Motor protein nano-biomachine powered by self-supplying ATP†

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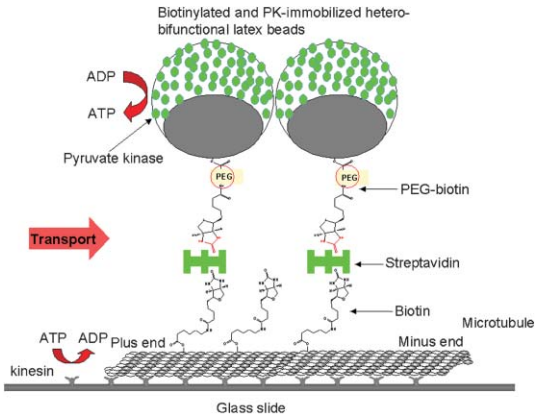
Received (in Cambridge, UK) 11th January 2005, Accepted 7th February 2005
First published as an Advance Article on the web 17th February 2005
DOI: 10.1039/b500327j

A new nano-biomachine has been created from microtubules (MTs) and hetero-bifunctional polymer particles bearing pyruvate kinase, which is propelled on glass surfaces coated with kinesin by use of self-supplying ATP.

In an eukaryotic cell, kinesin motor protein moves along filamentous structures called microtubules (MTs) using the chemical energy released by hydrolysis of adenosine 5′-triphospate (ATP) and is involved in transport of vesicles, chromosomes and protein complexes.[1,2] The conventional class of kinesin has two heads and two tail domains connected by a coiled coil stalk. The head domains are 7 nm × 4 nm × 4 nm in size,[3] and two heads in one molecules make alternating 8 nm steps along the MT surface[4] with a speed of roughly 500 nm s^{-1} (ref. 5) and a maximum force of 6–8 pN.[4,6] MTs have hollow cylindrical structures with a 25 nm diameter and various lengths in the micrometer range,[7] which result from polymerization of the α/β-tubulin heterodimer accompanied by hydrolysis of bound guanosine 5′-triphospate (GTP). An *in vitro* motility assay, where MTs glide over glass surfaces coated with kinesin, was firstly developed by Vale *et al.*[8] Recently, a variety of ATP-powered nano-biomachines were constructed to generate useful movements in a microscopic space.[9–15]

On the basis of all these studies, we designed a new MT nano-biomachine that has a cargo particle supplying ATP (Scheme 1). However, multivalent binding sites on both MTs and particles often resulted in severe aggregate formation upon mixing, while binding rates were sometimes too low due to large sizes of the two components. In an effort to design a new nano-biomachine comprising MTs and polymer particles, we attempted to solve those problems by using hetero-bifunctional latex particles possessing epoxy groups (white domain of particle in Scheme 1) and hydroxyl groups (gray domain of particle in Scheme 1)[16,17] to minimize aggregate formation between MTs and particles and by using polyethylene glycol (PEG)-based long linkers between MTs and particles to maximize efficient binding. These enabled us to assemble MT–particle complexes that move over kinesin-coated glass surfaces using ATP supplied by pyruvate kinase (PK) (green symbol on the white domains of particles in Scheme 1) immobilized on the particles. Possible applications of this novel nano-biomachine include transport and sensing systems of chemical compounds *via* the epoxy groups on the hetero-bifunctional particle surface in a microscopic space, while previously reported nano-biomachines were intended for surface imaging,[18] assembly and transport of molecules or nanoparticles,[19] and a force measurement.[20]



Scheme 1 Preparation scheme of kinesin motor protein nano-biomachine powered by self-supplying ATP.

For the *in vitro* motility assay of our new nano-biomachine (MT–particle), a flow chamber was constructed with a cover glass, a glass slide and double-sided tapes (see ESI Fig. 1†). The MT–particle complexes successfully moved when the concentrations of ADP and phosphoenol pyruvate (PEP) were 1 mM (Fig. 1 (a)–(c)) (and ESI Video. 1†). It is clear in Fig. 1 (a)–(c) that the MT–particle complex moved toward one direction; namely, the motion was not a thermal random walk. Fig. 1 (d) exhibits an image of the MT-particle complex, where green and yellow fluorescence dots represent the particles and about 90% of the polymer particles were contained in the MT-particle complexes. Time-average velocity of the MT-particle complex assembly is illustrated in Fig. 2 (a) as well as that of the maximum velocity. It is intriguing that the MT–particle started moving about 15 min after the substrate solution was introduced into the flow chamber. The average velocity was then steeply increased to 3 nm s^{-1}, followed by gradual reduction. The velocity decrease in the latter stage is not due to the decrease in the PEP concentration but possibly due to the damage of the kinesin-MTs motor by the excitation light to analyze the motion of MTs and the particles by observing their fluorescence emission, because the initial PEP concentration (1 mM) was sufficiently higher than the Michaelis constant for PEP (K_m = 256 μM).[16,17] On the other hand, it was found that the velocity is different in individual MTs; namely, the shorter MTs move faster than longer ones. Fig. 2 (b) shows the population of the MT–particle lengths in Fig. 1 (d), where the

† Electronic supplementary information (ESI) available: experimental details for modification of hetero-bifunctional latex beads, preparation of microtubules, binding between beads and microtubules and in vitro motility assay of kinesin motor protein. See http://www.rsc.org/suppdata/cc/b5/b500327j/

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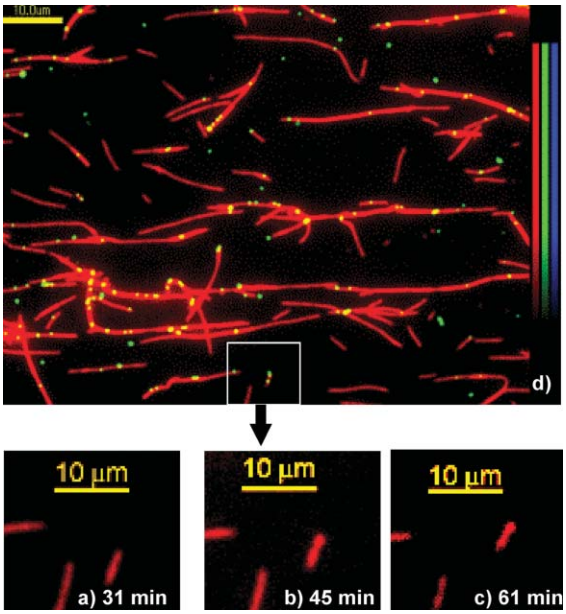

Fig. 1 Motion of MT–particle complex. (a) 31 min, (b) 45 min and (c) 61 min after starting the measurement; (d) about 90% of particles (green and yellow) are on MTs (red).

number average length is 11 μm and more than 50% of the MT–particles are shorter than 10 μm.

To understand the time-average velocity of the MT–particle complex, the time course velocity of MTs (without the particles) was investigated in the presence of ATP (initial concentration: 30 μM). It was found that the velocity was reduced exponentially with time (Fig. 2 (c)); *viz*., the relation is represented by $\ln v = -0.17t + 2.84$. Since the velocity at time 0 is 17.1 nm s^{-1}, the ATP concentration in the quasi-stationary stage in Fig. 2 (a) is estimated to be around 4 μM. An ATP producing reaction was independently conducted by using PK (labelled by BODIPY FL-X) immobilized on the beads in the presence of 0.1 mM ADP and 0.1 mM PEP. It was found that about 4 μM ATP was formed in 30 min (see ESI Fig. 2,† notice the produced ATP concentration is equal to produced pyruvate concentration), whose concentration is well compatible with the estimated value (*ca*. 4 μM) in the quasi-stationary stage (Fig. 2 (a)). The initial delayed period may be due to the lower ATP concentration under the detection threshold. Indeed, no motion of MTs was observed below 4 μM ATP. To observe the effect of ADP and PEP on the motion of MTs, 1 mM ADP and 1 mM PEP were added into the ATP solution, but no influence was found.

Streptavidin-coated particles and CdSe nanocrystal quantum dots (nQDs) have been coupled as a cargo to MTs through biotin–streptavidin linkage,[19,21] and colloidal gold was modified with actin to design the ATP-driven motility of the actin-based nanotransporter on a myosin coated surface.[22] In general, two key issues should be addressed for combining MTs/actin with particles.[21] One is undesired multiple aggregation between particles and MTs/actin, and the other is an inhibitory effect of a cargo particle on combining MTs with kinesin or combining actin with myosin. To solve these problems, we developed new hetero-bifunctional latex composite particles (*ca*. 200 nm average diameter with narrow size distribution) with epoxy and hydroxyl groups on the either side by soap-free seeded emulsion polymerization.[16,17] After biotinylation of the hydroxyl group of the particle, PK was immobilized on the particle through the epoxy group. The immobilized PK retained approximately half activity of the free PK. Here we combine the biotinylated and PK-immobilized particle with biotinylated MTs *via* streptavidin–biotin linkage, in which the immobilized PK catalyzes the supply of ATP (Scheme 1). The MTs used in this study were copolymerized from rhodamine-labeled tubulin and biotinylated tubulin with a 1:1 molar ratio. To confirm the composition of the prepared MTs, streptavidin labeled by Alexa Fluo 488 was further bound to the MTs. Fig. 3 (a) and (b) show the fluorescence image of MTs with rhodamine and Alexa Fluo 488, respectively. It is clear that the rhodamine-labeled tubulin and the biotinylated tubulin were homogeneously distributed along the MTs. At first, we tried to bind MTs with hetero-bifunctional particles[16,17] modified by 5-(*n*-succinimidyloxy-carbonyl)pentyl D-biotinamide. Contrary to our expectation the binding efficiency was very low, even when the reaction was carried out for a long time, and the MTs were fragmented into shorter tubules during the binding reaction. The binding efficiency was, however, markedly elevated by using biotin–PEG–CO_2–NHS modified particles (Fig. 1 (d)). This high efficiency should be, at least in part, due to the longer linker of polyethylene glycol (stretched length ~20 nm) between the biotin and succinimide portions, because the higher motional freedom of the longer linker has the possibility of accelerating the binding between poorly diffusive solid submicro-object (particle) and solid micro-object (MTs).

Our new particle has two advantages in the kinesin motor system. One is that the biotinylated area (hydroxyl group domain) is small enough to prevent undesired multiple aggregation between

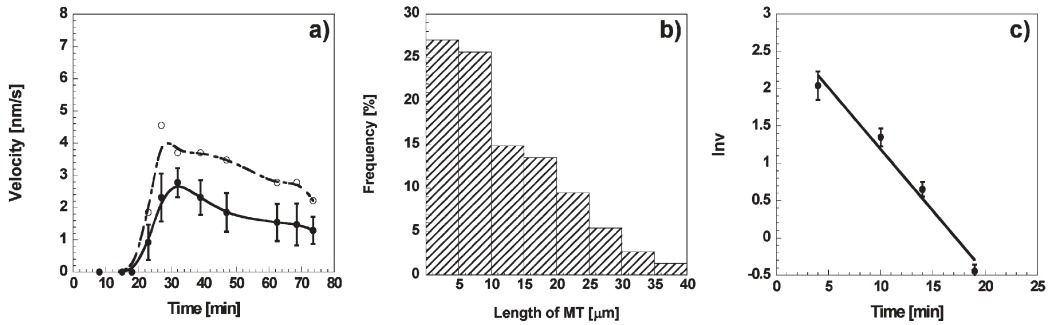

Fig. 2 (a) Time course velocity of MT–particle complex; average (•) and maximum (◦). (b) Population of MT–particle length. (c) Time course velocity of MT (without particle) in the presence of ATP (initial concentration: 30 μM).

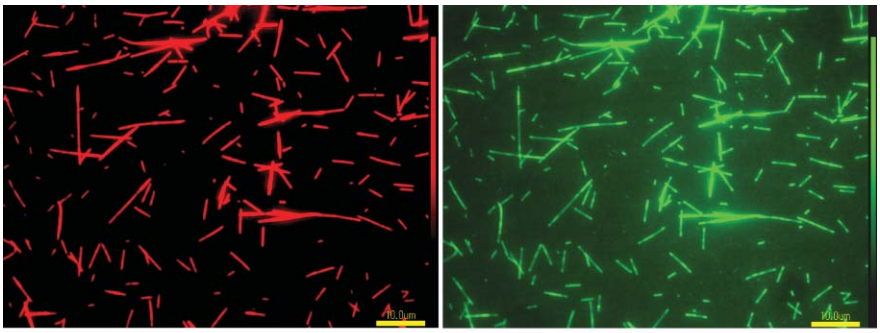

Fig. 3 MTs copolymerized from rhodamine-labeled tubulin and biotinylated tubulin (1:1 molar ratio). (a) Fluorescence image (red) of rhodamine-labeled tubulin; (b) Fluorescence image (green) of Alexa Fluo 488-streptavidin bound to biotinylated tubulin.

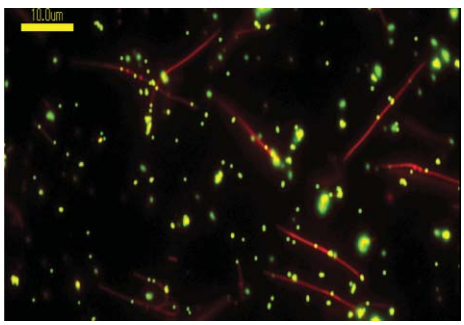

Fig. 4 Fluorescence image of binding of MTs with particles *via* linkage of streptavidin–biotin in solution.

the particles and MTs. Fig. 4 shows the binding image of MTs with particles *via* the linkage of streptavidin–biotin in solution. The particles were efficiently bound to MTs and no aggregation was found. To analyze the behavior of the MT–particle complex more clearly by accelerating its motion, we added higher concentration of ATP; that is, the solution containing 1 mM ATP and other required reagents (see ESI†) was charged into the chamber. The MT–particle complex moved smoothly and no crosslink was observed even when two MT–particle complexes collide with each other. Interestingly, the MT–particle complex was able to pick up a free particle owing to strong biotin–streptavidin binding (ESI video. 2†). The second advantage is that any chemical or biochemical functional molecules can be immobilized under mild conditions *via* the epoxy group of the particles ready for further tasks, whereas it is usually difficult to immobilize biomolecules such as proteins and DNAs to MTs with their functions being retained.

We have succeeded in creating an MT–particle complex with the ability of self-supplying ATP. Since the sliding velocities in our system are related to the concentrations of PEP in the medium, one possible application of this system is to measure concentrations of compounds that can produce ATP enzymatically, with a spatial resolution that is limited by diffusion of ATP.

Financial support from AIST Upbringing of Talent in Nanobiotechnology Course, and Promotion Budget for Science and Technology from MEXT (Minister of Education, Culture, Sports, Science and Technology, Japan) is gratefully acknowledged.

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