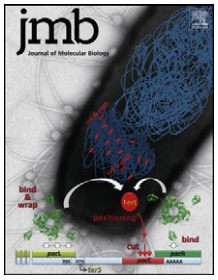

Interactions between Tau and Different Conformations of Tubulin: Implications for Tau Function and Mechanism

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Abstract

Tau is a multifaceted neuronal protein that stabilizes microtubules (MTs), but the mechanism of this activity remains poorly understood. Questions include whether Tau binds MTs laterally or longitudinally and whether Tau's binding affinity depends on the nucleotide state of tubulin. We observed that Tau binds tightly to Dolastatin-10 tubulin rings and promotes the formation of Dolastatin-10 ring stacks, implying that Tau can crosslink MT protofilaments laterally. In addition, we found that Tau prefers GDP-like tubulin conformations, which implies that Tau binding to the MT surface is biased away from the dynamic GTP-rich MT tip. To investigate the potential impact of these Tau activities on MT stabilization, we incorporated them into our previously developed dimer-scale computational model of MT dynamics. We found that lateral crosslinking activities have a much greater effect on MT stability than do longitudinal crosslinking activities, and that introducing a bias toward GDP tubulin has little impact on the observed MT stabilization. To address the question of why Tau is GDP-tubulin-biased, we tested whether Tau might affect MT binding of the +TIP EB1. We confirmed recent reports that Tau binds directly to EB1 and that Tau competes with EB1 for MT binding. Our results lead to a conceptual model where Tau stabilizes the MT lattice by strengthening lateral interactions between protofilaments. We propose that Tau's GDP preference allows the cell to independently regulate the dynamics of the MT tip and the stability of the lattice.

Introduction

Tau is a microtubule (MT)-binding protein that is predominantly expressed in neurons [1]. Tau has been the focus of much study because of its involvement in Alzheimer's disease and other "Tauopathies", a series of neurodegenerative diseases that have in common the appearance of abnormal Tau aggregates in the brain [2,3]. In healthy neurons, Tau appears to have a range of MT-related functions: it promotes MT polymerization *in vitro* [4–9], stabilizes MTs *in vivo* [4,7,8,10–12], and is also thought to space the MTs apart within the axon [9]. This spacing activity is important for neuronal function and cell signaling in densely packed axons [1,13–15]. Additionally, the binding of Tau to MTs has been reported to regulate motor proteins such as dynein and kinesin that use the MTs as pathways for cargo transportation [16–18].

To understand how Tau accomplishes these functions, it is necessary to determine how it interacts with MTs. Some aspects of Tau and its interactions are well established. Tau protein isoforms contain three or four MT-binding repeats resulting from alternative splicing [19,20]. Tau binds to the C-terminal tail of tubulin, a structure that is on the MT exterior [21–24], although it seems likely that there are also additional binding sites, perhaps including inside the MT [12,23,24]. This exterior binding is required for Tau's roles in MT spacing and motor regulation [9,16–18,25–28]. Recent biochemical data have shown that Tau also binds to unpolymerized tubulin [29], which

creates an additional complication in understanding the mechanism of how Tau regulates MT dynamics. Other aspects of Tau–MT interactions are less certain. For example, there is still a debate about the geometry of Tau–MT interactions: do the MT-binding repeats of Tau bind laterally across or longitudinally along MT protofilaments, or can both types of binding occur? In addition, it is clear that Tau binding occurs along the length of MTs (i.e., it is not end-specific) [25,30–32], but it is unclear why this occurs. One explanation for this binding behavior is that Tau is insensitive to the nucleotide state of the polymer. Alternatively, Tau might prefer to bind to the guanosine diphosphate (GDP) lattice, which is present everywhere except at the MT tip. Finally, it is not clear how Tau might interact with, cooperate with, or antagonize the elements of the MT plus-end tracking protein (+TIP) network, a set of proteins that is generally believed to be the "master regulator" of MT dynamics [33–36].

To establish the mechanism by which Tau stabilizes MTs, we need to understand Tau's binding geometry, but resolving this question has been challenging because Tau is largely "invisible" by standard electron microscopy methods [24]. Cryo-EM combined with helical image analysis led Al-Bassam *et al*. to conclude that Tau binds primarily along the MT protofilament ridges, that is, longitudinally [37]. In contrast, Santarella *et al*. showed that Tau can stabilize tubulin sheets; these data, together with other data, led them to suggest that Tau can bind both longitudinally (along) and laterally (across) protofilaments [24]. However, Schaap *et al*. used atomic-force-microscopy-based imaging and measurements of radial elasticity of Tau–MT complexes to again conclude that Tau binds mainly along protofilaments [31]. In addition, recent isothermal titration calorimetry studies by Tsvetkov and colleagues also led the authors to conclude that Tau binds primarily along protofilaments [38]. However, interpretation of their data is complicated by the recent demonstration that Tau binds to unpolymerized tubulin [29].

We set out to address some of these basic questions about Tau–MT interactions by determining the affinity of Tau for different types of tubulin polymer. We then investigated the potential effects of these different activities by incorporating them into our established dimer-scale computational model of MT dynamics [39,40]. Our experimental data show that Tau promotes the formation of stacks of Dolastatin-10 tubulin rings, providing strong evidence that Tau can strengthen lateral crosslinks between protofilaments. Taken together with previous evidence supporting longitudinal binding, this observation indicates that Tau can promote both lateral and longitudinal interactions between tubulin subunits, as might be predicted from Tau's flexible structure, multiple microtubule binding domains, and ability to interact with itself [22,41]. However, our computational simulation results suggest that it is Tau's ability to promote lateral interactions that plays the primary role in MT stabilization.

In addition, we found that Tau has a higher affinity for GDP tubulin than for guanosine-5′-[(α,β-)-methyleno] triphosphate (GMPCPP; GTP-like) tubulin. Although several other polymerization-promoting MT-binding proteins including end binding protein 1 (EB1) have been shown to bind preferentially to GTP-like tubulin conformations [42–45], to our knowledge, this is the first time that a MT stabilizer has been reported to prefer GDP tubulin. Finally, we found that Tau and the +TIP EB1 can compete for MT binding, consistent with recent results from other laboratories [36]. This observation suggests that the differential nucleotide preferences of Tau and EB1 might allow the cell to separately regulate the dynamics of the MT tip and the stability of the MT lattice. The sum of this information leads to a conceptual model where Tau binds to the MT surface in a heterogeneous manner that may include both lateral and longitudinal binding but stabilizes the MT primarily by strengthening the lateral interactions between protofilaments.

Results and Discussion

Tau binds tubulin protofilament rings with high affinity

As discussed above, controversy remains about the geometry of Tau–MT interactions. Most reports have

Fig. 1. Tau binds Dolastatin-10 tubulin protofilament rings. (a) Dolastatin-10 is a drug that polymerizes tubulin into rings that correspond to curved single protofilaments and model the ram's horns of a depolymerizing MT [49–51]. (b and c) Data from co-sedimentation assays showing that 1 μM Tau binds Dolastatin-10 tubulin rings (0–8 μM) with high affinity (fitted data yield a K_D of ≤35 ± 17 nM). (b) Representative SDS-PAGE gel that was quantified by densitometry using ImageJ to calculate the fraction of Tau bound to DL-10 rings as shown in Fig. 1c (data represent the average of ≥3 trials ± standard deviation). (d) Representative TEM image of Dolastatin-10 tubulin rings alone (1 μM). (e–h) Various structures seen by TEM when 1 μM Dolastatin-10 tubulin rings were incubated with 0.5 μM Tau. (e) Example of single rings observed in the Tau-containing samples; these appear nearly identical to tubulin rings alone, as might be expected from previous data showing that Tau is difficult to resolve by electron microscopy [24]. (f and g) Examples of Tau–tubulin ring stacks. These stacks do not form in the absence of Tau, suggesting that Tau is able to crosslink MT protofilaments laterally. The ring stack in (g) appears to be ~4–5 stacked rings. (h) An example of other structures that appeared in our TEM samples. Double-walled rings such as this one may result from Tau crosslinking rings with ring fragments, although other explanations are possible. Scale bars represent 50 nm; images shown are representative of three independent experiments. The cartoons in the right panels of (d–h) serve as a visual aid.