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Transport of actin-decorated liposomes along myosin molecules in vitro

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ABSTRACT

We examined whether actin filaments bound to positively charged liposomes could interact with myosin molecules and induce liposome motility. When liposomes were constructed from the mixture of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cationic *N*-[1-(2,3-Dioleoyloxy)propyl]-*N,N,N*-trimethylammonium (DOTAP), actin filaments bound to the liposomes. The actin-bound liposomes exhibited movement on myosin molecules in the presence of adenosine-5′-triphosphate (ATP). The displacement was almost linearly increased with time and the behavior differed from that of Brownian motion. Furthermore, the presence of 30% DOTAP in liposomes was most effective for transport. These data show that the actomyosin system was successfully integrated into the liposomes and possesses the ability to actively transport useful agents enclosed within the liposomes.

1. Introduction

In eukaryotes, various cytoplasmic organelles are transported throughout the cell by a class of transport molecules called motor proteins. Myosin motor proteins travel along actin filaments, whereas the kinesin and dynein motor proteins utilize microtubules for cellular localization. The movement of motor proteins along the cytoskeleton is dependent on both the mechanochemical nature of motor proteins and the structural polarity of the cytoskeletal filaments. Interestingly, these cellular trafficking systems can be reconstituted in vitro from purified motor proteins. For example, the movement of actin filaments along myosin molecules on a fixed glass surface can be observed under a fluorescent microscope [1]. Alternatively, methodologies have also been designed to visualize the movement of myosin molecules as they "walk" along glass-fixed actin filaments [2]. The utilization of these techniques facilitates the transport of materials, such as polystyrene beads, through these motor proteins [3]. Therefore, the motility functions of these motor proteins are thought to be valuable for spontaneous transport in the Lab-on-a-chips system [4,5]. To date, a number of in vitro transport systems that utilize actomyosins or kinesin-microtubules and their control systems have been developed for potential applications in nanoscale devices [6–12].

Phospholipid vesicles, namely liposomes, are widely utilized as drug delivery carriers. It is known that the mechanism of liposome delivery is mediated through interaction with the plasma membrane via the endocytic pathway [13]. In addition, the interaction between liposomes and actin molecules has been reported to mimic morphogenesis and motility in actual cells [14–18]. Among these reports, it has been determined that actin filaments can bind to positively charged liposomes composed of neutral and cationic lipids [15,16]. When this occurs, actin filaments are orientated on the surface of the liposome. These liposome-actin filament complexes may have the potential to transport materials or cargoes if the actin filaments that interact with liposomes can function to actively transport the liposome and its components.

In this study, we investigate the transport capability of a hybrid liposome-actomyosin system. The presence of cationic lipids in liposomes induced binding of actin filaments to the liposome surface. These actin-decorated liposomes are several micrometers in size and were successfully driven by heavy meromyosin (HMM) molecules via ATP hydrolysis.

2. Materials and methods

2.1. Chemicals and proteins

DOPC (1,2-Dioleoyl-sn-glycero-3-phosphocholine; product name: COATSOME MC-8181) was purchased from the NOF Corporation and *N*-[1-(2,3-Dioleoyloxy)propyl]-*N,N,N*-trimethylammonium (DOTAP) chloride was purchased from the Alexis Corporation. Actin and myosin molecules were prepared from rabbit skeletal muscle by the standard protocol [19]. HMM molecules were prepared by chymotryptic digestion of myosins. To remove the inactive HMM molecules irreversibly bound to actin filaments,

Abbreviations: ATP, adenosine-5′-triphosphate; DOPC, 1,2-Dioleoyl-sn-glycero-3-phosphocholine; DOTAP, *N*-[1-(2,3-Dioleoyloxy)propyl]-*N,N,N*-trimethylammonium; HMM, heavy meromyosin.

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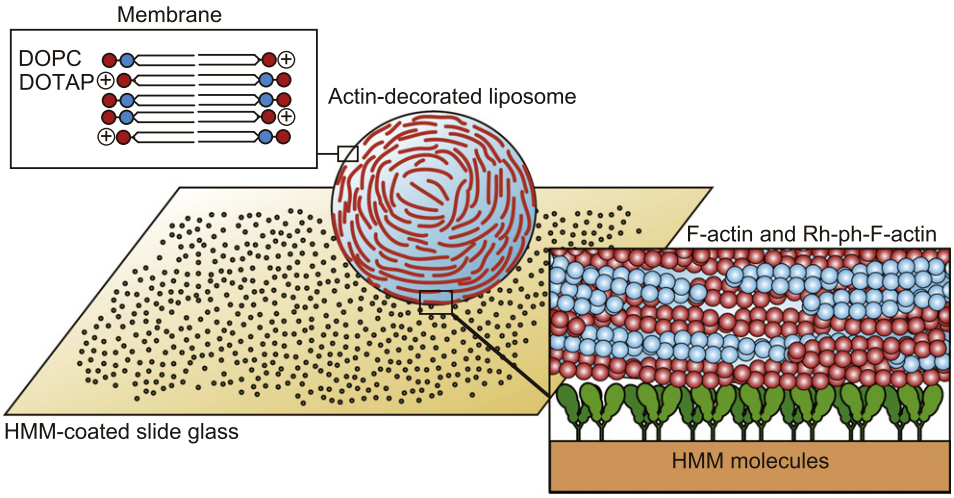

Fig. 1. A schematic of actin-decorated liposome driven by HMM molecules. The liposome consisted of DOPC (neutral lipid) and DOTAP (cationic lipid) at various molar ratios. After binding F-actin (the mixture of rhodamine-phalloidin-labeled and unlabeled actin filaments at molar ratio of 1:50) to the liposomes, the products were applied to the HMM-coated slide glass. Actin-decorated liposomes were 1–3 μm in radius.

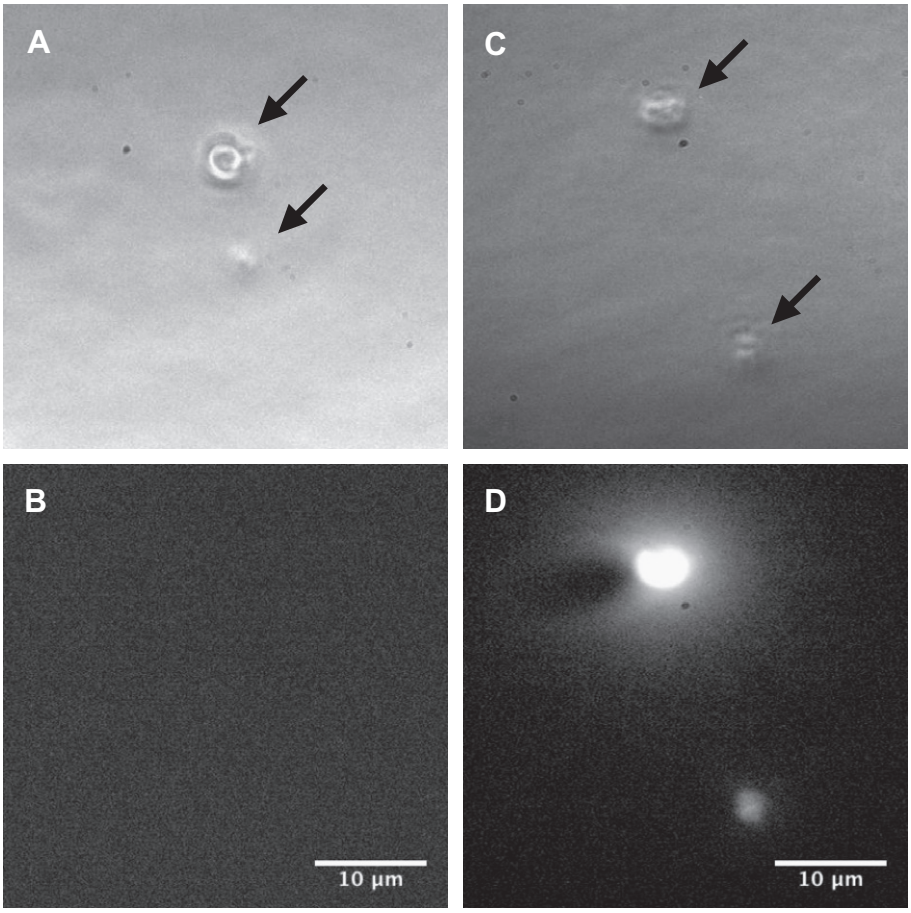

Fig. 2. Phase-contrast images (A and C) and fluorescence images (B and D) of liposomes. The liposome without DOTAP (A and B) or with 50% DOTAP (C and D) was mixed with the actin solution containing 2% of rhodamine-phalloidin-actin filament. Arrows indicate the liposomes. Scale bar indicates10 μm.

we performed co-sedimentation of HMM molecules and actin filaments in the presence of ATP via ultracentrifugation. The supernatant, which contained functional HMM, was used for the motility assay. Fluorescent actin filaments were prepared by binding TRITC-phalloidin (Sigma–Aldrich) to the filaments at equivalent molar ratio.

2.2. Preparation of actin-decorated liposomes

Using a hydration method by Bangham [20], liposomes were prepared from the mixture of DOPC and DOTAP as follows: DOPC and DOTAP, individually dissolved in chloroform, were mixed at various molar ratios (final concentration; 0.5 mM). The mixture

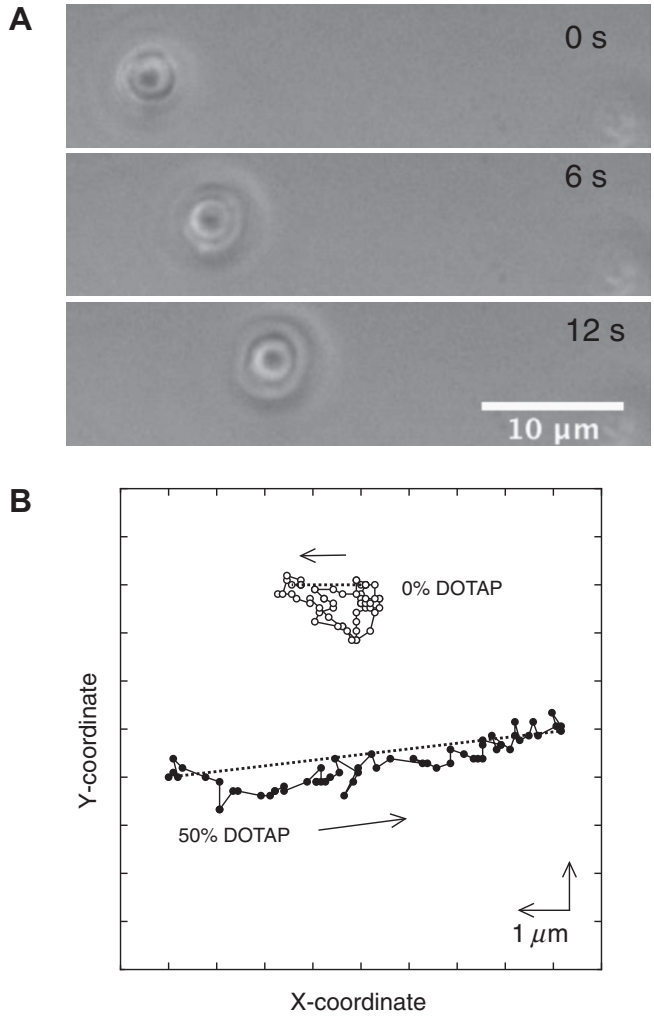

Fig. 3. (A) The sequential images actin-decorated liposomes movement driven by HMM molecules in the presence of ATP under a phase-contrast microscope. Liposome containing 50% DOTAP are shown. Images in each (top/middle/bottom) were taken at 6-s intervals. Scale bar indicates a distance of 10 μm. (B) Typical traces of moving liposomes. Open and filled symbols denote samples without DOTAP and samples with 50% DOTAP, respectively. Each data point was plotted at intervals of 0.5 s. The overall distance was defined as the sum of displacement at an interval of every 0.5 s for 30 s, as indicated by the connection between points. Straight dotted lines indicate the displacement defined as the distance between start point and end point. Arrows indicate the moving direction.

(500 μL) was placed into a sampling tube and was then dried under nitrogen gas. Subsequently, 500 μL of a standard solution [25 mM imidazole–HCl (pH 7.4), 2 mM $MgCl_2$] was added to the dried lipid film. After incubation for 24 h at 25 °C, liposomes spontaneously formed through the hydration of the lipid film.

To bind actin filaments to liposomes, 50 μL of actin solution [500 μg/mL F-actin, 10 μg/mL fluorescent F-actin, 25 mM imidazole–HCl (pH 7.4), and 2 mM $MgCl_2$] was mixed with 450 μL of the abovementioned solution containing liposomes. After incubation for 60 min, the samples were used for the motility assay.

2.3. Motility assay

An outline of our experimental system is shown in Fig. 1. HMM molecules were fixed on a collodion-coated glass slide (Matsunami, No. 1, 24 × 50 mm) by perfusion of HMM solution [0.1 mg/mL HMM, 25 mM imidazole–HCl (pH 7.4), 2 mM $MgCl_2$, 0.5% 2-mercaptoethanol] between the slide and the cover glass (Matsunami, No. 1, 18 × 18 mm) separated by 0.1 mm. Sixty seconds after perfusion, the solution was replaced with bovine serum albumin solution [3 mg/mL bovine serum albumin, 25 mM imidazole–HCl (pH 7.4), 2 mM $MgCl_2$, 0.5% 2-mercaptoethanol], to remove the unbound HMM molecules. The slide was then perfused with the actin-liposome solution and, immediately after, the ATP solution [25 mM imidazole–HCl (pH 7.4), 2 mM $MgCl_2$, 2 mM ATP, 0.5% 2-mercaptoethanol, 3 mg/mL glucose, 0.1 mg/mL glucose oxidase, 0.02 mg/mL catalase]. Once the ATP solution was applied, the movement of liposomes was observed under a phase-contrast microscope (Nikon, Ti-U, objective TIRF100×H). To visualize fluorescent actin, the microscope was equipped with a fluorescent illuminator unit (Nikon, TI-SFL) and optical rhodamine interference filters.

The microscopic images were acquired from a highly sensitive camera (Watec, WAT-120N) and recorded on a PC computer (Dell, Vistro 220) with video grabber board (Epix, PIXCI-SV5) using the StreamPix software (norpix). Spacing between the nearest-neighbor pixels in the image was 0.10 μm. The overall distance of movement of the liposomes was determined by measuring the signal displacement at intervals of 0.5 s, these data were quantified with the ImageJ software (NIH).

3. Results and discussion

3.1. Binding of actin filaments to positively charged liposomes

We constructed positively charged liposomes from the mixture of DOPC and DOTAP in order to bind actin filaments to these liposomes via electrostatic interactions. Fluorescently labeled actin filaments were utilized to verify actin-liposome binding. Fig. 2 includes phase-contrast and fluorescence images of the liposomes with 50% DOTAP that were found at the same location. In contrast, no fluorescence was observed in samples without DOTAP. The fluorescent intensity increased as the proportion of DOTAP increased (data not shown). This indicates that the binding of actin filaments to the liposomes occurs only if DOTAP is contained in the liposomes.

The binding of these actin-decorated liposomes to HMM molecules fixed on glass surface was also examined in the absence of ATP. The liposomes without DOTAP did not exhibit binding to the HMM-fixed surface, and thermally fluctuated. However, the liposomes containing DOTAP at proportions above 10% remained on the surface. As such, there seems to be strong binding between actin filaments decorated liposomes and the HMM fixed on the surface. It is likely that the presence of DOTAP in the liposomes induced an association with actin filaments, which enables their interaction with the HMM molecules.

3.2. Movement of actin-decorated liposomes driven by HMM molecules

Fig. 3A shows the movement of the actin-decorated liposomes on HMM molecules in the presence of ATP. A representative movement tracing is shown in Fig. 3B. The liposomes with 50% DOTAP traveled relatively straight over a long distance as compared to those without DOTAP. Fig. 4A indicates that the displacement between the start position and end point at each time was dependent on the proportion of DOTAP in liposomes. In the case of 0% DOTAP, the displacement was almost consistent with that of Brownian motion. However, the displacement for liposomes of 10–100% DOTAP linearly increased over time, thus, indicating that this phenomenon differs from Brownian motion. Moreover, the presence of 30% DOTAP was most effective for liposome transport in all the conditions tested.

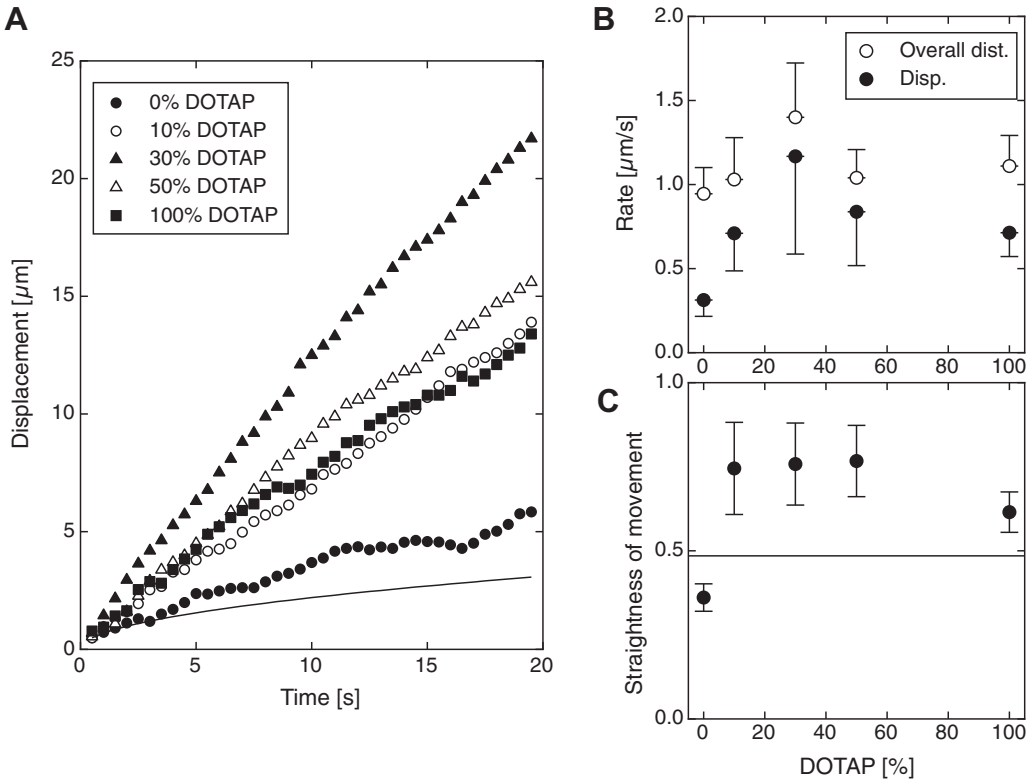

Fig. 4. (A) Time development of liposome displacement with various concentrations of DOTAP. Filled circle symbols denote the control without DOTAP. Open circle, filled triangle, open triangle, and filled square symbols denote the case with 10%, 30%, 50%, and 100% DOTAP, respectively. An average of 10 samples, which were 1–3 μm in radius, was plotted for each condition. The dotted curve line indicates theoretical values for the Brownian motion of a sphere of 2.0 μm in radius. (B) The rate of movement, that is, the distance or the displacement per second, and (C) the straightness of movement of actin-decorated liposomes containing DOTAP at various percentages. In panel (B), open circles and filled circles represent the overall distance and the displacement as described in Fig. 3A, respectively. These distances were divided by elapsed time (typically 30 s). Panel (C) shows the straightness of movement, which was defined by the ratio of displacement to the overall distance. Error bars indicate the standard deviations as generated from 10 samples. The horizontal dotted line indicates the level of a single actin filament moving in a standard in vitro motility assay.

Fig. 4B shows the rate of movement (displacement or distance per second) with various proportions of DOTAP in liposomes. The displacement was defined as the net distance connecting from the start point to end point during 30 s. The overall distance was defined as the successive distance collected at intervals of 0.5 s during the 30 s duration. Both moving rates were increased as DOTAP proportion was increased (up to 30%); DOTAP proportions above 30% decreased the distance travelled. The liposomes seemed to move straight along the HMM molecules. Fig. 4C shows the straightness of the movement of liposomes. This was defined as the ratio of displacement to overall distance. The straightness of movement of DOTAP-containing liposomes was higher than that of single actin filaments observed under a standard in vitro motility assay. However, the rate of movement (overall distance per second) of actin-decorated liposomes was approximately twice as slow as that of single actin filaments (approximately 2.5 μm/s in this experimental condition).

It is known that actin filaments, which intrinsically become negatively charged in neutral pH, are electro-statically bound to positively charged liposomes and have a tendency to be oriented on the membrane of the liposomes [15,16]. Therefore, the translatory movement of actin-decorated liposomes we observed may be associated with the orientation of actin filament on the liposomes. On the other hand, the slower rate was probably caused by the restraint of actin filaments on liposomes due to the binding and relevant steric hindrance to binding of myosin.

In the case of 100% DOTAP, the features of the liposomes appeared to be rough as the moving distance and straightness became lower than that of 30% DOTAP. A possible explanation would be that the excess of positive charge induced unstable formations of the liposome and disordered the arrangement of actin filaments on liposome, thus resulting in the failure of successive movement.

We also examined the correlation between of the rate of movement and liposomes size since there is a possibility that the liposome radius affects both the drag force and liposome surface area contact with HMM molecules. In samples without DOTAP, the rate of movement decreased as the radius increased. This result corresponds to the behavior seen with a diffusion limitation. However, the rate of movement of DOTAP-containing liposomes was almost independent of the radius. In fact, the drag force for an object of several micrometers in size is markedly smaller than the active force generated by individual myosin molecules [21]. Therefore, the transport of actin-decorated liposomes would be governed by myosin force. In other words, size-independent movement observed in DOTAP-containing liposomes is likely a result of actin-myosin interactions, the sliding velocity of which is independent of the length of actin filaments [3].

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