Experimental report

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Proposal: 8-02-790 **Council:** 10/2016

Title: Neutron fiber diffraction analysisof the structural dynamics of microtubules under physiological conditions

Research area: Biology

This proposal is a new proposal

Main proposer: Shinji KAMIMURA

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Samples: Microtubules

Instrument	Requested days	Allocated days	From	To
D16	8	4	02/03/2017	06/03/2017

Abstract:

Microtubules are key components of the cytoskeleton in eukaryotic cells, which structure is closely related to many cell functions, e.g., migration, shape changes, differentiation and mitosis, as well as to their abnormal functioning in the case of cancer cells. Here, using a new technique for the rapid shear-flow alignment of microtubule, we would like to investigate how the configuration change of microtubule subunits (tubulin) is occurring during the assembly of microtubules, chemical reactions (GTP hydrolysis, binding to paclitaxel) and disassembly. This novel approach we try here is expected to provide us with a new tool to investigate the structural dynamics of native microtubules including the effects of tubulin-targeting anti-cancer agents.

Experimental report

Neutron fiber diffraction analysis of the structure of native microtubules under physiological conditions

Scientific council 2016-10 Proposal number 8-02-790
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Microtubules are key components of the cytoskeleton in eukaryotic cells. Dynamic conversions between tubulin dimers (free protein unit before assembly in cytoplasm, MW=110,000) and assembled microtubules (polymerized state) occur in a controlled manner to modify intracellular microtubule networks, which varies concomitantly with the whole cell activities such as cell-migration, shape changes, mitosis and differentiation. One of the most fundamental questions is how such microtubule dynamics is associated with the molecular configuration of tubulin dimers, which is expected to be influenced by various factors, e.g., GTP-hydrolysis, inter-tubulin interactions, chemical modifications, structural heterogeneity among tubulin species, interaction with molecular motors, and the decoration by other MAPs (microtubule associated proteins). In addition, since microtubules are thought to be one of the most crucial targets to knockout abnormally proliferating cells, collecting new evidence how anticancer drugs such as paclitaxel are influencing the structure of tubulin dimer in solution is crucial from the biomedical point of view. Our approach is to address this issue by analyzing the structural dynamics of native microtubules that are assembled and kept in physiological solutions mimicking intracellular conditions. For such purposes, fiber diffraction is expected as one of the most powerful techniques since we can directly acquire the information of size, shape and structural periodicity from the diffraction signals using native biological structures, i.e., without freezing, staining or chemical modifications, fixation, crystallization or other artificial modifications.

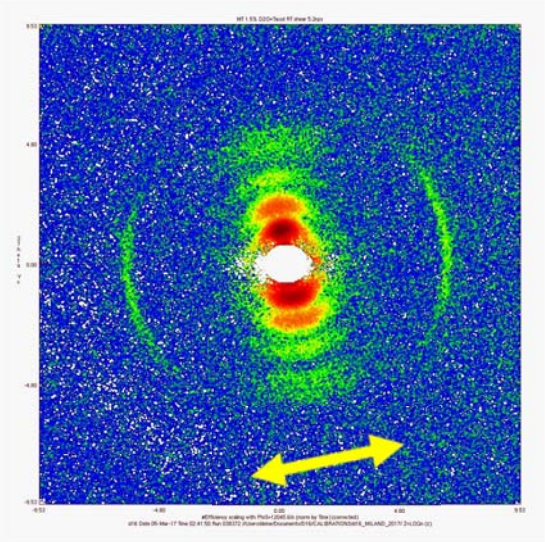

Figure 1. Example of neutron fiber diffraction image obtained with aligned microtubules in D_2O (92%) buffer solution containing 80 mM K-PIPES, 1 mM EGTA, 1 mM dithiothreitol, 0.2 mM Tris-HCl, 2 mM $MgCl_2$, 0.4 mM paclitaxel, 25 mg/mL tubulin, and 3 mM GTP, pH 6.9. During giving shear-flows (estimated shear-rate of ca. 20 s^{-1}), scattering diffraction images were collected at DM16 beam line with 950 mm detector position. Beam size, Φ4.5 mm. Exposure time, 2 hr. At room temperature (22ºC). Yellow arrow indicates meridional (longitudinal) axis of assembled microtubules.

In the present project, we applied our original technique[1-4] for the rapid shear-flow alignment of biological filaments. Using this technique, we expected to collect structural information from microtubules independently in axial (meridional, i.e., longitudinal direction) and diameter (equator, i.e., diameter direction) directions. This is the main advantage of this technique, comparing with SAXS[5-9] and SANS[10-12] experiments executed so far. In the present experiments of neutron fiber diffraction, we focused on these two signals.

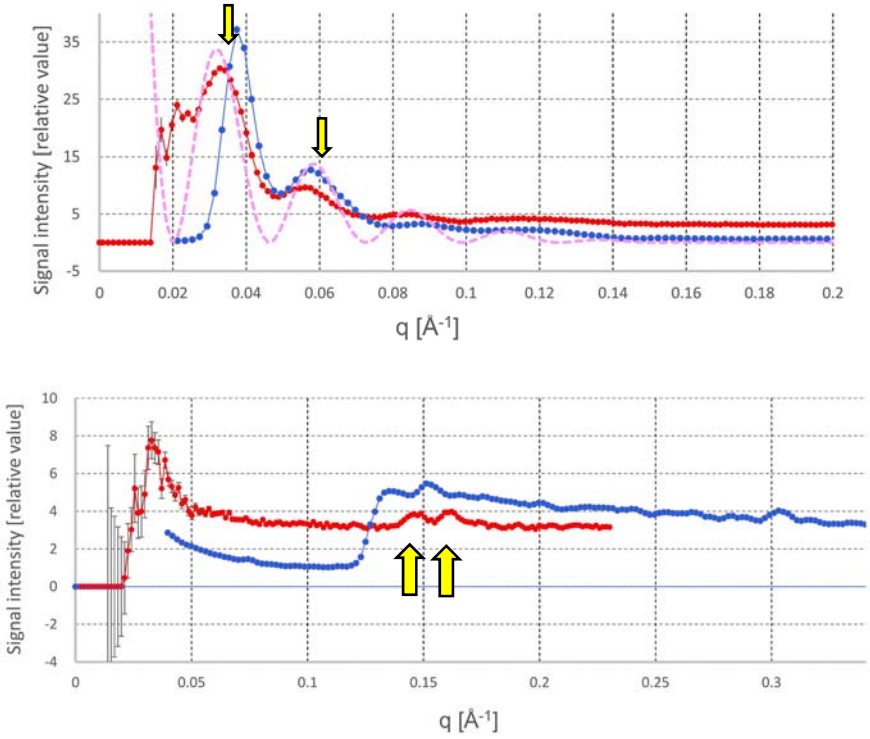

Figure 2. Signal profiles obtained by neutron fiber diffraction in the present study. (a), the profile of equatorial signals from center to higher angle area is shown (red) in a q-range of 0.02 to 0.2 Å^{-1}, which is superimposed with corresponding data we obtained previously with X-ray fiber diffraction (blue). A curve of simulated Bessel function is also shown (pink). Arrows indicates 0.035 and 0.05 Å^{-1} peak (20 and 12 nm) corresponding to the first two peaks of 0th-Bessel function. (b), the profile of meridional signals is shown (red) in a q-range of 0.02 to 0.35 Å^{-1}, which is also superimposed with the date by X-ray fiber diffraction. Arrows indicate the signals corresponding to the 4 nm-layer line.

As shown in Figure 1, we could obtain fiber diffraction signals that could be separately analyzed on meridional (yellow arrow) and equator. The intensity profile of equatorial signals is theoretically composed of the Bessel functions of 0th-order (reflecting mean microtubule diameter) plus 12-15th order (in higher angle area). The meridional signals, on the other hand, would be corresponding to 4-nm layer-line reflecting the longitudinal repeat of tubulin unit in assembled microtubules.

The obtained signal profiles were analyzed in more detail as shown in Figure 2. The results were not the same, but similar to those obtained by X-ray fiber diffractions[4]. By fitting the obtained equatorial signals (Figure 2a) to the 0th-Bessel function, we estimated the mean diameter of microtubule to be about 23.8 nm (23.2 nm for X-ray fiber diffraction data[4]). Still we must agree that we need to execute more careful fitting including real population of microtubules with different protofilament number as well as the structural factor of tubulin molecules (by tentatively putting 2.48 nm[4-5]) both estimates were almost the same to each other. Slight different of diffraction signals, i.e., neutron scattering appears at smaller angles, would be reflecting the size difference of core structure that interact

with incident beams as has been discussed[6] as well as the effects on microtubule structures by 92% D_2O in the buffer solution. Obtained meridional signals (Figure 2b) also showed similar peaks around q = 0.157 $Å^{-1}$ (corresponding to the 4-nm axial repeat of tubulin unit). The signals are split into two parts according to the helical arrangement of tubulin (3-start left-handed helix), i.e., composed of off-axial spots. This report is the first demonstration by neutron scattering (neutron fiber diffraction) showing the signals corresponding to 4-nm axial tubulin repeat which has been very difficult to detect from uniformly dispersed specimen in conventional methods of SANS[7-8].

In summary, it is revealed that the shear-flowing technique we had applied to X-ray fiber diffraction was also powerful for the neutron fiber diffraction of microtubules. Results shown here is still preliminary, however, it is clearly shown that both equatorial (diameter direction) and meridional (longitudinal unit repeat) structural information could be separately collected in native microtubules. Since the major advantage of neutron scattering is observation with no damages for biological specimen as well as the possibility to distinguish different molecules or intramolecular components within microtubules by utilizing hydrogen-deuterium exchange, we expect the present approach would provide us with a new tool in the future for the structural biology of microtubules.

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