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学 位 論 文 題 名

Dynamic Photocontrol of the Kinesin-Microtubule Motor Protein by a Photoisomerizable Monolayer Surface

(光異性化単分子膜表面によるモータータンパク質キネシン－微小管の光動的制御)

学位論文内容の要旨

To design nano assembly devices, smart biological materials and molecular sorting in the field of tissue engineering, microfluidics, mechanistic studies of substrate interaction with cells, peptides, DNA, proteins etc. using biomolecular motor as promising components is one of the hot topic in nanotechnology and bioengineering. Kinesins are prominent microtubule associated motor proteins, which play a central role in both structural and biological functions of the cells. The fundamental role of kinesin is to actively distribute the intracellular materials like organelles, vesicles, chromosomes along the microtubules and to drive the cell division process by converting the chemical energy of adenosine triphosphate (ATP) into mechanical energy and can move towards the fast polymerizing plus ends of microtubules. Among the molecular motors, the kinesin-microtubule system is used widely for developing nanoscale biodevices, because the motility can be regenerated on a surface comparatively easily. The kinesin-microtubule system has other advantages such as, its small size, processivity, and the linear movement of single kinesin along the microtubule compared to other molecular motor proteins.

The ability to reversibly control the speed of microtubule gliding and the direction of movement on the surface of kinesin by external stimuli would greatly improve the sophistication of nano devices. Several methodologies have been used for the artificial control of microtubule function which includes microlithographic tracks, electric and magnetic fields, antibody and so on. When considering the biodevices in the future, photochemical energy inputs offer advantages compared to chemical energy inputs such as (i) light does not generate waste products; (ii) it can be switched on/off easily and rapidly; (iii) photons, besides supplying the energy to the system, can also be useful to "read" the state of the system and thus to control and monitor the operation of the machine etc.. Higuchi et al. used the caged ATP as photo-controlled switch, because their inactive caged states (OFF state of motility) can be converted to active uncaged states (ON state of motility) by UV light irradiation. Another group successfully used the photolysis of caged ATP to develop molecular shuttles on engineered kinesin tracks. On the other hand Tatsu et al. demonstrated the switching of kinesin's activity from the ON state to the OFF state by the photolysis of caged peptide derived from the kinesin C-terminus domain. All these methods can either trigger the initiation of the movement or cessation of the microtubule motility. To design the more useful nanoscale biodevices, it is important that the

system should provide ON and OFF switching of gliding motility at any desired time and at any desired position in space.

We reported that, the reversible and repeated regulation of the motility of microtubule by the photoisomerization of the underlying monolayer using two different wavelengths of light. For the fabrication of such photoresponsive monolayer, we employed a derivative of azobenzene; one of the most studied photochromic compounds due to its strong photo-switching effect, reversibility and simplicity of incorporation, with a triethoxy silane group which react with the glass surface to be anchored and a lysine group which can interact with motor proteins. Using this approach, we described the reversible and repeated control of the gliding velocity of microtubules driven by kinesin on the azobenzene monolayer (with a maximum of 15% difference in velocity) upon irradiation with UV and visible light.

In **Chapter 2,** a novel platform for the reversible control of microtubules adhesion on a molecularly well-defined surface by external stimuli is described. Photochemical isomerization of azobenzene functionalized surfaces was used to induce attachment/detachment of microtubule on the surface and allowing for the controlling of the binding of microtubule on the surfaces. The surface had a lower affinity for the microtubules in the Z form of the lysine-presenting azobenzene monolayer than it did in its E form.

In **Chapter 3**, the repeated regulation of fast and slow modes of microtubule gliding motility by the reversible photoisomerization of the underlying monolayer using light of two different wavelengths is described. For the photoresponsive components, a series of derivative of azobenzene monolayer surface, one of the most studied photochromic compounds, with various terminal groups is employed. The gliding velocity of the microtubules, driven by kinesin immobilized on the positively charged terminal group monolayer could be controlled repeatedly between fast and slow modes upon irradiation with UV and visible light, respectively.

In this dissertation, the generality and the core mechanism underlying in this in-vitro control of kinesin activity and microtubule motility on a molecularly well-defined surface using a series of light responsive azobenzene monolayer depending on the conformation of azobenzene moiety in the monolayer was demonstrated.

学位論文審査の要旨

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博士学位論文審査等の結果について（報告）

近年、生体モータータンパク質を、ナノテクノロジーにおける高精度な物質移動へと応用しようという動きがある。そのためには生体モータータンパク質を望みの場所で望みのタイミングで働かせるように人為的に制御しなくてはならない。光刺激によって、生体モータータンパク質を何度でも制御できれば有用な方法となるが、これまで光刺激でモータータンパク質の活性を可逆的に制御する研究は行われてこなかった。

本論文は、このような現況にあるモータータンパク質活性の繰返し制御について、基板上のフォトクロミック単分子膜を利用する方法を考案し、モータータンパク質の一つであるキネシン－微小管系を人為的に制御する新しい仕組みを実現することを目的としたものである。

種々の分子構造の単分子膜を系統的に合成し、その光異性化反応に於ける、モータータンパク質キネシンの活性およびキネシン－微小管系の運動特性への影響を研究した結果、フリーのアミノ基を有するアミノ酸残基を末端に含むアゾベンゼン単分子膜の光異性化反応によって、その上に配されたキネシンの活性が可逆的に変化することを世界で初めて見出した。さらに、実際にキネシンの運動活性の可逆的変化をATPの加水分解速度と微小管の滑走速度の可逆的変化として観測・可視化することに成功した。

これを要するに、著者は、モータータンパク質の運動活性およびその人為的制御の方法に関して新知見を得たものであり、生命科学と材料化学もしくは合成化学の境界領域に貢献するところ大なるものがある。

よって著者は、北海道大学博士（理学）の学位を授与される資格あるものと認める。