Photo-control of kinesin-microtubule motility using caged peptides derived from the kinesin C-terminus domain†

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To design a nanoscale biodevice that can be controlled by an external stimulus, we have introduced photochemical switching peptides derived from the kinesin C-terminus domain into the kinesin-microtubule *in vitro* motility system.

The design of nanoscale devices using biomolecules as building blocks is one of the current topics in nanobiology and bioengineering. Kinesin, one of the motor proteins, transports intracellular materials (*e.g.* vesicles, chromosomes) along the cytoskeletal networks composed of microtubule filaments using the chemical energy obtained from the ATP hydrolysis.[1–3] The kinesin-microtubule system may be ideal for developing nanoscale biodevices because the motility can be reconstructed on a glass surface relatively easily; *i.e.*, microtubules are able to glide across the kinesin-coated glass surface.[4] This system has been favored also due to its small size, processivity, and the linear movement of kinesins along the microtubule. To make the nanoscale biodevices more useful, however, it is important to guide the direction of microtubule movement and even switch on and off the movement by external means at will. To this ultimate end, various methodologies have been developed so far. These include the utilization of an electric field,[5,6] a magnetic field,[7] microlithographic tracks,[8,9] an antibody,[10] an enzyme[11] and so on. These methodologies successfully can control the direction and switch "ON", but cannot switch "OFF". The switch "OFF" technique of microtubule movement is required for effective transport of cargos to target sites. Furthermore, when considering the miniaturization of biodevices in the future, photo-control would be most favorable because of its rapidity, restricted target-site specificity, and non-invasiveness.

Various caged compounds have been intensively investigated as a photo-controlled switch, because their inactive "caged" states can be converted to active "uncaged" states by UV-light irradiation. By using caged peptides, we have already succeeded in controlling the hydrolases[12,13] and sperm-activation.[14] As successful applications of caged compounds in kinesin-microtubule systems, Higuchi *et al.* demonstrated stepping motion by kinesin molecules initiated by the photolysis of caged ATP.[15] Vogel's research group developed "molecular shuttles" using the kinesin-microtubule system, which move on engineered tracks by photolysis of the caged ATP.[16] In both systems, photolysis of the caged compounds triggers "initiation" of the movement. To the best of our knowledge, there is no report on the "cessation" of the microtubule motility by photolysis of a caged compound.

It has been previously reported that the kinesin C-terminus domain suppresses the ATPase activity of the motor domain of kinesin.[17–19] Based on these reports, we have discovered that 20- or 30-mer peptides derived from the kinesin C-terminus domain effectively inhibit the kinesin-microtubule motility.[20] In this report, we have caged amino acid residues within these inhibitory peptides and demonstrated that the kinesin-microtubule motility can be suppressed by photolysis of the caged inhibitor peptides.

A caging group can be introduced into limited amino acid residues, such as Ser, Tyr, Cys, and Lys by solid-phase peptide synthesis.[21] The peptide from the kinesin C-terminus domain contains Lys residue. Therefore, we prepared three peptides having the caged Lys at positions 922, and 925 and both as candidates for photo-controlled inhibitors (Fig. 1a). The Arg residue at position 925 was substituted with caged Lys because Arg and Lys both bear a cationic side chain. All peptides were synthesized by the Fmoc solid-phase method using the caged lysine[12] instead of a normal Fmoc lysine (Fig. 1b) and characterized by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The former NB922 and the latter two peptides, NB925 and NBs922/925, generate WT and R925K upon UV-light irradiation, respectively, which was confirmed by a mass spectroscopic analysis (see Supplementary Information).

(a) Amino acid sequences

		914						933	
WT	: H-	RG	HSA	QIA	KPI	RPG	QHP	AAS	-NH$_2$
R925K	: H-	RG	HSA	QIA	KPI	KPG	QHP	AAS	-NH$_2$
NB922	: H-	RG	HSA	QIA	**K**PI	RPG	QHP	AAS	-NH$_2$
NB925	: H-	RG	HSA	QIA	KPI	**K**PG	QHP	AAS	-NH$_2$
NBs922/925	: H-	RG	HSA	QIA	**K**PI	**K**PG	QHP	AAS	-NH$_2$

(b) Caged Lys

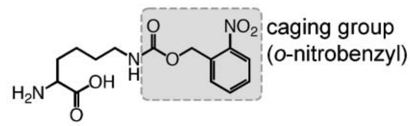

Fig. 1 (a) Amino acid sequences of inhibition peptides. WT is a part of the human kinesin C-terminus domain. The caged amino acid residues are represented by bold letters. (b) A caged amino acid (Lys). The caging group is highlighted.

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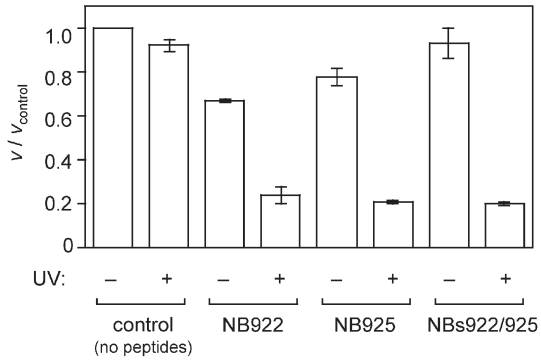

Fig. 2 Microtubule motility in the presence of the caged peptides. Exposure to UV light for 30 s. The data were acquired at 27 °C. [peptides] = 10 μM. The velocity of the control (no peptides, no UV): 0.63 ± 0.03 μm/s.

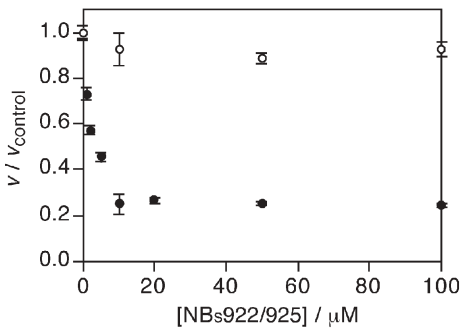

Fig. 3 Microtubule motility in the presence of NBs922/925 (open circles) and the photolysate (closed circles) at 27 °C. The velocity of the control (no peptides): 0.63 ± 0.03 μm/s.

The efficiency of "cessation" by each caged peptide against the kinesin motility was evaluated by monitoring the motility of the fluorescent-labeled microtubules using fluorescence microscopy.[4,20] Before the UV-light irradiation, NBs922/925 exhibited only a 7% inhibitory activity, while the inhibitory activities of NB922 and NB925 were 33% and 22%, respectively (Fig. 2). Thus, it was demonstrated that the introduction of two caging groups is effective for suppressing the inhibitory interaction between the peptide and microtubules and/or kinesin. Fig. 3 shows the concentration dependence of NBs922/925 and the photolysate on the inhibitory activity. The plot revealed the high inhibitory activity of the photolysate of NBs922/925 with IC_{50} of 5 μM. In contrast, the caged peptide showed only a slight inhibitory activity even at 100 μM, which indicates that an excess amount of NBs922/925 does not interfere with the kinesin-microtubule system.

The UV-irradiation was examined at 330–385 nm on the same microscope for the normal motility assays using a mercury lamp (Olympus U-LH100HGAPO, 19 V, 100 W) with a band-pass filter (Olympus BP330-385). The velocity of the microtubules was measured before and after exposure to UV-light (1.68 mW/cm^2 at 360 nm) in the assay buffer containing a caged peptide instead of an inhibitory peptide. All other conditions followed those of the normal motility assay.[4,20] Upon UV-light irradiation in the presence of caged peptides, the velocity of the motility of the microtubules decreased to *ca.* 20% (Fig. 2). The photolysates of NB925 and NBs922/925 exhibit a higher inhibitory activity than the photolysate of NB922.

Fig. 4 shows the fluorescence micrographs of the rhodamine-labeled microtubules in the presence of NBs922/925. The microtubule motilities before and after the UV-light irradiation are shown in Fig. 4a and 4b, respectively (see microtubules highlighted by arrows). In this time range, the microtubules mostly stopped after the UV-light irradiation. The number of microtubules decreased to almost half after the UV-irradiation, which indicates detachment of some microtubules from the glass surface (Fig. 4b). The binding of the uncaged inhibitory peptide to the kinesin and/or microtubule is one probable reason for this detachment. Damaging of the kinesin and/or microtubules by UV light is another possibility. To assess the latter possibility, we monitored the kinesin-microtubule motility in the absence and

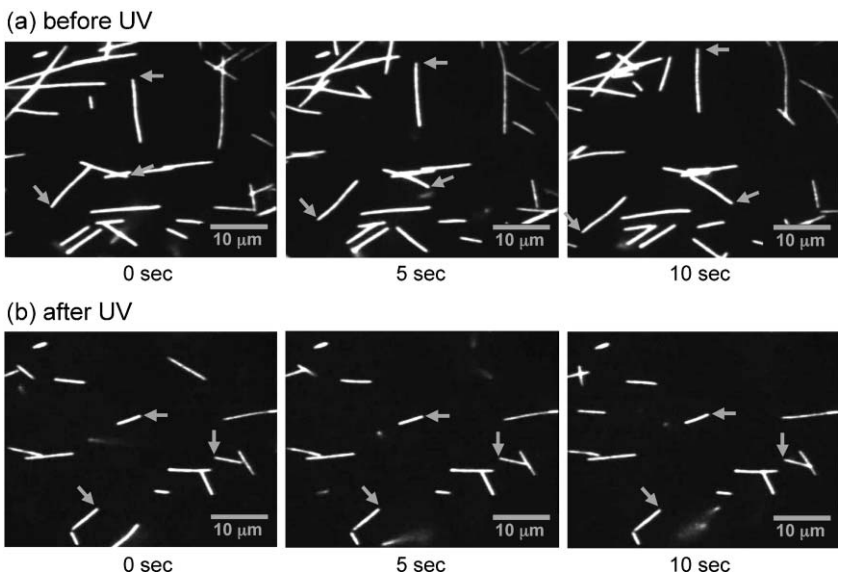

Fig. 4 Fluorescence micrographs of rhodamine-labeled microtubules with NBs922/925 (a) before and (b) after UV-irradiation. UV-irradiation: 30 s, [NBs922/925] = 10 μM, temperature: 27 °C.

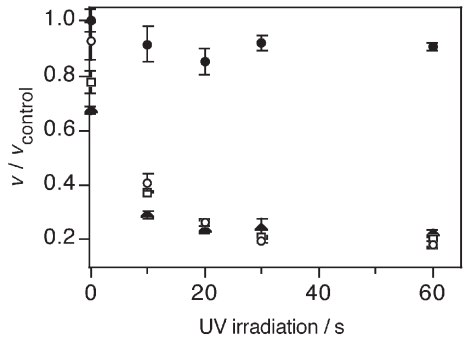

Fig. 5 UV-irradiation time dependence of microtubules motility: no peptides (closed circles), NB922 (closed triangles), NB925 (open squares), NBs922/925 (open circles). The data were acquired at 27 °C. [peptides] = 10 μM. The velocity of the control (no peptides, no UV): 0.63 ± 0.03 μm/s.

presence of the caged inhibitory peptide during UV-irradiation for 60 s. As shown in Fig. 5, the motility decreased to *ca.* 20% in 20–30 s in the presence of the caged inhibitory peptide. In contrast, the velocity and number of microtubules only slightly changed in the absence of the peptide after 30 s (see also Movie (a) in Supplementary Information). These results exclude the possibility that UV-light irradiation damages the kinesin and/or microtubules, and unequivocally demonstrate that the uncaged inhibitory peptides interfere with the interactions between kinesin and microtubule.

In summary, we have successfully demonstrated that the photolysis of the caged peptides whose sequence is derived from the kinesin C-terminus domain can suppress the kinesin-microtubule motility. This is a prototype of the "cessation" device and the efficiency could be improved by the optimization of the peptide sequence. The method developed here should be applicable in forthcoming nanoscale biodevices based on the kinesin-microtubule system. The combination of the photo-triggered "initiation"[16] and "cessation" of the kinesin-microtubules motility would lead to more sophisticated nanoscale biodevices.

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