Ultra-Small-Angle X-ray Scattering from Drug-Induced Tubulin Ring Polymers

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Introduction

The protein tubulin, of which there are several isomers, is ubiquitously distributed in eucaryotic cells. The αβ-tubulin dimer is considered to be the primary building unit of various polymeric structures observed in nature. The most widely studied of these are microtubules, which are implicated in cell division, cell motility, intracellular transport, and other important biological functions. Under appropriate *in vitro* conditions, the addition of the nucleotide GTP induces the formation of microtubules, whereas GDP favors the formation of closed double-walled rings of diameter close to 46 nm.

Recently, a new class of marine natural products has attracted the attention of researchers because of their remarkable effect on tubulins and their ability to modify cell function. Electron micrographs taken on tubulin samples mixed with these putative drugs showed the formation of a different kind of structure: single-walled rings. Complementary studies with other techniques (small-angle neutron scattering and dynamic light scattering) confirmed this observation and allowed a quantitative characterization of these rings. By varying solution conditions (e.g., modifying the magnesium ion concentrations), a strong increase in the optical turbidity of the solution is observed, indicating the possible formation of large aggregates from these rings. In this present on-going work, we have been interested in identifying and characterizing these aggregates and, more importantly, in understanding the assembly process of these structures and comparing the results with those obtained so far for double-walled rings and microtubules.

Methods and Materials

We have applied ultra-small-angle x-ray scattering (USAXS) to measure *in situ* the intensity profiles of the scattering entities present in several samples by employing the Bonse-Hart USAXS-instrument installed at the 33-ID beamline at the Advanced Photon Source at Argonne National Laboratory. This instrument, which uses a high-intensity synchrotron beam with a flux of 1013 photon/s on a 0.5x1 mm^2 area, yields a particularly good signal-to-noise ratio. Its wavevector range, $0.0001<q<0.01$ A^{-1}, is appropriate to probe length scales (up to few microns) usually inaccessible by traditional SAXS and visible-light scattering instruments, allowing us to examine the tubulin samples in an entirely new range. Hence, we prepared several samples from purified rat-brain tubulin dissolved in morpholinoethane sulfonic a cid (MES), EGTA, and $MgCl_2$ at pH=7.0 and, in preliminary experiments, tested three drugs—Dolastatin, Cryptophycin, and Hemiasterlin—for their effects on the tubulin solutions. We collected the intensity profiles of these samples at different times following the mixing of the solutions. The intensity profiles are generally plotted as function of the wavevector defined as $q=(4\pi/\lambda)\sin(\theta/2)$, with $\lambda=1.12$ Å being the wavelength of the x-ray beam and θ being the scattering angle.

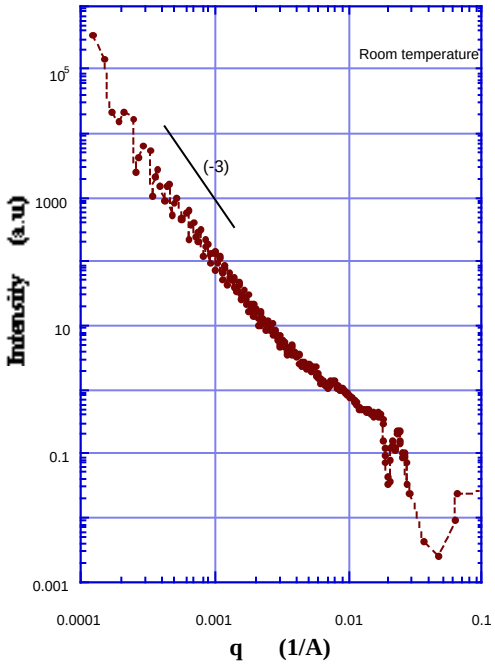

FIG. 1. Measured slit-smeared USAXS intensity profile of a solution of [tubulin]=40 μM, [$MgCl_2$]=0.5 mM, [EGTA]=0.1 mM, treated with [Dolastatin]=50 μM under pH=7.0.

Results

Although the samples were prepared with the same concentrations and under the same conditions, the Dolastatin sample shows the most remarkable features in its scattering profile (Fig. 1). Also, unlike the other drugs, Dolastatin causes a strong increase in optical turbidity immediately following the mixing of the sample. This is a signature for a possible aggregation phenomenon. The oscillatory behavior of the intensity profile seen in Fig. 1, especially at low-q, is particularly intriguing. One plausible explanation is the formation of large, spheroidal particles from the rings. So, in a first attempt to analyze the data, we model these particles as spheres with the form factor expressed by $F(q, R) = A[\sin(qR)–(qR)\cos(qR)]^2/(qR)^6$, with R and Abeing the radius and a scaling factor. From the measured F(q) we estimate R to be approximately 3.5 microns, as shown in Fig. 2. where we plot the calculated scattering profile of a Gaussian distribution of spherical particles with a 2% width dispersity and slit smeared to account for the experimental effect of the width of the beam. Good agreement is noted between theory and experimental data.

At high-q ($q>0.004$ A^{-1}), the oscillatory behavior is obscured as we probe length scales comparable to the size of individual rings which, from earlier SANS and electron microscopy, is close to 47 nm. However, in this q range (q > 0.01 A-1), Bessel peaks characteristic of scattering from single rings might be observed. Indeed, in Fig. 1 we notice a peak near $q = 0.025$ A^{-1}, which is approximately the value of the amplitude of the wavevec-

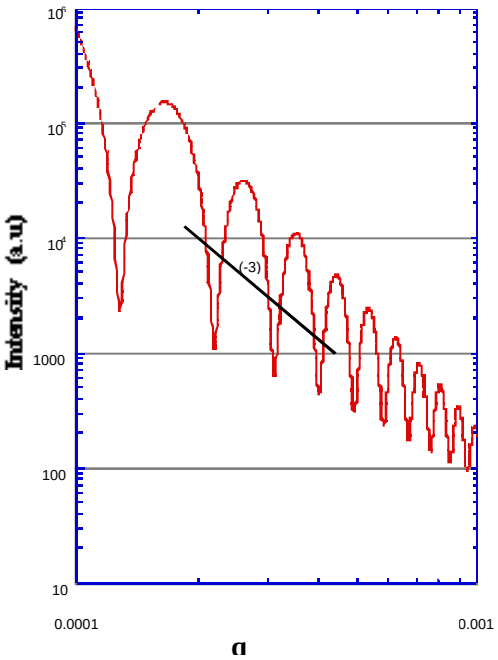

FIG. 2. Calculated slit-smeared scattering profile of a Gaussian size distribution of spherical particles with maximum radius R_{max} =3.5 μm and 2% size dispersity.

tor of the second SANS peak of isolated 47 nm rings. The oscillatory behavior disappears at later times as gravity-induced sedimentation becomes significant and drives the larger particles to settle in the bottom of the sample cell away from the beam.

Discussion

We note that the observation of the oscillatory behavior of the scattering profiles in the Dolastatin sample was possible only with the use of the USAXS instrument; traditional SAXS and SANS instruments cover, generally, higher q-ranges ($q > 0.003$ A^{-1}). Preliminary analysis of the data shows that this oscillatory behavior can be attributed to the formation of large spheroidal aggregates (~6-8 microns in average size). Interestingly, these spheroidal particles show a relatively narrow dispersity in sizes. An interesting question is the supramolecular conformational structure of the rings within the 6-8 micron particles. It is unclear at this point whether the rings are randomly oriented or are organized in a peculiar phase similar to the columns observed in some discotic liquid-crystal phases. Additional measurements have been planned that address this and related questions. Also, for consistency it would be interesting to extend the q-range of the present USAXS profiles to overlap with that of the SANS measurements.

Acknowledgement

Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. We would like to extend our thanks to the UNI-CAT team for their assistance in performing these experiments.