ESRF	**Experiment title: Structural dynamics of tubulin dimers based on wide-angle X-ray fiber diffraction analysis of aligned native microtubules**	**Experiment number**: L26-02 852
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ABSTRACT

In the present study, we focused on the observation of WAXS signals from aligned microtubules. For this purpose, we tentatively chosen the energy of 12 keV and the camera length of 0.67 m to cover the layer line signal up to 0.45 nm in reciprocal space. We first tested structural changes during X-ray irradiation from 1 s to 16 min. During the exposure time up to 8 min, there were no apparent damage to the structure of microtubules. Even without any noise reduction by background subtraction/averaging, we could detect clear 1-nm layer line signal, which corresponds to the 4th-order diffraction due to regular axial repeat of tubulin within microtubules. With the present WAXS setting, higher order reflections from tubulin, i.e., 0.8, 0.667, 0.57 and 0.5 nm (in reciprocal space) could be detected after image-processing. This is the first report to show WAXS-range observation of X-ray fiber diffraction of aligned microtubules in solution. In addition, new meridional signals of 0.60, 0.56 and 0.46 nm, were detected in microtubules, which we speculated to be coming from internal structure of tubulin molecules.

BACKGROUND

Microtubules are key components of the cytoskeleton in eukaryotic cells. Dynamic conversion between tubulin dimers (free unit protein before assembly in cytoplasm, MW=110,000) and assembled microtubules (polymerized state) occurs in a controlled manner, which modifies intracellular microtubule networks along with the whole cell activities such as cell-migration, shape changes, mitosis, differentiation and so on. Since microtubules are one of the most crucial targets of anti-cancer chemicals (e.g. paclitaxel) that knockout cancer cells, our question is how such tubulin-binding drugs and related derivatives affects the structure of microtubules depending on the states of tubulin dimers during chemical reaction of GTP-hydrolysis. For such purposes, we applied our original technique for the rapid shear-flow alignment of biological filaments (Sugiyama et al., 2009; Kamimura et al 2016) to observe diffraction signals from microtubules at higher resolutions (up to 0.45 nm), which angle area is expected to provide us with the signals derived from the internal structure of tubulin (Kellogg et al., 2017; Makowski, 2010; Suering et al, 2018; Serpell & Smith, 2000) as well as those from tubulin lattice arrangement within microtubules.

RESULTS & DISCUSSION

We first tested experimental setup to test whether the original shearing machine for microtubules (Kamimura et al, 2016) could be applied to SAXS/WAXS analysis at BM26B. We used a shear-flow machine which is equipped with two flat round-plates placed parallel and coaxially to each other with a small gap of 0.35 mm. One of the plates was made of a round quartz window (thickness 0.2 mm, diameter 18 mm). The other flat part was made of a copper plate that enabled us temperature control (37ºC), on which a small window of 3-mm in dimeter (with a CDV plate, thickness 0.2 mm) had been previously perforated at a site 7.5 mm off from the center. After applying microtubule suspension (PIPES buffer, protein concentration, 5-10 mg/mL, volume 0.1 mL) between the two plates, the quartz part was started to spin by 10-20 rps. Since the other copper plate is fixed on a holder placed on the beam line, continuous flows are made in circumferential directions between the two parallel plates, which produced shearing-flow to the buffer solution containing microtubules. X-ray beam, which was designed to be incident into the off-centered CDV window, shows diffractions with an orientation specific for the direction of aligned microtubules in shear flow. First, we tested the effects of continuous X-ray irradiation to the specimen. Our specimen of 5-10 mg/mL tubulin contains roughly 0.5 million filaments of microtubules (presumed mean length of 10 μm) in 7 nL of specimen buffer volume which was estimated from the beam-size (0.1 x 0.2 mm) and the specimen thickness (0.35 mm). Under the present condition with continuous X-ray exposures, irradiated volume should correspond to only 0.007 % of total volume. This would be the reason why we could prolong the exposure time without apparent accumulation of X-ray damages up to 8 min. Wcould also observe the 1-nm layer line signal corresponding to the 4th-order diffraction of axial tubulin repeat (approximately 4 nm) was clearly observed on the display without any image processing of averaging and background subtractions.

With the shorter camera-length of 0.65 m we used, we could detect higher-order diffraction up to 0.5 nm (8^{th}-order diffraction by tubulin axial repeat). This is the first case to detect the signlas of X-ray fiber-diffraction up to the range of 0.5 nm (Fig. 1). In addition to these higher-order signals, 0.60, 0.56 and 0.46 nm signals were also observed. Although we need to continue observations carefuly to prove from where these signals are derived, we expect that our observation is the first case to detect signals coming from internal structures of tubulin dimer, i.e., secondary structure of helices (0.54 nm) or parallel strands (0.7 nm), in protein specimen under physiological solution conditions. In this area, however, in high angle WAXS range, scattering from the window materials could not be neglected (Fig. 1) in addition to the Kossel-Kikuchi line derived from the window material (CDV) in the chamber. Improvement of machines and modification of optics to avoid high-angle scattering would be required.

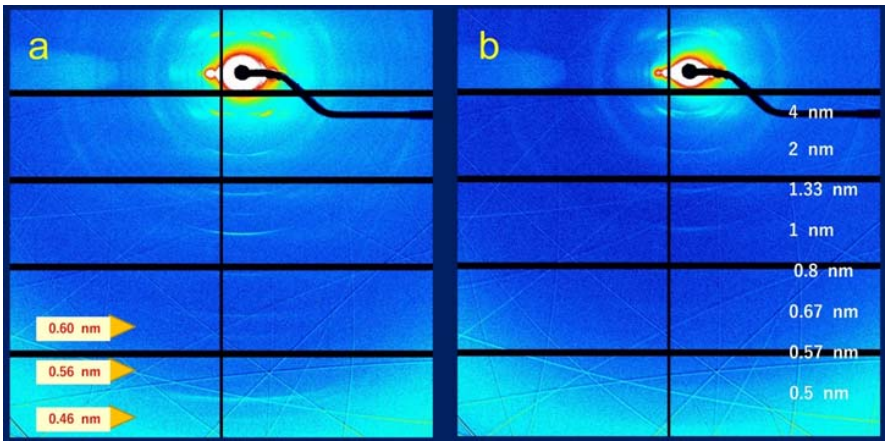

Fig. 1 An example of X-ray fiber diffraction by a WAXS setup. **a** and **b**, diffraction from microtubules without and with paclitaxel, respectively. Numbers on the right side indicate the layer line signals due to approximately 4-nm axial repeat of tubulin within microtubules. Sharp arc lines in the WAXS range are Kossel-Kikuchi lines from the window materials (CDV) we used.