学 位 論 文 の 要 約

博士の専攻分野の名称　　博士（生命科学）　　氏　名　　Amrutha A S

学 位 論 文 題 名

Structure-Property Relationship Studies of the Photoresponsive Inhibitors of Kinesin-Microtubule Motor System

（キネシン - 微小管モーター系に対する光応答性阻害剤の構造 - 物性相関研究）

The ATP fuelled linear motor protein kinesin is responsible for the microtubule based transportation of the cellular cargoes such as vesicles, organelles etc. in a living cell. These "molecular shuttles" can be integrated with the synthetic components for the applications in the artificial nanotransportaion systems. To adopt these naturally evolved nanomachines in the artificial environment, one needs to have precise spatiotemporal control over their motor activity. Recently our group demonstrated the complete and reversible regulation of the kinesin motor activity using azobenzene tethered peptide (Azo-peptide; Azo-Ile-Pro-Lys-Ala-Ile-Gln-Ala-Ser-His-Gly-Arg). The azo-peptide in the *trans* state of the azobenzene unit completely stopped the kinesin driven gliding motility of microtubules and in its *cis* rich state allowed the motility. Yet we have not previously been in a position to explain the modes of interaction involved in the inhibition by this reverse-ordered peptide; in addition , we had not identified the critical peptide sequence required for inhibition, not had we examined the effects of substituent groups present on the azobenzene moiety on the inhibitory activity and photoswitchability. For practical applications more efficient inhibitors that function at lower concentrations with greater photoswitchability were required. Developing the photoswitches which can make use of only visible light for isomerization and achieving the selective manipulation of single microtubules were also our important objectives.

I studied the structure-property relationships of the photoresponsive inhibitors of kinesin motor through the systematic variations in the structure of the peptide unit and also through the various substitutions on the photoresponsive azobenzene unit. The important amino acids responsible for the inhibition, modes of interactions involved in inducing the inhibition and also the substituent group on azobenzene unit enhancing the photoswitchability were identified. As a result a new, more efficient inhibitor with shorter peptide sequence (-Arg-Ile-Pro-Lys-Ala-Ile-Arg) coupled to the azobenzene unit substituted with an OMe group at the *para* position was obtained. This novel inhibitor completely stopped the microtubule movement at 750 μM concentration in the *trans* form and provided a higher recovery of velocity in motility (86%) in the *cis* form after UV irradiation.

I also established a new, simple optical set up through which I successfully demonstrated the local concentration and dispersion of the microtubules at any desired position and time by irradiating a local area of the motility system at one wavelength, while irradiating the entire area at another wavelength, to enrich either *cis* or *trans* isomers of photoswitches in the selected region. Furthermore, various manipulations (e.g., driving, bending, and cutting) of single microtubules were made possible by arresting the activity of ambient microtubules. All these were achieved without the need for any surface patterning.

Further, I developed a new class of photoresponsive inhibitors which undergo *trans* to *cis* isomerization by the absorption of visible light and revert back to the *trans* state from

the *cis* state by the fast thermal relaxation. Thus the reversible control of kinesin motor activity is now possible with the use of only one wavelength of visible light. These photoswitches have made it easier to manipulate the single microtubules because the system with previously reported class of photoresponsive inhibitors required the optimization of the intensities of the two wavelengths (UV and visible) which was a challenging job. I expect that the new class of photoresponsive inhibitors developed will remove the roadblocks in successful implementation of the motor protein kinesin and its associated filaments microtubules in the artificial nano transportation system.