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Small-angle neutron scattering studies of tubulin ring polymers

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Abstract

We applied SANS to study nanoscale structures formed from tubulin and either of two similar peptides, cryptophycin1 and dolastatin10. A simple bead ring model adequately describes the cryptophycin–tubulin data, indicating high monodispersity and non-association of the rings. In contrast, dolastatin–tubulin samples show secondary assembly of larger structures. Analysis indicates that these macrostructures may contain locally stacked rings.

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1. Introduction

Tubulin is the dimeric protein that is the subunit of microtubules, which are cylindrical polymers essential to many important processes in biological cells including cell division [1]. The two monomers in the tubulin dimer are very similar in chemical sequence as well as in shape (4.6 nm × 4.0 nm × 6.5 nm) and molecular weight (~50 kDa) [2]. Many clinically important drugs interfere with cell division by altering the polymerization of tubulin to microtubules [3].

Several small peptides have been shown to inhibit polymerization of microtubules and instead cause formation of single-walled tubulin ring polymers [3]. Among these are cryptophycin-1 and dolastatin-10, which induce nanoscale rings of 8 and 14 tubulin dimers, respectively [3–5]. While these ring polymers are very similar in chemical composition, upon initiation of polymerization under standard conditions a striking difference in behavior is observed. It appears that the cryptophycin–tubulin rings (Cr1) do not interact whereas dolastatin–tubulin rings (D10) form macrostructures that settle under the effect of gravity. It is unclear from available data whether the interactions of D10 rings are random or specific. We previously used SANS to investigate microtubules [6], and now demonstrate that SANS can be used to better characterize these tubulin ring polymers.

2. Methods

Samples were prepared by mixing 40 μM tubulin with 50 μM of either cryptophycin or dolastatin in PIPES buffer (0.1 M PIPES, 1 mM $MgCl_2$, pH7.0)

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at room temperature. Samples were loaded into 1-mm path-length quartz scattering cells and kept at 33°C.

SANS measurements were carried out at the 30 m SANS instrument at the NIST Center for Neutron Research at the National Institute of Standards and Technology in Gaithersburg, Maryland, USA [7]. The wavelength of the neutron beam was set at $\lambda = 6$ Å. Scattered neutrons were collected by a two-dimensional ($64 \times 64\,cm^2$) detector located at different distances from the sample, covering the wave vector range $0.003 < Q < 0.27\,Å^{-1}$. (The amplitude of the wave vector is defined as $Q = (4\pi/\lambda)\sin(\theta/2)$, θ being the scattering angle.) Raw data were corrected for background and detector efficiency, and then circularly averaged to yield the intensity profile, I(Q) vs Q. Finally, the data were "desmeared" by deconvolution with a known resolution function, removing hence the wavelength spread and angular divergence of the neutron beam [7].

We based our data analysis on an "NXbead ring model", defined as a circular ring of N contiguous spherical beads [8]. We used the computational code, HYDRO, to calculate the scattering cross sections pertaining to this model [9].

3. Results and discussion

3.1. Cryptophycin–tubulin polymers

We first examined samples prepared from cryptophycin and tubulin (Fig. 1). Several peaks or bands can be discerned at $Q \simeq 0.031$, 0.059, 0.09, $0.120\,Å^{-1}$. These features can be attributed to highly symmetric scattering structures such as rings. Thus, in accord with earlier observations [4,5], we calculated the scattering profile for the NXbead ring model with $N = 16$. We varied the bead diameter over a reasonable range (4–5 nm) to find the most appropriate ring diameter that produces the observed number of peaks and their locations. In Fig. 1, we compare the measured profile with that calculated with a bead diameter of 4.75 nm, which yields a value of 29.1 nm for the outer diameter of the ring. This bead diameter

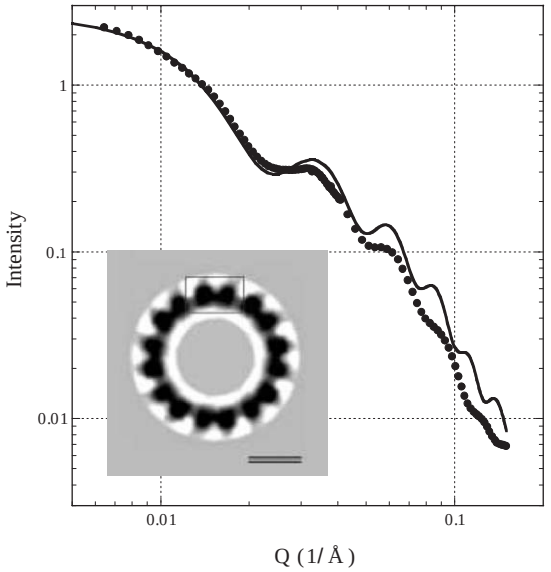

Fig. 1. SANS profiles of cryptophycin–tubulin samples: (•) measured profile; (–) calculated profile for a 16 × bead ring with a bead diameter of 4.75 nm. Inset: averaged image of Cr1 rings from cryoelectron microscopy [4]. The box encloses one tubulin dimer and the bar represents 10 nm.

(4.75 nm) is close to the geometrical mean value (4.8 nm) derived from the known dimensions of a tubulin monomer [2]. The first four calculated peaks are located at $Q \simeq 0.033$, 0.058, 0.083, $0.107\,Å^{-1}$ and should be compared with those listed above. We thereby note that the simple 16Xbead ring model appears to capture the main characteristics of the data.

3.2. Dolastatin–tubulin samples

D10 samples showed behavior different from that of the Cr1 samples. After a 10–20 minute period following the mixing of tubulin and dolastatin, the samples became optically turbid, a signature of aggregation/polymerization. Further, at later times the aggregates became so large that they settled to the bottom of the scattering cell under the effect of gravity.

In Fig. 2, we plot the measured scattering profile of the D10 samples. There are two pronounced peaks at $Q \simeq 0.017$ and $0.035\,Å^{-1}$ and three discernable bands at $Q \simeq 0.051$, 0.067, and $\simeq 0.090\,Å^{-1}$. These peaks and bands also indicate

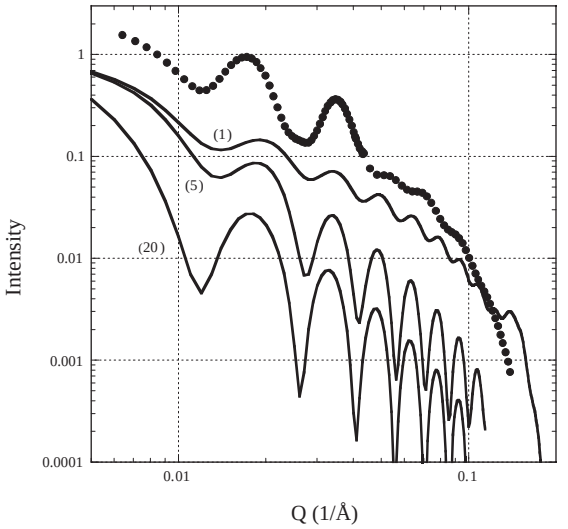

Fig. 2. SANS profiles of dolastatin-tubulin samples: (•) measured profile; (–) profiles calculated for different numbers of stacked 28Xbead rings with a bead diameter of 4.75 nm. Note the shift of the peaks to lower Q-values, as well as the sharpening of the peaks, when the number of stacked rings increases. Each calculated curve is labelled to indicate the number of stacked rings.

the presence of highly symmetrical structures. In Fig. 2, we include the calculated profile of a 28Xbead ring with a bead diameter of 4.75 nm, corresponding to the 14 tubulin dimers determined previously for these rings [4]. It appears that the model fails to account adequately for the locations of the first two peaks, and the measured peaks are more prominent than those calculated for independent rings. Even if the actual D10 structures are perfect rings, the observed peaks should be broadened by various experimental factors (residual effects of beam size, detection resolution, etc). The extent of this broadening is evident in the Cr1 samples (Fig. 1).

To appropriately interpret the SANS data of the D10 samples, we propose a stacking of primary dolastatin–tubulin rings to yield the large macrostructures observed in these samples. In Fig. 2 we also plot scattering profiles calculated for several columnar stacks made with different numbers of $28\times$ bead rings. As already noted [10], the peaks shift to smaller Q-values and become sharper as the number of stacked rings is increased. Thus, the stacking may explain the sharpness and positions of the observed peaks. In contrast, rings that are assembled edge-on to form sheets, or rings whose orientation is totally random, will not produce the observed features. We note that similar stacking has been observed in computer simulations and experimental studies of other asymmetrically shaped particles [11,12]. The asymmetric shape ratio of the D10 rings (ring thickness/ring diameter $\simeq 0.14$) is in the range of that found as a condition for the formation of columnar phases [11].

In conclusion, the tubulin rings described here can be used as a model system for studies of closed–ring polymers and their assembly. The Cr1 samples are composed of highly monodisperse and non-interacting rings; this monodispersity can be exploited to investigate various physical properties of nanoscale ring polymers. The D10 samples represent an example of highly interacting rings that combine to form large macrostructures, which appear to be locally ordered. We have demonstrated that SANS can provide useful insight into the structure of independent tubulin rings as well as their supramolecular interactions.

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