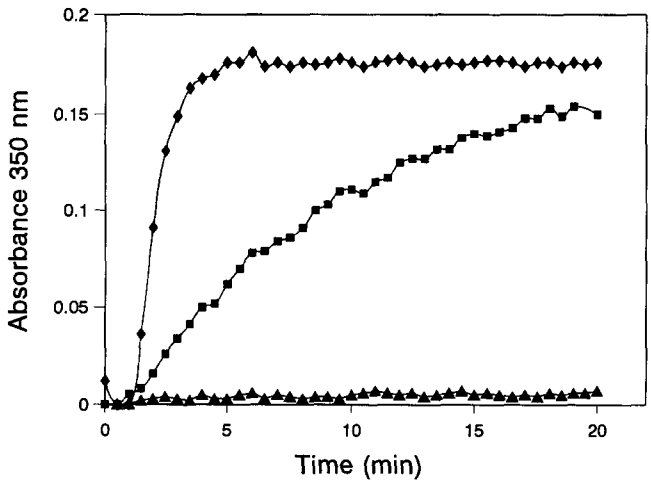

Fig. 1. Polymerisation of purified tubulin in the absence or in the presence of MAP1B and MAP2. FPLC-purified tubulin (1.5 mg/ml) in MES buffer, containing 40 μM GTP, was incubated at 30°C either in the absence of MAPs (▲) or in the presence of 0.2 mg/ml MAP1B (◆) or 0.18 mg/ml MAP2 (■). Polymerisation was monitored by the change in absorbance at 350 nm in a Shimadzu UV2100 spectrophotometer fitted with a temperature- controlled compartment.

and MAP2-promoted assembly typically exhibited an initial lag phase, representative of nucleation events. The lag-phase was followed by rapid elongation and achievement of a more or less steady plateau (Fig. 1). While the final extent of polymerisation was similar for both MAP1B and MAP2, and was confirmed by pelleting the assembled microtubules and determination of protein content, MAP1B promoted-assembly tended to achieve a plateau much more rapidly compared with MAP2.

At the end of the incubation period aliquots were removed and either centrifuged to pellet the microtubules or alternatively fixed with 0.1% glutaraldehyde and negatively stained for electron microscopy. SDS-PAGE analysis of the pelleted protein showed that both MAP1B and MAP2 co-sedimented with assembled microtubules (Fig. 2A), the stoichiometry MAP1B and MAP2 to tubulin dimers was roughly 1 mol MAP1B:13 mol tubulin dimers, and 1 mol MAP2:8 mol tubulin dimers. The MAP2 stoichiometry was similar to that previously reported [11]. Electron microscopy confirmed that 'normal' microtubules were present in samples containing MAP1B (Fig. 2B) and MAP2 (Fig. 2C); no microtubules were observed in the pure tubulin sample (not shown). These data argue that the increase in turbidimetry was therefore due to the formation of microtubules. Measurement of microtubule length showed that on average MAP1B-microtubules were about 1.5-fold longer when compared with MAP2-microtubules (25 μm vs. 17 μm).

MAP1B and MAP2 assembly kinetics were further examined by pseudo-first-order reaction plots which can be used to determine the net association rate [15,16]. In such plots the slope represents the pseudo-first-order constant which is a sum of the association rate constant and the number concentration of nucleation seeds. Upon completion of the nucleation phase both MAP1B- and MAP2-promoted assembly was described by a single reaction (Fig. 3) with an apparent rate of about 14.5×10^{-3} s^{-1} and 1.9×10^{-3} s^{-1}, respectively. The apparent reaction rate corrected for the number concentration of ends yielded association rate constants of 200×10^6 $M^{-1} \cdot s^{-1}$ for MAP1B and 18×10^6 $M^{-1} \cdot s^{-1}$ for MAP2. The steady-state plateau and the amount of polymerised protein was similar for MAP1B- and MAP2-promoted assembly.

4. Discussion

Despite the importance of MAP1B in neurogenesis, studies on its interaction with microtubules have been hampered by the lack of suitable purification protocols. We have recently described a procedure for the isolation of bovine brain MAP1B in milligram quantities and have characterised the purified protein and its interaction with microtubules [12].

It has been a generally accepted belief that MAP1B is less efficient at promoting microtubule assembly and in stabilising microtubules [2]. This notion derives from observations that: (a) MAP1B, in the presence of other MAPs, cycles inefficiently with microtubules in vitro: (b) MAP2 can efficiently displace MAP1B from microtubules [12,17]; and (c) drug resistance ex-

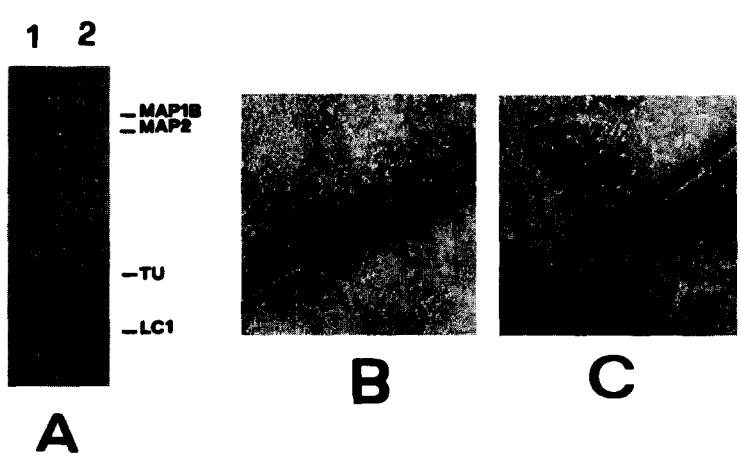

Fig. 2. SDS-PAGE and electron microscopy. At the end of the incubation period the MAP1B or MAP2 assembled protein (see Fig. 1) was either centrifuged or fixed and stained for electron microscopy. After resuspension the protein in the pellet was fractionated by SDS-PAGE on a 4–15% acrylamide gel and stained with Coomassie brilliant blue (A): lane 1 = MAP2-microtubules; lane 2 = MAP1B-microtubules. Negatively stained MAP1B-microtubules (B) and MAP2-microtubules (C) are shown at a magnification of ×200,000.

Microtubule associated protein 1B (MAP1B) promotes efficient tubulin polymerisation in vitro

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Abstract The effect of MAP1B on tubulin polymerisation has been examined in reconstitution experiments using purified tubulin and MAP1B. Under the assembly conditions used, tubulin alone was incapable of polymerising, but addition of MAP1B resulted in rapid assembly into microtubules. The kinetics of MAP1B-promoted microtubule assembly examined using pseudo-first-order plots show that assembly is described by a single reaction rate. The calculated association rate constant for MAP1B was about 200×10^6 $M^{-1} \cdot s^{-1}$ and this constant was one order of magnitude higher when compared with that for MAP2-promoted assembly.

Key words: MAP1B; High-molecular weight MAPs; Tubulin; Cytoskeleton; Microtubule

1. Introduction

A heterogenous group of proteins (MAPs) normally associated with microtubules have been suggested to play an important role in the growth and maintenance of neuronal processes [1]. Microtubule associated protein 1B (MAP1B, also known as MAP1X, MAP1.2, MAP5; [2])differs from most high molecular weight MAPs, e.g. MAP2, in that it is mainly present in neurons at early developmental stages and in the relative inefficiency with which it co-sediments with microtubules in vitro. However, antisera against MAP1B immunostain microtubule networks in neuronal cells, axons and dendrites, and non-neuronal cell lines [1]. Immunoaffinity purified MAP1B has also been shown to bind to microtubules and to form long filamentous projections from the microtubule surface [3].

MAP1B is one of the earliest MAPs to appear during brain development and is abundant in fetal and neonatal brains [4,5] but is found at only low basal levels in adult brain. The strong developmental regulation of MAP1B and its prominence in newly forming axonal processes suggest a role in neurogenesis and process plasticity [2,6]. More recently, alterations in the expression of MAP1B have been implicated in schizophrenia [7] and hyperphosphorylated MAP1B has been found associated with neurofibrillary tangles in Alzheimer's disease [8].

The nucleotide sequence of MAP1B [9] shows a significant sequence homology with another high molecular weight protein MAP1A [10]. Positively charged KKE repeat motifs in the N-terminal portion of MAP1B, similar to those in MAP1A, have been suggested to constitute the microtubule binding site [9,10]. Both proteins contain two common associated light chains, LC1 and LC3, of 32 kDa and 18 kDa [5,11,12].

The lack of satisfactory purification procedures has limited any detailed biochemical studies with MAP1B. Our laboratory has recently described procedures for the purification of MAP1A and MAP1B and demonstrated that MAP1A and MAP1B can bind to microtubules [11,12]. In this report we have further examined the ability of MAP1B to promote tubulin polymerisation and describe the kinetics of MAP1B-promoted microtubule assembly.

2. Materials and methods

2.1. Protein purification and composition

Calf-brain microtubule protein was prepared by two cycles of temperature-dependent assembly/disassembly according to the procedure of Pedrotti et al. [13]. Tubulin, MAP2, and MAP1B were purified as described in detail previously [11,12].

Proteins were fractionated by denaturing SDS-PAGE using the Pharmacia Phast system. The gels were stained and the integrated peak areas determined as described previously [11]. Protein concentration was determined using the Bradford method (Bio-Rad Protein Reagent kit), bovine serum albumin was used as the standard.

2.2. Tubulin polymerisation

Purified tubulin, in MES buffer (MES 0.1 M, EGTA 2.5 mM, $MgCl_2$ 0.5 mM, EDTA 0.1 mM and DTT 1 mM, pH 6.4 with NaOH) containing 40 μM GTP, in the absence or presence of MAPs was degassed, and transferred to warmed cuvettes in a Shimadzu 2100 spectrophotometer fitted with a temperature-controlled compartment. The kinetics at 30°C were monitored by absorbance change at 350 nm [11,13]. At the end of the incubation period aliquots were removed and either fixed by dilution in 0.1% (v/v) glutaraldehyde and negatively stained or centrifuged at $100,000 \times g$ for 25 min at 37°C in a TL-100 Beckman ultracentrifuge [11,13] to pellet the microtubules. Determination of seed concentration and electron microscopy were performed as described previously [11,13].

All biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals used were of Analar grade.

3. Results

The ability of MAP1B to promote microtubule assembly was investigated in reconstitution experiments and compared with that of MAP2. Pure tubulin was incubated either in the absence of associated proteins or in the presence of stoichiometric amounts of MAP1B or MAP2 and assembly monitored by turbidimetry. In the absence of added MAPs little or no polymerisation of pure tubulin was observed (Fig. 1). By contrast, rapid polymerisation was observed both in the presence of MAP1B and MAP2. Microtubule assembly conforms to a linear condensation polymerisation reaction [14] wherein a nucleation stage precedes the elongation events and both MAP1B-

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Abbreviations: EGTA, ethylene glycol bis (β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; GTP, guanosine 5'-triphosphate; MAP(s), microtubule-associated protein(s); MES, 2-(*N*-morpholino)-ethanesulphonic acid; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis.

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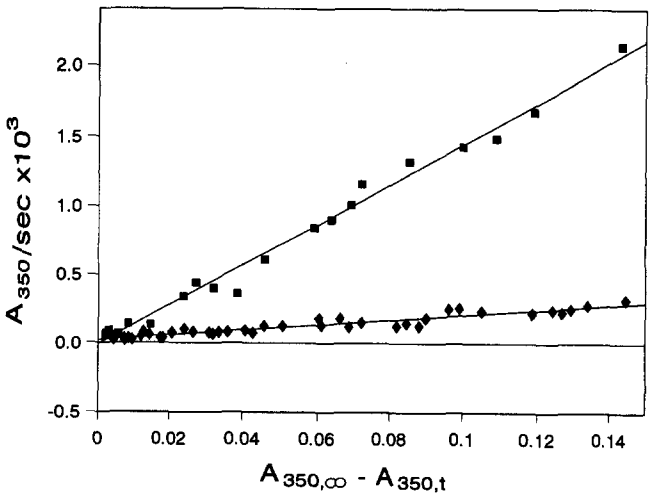

Fig. 3. Pseudo-first-order plots of MAP1B (■) and MAP2 (◆) promoted polymerisation. The rate of tubulin polymerisation (A350/S) is plotted against the instantaneous free subunit concentration ($A_{350nm,\infty} - A_{350nm,t}$). Data from the nucleation phase have been omitted. The increase in absorbance as a function of time for MAP1B- and MAP2-promoted polymerisation is shown in Fig. 1.

periments with nocodazole using transfected cell lines [18] show that MAP2 and tau are more efficient stabilisers of microtubules compared with MAP1B. However, MAP1B, like MAP2, is able to promote the nucleation and elongation of pure tubulin under conditions where pure tubulin alone is incapable of polymerisation. Interestingly, MAP1B is much more efficient at promoting elongation when compared with MAP2: association rate constant for MAP1B is about an order of magnitude higher. We have also shown that MAP1B and MAP2 compete for microtubule binding suggesting similar or overlapping sites on the tubulin molecule for both MAPs, and MAP2 exhibits a higher affinity [12]. The higher association rate would, however, argue that the interaction is not equivalent. In this context, it is important to mention that the MAP1B microtubule binding site composed of KKE sequences may be helical [19], while the MAP2 microtubule binding repeats contain helix breakers [20]. The higher association rate and lower affinity of binding, and probably lower stabilisation of microtubules [18], may suggest that MAP1B plays a specialised role in permitting rapid assembly of microtubules but that these microtubules are more dynamic than those observed with the classical MAPs, e.g. MAP2 and tau. This is further supported by the observation that MAP1B-microtubules were only 1.5-fold longer when compared with MAP2-microtubules. Indeed, MAP1B is one of the earliest MAPs to appear during development and is concentrated in the distal region of the growing axon, regions which represnt major sites of microtubule assembly dynamics in growing axons [21].

MAP1A, like MAP1B, also contains KKE motifs (their role in microtubule binding still remains to be defined) and promotes microtubule assembly but with an association rate constant which is about 5-fold lower than observed with MAP1B [11]. Furthermore, unlike MAP1B, MAP1A colocalises on the same microtubules with MAP2 suggesting that it binds to different binding sites [11,12,22]. The identification of a novel acidic microtubule binding domain in MAP1A [22] coupled with the observed differences in the microtubule assembly behaviour of MAP1A and MAP1B may suggest that MAP1A binds to microtubules via its acidic binding domain while MAP1B does so with the KKE domain. Considering the complementary expression of MAP1A and MAP1B [2,4] during brain development these differences between the MAP1 proteins may be important in cells during the transformation changes from a 'plastic' to a more 'stable' stage in neuronal processes.

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