学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study of the Effect of Tubulin C-terminal Tail on Mechanical Properties of Microtubule and Interaction with its Associated Motor Protein

(微小管の機械的特性および関連モータータンパク質との相互作用に対する チューブリン C 末端尾部の効果研究)

Microtubules, polymerized from αβ tubulin heterodimers, are essential for cellular functions like maintaining cell shape, cell movement, mitosis, intracellular cargo transport, etc. Each tubulin of a microtubule is composed of an ordered core and an extended flexible segment called the C-terminal tail (CTT). Both the ordered core and the disordered CTT of tubulin undergo modifications while microtubules perform their diversified functions. Compared to the tubulin cores, the CTTs that protrude from the microtubule surface are more prone to such modifications. Previous studies show that the removal or addition of one or more residues on the tubulin CTT can alter the microtubule properties and interaction with its associated proteins for example, dynein, tau, etc. The tubulin CTTs have also been reported to regulate the electrical properties of microtubules. However, the effect of the absence of the tubulin CTT on microtubule mechanical properties, for example, bending rigidity and their interaction with one of the most studied motor proteins, kinesin remains elusive. In this dissertation, I investigated the role of tubulin CTTs in altering the bending rigidity of microtubules. I further explored if the tubulin CTTs alter the interaction between microtubules and kinesin. Using the all-atom Molecular Dynamics (MD) simulation, I explore the mechanism of the contribution of tubulin CTT in regulating the interaction between microtubule and kinesin at the molecular level.

In **Chapter 1,** I described the purpose and the background of the study of this dissertation.

In **Chapter 2,** I investigated the effect of CTTs of tubulin on the mechanical properties of microtubules. For this, I used the subtilisin treatment to remove tubulin CTTs (fig. 1A). The successful removal of tubulin CTT was confirmed using SDS-PAGE and MALDI-TOF mass spectroscopy techniques. To understand the effect of tubulin CTT on microtubule mechanical properties, I determined the persistence lengths of microtubules polymerized from native and modified tubulin. The persistence length of microtubules with intact tubulin CTTs was significantly higher than that without CTTs (fig. 1B). I also studied the response of both the microtubules to mechanical stress, in this case, compressive stress. I estimated the bending rigidity of microtubules prepared from native and modified tubulin from both observations and found that the microtubules with intact tubulin CTTs exhibit twice the rigidity compared to the microtubules that lack CTTs of tubulin. These results suggest that the absence of tubulin CTTs softens microtubules.

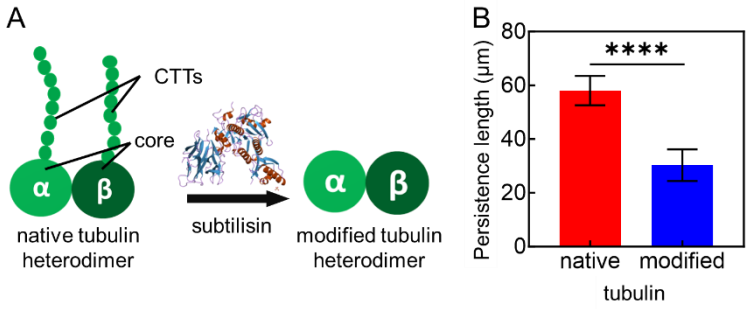

Fig. 1 A) *Subtilisin treatment of tubulin to remove the CTTs.* ***B)*** *Mean persistence lengths of microtubules prepared from native and subtilisin-modified tubulin. ****P<0.0001 using Student's t-test.*

In **Chapter 3,** I investigated the role of tubulin CTT on the interaction of microtubules with its associated motor protein, kinesin. For this, I studied kinesin-driven cargo (quantum dot) transport along microtubules polymerized from native and modified tubulins in the presence of ATP (fig. 2). The cargo transport dynamics by kinesin along microtubule were facilitated when tubulin CTTs were intact. To better understand the kinesin-tubulin interaction, I also performed the inverted gliding assay of microtubules polymerized from native and modified tubulin on kinesin-coated glass substrate. I found that the removal of the tubulin CTTs caused the microtubule gliding to slow down. These findings conclude that the absence of tubulin CTT impedes tubulin-kinesin interaction.

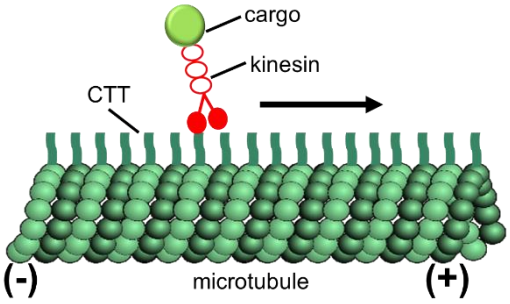

***Fig. 2** Schematic illustration of kinesin-driven cargo transport assay along microtubule polymerized from native tubulin.*

In **Chapter 4,** I investigated the mechanism from the energetic aspect by which the tubulin CTT influences the interaction between the kinesin and tubulin. I performed all-atom MD simulations of the protein complex systems consisting of a tubulin dimer with and without CTTs and a motor domain of kinesin (fig. 3A). I analyzed the interaction energies (fig. 3B) between tubulin heterodimer and kinesin in the presence and absence of CTTs. The total interaction energy of the system in the presence of CTTs was significantly higher than that of the system where no CTTs were present. These results support the experimental observation and shed light on the effect of CTT on the binding of kinesin with tubulin.

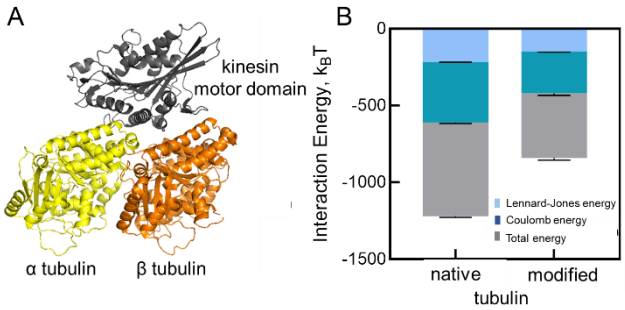

***Fig. 3 A)** Tubulin-kinesin model for MD simulation. **B)** Interaction energy between tubulin heterodimer and kinesin in the presence and absence of tubulin CTT.*

In **Chapter 5,** I summarized all the important outcomes and discussed the prospects of this research.

In this dissertation, I investigated the role of tubulin CTT in modulating the properties and functions of microtubules. I compared the mechanical property of bending rigidity of microtubules with and without intact tubulin CTTs using two different approaches and concluded that the removal of CTT softens the microtubule. I also investigated the interaction of microtubules with kinesin, by studying kinesin-driven cargo transport assay and inverted gliding assay of microtubules in the presence and absence of tubulin CTTs and found that the removal of tubulin CTT impedes the tubulin-kinesin interaction. The mechanism of this experimental finding was unveiled using all-atom MD simulation results showing that CTT facilitates the movement of kinesin along the microtubule by enhancing the interaction between tubulin and kinesin. This study will provide important insights into different cellular processes that involve the modification of microtubules at the tubulin CTTs and lead to novel therapeutic approaches for neurodegenerative diseases with altered microtubule properties.