Kinesin-1 moves processively along microtubule by alternately moving two motor domains, but the mechanism of the preferential forward stepping is still controversial. The “neck linker-docking model” proposes that the neck linker docking of the microtubule-bound head generates forward bias of the tethered head. However, our recent structural analysis of kinesin dimer (Makino et al.) suggested an alternate model in which the tethered head position does not necessarily be biased because the tethered head is not allowed to bind to the rear tubulin-binding site due to a steric constraint on its neck linker and can only release ADP at the forward binding site (“biased-binding model”). To distinguish these mechanisms as alternate steps, we engineered two-headed monomer kinesin by joining two motor heads in tandem on a single polypeptide, in which the neck linker of first head (N-head) is connected to second head (C-head) so that it can propel C-head forward, whereas the neck linker of C-head is free. Single molecule fluorescence observation showed that this two-headed monomer moves processively along microtubules although the velocity was smaller than wild-type dimer by four-fold. In addition, FIONA measurement of individual head showed that both heads takes discrete 16 nm steps, illustrating that this monomer moves by alternately exchanging two heads. Then we measured the dwell time of alternate steps using single molecule FRET and found that forward-stepping of C-head presumably driven by the neck linker docking was less efficient than the forward-stepping of N-head, because the tethered C-head often rebinds to the rear-binding site. These results suggest that biased-binding mechanism is more efficient to drive forward stepping, because rebinding of the tethered head to the rear-binding site is effectively prohibited.

Kinesin is known as a dimeric motor protein, which carries cellular cargoes along microtubules by hydrolyzing ATP. Calmodulin (CaM) is a calcium binding protein that participates in cellular regulatory processes. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. We have previously demonstrated that kinesin fused with CaM at the C-terminal binds reversibly to M13-Qdots in a calcium dependent manner. In this study, we tried to make the calcium dependent reversible dimerization of kinesin utilizing CaM-target peptide M13 fusion binding system in order to control motility of kinesin. First we designed and prepared the cDNA of the truncated kinesin (355 amino acids) that does not form dimers, then prepared cDNA encoding two kinesin chimeric proteins in which C-terminal of kinesin355 was fused with calmodulin (K355-CaM) and fused with M13-Cys (K355-M13-cys). The cDNAs of the kinesin chimeras were cloned into expression vector pET21a and transformed into E.coli BL21 (DE3). The kinesin chimeras were successfully expressed and purified by C-Batchel column. These kinesin chimeras showed normal ATPase activities. Furthermore, K355-CaM bound to M13-YFP in a calcium dependent manner. The calcium dependent interaction between K355-CaM and K355-M13-Cys was examined using Size-exclusion chromatography (SEC)-HPLC, whereas two chimeras did not bind in the absence of Ca++ . In vitro motility assay demonstrated that the dimerized chimera induced microtubule gliding in the presence of Ca++.