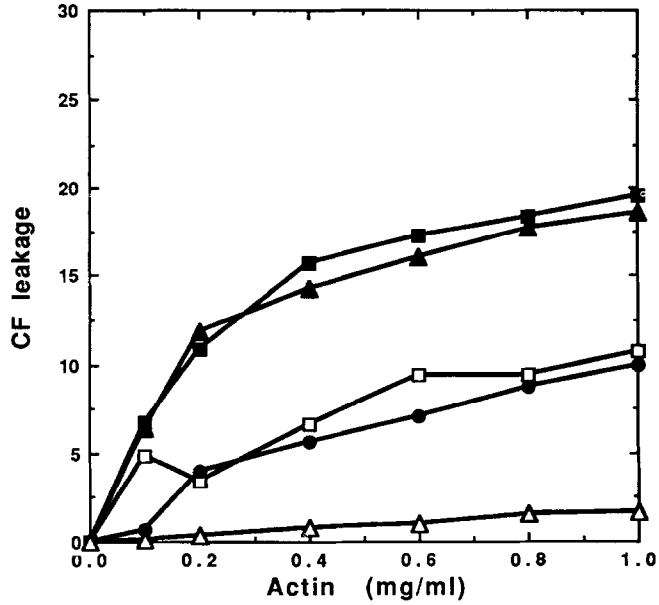

Figure 3
Permeability of liposome to carboxyfluorescein as a function of actin concentration in the absence of divalent cation. □ PC; ● PG; ■ CO; ▲ AO; △ PS.

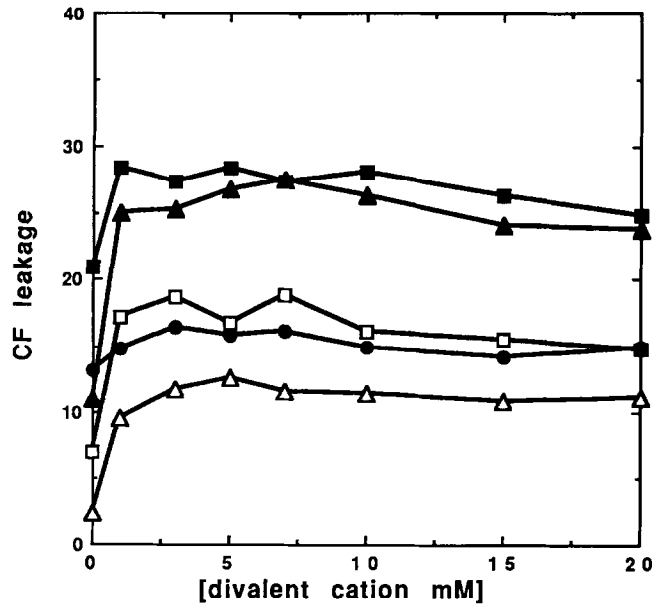

Figure 4
Effect of increased concentration of divalent cation on carboxyfluorescein leakage induced by 0.3 mg/ml actin. □ PC; ● PG; ■ CO; ▲ AO; △ PS.

concentrations do not. Divalent cations alone have no effect on the permeability of the liposome membrane to carboxyfluorescein. There seems to be a synergistic effect between divalent cations and actin on liposome permeability.

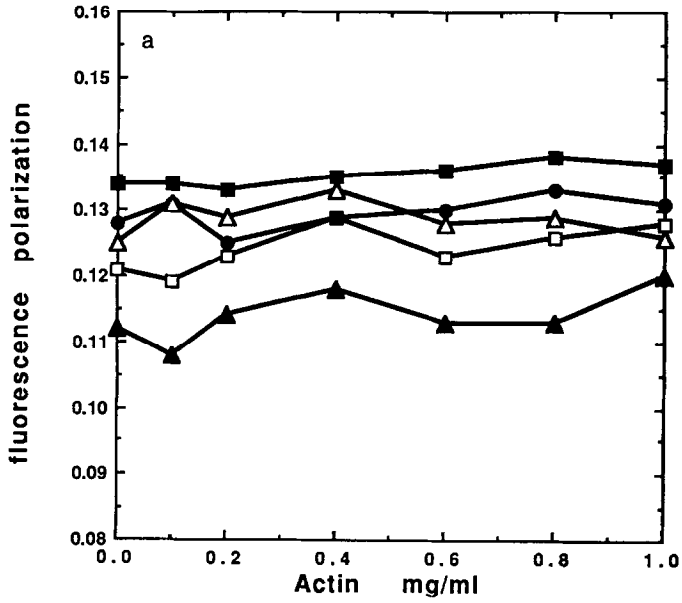

Figure 5
Effect of actin and divalent cation on fluorescence polarization of the DPH probe incorporated in the lipid bilayer of the liposome. a) Liposome and actin without divalent cation. b) Liposome and divalent cation without actin. c) Liposome + 0.3 mg/ml actin + divalent cation. □ PC; ● PG; ■ CO; ▲ AO; △ PS.

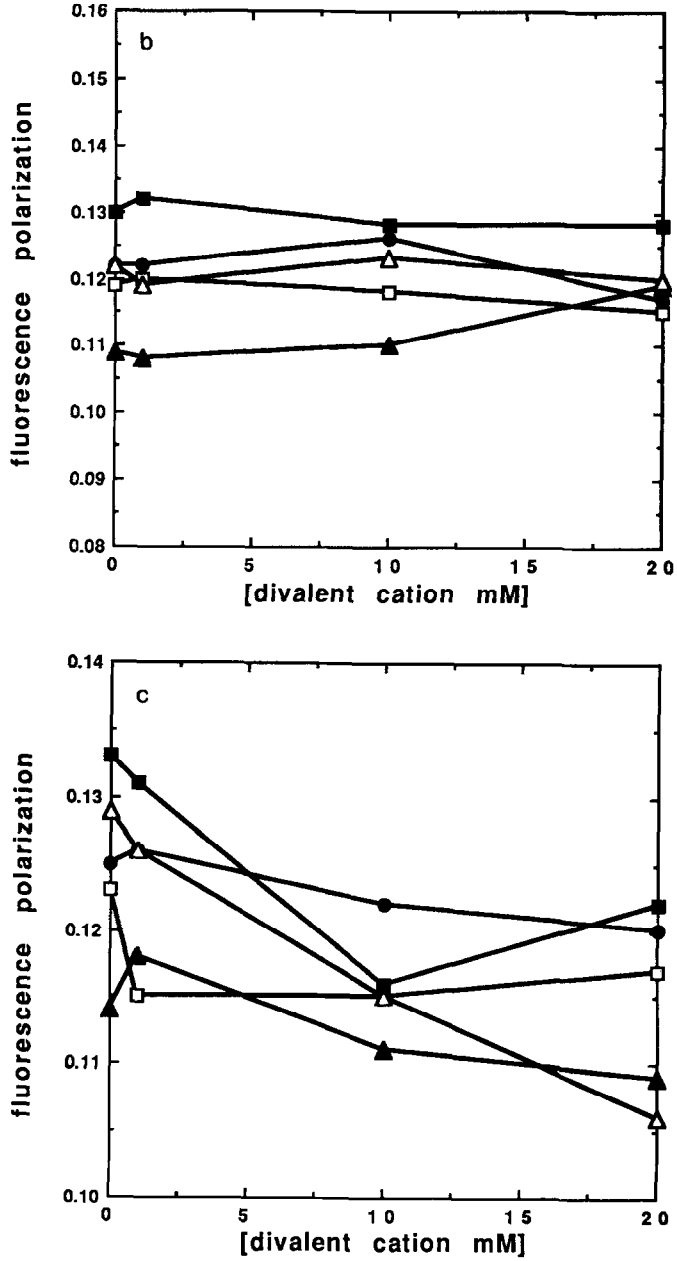

Figure 5-Continued

In order to determine whether the interaction of actin with liposome causes a disorganization of the acyl chains of the lipids, we measured the polarization of fluorescence of the DPH probe incorporated into the lipid bilayer. Actin alone in the range 0-1 mg/ml, or divalent cations in the range 0-20 mM do not change the polarization of the fluorescence of the DPH probe incorporated into the lipid bilayer (Fig. 5a, b). However, when divalent cations which may be either Ca^{++} or Mg^{++} at concentrations near 10 mM are

added to liposomes and actin, the polarization of the fluorescence of the DPH probe is significantly decreased (t sample paired test < 0.05) (Fig. 5c). This shows that, in the presence of high divalent cations concentration, the interaction of actin with lipids produces a disorganization of the acyl chains in the lipid bilayer.

DISCUSSION

Our results clearly establish the fact that pure actin is able to interact directly with membranar lipids of different compositions in the absence of any intermediate proteins. This observation is in opposition with the traditional understanding in this field of research which stresses the necessity of integral actin-binding-proteins to anchor actin filaments to the cell membrane. (cf. Niggli, V. and M. Burger (22). This belief is partly based on the fact that actin is a highly hydrophilic protein and it seems unlikely that it would interact with phospholipids. However, several other hydrophilic proteins also interact with lipids including lysozyme, cytochrome c, ribonuclease, albumin and spectrin (23, 24). In these cases, calcium in the millimolar range has a synergistic effect upon the interaction and it has been established that electrostatic attraction and conformational change of the protein mediate its penetration into the lipid bilayer.

In previous publications, several authors have indicated the possibility of a direct actin-lipid interaction: Wenstein et al. (13) working on interactions between proteins and lipids show that actin induces phase transition release of carboxyfluorescein from liposomes, but experimental conditions are not specified. Okimasu et al. (14) also established that cholesterol inhibits the fixation of soluble cytoplasmic proteins on liposomes, including actin. However, these works are not often cited in the literature.

The interaction of actin molecules with the lipid bilayer seems to involve two mechanisms which depend on the concentration of divalent cations:

1) In the absence of divalent cation, actin does not cause liposome aggregation, but an interaction does occur, indicated by the increase in liposome permeability to carboxyfluorescein. The ability of proteins to increase the permeability of lipid membranes has been correlated with their degree of penetration into the lipid bilayer (24, 25). Therefore, in the absence of a divalent cation, actin partially penetrates the lipid bilayer by a mechanism which may involve hydrophobic associations. However, this interaction does not disorganize the acyl chains in the lipid bilayer since the polarization of the DPH probe is not modified.

2) In the presence of a low (1 mM) concentration of divalent cations, the effect of actin on membrane permeability is increased. Two possible scenarios may explain the potentialization by divalent cations. Firstly, divalent cations may neutralize some anionic sites on actin and on the lipids, thus decreasing the repulsion between both components. Secondly, small concentrations of divalent cations may induce a change in conformation of the actin molecule, making it more favorable to directly interact with lipids. This augmentation of the interaction of actin molecule with the lipid bilayer results in a perturbation of the arrangement of some acyls chains, since the polarization of the DPH probe is decreased. Therefore, the actin molecule first binds via a hydrophobic interaction to the lipid bilayer, which is reinforced, in the presence of divalent cations, by additional electrostatic phenomena.

We found a direct interaction between actin and lipids *in vitro*. Does this phenomenon have any significance *in vivo?* The interaction is dependent upon the concentration of a divalent cation which may be either calcium or magnesium. In cells, concentration of calcium is far below the 1 millimolar range but magnesium is estimated to be between 1 - 8 mM (26). Consequently, it seems possible that a direct interaction between actin and lipids also exists in the cell.

There is evidence that some actin is acylated *in vivo* by palmitic acid (27), or isoprenoid residues (28). The function of this acylation is unknown. Since acylation increases actin-lipid affinity, it may be a mechanism to control the interaction between actin and lipids.

Previous works (6, 9) have clearly established that actin may attach to the membrane by actin-binding-proteins. Our results are not incompatible with these works and lead to the conclusion that two types of interaction may be involved in the anchoring of actin to membranes: direct interaction with the phospholipids and attachment via integral proteins.

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