Microtubule-Cyclodextrin Conjugate: Functionalization of Motile Filament with Molecular Inclusion Ability

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Received November 15, 2004; Accepted December 8, 2004

A microtubule-β-cyclodextrin conjugate was prepared on a kinesin-adsorbed glass surface by chemical and biochemical means. Fluorescence microscope observation and a motility assay indicated that the conjugate simultaneously expressed an inherent motor function and an inclusion property.

Key words: microtubule; cyclodextrin; inclusion ability; motility assay; chemical modification

Microtubules, tubular polymer filaments assembled from α- and β-tubulin proteins, play important roles as “roads” along which kinesin motor proteins carry cargoes in nerve axons. Pioneering study by Howard and co-workers demonstrated that microtubules move on a kinesin-absorbed glass surface. Since that time, the microtubule-kinesin system has received intense interest as a platform for a nanoscale transport system. Recent efforts to develop this system have focused on guiding microtubule movement. Another task is to establish methods for loading and unloading cargoes. Previous studies have demonstrated the feasibility of utilizing avidin-biotin binding to load cargoes onto microtubules, but this biological binding method is inadequate for a loading/unloading system, since dissociation of the binding is essentially impossible. Thus one of the greatest challenges in this area is to devise methods for unloading cargoes.

Our approach to this problem is based on utilizing the reversible inclusion phenomena provided by cyclodextrin (CD). We hypothesize that CD attached to microtubules can capture guest molecules and release them according to external stimuli. Although this strategy appears promising, several fundamental issues regarding the nature of CD and of the microtubules remain to be clarified. Will CD exert its inclusion ability even on a microtubule? Will a microtubule move even with CD conjugated? The primary aim of the study reported here was to examine these questions by observing fluorescence images of an enclosed guest and measuring the motility of the microtubule-CD conjugate. In this paper, we provide experimental evidence for simultaneous expression of a motor function and an inclusion property provided by the CD-functionalized microtubule. On the basis of its potential loading/transporting/unloading functions, we term such a conjugate a molecular courier.

The design principle used for constructing the CD-functionalized microtubule incorporates three structural elements (Fig. 1). Specifically, it includes a biotinylated microtubule, biotinylated β-CD (1), and streptavidin to connect them. The synthesis of 1 was straightforward. Twenty-five mg of 6-monodeoxy-6-monoamino-β-CD (CycloLab) and 10 mg of biotin carboxylic acid succinimidyl ester (Pierce) were stirred in dry pyridine at room temperature for 18 h. Then evaporation of pyridine at 313 K and subsequent purification via ion-exchange chromatography (TOYOPEARL CM-650) produced the requisite compound. Successful preparation was confirmed by 1H-NMR and TOF-MS. A long, flexible linker was employed for 1 so that the CD could exert its inherent inclusion ability without steric hindrance and unexpected interplay by the streptavidin or the microtubule.

The experimental procedure used for constructing microtubule-CD conjugates in a flow cell consisted of (i) placing the biotinylated microtubules on a kinesin-adsorbed glass surface, (ii) adding the streptavidin, and (iii) adding the biotinylated β-CD through procedures similar to those previously reported. In essence, a kinesin solution (2 mg/ml) containing casein was perfused into the flow cell. After 2 min, the solution was exchanged with a buffer to wash out the non-adsorbed kinesin. Then rhodamine-labeled and biotinylated microtubules (1 mg/ml), obtained by polymerization of a 1:1 mixture of rhodamine-labeled and biotinylated tubulin (1 mg/ml), were perfused and incubated for 2 min. Next, streptavidin, Alexa Fluor conjugates...
Molecular Probes) (1 mg/ml) replaced the flow cell and were incubated for 10 min to form avidin-biotin complexes. After washing, 1 (25 mM) was perfused and incubated for 30 min, and finally albumin (4 mg/ml) replaced the cell after washing and was incubated for 1 h to protect nonspecific adsorption on the surface of the microtubules.12)

To judge the ability of the conjugated β-CD to enclose guests, we measured the fluorescence image of a fluorescent probe that could be accommodated within the cavity. Here, 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS) was chosen as a probe based on its ability to be captured by β-CD and to enhance its fluorescence within the cavity,13) thus permitting fluorescence microscope observation. Hence 1,8-ANS solution (27 μM) was added to replace the cell containing the microtubule-β-CD conjugates, and incubated for 1 h. This was followed by thorough washing with a buffer. Figure 2A shows a fluorescence image of rhodamine attached to microtubules. The striated red fluorescence indicates successful preparation of the microtubules under the current experimental conditions. A similar striated green fluorescence image of Alexa488 was observed at the same place (data not shown), indicating that the streptavidin combined with the biotinylated microtubules. Then we observed the same field with another filter appropriate for the blue fluorescence of 1,8-ANS. As can be seen in Fig. 2B, the fluorescence image of 1,8-ANS overlapped with that of rhodamine. These results imply that the 1,8-ANS was captured by the β-CD. To eliminate the possibility of 1,8-ANS’s nonspecific adsorption onto the microtubules, we observed a fluorescence image of microtubules without β-CD. Streptavidin-loaded microtubules were prepared using procedures similar to those used for the microtubule-β-CD conjugate. The striated fluorescence image of rhodamine (Fig. 2C), together with the fact that the Alexa488 image overlapped that of rhodamine (data not shown), confirmed preparation of the desired filament. Then we examined the 1,8-ANS image of the streptavidin-loaded microtubules after adding the fluorescence probe. As shown in Fig. 2D, dim fluorescence was observed. Thus the results obtained from this control experiment support the conclusion that 1,8-ANS did not adsorb on the conjugate nonspecifically. Taken together, these microscopy assays indicate that β-CD exerted its inherent ability to enclose guest molecules when conjugated with microtubules.

Another central question of considerable concern is whether the functionalized microtubules would move in a manner similar to that previously reported for unmodified microtubules. Hence we examined the motility of the microtubule-β-CD conjugates (1.0 mM ATP, 300 K). Figure 3 depicts sequential micrographs of the conjugates tracing the fluorescence of Alexa488. Each microtubule moved randomly on the kinesin-adsorbed surface at a constant speed; the average speed was 0.03 μm/sec, which is about one-tenth of an unmodified microtubule’s speed. A plausible explanation for the decrease in motility is steric hindrance, or a strong interaction between microtubules and additives including β-CD and albumin.

Fig. 2. Fluorescence Images of (A) a Rhodamine-Labeled Microtubule-β-CD Conjugate and (B) 1,8-ANS, Along with Control Fluorescence Images of (C) Rhodamine and (D) 1,8-ANS without I. The overlapped fluorescence images of rhodamine and 1,8-ANS indicate that the guest was captured by the β-CD as designed in Fig. 1B.
Despite the considerable effort expended in function- 
alizing the kinesin-microtubule system by biological and 
physical approaches, there appears to be considerable 
room for expanding its functionality. The results 
reported here are the first example of providing micro-
tubules with a supramolecular function via 
chemical means. This study provides further incentive for chemi-
cally modifying microtubules to expand their function-
ality. Efforts aimed at developing a molecular courier, i.e., a cargo loading/transporting/unloading system 
based on the microtubule-β-CD conjugate, are currently in progress.

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