MICROTUBULE-ASSOCIATED PROTEIN MAP1 PROMOTES MICROTUBULE ASSEMBLY IN VITRO

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1. Introduction

Several proteins co-purify with tubulin through polymerization–depolymerization cycles. These proteins are called microtubule-associated proteins (MAPs), and some of them have been found to be really associated with cellular microtubules (review [1]). It is MAPs that are thought to be responsible for microtubule functions and interactions with other cellular components.

Three MAPs have already been purified. These are MAP2 [2–4] and τ [5] from mammalian brain and the 210 000 M_r protein from HeLa cells [6,7]. These proteins were found to promote microtubule assembly in vitro. Their assembly-promoting activity is heat-resistant.

Little has been known about the activities of the heaviest of MAPs, MAP1, which comprises a significant part of brain MAPs. There was only the report [8], which concluded that MAP1 does not possess any assembly-promoting activity. This conclusion was based on the fact that the removal of MAP1 from microtubule proteins had no effect on their polymerization.

We have developed a method for MAP1 isolation from bovine brain [9]. Using a purified protein, we show here that MAP1 promotes assembly of brain tubulin into microtubules. Unlike the activity of MAP2 and τ, the assembly-promoting activity of MAP1 is not thermostable.

Abbreviations: EGTA, ethyleneglycol-bis(aminoethyl ether)-*N,N'*-tetraacetate; EDTA, ethylenediaminetetraacetate; GTP, guanosine 5'-triphosphate; SDS, sodium dodecylsulphate; MAP, microtubule-associated protein; M_r, relative molecular mass

2. Materials and methods

Tubulin was prepared from bovine brain by an assembly–disassembly procedure [10], modified as in [2], and separated from MAPs by chromatography on phosphocellulose (Whatman P-II) [11] in a buffer containing 50 mM imidazole–HCl (pH_{20} 6.7), 50 mM KCl, 0.5 mM $MgCl_2$, 0.1 mM EDTA and 1 mM 2-mercaptoethanol (buffer A). Methods for MAP1 and unheated MAP2 purification were as in [9].

Tubulin was polymerized at 37°C in buffer A supplemented with 1 mM GTP and 1 mM EGTA. Polymerization was followed by monitoring the light scattering of the solution at 330 nm [12]. The morphology of tubulin polymers was studied by electron microscopy after thin-sectioning or negative-staining of the samples with 1% aqueous uranyl acetate.

For determination of the protein composition of the polymerization products they were pelleted by centrifugation at 200 000 × *g* through a cushion of buffer A containing 4 M glycerol and 1 mM EGTA, and the pellets were analysed by SDS gel electrophoresis.

SDS gel electrophoresis was performed as in [13] in 10% polyacrylamide slab gels at the acrylamide to *N,N'*-methylenebisacrylamide ratio of 100:1 (w/w).

Protein concentration was determined as in [14] with bovine serum albumin used as a standard.

3. Results

Fig.1 shows changes in the turbidity of tubulin–MAP1 mixtures during incubation at 37°C in the presence of GTP and EGTA. MAP1 concentrations in the mixtures were 0–0.3 mg/ml. Whereas the turbidity

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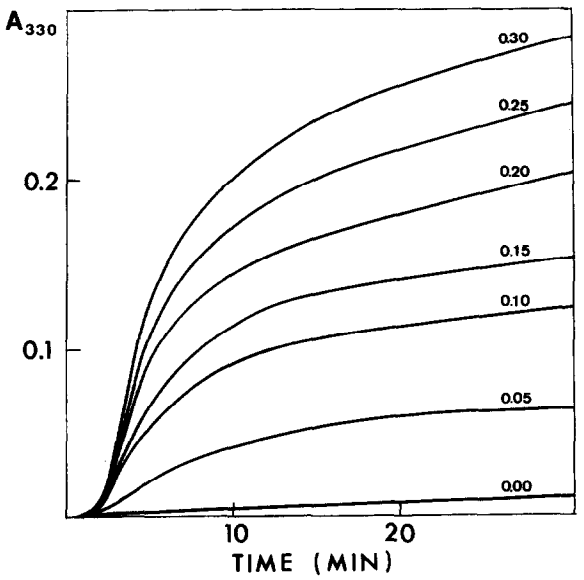

Fig.1. Tubulin polymerization stimulated by a purified MAP1 fraction: [tubulin] was 0.9 mg/ml; [MAP1] (mg/ml) is indicated by the numbers above the turbidity curves.

of phosphocellulose-purified tubulin did not change, that of tubulin supplemented with MAP1 increased, which indicates that tubulin polymerizes in the presence of MAP1. The extent of polymerization was the higher the more MAP1 was added to tubulin. Evident stimulation of tubulin polymerization was observed at as low as 0.05 mg MAP1/ml. Tubulin polymerization induced by MAP1 was GTP-dependent and failed to occur in the cold. At 0.3 mg MAP1/ml, the critical tubulin concentration for polymerization was 0.1 mg/ml.

As judged by electron microscopy of negatively stained or thin-sectioned samples, tubulin polymers formed in the presence of MAP1 were normal microtubules (fig.2).

When polymers formed in the tubulin–MAP1 mixtures were pelleted through a glycerol cushion and analysed by SDS gel electrophoresis, both tubulin and MAP1 were found in the pellet (fig.3C). In the control samples containing only tubulin or only MAP1, no protein sedimented through the cushion after incubation under the assembly conditions. Thus the MAP1 preparation promotes tubulin assembly in vitro and the resulting microtubules contain MAP1.

The interpretation of these data is complicated because MAP1 obtained by our method [9] is often contaminated with trace amounts of MAP2, which is itself capable of promoting microtubule assembly. To decide whether MAP2 contamination was indeed responsible for the assembly-promoting activity of our MAP1 preparation, it was useful to compare some characteristics of MAP1 and MAP2 activities. As MAP2 is thermostable [2–4], we studied the sensitivity of the MAP1 preparation to high temperatures. We incubated MAP1 for 10 min at 100°C in buffer A with 1 M KCl (i.e., under the same ionic conditions as with MAP2 [2]), dialysed it against buffer A and added to tubulin. MAP1 ability to stimulate tubulin polymerization was greatly reduced by heating (fig.4 (A,B)). Only occasional short microtubules and numerous amorphous aggregates were seen in the electron micrographs of tubulin incubated with heated MAP1 under the assembly conditions (not shown). Therefore, in contrast to MAP2, our MAP1 preparations were not thermostable.

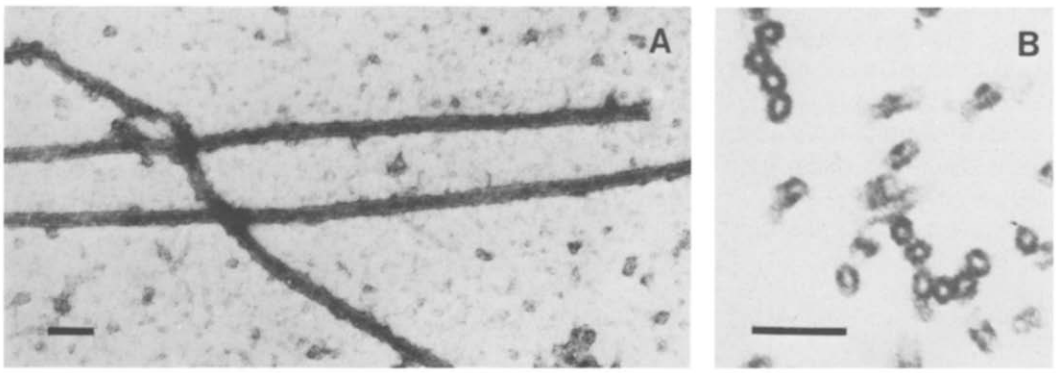

Fig.2. Electron micrographs of the microtubules assembled with MAP1: (A) sample negatively stained with 1% aqueous uranyl acetate; (B) thin section of the microtubule pellet fixed with 1% glutaraldehyde; bars, 1000 Å.

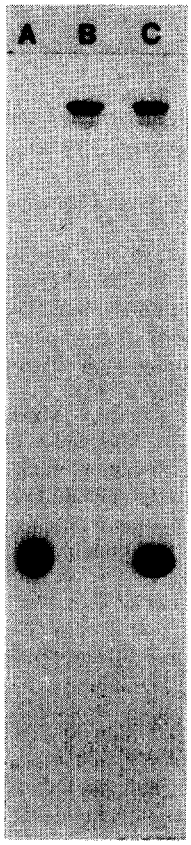

Fig.3. SDS gel electrophoresis of the pelleted microtubules assembled in the tubulin–MAP1 mixture: (A) tubulin used in these experiments; (B) MAP1 used in these experiments; (C) pellet of microtubules polymerized in the mixture, containing 1 mg tubulin/ml and 0.3 mg MAP1/ml.

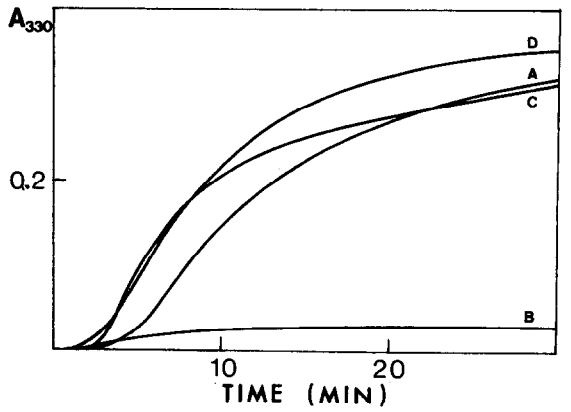

Fig.4. Effect of heating on the ability of MAP1 and MAP2 to induce microtubule assembly. The additions of MAPs were: unheated MAP1 (A); heated MAP1 (B); unheated MAP2 (C); heated MAP2 (D). In the mixtures, tubulin was 1.1 mg/ml; MAPs were 0.4 mg/ml.

It is necessary, however, to keep in mind that the reported data on MAP2 thermostability were obtained for the protein which had been heated during purification. However, MAP2 contaminating the MAP1 preparation was not heated. Therefore, the thermostability of unheated MAP2 had to be tested. We have developed a method for separation of MAP2 from other MAPs by DEAE-Sephadex and hydroxyapatite chromatography [9]. Thermoprecipitation is not used in our procedure. As shown in fig.4 (C), the addition of unheated MAP2 purified by our procedure to tubulin under the assembly conditions induced the increase in the turbidity of the solution. Electron microscopy confirmed the assembly of microtubules in the unheated MAP2–tubulin mixture (not shown). Incubation of purified unheated MAP2 for 10 min at 100°C in buffer A supplemented with 1 M KCl produced no decrease in its polymerization-promoting activity, as judged by turbidity assay (fig.4D) and electron microscopy (not shown).

These data make it evident that the assembly-promoting activity of our MAP1 preparation cannot be accounted for by contamination of MAP1 with MAP2.

4. Discussion

We have demonstrated here that:

1. Purified brain high-M_r protein MAP1 is able to promote tubulin assembly into microtubules;
2. MAP1 assembly-promoting activity is thermolabile.

The latter property distinguishes MAP1 from MAP2 and other known assembly-promoting MAPs, the activity of which is thermostable [2–5,7]. This difference shows that the ability of our MAP1 preparations to induce tubulin polymerization was not due to contamination with MAP2 or other thermostable MAPs.

MAP2 thermostability has been reported in [2–4]. In [2–4], however, MAP2 was purified by thermoprecipitation, and it is not excluded that one dealt with some residual MAP2 activity or a small thermostable fraction of MAP2 whereas a considerable part of the initial assembly-promoting activity of native MAP2 was lost at high temperature. Here we have studied the thermosensitivity of 'native' MAP2 purified without heating. We have found that 'native' MAP2 retains

its assembly-promoting activity after incubation at 100°C.

The results of this work are at variance with the conclusions in [8]. It was reported [8] that the removal of MAP1 from microtubule proteins had no effect on microtubule assembly, while the removal of both MAP1 and MAP2 prevented tubulin polymerization; it was concluded that MAP1 does not induce polymerization of tubulin [8]. However, using the isolated protein, we have directly demonstrated that MAP1 does promote microtubule assembly. A possible explanation of this discrepancy is that brain microtubule proteins purified by assembly–disassembly cycles (as was the case in [8]) are saturated with MAPs, so that the partial removal of MAPs (MAP1 or MAP2) would not markedly influence tubulin polymerization.

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