Synchrotron Small Angle X-Ray Scattering Quantitatively Detects Angstrom Level Changes in the Average Radius of Taxol-Stabilized Microtubules Decorated with the Microtubule-Associated-Protein Tau

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Abstract. With the emerging proteomics era the scientific community is beginning the daunting task of understanding the structures and functions of a large number of self-assembling proteins. Here, our study was concerned with the effect of the microtubule-associated-protein (MAP) tau on the assembled structure of taxol-stabilized microtubules. Significantly, the synchrotron small angle x-ray scattering (SAXS) technique is able to quantitatively detect angstrom level changes in the average diameter of the microtubules modeled as a simple hollow nanotube with a fixed wall thickness. We show that the electrostatic binding of MAP tau isoforms to taxol-stabilized MTs leads to a controlled increase in the average radius of microtubules with increasing coverage of tau on the MT surface. The increase in the average diameter results from an increase in the distribution of protofilament numbers in MTs upon binding of MAP tau.

1. Introduction

Microtubules (MTs) are nanometer scale hollow cylinders derived from the eukaryotic cell cytoskeleton [1,2]. The hollow cylinders are comprised of globular tubulin subunits, which align end-to-end to form linear protofilaments (figure 1). The lateral interactions between protofilaments stabilize the tubular wall. In cells, MTs and their assembled structures are key components in a broad range of functions from providing tracks for the transport of cargo (organelles, vesicles containing neurotransmitter precursors, etc.), to forming the spindle structure in cell division, and to imparting mechanical stability in the long axons of neurons [1-4]. From a biomolecular materials perspective MTs may also be considered as model nanotubes with a well-
defined inner ($\approx 15$ nm) and outer ($\approx 25$ nm) diameter, which can be fine-tuned depending on the solution conditions. Nanotubes and bundles of nanotubes are of high interest because of their potential technological, biomedical, and biotechnological applications [5-7].

Microtubules are highly dynamical biopolymers. They are usually found in two types of populations: Unstable MTs rapidly shrink (depolymerize) and grow (polymerize) (an important cellular property, which enables, for example, MTs to “search” and “capture” chromosomes in dividing cells during mitosis), whereas, in non-replicating mature neurons MTs are longer-lived in axons and dendrites. Distinct members of microtubule-associated-proteins (MAPs) control these two populations through MT-MAP interactions, although the precise manner in which MAPs affect MT structure and dynamics and MT-MT interactions is not well understood. The current model is that in neuronal axons, different isoforms of the microtubule-associated-protein (MAP) tau (shown in Figure 2) enhance tubulin assembly and regulate MT dynamics although much of the details remain unknown.

Figure 1. The $\alpha/\beta$ tubulin dimer is the building block of protofilaments. Protofilaments interact laterally in stabilizing the tubular wall of the microtubule. The cartoon of the microtubule (redrawn from [3]) highlights a tubulin dimer and a protofilament.

The very high interest in elucidating the precise nature of the interactions between MAP tau and MTs is the well known fact that altered tau-MT interactions, which changes the balance in the fraction of tau bound to the MT surface versus soluble tau, leads to MT depolymerization and tau tangles. This behavior, in turn, has been implicated in a large number of neurodegenerative diseases, including fronto-temporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), late stage Alzheimer’s disease, Pick’s disease, and supra-nuclear palsy [8-12].

2. Experimental Results and Discussion
Human MAP tau exists in the form of six isoforms, those containing four C-terminal “imperfect” repeats (constituting the cationic MT binding domains) labeled 4R-tau, and those with three such repeats (3R-tau) (figure 2) [13]. Each family contains three distinct alternatively spliced
isoforms, yielding N-terminal short (S), medium (M), and long (L) projection domains (figure 2). The six tau isoforms are thus labeled: 4RS, 4RM, 4RL, 3RS, 3RM, and 3RL.

Figure 2. Schematic of a microtubule (MT) decorated with adsorbed MAP tau isoforms and two tau proteins in solution. MTs are nanotubes (outer diameter \( \approx 25 \) nm) and are overall negatively charged. In their biologically active state tau isoforms are unstructured polypeptides, which possess either 3 “imperfect” repeat (3R) or 4 “imperfect” repeat (4R) cationic binding domains (colored rectangular boxes). The isoforms also differ in their N-terminal projection domain by possessing either zero, one, or two 29 amino acids, thereby generating short (S-), medium (M-), or long (L-) isoforms.

Synchrotron small angle x-ray scattering (SAXS) was used to quantitatively study the assembly structure of taxol-stabilized microtubules mixed with the six human MAP tau isoforms [14]. Figure 3 (Left) shows SAXS profiles of taxol-stabilized microtubules in the presence of the 4RL-tau isoform as a function of \( \Phi \) defined to be the molar ratio of tau to tubulin-dimer in the reaction mixture. The observed intensity oscillations as a function of scattering wave vector \( q \) may be quantitatively described in terms of the MT form factor \( F_{MT} \) corresponding to the fourier transform of the MT electron density (figure 3, Middle). The MT was modeled as a simple nanotube with a uniform wall electron density (figure 3, Middle). The SAXS intensity \( I(q) \) is therefore proportional to \( |F_{MT}|^2 \) averaged over all directions in q-space [14].

The SAXS profiles are found to quantitatively fit the form factor model of a microtubule as can be seen from the colored solid lines going through the data in figure 3 (Left). We used a MT wall thickness of 49Å and a mean electron density of 0.07817e/Å\(^3\) consistent with previously published papers [15-17]. Therefore the inner MT radius \( <R_{MT}^{in}> \) was the only fitting parameter. This model was also used previously to describe SAXS data of microtubules in the absence of MAP tau [18,19]. Consistent with earlier work we subtracted a background, which was a polynomial equation that goes through the minima observed in the SAXS profiles [14]. Figure 3 (Left) shows that the MT form factor is shifted with the addition of tau towards lower q values. This is consistent with an increase in the inner diameter of the microtubule.

The results of \( <R_{MT}^{in}> \) obtained from the fits of the SAXS profiles to the data are shown in figure 3 (Right). The data is plotted both as a function of \( \Phi \) and \( \phi \). While the former is the tau to tubulin-dimer molar ratio in the reaction mixture, the latter corresponds to the same ratio but only counting the tau molecules, which are bound to the MT surface (i.e. without the soluble tau fraction). The increase in the MT radius results from an increase in the average number of protofilaments in MTs (i.e. due to a shift in the distribution of MT protofilament numbers \( <N_p> \)
also shown in figure 3 (Right). Significantly, $<R_{in}^{MT}>$ was observed to rapidly increase for $0 < \phi < 0.2$ and saturate for $\phi$ between 0.2 and 0.5. Thus, a local shape distortion of the tubulin dimer upon tau binding, at coverages much less than a monolayer, is spread collectively over many dimers on the scale of protofilaments. Our findings imply that MAP tau regulates the shape of protofilaments and thus the spontaneous curvature of microtubules leading to changes in the curvature.

Figure 3. SAXS data showing that MAP tau regulates the mean radius of taxol-stabilized MTs, or equivalently, the microtubule (MT) protofilament number (N_pf). LEFT: Synchrotron SAXS profiles of MTs with bound 4RS tau as a function of tau to tubulin-dimer molar ratio in the reaction mixture ($\Phi$). With increasing tau, the SAXS profiles shift to lower $q$ implying an increase in the MT radius. Colored lines are results of fits of the data to a model consisting of a hollow MT nanotube with the inner radius $<R_{in}^{MT}>$ being the fitting parameter. MIDDLE: The electron density profile of the wall of the microtubule used to calculate the form factor of the microtubule. RIGHT: The inner radius $<R_{in}^{MT}>$ for 4RL-tau isoforms plotted versus $\Phi$ (TOP) and $\phi$ (BOTTOM), where $\phi$ is the molar ratio of tau adsorbed to the MT surface to the tubulin dimer (i.e. it is proportional to the number of tau molecules bound to the MT surface per unit area). The radial size of MTs increases as a function of increasing tau. The increase in $<R_{in}^{MT}>$ is a reflection of the shift (towards larger protofilament numbers) in the distribution of protofilaments in MTs with increasing tau binding [adapted from 14]. The SAXS profiles for $\Phi = 1/2$, 1/6, 1/10, 1/20, 1/100, and no tau are adapted from [14]. The SAXS profiles for $\Phi = 1/4$, 1/7, 1/8, 1/40, which show the same trend as the previously published data reported in [14] are new SAXS data.

3. Concluding Remarks
Although electron microscopy remains a very important probe of the structure of microtubules, Synchrotron SAXS, which yields statistically averaged structures, is a highly quantitative probe of the parameters characterizing individual microtubules such as the wall thickness and the electron density of the microtubule. Thus, the combination of these complementary techniques can quantitatively yield the structure of both isolated MTs and their assemblies [14,18,19]. Aside from the biophysical interest in the behavior of microtubules and associated proteins, microtubules may also be considered as model nanotubes and thus of high interest in applications. For example, Nanotubes are indispensable components in the development of future miniaturized materials. They have applications in diverse areas from uses as chemical, drug, and gene encapsulation vehicles, to biosensors and circuitry components. Thus, an area of intense current
research in nanoscience is in elucidating the key parameters, which control the self-assembling properties of nanometer scale tubes. In this paper we described how MAP tau isoforms regulate the average radial size of taxol-stabilized MTs at the ångstrom scale. In particular, we found that tau regulates the distribution of protofilament numbers in MTs as reflected in the observed increase in the average radius <R_{in}\text{MT}> of MTs with increasing coverage of MTs by tau. The results may impact biomolecular materials applications requiring radial size controlled nanotubes.

**Acknowledgments**

CRS, YL, MCC, UR, DJN acknowledge support by the U. S. DOE BES DE-FG02-06ER46314 (plasmid preparation and protein binding and self-assembly) and the U. S. NSF DMR-0803103 (protein phase behavior). SCF was supported by the U. S. NIH NS35010 and LW and HPM were supported by the U. S. NIH NS13560. MCC further acknowledges support in part from the Korean Foundation Grant KRF-2005-2214-C00202. UR was also supported by the Human Frontiers Science Program Organization (CDA 0059/2006) and the Israel Science Foundation (grant number 351/08). DJN was further supported by a Human Frontiers Program Grant number RGP0034/2010 and the U. S. NSF grants DBI-0959721, PHY-0847188, and the Harvard MRSEC DMR-0820484. UR and DJN were jointly supported by the US-Israel Bi-national Science Foundation (grant number 2009-279). MK was supported by Korea Health 21 R&D Project MOHW and the WCU (World Class University) program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology No. R33-2008-000-10163-0. The Stanford Synchrotron Radiation Laboratory, where the x-ray scattering work was performed, is supported by the U.S. Department of Energy. CRS acknowledges useful discussions with KAIST Faculty where he has a World Class University Visiting Professor of Physics appointment.

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