



TAU PROTEIN DETECTION BY USING DEVELOPED MICROTUBULE-KINESIN SYSTEM

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Lack of intraneuronal transport is a common hallmark of many neurodegenerative diseases including Alzheimer's disease (AD) [1, 2]. This often involves dysfunction of microtubule associated proteins such as tau, which normally protect microtubule stability and regulate molecular transport along the microtubules. Tauopathies, a large group of age-related neurodegenerative diseases including AD, is characterized by abnormal accumulation of tau protein [3-5]. A reliable and sensitive in vitro assay for rapid analysis of the components and conditions affecting intraneuronal microtubule-based transport would be crucial.

Conventional bead assay uses microtubules immobilized on a solid surface [6]. However, the issue of whether full attachment negatively affects kinesin motion was not previously investigated. In this work, an alternative bead assay design employing microtubules immobilized between two parallel walls (suspended microtubules) to prevent substrate interferences was described and the performance of the "suspended" bead assay in comparison to a conventional bead assay using microtubules immobilized on a glass surface (attached microtubules) was examined.

Experiments were conducted in flow cells to optimize the detection system. Two different immobilization configurations were compared: attached and suspended microtubule cases. Microtubules were immobilized on a poly-l-lysine (PLL) coated surface in the attached microtubule configuration. For the suspended microtubule case, microtubules were bridged in between high parallel walls. After immobilization of microtubules, kinesin-coated beads were inserted in the flow cells. By adding ATP solution, the kinesin motion was activated.

Motion of the kinesin-coated bead was investigated along both attached and suspended microtubules. The average kinesin-coated bead velocity for the attached microtubules was 37% slower ($p < 0.0001$) than that for the suspended microtubules. Next, the sensitivity of the assays for the tau binding experiments was investigated. The suspended microtubules demonstrated higher significance ($p = 0.014$) than the attached microtubules ($p = 0.065$) and the significant difference ($p < 0.05$) was achieved only along the suspended microtubules indicating higher sensitivity of the "suspended" assay.

In this work proposed "suspended microtubules" configuration with the commonly used "attached microtubules" configuration has been compared and the proposed suspended system shows higher sensitivity in the microtubule-tau attachment experiments has been demonstrated. As the kinesin-microtubule system is an essential component of the intraneuronal transport, development of the reliable and robust in vitro system permitting functional analysis of its components is of paramount importance for the development of novel diagnostic and therapeutic approaches selecting tau born neurodegenerative diseases including AD.

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