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The Determinants That Govern Microtubule Assembly from the Atomic Structure of GTP-Tubulin

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Tubulin alternates between a soluble curved structure and a microtubule straight conformation. GTP binding to αβ-tubulin is required for microtubule assembly, but whether this triggers conversion into a straighter structure is still debated. This is due, at least in part, to the lack of structural data for GTP-tubulin before assembly. Here, we report atomic-resolution crystal structures of soluble tubulin in the GDP and GTP nucleotide states in a complex with a stathmin-like domain. The structures differ locally in the neighborhood of the nucleotide. A loop movement in GTP-bound tubulin favors its recruitment to the ends of growing microtubules and facilitates its curved-to-straight transition, but this conversion has not proceeded yet. The data therefore argue for the conformational change toward the straight structure occurring as microtubule-specific contacts are established. They also suggest a model for the way the tubulin structure is modified in relation to microtubule assembly.

Introduction

Microtubules are hollow cylindrical assemblies of straight, parallel protofilaments made of αβ-tubulin heterodimers (tubulin). In the microtubule wall, longitudinal contacts are established between tubulins arranged head to tail along the protofilament axis, whereas adjacent protofilaments interact laterally.[1,2] The dynamics of microtubules is linked to a GTP/GDP cycle.[3] Whereas a structural GTP is always bound to the α subunit, for microtubule assembly, the β monomer should also be loaded with GTP (GTP-tubulin); hydrolysis of this nucleotide to GDP follows incorporation in the polymer. By contrast with their straight microtubular counterpart, protofilaments at the ends of disassembling microtubules or made from pure GDP-tubulin are curved.[4,5] The curvature of non-microtubular tubulin assemblies is variable; it has been described in most details in crystals of the complex (T_2R) of tubulin with the stathmin-like domain (SLD) of the RB3 protein ($RB3_{SLD}$), which display 12° bends between the tubulin subunits.[6] This is accompanied by changes within monomers compared to their microtubular conformation. Each of these monomers comprises two globular domains that respectively consist of an N-terminal nucleotide-binding module together with a C-terminal helical hairpin and of an intermediate domain;[7] in-between runs a central helix. Upon disassembly, a relative rotation of the two globular domains is observed together with a sliding of the central helix and local movements.[8,9] By contrast, the triggering of the reverse conversion is poorly understood. The debate focuses on the respective roles of GTP binding and microtubule assembly in this process.[5,10,11]

The reason that GTP-tubulin, as opposed to GDP-tubulin, assembles in microtubules has long been thought to be due to a difference of their overall shapes. GTP-tubulin in solution would be straight, whereas GDP-tubulin would be curved. More recently though, based on electron microscopic observations of large multiprotofilament assemblies of tubulin, it has been proposed that GTP binding to

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Abbreviation used: SLD, stathmin-like domain; GMPCPP, guanylyl-(α,β)-methylene-diphosphonate.

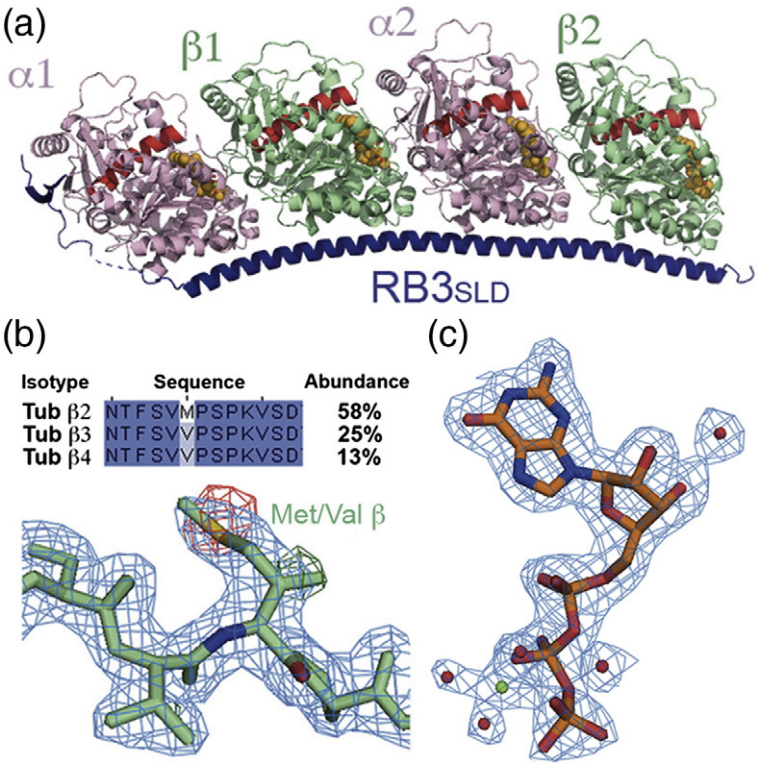

Fig. 1. The sT_2R structure. (a) Overview of GTP-sT_2R. The complex comprises one copy of $RB3_{SLD}$ and two tubulin αβ heterodimers; in each of the subunits, the central helix (red) and GTP (orange) are highlighted. (b) The 2.1 Å resolution $F_{obs}-F_{calc}$ map reflects the Met/Val polymorphism at β-tubulin position 172. An alignment of the most abundant isotypes in mammalian brain tubulin is shown together with their natural abundance.[15] The $2F_{obs}-F_{calc}$ map is contoured at the 1 σ level (blue). The $F_{obs}-F_{calc}$ map calculated with a methionine at position β172 is contoured at the +2.5 σ (green) and −4 σ (red) levels. (c) β2 GTP electron density. The 2.5 Å resolution $2F_{obs}-F_{calc}$ map is calculated in the structure with full GTP occupancy and contoured at the 1 σ level. Mg^{2+} (green), coordinated by two oxygen atoms of the β and γ phosphates and water molecules, and other water molecules (red) bound to the nucleotide are shown.

tubulin triggers long-range conformational changes that yield a straighter conformation.[4,11] Several lines of evidence have challenged this view. For example, it has been shown that colchicine site ligands, which do not bind to microtubules, have very similar affinities for GDP- and GTP-tubulins.[10,12] Atomistic molecular dynamics simulations of short protofilaments also suggest that GDP- and GTP-bound filaments in solution have a very similar curved conformation.[13] Likewise, the small-angle X-ray scattering profiles of GDP- and GTP-tubulins are virtually identical, suggesting very close shapes.[10] It has therefore become clear that, to resolve the issue of the role of the nucleotide, one would need high-resolution structural data to spot differences that, despite their significance, might be small. These should define the changes upon GTP binding and their effects on tubulin intermolecular interactions. Here, we determined the structures of GDP-tubulin and GTP-tubulin at resolutions that allow us to address these issues.

Results and Discussion

A tubulin structure at 2.1 Å resolution

We determined the structures of T_2R complexes in which the C-terminal tails of α and β subunits are cleaved by subtilisin[14] (sT_2R, Fig. 1a). The crystals belong to the $P2_12_12_1$ space group and diffract to up to 2.1 Å resolution (Table 1), which is a significant improvement over the 3.5 Å resolution of the formerly determined T_2R structure in the $P6_5$ space group.[9] This yields electron density maps that convincingly agree with the isotype content of mammalian brain tubulin.[15] This is most obvious when the differences of substituted amino acids are substantial (Fig. 1b). In addition, many water molecules are defined including, for example, those that constitute the coordinating shell of the Mg^{2+} bound to GTP (Fig. 1c). The quality of the model is also reflected by the Ramachandran statistics (see Materials and Methods). The overall shape of tubulin in sT_2R is very similar to that in the previously described T_2R structure,[9] the root-mean-square deviation (rmsd) of C^{α} atoms of the first tubulin ($\alpha_1\beta_1$) of T_2R and sT_2R after superimposition being 0.82 Å (the rmsd of the second tubulin, $\alpha_2\beta_2$, after superposition is 0.87 Å). This is close to the rmsd of individual subunits taken separately, which ranges from 0.6 Å to 0.9 Å. However, when whole complexes are superimposed, the rmsd is significantly higher (2 Å). This is due to different arrangements at their inter-tubulin interface that result in different curvatures. While bends at the tubulin–tubulin interface are of similar amplitudes in the two structures (12°), they are about axes that are misaligned by 10°. These differences may be