

Transformation of Actin-Encapsulating Liposomes Induced by Cytochalasin D

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ABSTRACT Liposomes encapsulating actin filaments were prepared by swelling at 0°C lipid film consisting of a mixture of dimyristoyl phosphatidylcholine and cardiolipin (equal amounts by weight) in 100 μM rabbit skeletal muscle actin and 0.5 mM CaCl₂ followed by polymerization of actin at 30°C. Liposomes initially assumed either disk or dumbbell shape, but when cytochalasin D was added to the medium surrounding the liposomes, they were found to become spindle shaped. Liposomes containing bovine serum albumin that were given cytochalasin D and actin-containing liposomes that were given dimethylformamide, the solvent for cytochalasin D, did not transform. These results indicated actin-cytochalasin interaction is involved in the transformation process. Falling-ball viscometry and sedimentation analysis of actin solution indicated that cytochalasin cleaved actin filaments and caused depolymerization. The observation of polarized fluorescence of encapsulated actin labeled with acrylodan indicated that the actin filaments in the transformed liposomes aligned along the long axis of the liposomes. Because the actin filaments in the disk- or dumbbell-shaped liposomes formed bundles running along the liposome contour, the transformation was likely to be accompanied by the change in the actin filament arrangement in the liposomes, which was induced by actin-cytochalasin interaction.

INTRODUCTION

Involvement of actin in dynamic activities at cell periphery, such as formation and retraction of pseudopods, has long been suggested. Existence of well-developed meshwork mainly consisting of actin filaments at the cell periphery (Nachmias, 1980; Small, 1988), or alteration of actin assembly concomitant with morphological changes of chemically stimulated cells (Condeelis et al., 1988; Downey et al., 1991; Coates et al., 1992) have been reported. Biochemical investigations (Jennings et al., 1981; Hartwig and Janmey, 1989; Watts et al., 1991) have provided evidence for an increase in the amount of actin filament or actin nucleation activity in chemically stimulated macrophage or neutrophils, indicating the importance of actin polymerization in peripheral dynamic activities.

Perhaps one of the strongest pieces of evidence that actin is involved in the cellular activities has come from in vivo studies utilizing cytochalasins, a group of fungal metabolites that act specifically on actin (Schliwa, 1982; Yahara et al., 1982; Forscher and Smith, 1988). A more recent study (Ohmori et al., 1992) has demonstrated that effects of cytochalasin on the cellular activities or supramolecular structures of actin involve direct interaction of cytochalasin with actin. In vitro experiments have shown that cytochalasin changes the polymerization kinetics of actin (Brenner and Korn, 1980; Flanagan and Lin, 1980; Goddette and Frieden,

1986; Bonder and Mooseker, 1986; Cooper, 1987; Sampath and Pollard, 1991), binds to actin monomer (Goddette and Frieden, 1986), and promotes conversion of monomer-bound ATP to ADP (Sampath and Pollard, 1991). The drug also alters the physical properties of actin solution, as manifested in simultaneous reduction of actin filament length and its amount by combination of cleaving and depolymerizing activity (Lanni and Ware, 1984; Walling et al., 1988; Urbanick and Ware, 1989).

Whereas the above studies strongly indicate that actin plays the pivotal role as a mechanical element in the cellular dynamic activities, the question of how actin is involved still remains unanswered (Cooper, 1991). This is presumably caused partly by the lack of an adequate model system. Recently, however, several model systems, which were intended to mimic cellular activities, have been developed: tubulin, a subunit of microtubules, actin or actin plus other cytoskeletal components have been encapsulated in liposomes, closed vesicles of phospholipid membrane (Cortese et al., 1989; Hotani and Miyamoto, 1990; Barmann et al., 1992; Janmey et al., 1992; Miyata and Hotani, 1992). Polymerization of the encapsulated tubulin or actin was expected to cause shape change of the liposomes, and light microscopy has shown this is indeed the case. The simplicity of the liposome system is advantageous in clarifying essentials of the mechanical role of actin in cellular morphological change.

We have shown that actin-containing liposomes assumed disk or dumbbell shape and became extremely rigid (fluctuation of the lipid membrane was suppressed) after actin polymerization was complete (Miyata and Hotani, 1992). This rigidity, attributed to continuous actin bundles that spontaneously formed and underlay the liposome membrane, implied that no further shape change would be possible unless this bundle structure was altered. Thus, we have been trying to manipulate actin inside the liposomes to induce

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shape changes. In this paper we describe microscopic observations that liposomes containing polymerized actin undergo drastic shape change with the addition of cytochalasin D, and this change is perhaps induced by a change in alignment of actin filaments in the liposomes.

MATERIALS AND METHODS

Proteins, lipids, and other chemicals

Actin was prepared from acetone powder (Spudich and Watt, 1971) and further purified by gel filtration over a column (Pollard and Cooper, 1982) packed with G-150 (Pharmacia; Uppsala, Sweden). Purified actin was stored on ice in G-buffer: 2 mM Tris(hydroxymethyl)aminomethane, 0.2 mM ATP, 0.2 mM CaCl_2 , 0.3 mM NaN_3 , and 5 mM 2-mercaptoethanol. Cytochalasin D and bovine serum albumin (BSA) were from Sigma Chemical Co. (St. Louis, MO). Dimyristoyl phosphatidylcholine and cardiolipin (bovine heart, sodium salt) were from Avanti Polar Lipids Co. (Alabaster, AL). Acrylodan was from Molecular Probes, Inc. (Eugene, OR). All other chemicals were of analytical grade. Polystyrene beads (0.5 μm diameter) were from Polyscience Inc. (Warrington, PA).

Actin labeling

Actin was labeled with acrylodan according to the method described by Marriott et al. (1988) and further purified by gel filtration over G-150. Hereafter, we call the labeled actin prodan-actin. A recent report (Coppin and Leavis, 1992) has indicated that the emission dipole of actin-bound prodan is nearly perpendicular to the axis of an actin filament, demonstrating that this label is useful in assessing orientation of actin filaments. This label can also be used to estimate the degree of actin polymerization, because the emission spectrum of the prodan-actin exhibits a blue shift (~ 20 nm) and an increase in the peak intensity (1.7 times) upon actin polymerization (Marriott et al., 1988).

Preparation of actin-containing liposomes

Detailed procedures have been described elsewhere (Miyata and Hotani, 1992). Briefly, a mixture of dimyristoyl phosphatidylcholine and cardiolipin (1:1 by weight) in chloroform was dried at the bottom of a small test tube in vacuo, and the resultant lipid film was swollen on ice for 1 h to form liposomes in G-buffer supplemented with 100 μM G-actin, 89 mM sucrose, and 0.3 mM CaCl_2 . It was necessary to maintain the low temperature to retard the actin polymerization. After the low temperature incubation, this concentrated liposome solution was diluted 50 times with G-buffer supplemented with 89 mM glucose, 0.3 mM CaCl_2 , and 100 μM BSA. Then this dilution was warmed to 30°C to induce actin polymerization; the dilution was necessary to prevent actin polymerization outside liposomes at 30°C. Under the ionic conditions employed, liposome-encapsulated actin (100 μM) was able to polymerize at 30°C, but actin outside liposomes (2 μM) was not (Miyata, unpublished observation). After 1 h incubation at 30°C, the liposome solution was allowed to cool down, so that the solution temperature equilibrated with the room temperature (~ 20 – 22°C) at which the observations were made. The 30°C incubation facilitated the actin polymerization, but the lowering of the temperature did not cause depolymerization, as checked with the emission spectrum of prodan-actin: when 100 μM actin was polymerized with 0.5 mM CaCl_2 and then left at room temperature for 36 h, no peak shift and only a slight ($\sim 5\%$) decrease in the emission spectrum was observed.

Not all the liposomes prepared in the above manner assumed disk or dumbbell shape. We roughly estimated that ~ 10 – 20% of the liposomes were disk-shaped and ~ 1 – 2% were dumbbell-shaped. The dimension of most of the liposomes observed in our experiments was < 20 μm irrespective of their shapes, and those having larger dimension were rare (but see Fig. 1d). We could only assess the shape of the liposomes having maximum dimension of > 5 μm , because Brownian motion of smaller ones was too fast to determine their shapes. Liposomes other than disk- or dumbbell-

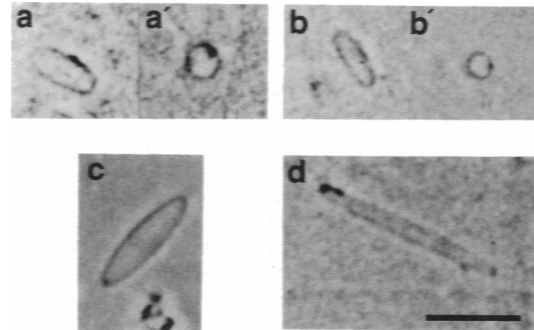


FIGURE 1 Phase-contrast images of four spindle-shaped liposomes, which were taken 15–35 min after the addition of the drug. (a, a', b, b') The images of two liposomes with different view angles demonstrate that they are spindle-shaped. Cytochalasin D concentration was 20 μM . (c) Another spindle-shaped liposome observed at 20 μM cytochalasin D. (d) A spindle-shaped liposome having particularly large axial ratio observed at 100 μM cytochalasin D. This liposome rotated around its long axis, but its width did not change, indicating that it was spindle-shaped. Bar, 10 μm .

shaped ones possessed a wide variety of shapes (from nearly spherical to a more elongated, sausage-like shape) and relatively flaccid. Most of these liposomes probably did not contain a sufficient amount of actin and were formed after the dilution. Encapsulation of the prodan-actin supported this notion: we sometimes observed the liposomes only emitting dim or even no fluorescence. Despite a high content of cardiolipin having negative charges (New, 1989), the swelling of the lipid was not complete, as aggregates of the lipid were occasionally observed in the diluted liposome solution: these aggregates are, we suspect, the source of the liposomes containing little actin. The lamellarity of the liposomes was also variable as judged from the contrast of the lipid membrane. We only chose the liposomes with an apparently thin membrane exhibiting relatively low contrast, but with this criterion alone we cannot distinguish unilamellar liposomes from oligolamellar ones (Servuss et al., 1976; Kwok and Evans, 1981).

When prodan-actin was used, the procedures for liposome preparation were the same, but all the buffers were degassed and supplemented with 20 mM dithiothreitol to reduce photobleaching of the dye that occurs under the intense excitation under the microscope. The inclusion of dithiothreitol did not affect liposome morphology (not shown). When liposomes containing BSA were prepared, the same procedure as above was carried out, but actin was replaced with 100 μM BSA. When polystyrene beads were incorporated along with actin, the beads were washed several times in G-buffer and added to the solution in which liposomes were formed. The bead concentration was 0.1% in this solution.

Addition of cytochalasin D

To observe the effect of cytochalasin D on liposome shape, either the drug dissolved in dimethylformamide (DMF) (at the concentrations of 10–50 mM) was mixed with the diluted liposome solution prior to the observation, or it was delivered from a capillary to the medium surrounding a liposome during the observation. In the former case, a time interval of about 3 min was required between the cytochalasin addition and the start of observation. In the latter case, 10–20 μl of cytochalasin D (50–200 μM , diluted in G-buffer supplemented with 89 mM sucrose, 0.3 mM CaCl_2 , and 100 μM BSA) was delivered from a capillary (inner diameter 1 mm) attached to a micromanipulator (Narishige, Tokyo). Leakage of the drug was prevented by drawing 5 μl of G-buffer supplemented with 89 mM glucose, 0.3 mM CaCl_2 , and 100 μM BSA into the capillary. The capillary was placed at the nearest possible location to the liposome but still far enough away to avoid interference of illumination by the capillary tip.

Liposome observation

Liposomes were observed through a 100 \times , oil-immersion objective lens (NA 1.3, Fluor Ph4DL; Nikon, Tokyo) attached to an inverted microscope

(Diaphoto TMD; Nikon, Tokyo) equipped with a high-pressure mercury lamp (100 W) for epifluorescence microscopy and a tungsten lamp (50 W) for phase-contrast microscopy. To this microscope was attached a W-microscopy assembly (Kinosita et al., 1991) for observation of polarized fluorescence. All the images were detected with a silicone-intensified target camera (C2741; Hamamatsu Photonics Inc., Shizuoka, Japan) and recorded with a videotape recorder (A-VS-1; Toshiba, Tokyo or EVO9650; Sony, Tokyo). Contrast enhancement and background subtraction were carried out with an image processor (DIPS; Hamamatsu Photonics Inc.).

Phase-contrast microscopy was carried out as follows. When final liposome morphology was concerned, liposome solution (3–5 μl), which was treated with cytochalasin D, was placed on a coverslip (24 \times 36 mm²), covered with an 18 \times 18 mm² coverslip and sealed with silicone grease. When the transformation process was concerned, 100–200 μl liposome solution was placed in an open chamber, which was constructed from two pieces of rectangular-shaped glass (1 mm thickness) fixed in parallel with silicone grease on a 24 \times 36 mm² coverslip. This chamber was placed on the microscope stage and covered with a petri dish with an opening for the access of the capillary for the addition of the drug. A piece of wet tissue paper was fixed inside the petri dish to prevent evaporation from the surface of the liposome solution. Evaporation was negligible during the time required for the addition of cytochalasin and subsequent observation. Stage temperature was 22 \pm 2°C during the period of each observation (~20–40 min).

When prodan-actin was used, excitation of and collection of the fluorescence of the liposome sample were performed with a filter assembly (Nikon, Tokyo) for indo-1 fluorescence measurement (excitation and emission wavelength were 340 nm and \geq 400 nm, respectively). A W-microscopy assembly with fluorescence and phase-contrast mode was used to observe liposome shape and polarized fluorescence simultaneously. The fluorescence image of a liposome was split in the assembly into two images based on their polarization, namely vertical and horizontal components.

Evaluation of the effects of cytochalasin D on actin solution

The effect of cytochalasin D on actin solution has been examined in test tube experiments under ionic conditions different from ours: MgCl₂ or a combination of KCl and MgCl₂ were used to polymerize actin, whereas CaCl₂ was used in our case. For this reason, we examined effects of the drug on F-actin solution under our ionic conditions by viscometry and sedimentation experiment.

Viscosity of actin solution in the presence or absence of cytochalasin D was measured by rolling-ball viscometry according to the method described by Pollard and Cooper (1982). Time required for a steel ball (diameter 0.6 mm) to roll 4 cm in a capillary (inner diameter 1.68 mm, Drummond; Broomall, PA) held at an angle of 30° was measured. The standard rolling times were obtained with a series of mixtures of glycerol and water. The rolling times in the actin solutions were converted with these values into the viscosities relative to that of water at 20°C.

Degree of actin polymerization in the presence or absence of cytochalasin D was estimated by the sedimentation analysis (Cooper and Pollard, 1982). G-actin (100 μM) was polymerized by adding 0.3 mM CaCl₂ at 30°C for 1 h in several centrifuge tubes. To these tubes were added an appropriate amount of cytochalasin D dissolved in DMF or DMF alone, and the final mixtures were gently homogenized, incubated for 10 min at room temperature, and ultracentrifuged in TL-100 ultracentrifuge (Beckman, Palo Alto, CA) at 340000 \times g for 1 h at 20°C. Actin amounts in the supernatants and pellets were measured by the method described by Bradford (1976).

RESULTS AND DISCUSSION

Morphologies of the liposomes observed upon addition of cytochalasin D

Before the addition of cytochalasin D, the actin-containing liposomes assumed either disk or dumbbell shape and pos-

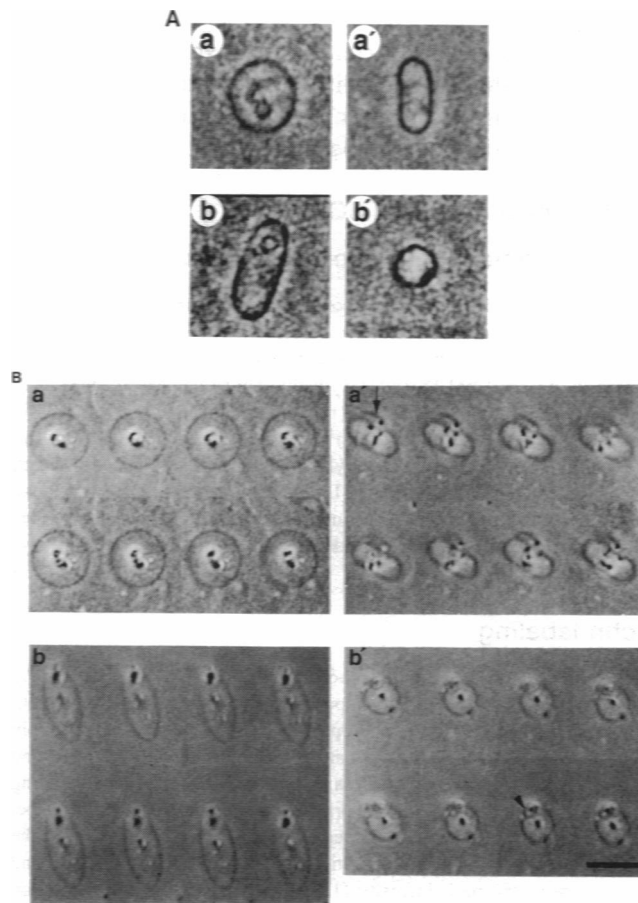


FIGURE 2 Phase-contrast images of a liposome before and after the transformation. (A) Cytochalasin D was delivered to the medium surrounding this liposome from a capillary situated nearby. Concentration of the drug in the capillary was 60 μM . (*a* and *a'*) Two different views of the liposome before the cytochalasin addition, demonstrating that this liposome is disk-shaped. (*b* and *b'*) Two different views of the same liposome after the completion of the transformation, demonstrating that this liposome is spindle-shaped. Because the transformation was fairly quick (<1 min), we could not obtain clear still images of the liposome in the middle of the transformation. This liposome contained several smaller liposomes, one of which is seen as a smaller circle in (*a*) and (*b*). These "child liposomes" did not seem to affect the transformation process. (B) Another example of the transformation. (*a* and *a'*) Phase-contrast images of the liposome before the transformation. (*b* and *b'*) Those after the transformation. The images in each panel (from top left to bottom right) were recorded every 0.13 s to show the Brownian motion of the encapsulated beads (appearing as black and white dots in the liposome). In some images the edge of the liposome was out of focus because of Brownian motion. Without any restriction, a bead with a diameter of 0.5 μm travels a distance of approximately 0.5 μm during 0.13 s in water at 20°C by Brownian motion. In *a* and *a'*, beads in the liposome changed their position relative to each other and relative to the liposome contour (it is difficult to fully represent the beads' motion with still images), whereas in *b* and *b'*, the beads did not seem to change their positions. One smaller liposome attached to this liposome (indicated with an *arrow*, before the transformation and with an *arrowhead*, after the transformation, which is barely visible because of different focus levels) did not seem to affect the transformation. A bead was trapped in this accessory liposome. *Bar*, 10 μm .

sessed rigid contours (Miyata and Hotani, 1992). Some disk-shaped liposomes were thick and slightly discoid (as shown in Fig. 2, A and B, *a* and *a'*), and some were more flat (Miyata

and Hotani, 1992). The ratio of the width of the expanded portion to the length of the connecting bar portion of the dumbbell-shaped liposomes was also variable. In most cases the diameter of the disk and the length of the dumbbell was $\sim 10 \mu\text{m}$ and the number of large ($> 20 \mu\text{m}$) ones was small; the shapes of small ones ($< 5 \mu\text{m}$) were difficult to observe, as stated above. But when cytochalasin D was added to the liposome solution, liposomes with different shapes appeared (Fig. 1, *a-d*). We call this type of liposome, characterized by rigid elliptical contours and axial ratios ≥ 2 spindle-shaped. The spindle-shaped liposomes, never being observed before the addition of the drug, became observable as soon as observation was started (about 3 min after the addition of cytochalasin D), and at a concentration as low as $20 \mu\text{M}$. The spindle-shaped liposomes were found as frequently as the disk-shaped liposomes. The proportions and sizes of the spindle-shaped liposomes were diverse: some possessed rather sharp ends, whereas others possessed very rounded ends; in some cases the sharpness differed between the opposing ends of the same liposome. Length of the spindle-shaped liposomes ranged from 5 to $15 \mu\text{m}$ in most cases. The axial ratios of the spindle-shaped liposomes did not strongly depend on the drug concentration: the axial ratio at $20 \mu\text{M}$ was between 2.0 and 12.7, and it was between 2.7 and 18.6 at $100 \mu\text{M}$. The spindle-shaped liposomes could be observed for at least the period of the observation (~ 20 – 40 min.)

The liposomes that did not contain actin (Servuss et al., 1976) or contained BSA (Miyata and Hotani, 1992) were not completely spherical and sometimes even possessed very elongated shapes (axial ratio > 5). But the elongated liposomes were very flexible and their shape was sausage-like rather than elliptical; thus, they were easily distinguished from the spindle-shaped liposomes.

Control experiments

Because both cytochalasin D and DMF could interact with the lipid membrane according to their chemical nature (the very low solubility of cytochalasins in water (Cole and Cox, 1981), and the miscibility of DMF with most organic solvent (Budavari, 1989), we carried out experiments to check whether interaction of each of these reagents with the lipid membrane brought about the spindle shape. Neither addition of up to $100 \mu\text{M}$ cytochalasin D (in DMF) to BSA-containing liposomes nor addition of DMF (1%) alone to actin-containing liposomes brought about any spindle-shaped liposomes, indicating that their appearance requires both cytochalasin D and actin. The added cytochalasin D was probably readily (< 3 min) incorporated into the lipid membrane and then interacted with the actin in the liposomes to bring about the spindle-shaped liposomes.

An osmotic pressure difference between inside and outside liposomes can drive liposome transformation (Hotani, 1984). In our case DMF could have increased the outer osmotic pressure, although the permeability of DMF across the phospholipid membrane is probably rather high (Orbach and Finkelstein, 1980; Walter and Gutknecht, 1986). However,

the osmotic pressure is unlikely to be the cause for the transformation, because the addition of DMF alone had no effect, as stated above. To further check this point, we also carried out an experiment in which the actin-containing liposomes were diluted and incubated at 30°C in a medium of higher osmolarity (containing 137 mM sorbitol). We observed no spindle-shaped liposomes.

Effects of cytochalasin D on actin filaments demonstrated in vitro

Cytochalasin D decreased the viscosity of the actin solution and the amount of pelletable actin as revealed in in vitro assay (Table 1). A large decrease in the apparent viscosity and the amount of the sedimented actin was observed at the lowest drug concentration tested ($10 \mu\text{M}$). Further increase in the drug concentration brought about significantly fewer changes in both quantities. The decrease in the apparent viscosity seems to be attributable to the cleavage of the actin filaments, and the increase in the amount of unpelleted actin seems to be attributable to depolymerization caused by capping the barbed end (Lanni and Ware, 1984; Urbanick and Ware, 1989) and/or by promoting hydrolysis of ATP bound to an actin monomer (Sampath and Pollard, 1991). The cleaving probably also increased the amount of the unpelletable actin. The viscosity exhibits much stronger dependence on the cytochalasin concentration probably because it is highly sensitive to the length of actin filaments (Pollard and Cooper, 1982).

Observation of the transforming process

In order to observe the process of transformation, we delivered cytochalasin D to the medium surrounding a single liposome. Shown in Fig. 2 *A* are the images of a liposome before and after transformation. The flow from the capillary and Brownian motion occasionally changed orientation of the liposome so that views from different angles could be obtained. Fig. 2 *A*, *a* and *a'*, demonstrate that this liposome assumed a disk shape before the addition of the drug, whereas Fig. 2 *A*, *b* and *b'*, demonstrate that the same liposome assumed a spindle shape after the transformation. In control experiments DMF alone was delivered, but no transformation occurred.

TABLE 1 Effect of cytochalasin D on apparent viscosity and amount of pelleted F-actin polymerized in 0.5 mM CaCl_2

| Cytochalasin D (μM) | Actin in the pellet (% of total protein) | Apparent Viscosity (η/η_0) [*] |
|----------------------------------|--|---|
| 0 [†] | 86.8 | 50.0 |
| 10 | 74.3 | 5.8 |
| 20 | 74.6 | 3.0 |
| 50 | 76.6 | 2.3 |
| 100 | 76.2 | 2.2 |

Actin concentration was $100 \mu\text{M}$.

^{*}: viscosity of water at 20°C .

[†] 1% (by volume) DMF alone was added.

The transformation of the disk-shaped liposomes occurred in the following way. Some time after the arrival of cytochalasin D, (~ 1 – 3 min, which varied from liposome to liposome) the liposome suddenly started changing its shape in such a way that the width of the disk became smaller in one of its radial directions and at the same time became larger in another direction, which was perpendicular to the former one. The transformation process, once it became noticeable, was complete within about 1 min.

Of 21 liposomes that were given the drug, 9 transformed into the spindle shape, some became spherical ($n = 4$), and the rest ($n = 8$) did not change shape. We suspect that in those liposomes that became spherical, length of actin filaments may have become too short to maintain the elongated shape of the liposomes, as suggested from the experiment in which the actin-containing liposomes became more round when shorter actin filaments were developed with increasing amount of gelsolin (Cortese et al., 1989). We do not have a clear explanation for the last result, but we might have chosen liposomes containing little actin.

We also tried to observe the transformation process of the dumbbell-shaped liposomes, but the number of these liposomes was much smaller than that of the disk-shaped ones (about $1/10$, Miyata and Hotani, 1992), and we could only observe three examples, all of which transformed into the spindle-shape (not shown). In these cases the transformation occurred in the following manner: enlarged portions of the liposome became tapered and finally formed the end portions, while the connecting bar portion became thicker; thus, the spindle-shape was formed.

In other experiments we encapsulated polystyrene beads in the actin-containing liposomes to facilitate observing the changes inside the liposomes during the transformation. Fig. 2 B, *a* and *a'*, before the transformation and Fig. 2 B, *b* and *b'*, after the transformation, show the images recorded every 0.13 s. Before the transformation, beads occupied the central part of the liposome (Fig. 2 B, *a* and *a'*), and exhibited Brownian motion; the beads' motion was restricted within a space with a dimension of roughly $1/3$ of the liposome diameter. The Brownian motion was possible presumably because of the low actin content in this area, and the restriction was caused by the actin bundles running around the central part of the disk-shaped liposomes (Miyata and Hotani, 1992). When the transformation was complete, the Brownian motion ceased (Fig. 2 B, *b* and *b'*). The transformation seemed to be accompanied by some alteration of the actin bundle structure.

In three cases, we were able to estimate changes of surface area and volume during the transformation: significant decreases in volume (19, 17, and 28%) compared with the corresponding changes (-9 , -2 , and $+5\%$) in surface area were found. On the other hand, DMF alone did not cause the shape change as stated above, suggesting that the volume decrease was associated with the cleavage and depolymerization of the actin filaments. This might be analogous to the situation in which solution containing high-actin filaments concentration resists volume decrease when an osmotic stress is applied, and this resistance is lost upon severing of F-actin with gelsolin (Ito et al., 1987).

Arrangement of actin filaments in the transformed liposomes

The rigid shapes of the transformed liposomes and the cessation of the Brownian motion of the encapsulated beads in these liposomes (Fig. 2 B) suggested the existence of some structure such as actin bundles, but in some form different from that in the disk- or dumbbell-shaped liposomes. To further investigate the actin arrangement in the transformed liposomes, we encapsulated prodan-actin and observed the transformed liposomes by fluorescence W-microscopy.

Fig. 3A shows a control experiment in which we examined the polarization of fluorescence of actin-bound acrylodan relative to the actin filament axis by developing paracrystal

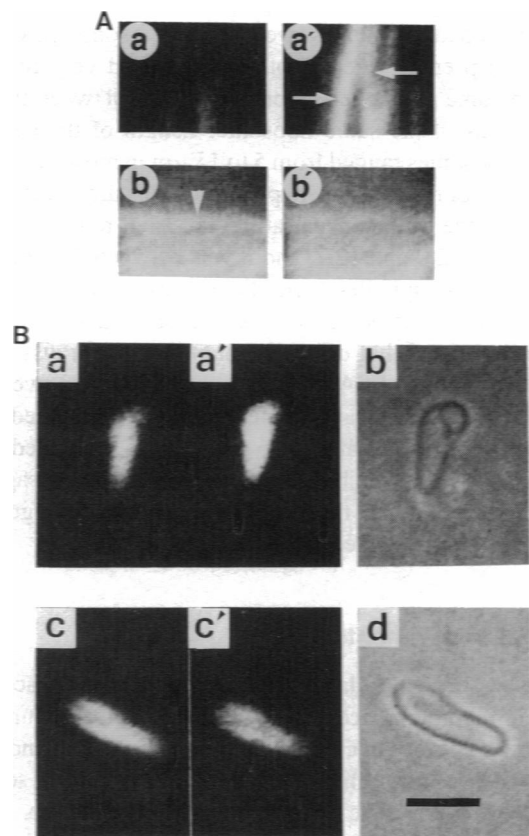


FIGURE 3 (A) Bundles of prodan-actin filaments developed in 20 mM $MgCl_2$ and observed with fluorescence W-microscope. (*a* and *a'*) The bundles (*arrows*) are running vertically. (*b* and *b'*) The bundle runs horizontally (*arrowhead*). (*a* and *b*) The fluorescence images of vertically polarized fluorescence. (*a'* and *b'*) The images of horizontally polarized emission. These images indicate that the emission dipole of the actin-bound acrylodan is perpendicular to the axis of an actin filament. Actin concentration was $100 \mu M$. (B) Polarized fluorescence and phase-contrast images of two transformed liposomes containing prodan-actin observed in the presence of $50 \mu M$ cytochalasin D. (*a*, *a'*, and *b*) One set of the images. (*c*, *c'*, and *d*) The other set. (*a*, *a'*, *c*, and *c'*) Fluorescence images. (*b* and *d*) Phase-contrast images. (*a* and *c*) Images of vertically polarized fluorescence. (*a'* and *c'*) Images of horizontally polarized fluorescence. The liposome in the first set (upper panels) is almost vertical, whereas that in the second set (lower panels) is not completely horizontal. Because of this slight tilt, difference in the intensity of the vertically and horizontally polarized fluorescence in the second set is not as prominent as in the first set. *Bar*, $10 \mu m$.

from 100 μM actin with 20 mM MgCl_2 . The polarization was found to be perpendicular to the filament axis, confirming the previous report (Coppin and Leavis, 1992).

Fig. 3 B shows two sets (*upper and lower rows*) of images of the transformed liposomes encapsulating prodan-labeled actin. In each set a pair of the fluorescence images (*a, a', c, c'*) are presented: (*a*) and (*c*) are the images of vertically polarized fluorescence, whereas (*a'*) and (*c'*) show horizontally polarized fluorescence. Comparison of these images with those shown in Fig. 3 A indicates that the actin filaments in the transformed liposomes align along the longer axis of the transformed liposomes.

Comparison of the phase-contrast and the fluorescence images in Fig. 3 B indicated that the aligned actin filaments filled the whole volume of the transformed liposomes: presumably, the actin filaments formed a bundle-like structure filling the inside to maintain the rigid and elongated shape of the liposome. This alignment was different from that of microtubules in the lemon-shaped liposomes (Hotani and Miyamoto, 1990), in which a few microtubules seemed to run like a strut along its central axis. The difference was presumably caused by the fact that an actin filament is less rigid than a microtubule (Mizushima-Sugano et al., 1983; Yanagida et al., 1984; Egelman, 1985; Gittes et al., 1993).

CONCLUSION

We have demonstrated that the actin-containing liposomes, originally assuming rigid and disk or dumbbell shape, transformed into the spindle shape when cytochalasin D was added outside the liposomes. The arrangement of the actin filaments in the transformed liposomes were found to be different from that in the disk- or dumbbell-shaped liposomes. Our tentative explanation for this phenomenon is that the cytochalasin D altered the actin bundle structure by cleavage and depolymerization, and this change induced the new liposome shapes. We presume that the initial liposome shapes, disk and dumbbell, were achieved as a result of a balance among the lipid membrane tension, elastic stress of the actin bundles, osmotic pressure difference across the lipid membrane, and gel osmotic stress of the actin bundle; in our experiment these factors were altered by the cleavage and depolymerization of actin, which destroyed the initial force balance, and thus the transformation occurred. No doubt this explanation should be evaluated in future works.

The elastic nature of the actin filaments (Egelman, 1985) and the liposome membrane (Kwok and Evans, 1981) would have contributed to the process of rearrangement of actin filaments through their mutual interaction. The tendency of actin filaments to align spontaneously at the actin concentration used in our experiment (Cortese and Frieden, 1988; Kerst et al., 1990; Suzuki et al., 1991; Coppin and Leavis, 1992) might have also contributed to the process, but it is difficult to evaluate the extent of these contributions.

It is difficult to estimate the amount of the drug that had entered the liposomes, but even if the amount of entered drug is small, it must have strongly affected the encapsulated actin

filaments for the following two reasons. First, relatively small amounts of cytochalasin D drastically changed the physical properties of the actin solution as shown in Table 1. Second, micromolar concentrations of cytochalasin D have been shown to catalytically and rapidly convert monomer-bound ATP into ADP (Sampath and Pollard, 1991). Because of a limited amount of ATP in the limited volume of liposomes, it is possible that ADP-binding monomers were generated almost irreversibly and hence, a significant proportion of the encapsulated actin became ADP-binding monomer having higher critical concentration than ATP-binding monomer. At present, we are unable to estimate the amount of ADP-binding monomer in the liposomes. Checking the reversibility of cytochalasin effects on liposome morphology is one way to confirm that the effect was caused by the interaction between the drug and the encapsulated actin (Forscher and Smith, 1988), but the abovementioned irreversibility seems to make the point difficult to be checked.

It is obvious that the liposome system is far simpler than any real cell, not to mention that the system consists of only two major components. For example, there is no linkage between the encapsulated actin and the liposome membrane, in contrast to real cells (Schwartz and Luna, 1988; Tranter et al., 1991), nor any regulatory factors that have been shown to play roles in the cellular dynamic activities (Oster and Perelson, 1987; Stossel, 1993). Nevertheless, the observations with this system suggest two possible ways in which actin may be used as a mechanical element in the cellular dynamic activities (Condeelis, 1993). One is the polymerization (Cortese et al., 1989; Barmann et al., 1990; Janmey et al., 1992; Miyata and Hotani, 1992), and the other is the alteration of the preexisting actin structure (a bundle-like one in the present case) in the liposomes. By utilizing the regulatory factors, these two ways of actin usage may be spatially and temporarily controlled in a real cell (Condeelis, 1993), or, as in the case of the elongation of acrosomal process (Tilney and Inoue, 1982), one of the two mechanisms may dominate. Whether this notion is correct should be evaluated in future studies.

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