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Modulation of microtubule shape in vitro by high molecular weight microtubule associated proteins MAP1A, MAP1B, and MAP2

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Abstract The effect of microtubule associated proteins on microtubule shape has been investigated in reconstitution experiments using purified tubulin and purified MAP1A, MAP1B, and MAP2. Microtubules assembled in the presence of these MAPs were fixed with 0.1% glutaraldehyde and, after negative staining, were examined by electron microscopy. The results show that MAP1A microtubules were generally short and 'straight' while those assembled with MAP1B were longer and 'bendy'. MAP2 microtubules showed both types of morphologies even though straight microtubules were more abundant. These data suggest that MAPs may modulate not only microtubule dynamics but also microtubule shape which may be important in their spatial distribution and/or role in specific neuronal areas.

Key words: High-molecular weight MAP; Microtubule; Cytoskeleton; MAP2; MAP1A; MAP1B

1. Introduction

Microtubule rigidity is required for the support functions in the cell architecture although specific cellular functions may require more flexible features and different conformations. For example, bundles of microtubules tend to run parallel down the axons and dendrites while during axon elongation, microtubules in stationary growth cones tend to be organized in splayed arrays and wavy profiles, often forming loops which are rapidly bundled in linear arrays when an event of growth cone migration occurs [1]. Similarly, microtubule turning is an early event in determining growth cone re-orientation and precedes any change in the overall morphology during the establishment of new migrating directions [2].

Factors responsible for the different morphologies displayed by microtubules are not known but the spatial and temporal distribution of microtubule associated proteins (MAPs) makes them potential candidates for such a role [3,4]. Indeed, MAPs have been implicated in both increasing microtubule rigidity and altering microtubule dynamics [3,5,6] and several MAPs have been shown to be spatially and temporally regulated [4]. For example, high molecular weight MAP2 is found essentially in dendrites and transfection of MAP2 in non-neuronal cell lines causes bundling of microtubules and increased rigidity [7], an ability which may be important for promotion of process outgrowth. It has been further suggested that MAP2 interaction with multiple tubulin dimers may be correlated with bundling and stiffening of microtubules and process outgrowth [6,8,9].

The MAP1 class of proteins, MAP1A and MAP1B, exhibit a widespread distribution but show a complementary expression during development [3,4,10]. Thus, MAP1B is expressed at high levels during the early stages of neuronal differentiation and is concentrated in the distal edge of the growing axons [11] while MAP1A is expressed at later stages when neuronal differentiation reaches completion and MAP1B levels decrease [4]. MAP1B and MAP2 appear to bind to similar sites on the microtubule although the binding affinity of MAP1B is lower [12–14]. In spite of the reduced strength of the microtubule:MAP1B interaction, MAP1B induces neurite outgrowth [15] even though the microtubules are less stable when compared with those formed in presence of MAP2 or tau [16].

MAP1A, which exhibits a significant sequence homology with MAP1B, binds to different sites on the microtubule compared with MAP1B and MAP2 both in vivo [17] and in vitro [18]. A novel acidic binding domain has been identified in MAP1A [19] and transfection studies, with a truncated form of MAP1A containing this site, in cell lines have shown that expression of MAP1A leads to microtubules becoming somewhat shorter and much more straight [19].

It has been suggested either that microtubule flexibility is regulated by MAPs [6,7,20] or that MAPs have no effect on flexibility [21]. On the other hand, the different morphologies of microtubules in vivo are thought to be modulated by cellular factors such as shear-forces, cell size, etc. (see [6]). In this report we have investigated whether microtubule shape could be modulated by MAPs. We now show that microtubules reconstituted in the presence of MAP1A, MAP1B, and MAP2 assume distinct morphologies suggesting that these MAPs may play a role in determining microtubule shape.

2. Materials and methods

All protein purification methods and successive experiments were performed in MES buffer (MES, 0.1 M; EGTA, 2.5 mM; $MgCl_2$, 0.5 mM; EDTA, 0.1 mM; pH 6.4 with NaOH).

2.1. Protein procedures

Twice cycled calf brain microtubule protein was prepared according to Pedrotti and Islam [22].

Tubulin, MAP2, MAP1A and MAP1B were purified as described by Pedrotti and Islam [18,22]. Protein concentration was determined using Bio-Rad (Rockville Centre, NY) protein reagent and bovine serum albumin as standard.

SDS-PAGE was performed using the 'Phast System' (Pharmacia, Piscataway, NJ) and gels were stained with Coomassie blue R-250 [23].

2.2. Assay procedures

Purified tubulin was polymerised in MES buffer at 37°C by addition

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Abbreviations: GTP, guanosine 5'-triphosphate; MAPs, microtubule-associated proteins; MES, 2-(*N*-morpholino)ethanesulphonic acid; PMSF, phenylmethanesulphonyl fluoride.

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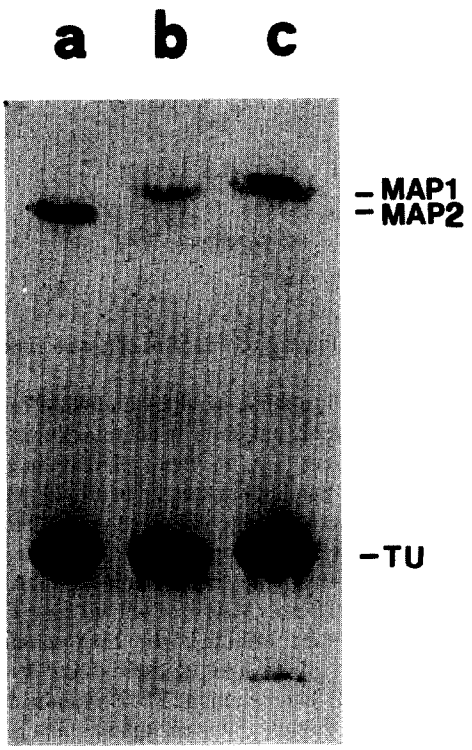

Fig. 1. Protein composition of reconstituted microtubules. Microtubules polymerised in the presence of the different MAPs were pelleted by centrifugation. After resuspension, the protein was fractionated by SDS-PAGE on 4–15% acrylamide gels and stained with Coomassie brilliant blue: (a) MAP2 microtubules, (b) MAP1A microtubules, and (c) MAP1B microtubules.

of 40 μM GTP and either MAP2, MAP1A, or MAP1B. The kinetics were monitored by the absorbance change at a wavelength of 350 nm in a Shimadzu 2100 spectrophotometer [18,24]. Upon achievement of steady state, aliquots were removed and either fixed by dilution in (0.1% v/v) glutaraldehyde and negatively stained with 0.25% uranyl acetate or centrifuged at 100000×*g* for 25 min at 37°C in a TL-100 Beckman ultracentrifuge to pellet the microtubules [18,24].

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

3. Results

Polymerisation of purified tubulin in either the absence or presence of pure MAP2, MAP1A, or MAP1B, was induced by raising the temperature to 37°C and assembly monitored spectrophotometrically. Under these conditions pure tubulin was not able to polymerise but efficient assembly was observed in the presence of stoichiometric amounts of each MAP (data not shown, see [18,24]). Upon attainment of steady state, generally between 15 and 30 min depending on the specific MAP (see [18,24], aliquots were removed and either sedimented by centrifugation or, after fixation with 0.1% glutaraldehyde, negatively stained for electron microscopy.

After centrifugation, the pelleted microtubules were resuspended and analysed by SDS-PAGE on 4–15% gradient gels. As shown in Fig. 1, MAP1A, MAP1B, and MAP2 co-sedimented with assembled microtubules confirming that in each case the microtubules formed consisted of tubulin and the appropriate MAP.

Electron microscopy (Fig. 2: insets) showed that normal microtubules, possessing a typical diameter of about 25 nm, were formed in the presence of each MAP and open or aberrant microtubular structures were not detected. However, the microtubules displayed quite different morphologies depending on the nature of the MAP used to promote tubulin polymerisation (Fig. 2). A striking difference was observed between MAP1B microtubules and those assembled with MAP1A and MAP2 (cf. Fig. 2b and a,c) in that microtubules formed in the presence of MAP1B exhibited a marked 'bendiness' with multiple bends along the entire length of individual microtubules. Such an effect was not observed with MAP1A microtubules which appeared to be essentially 'straight' and rod-like structures. On the other hand, MAP2 microtubules exhibited both straight and 'bendy' shapes, the latter being less frequent and generally did not exhibit multiple bends along a single microtubule.

The lengths of MAP1A, MAP1B and MAP2 microtubules were also determined from E-M photographs. About 350 microtubules were measured for each MAP and the number of microtubules were plotted as a function of microtubule length

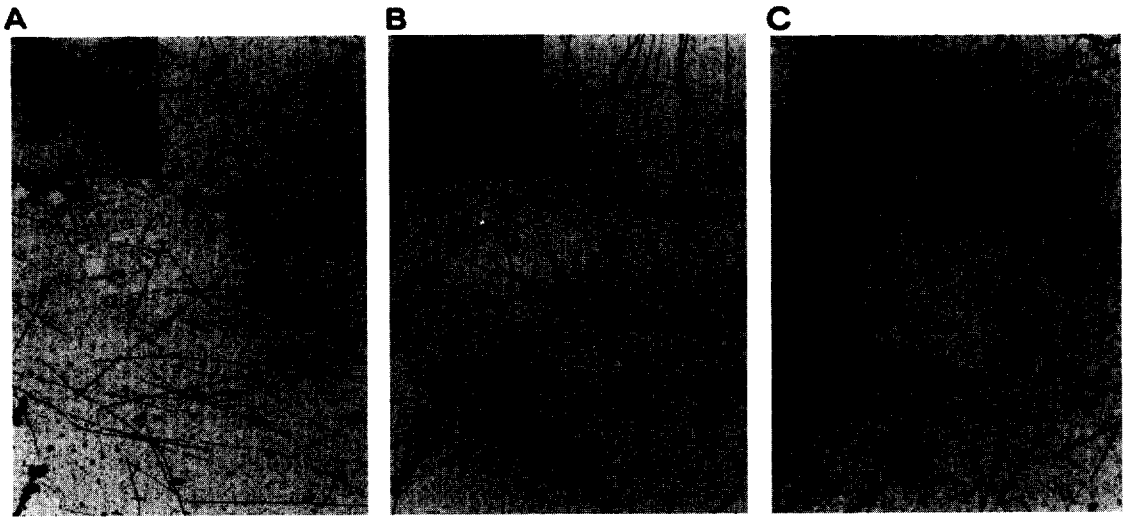

Fig. 2. Electron microscopy. Microtubules assembled in the presence of (A) MAP1A, (B) MAP1B, and (C) MAP2, were fixed with glutaraldehyde and negatively stained and observed using a JOEL 80 electron microscope. Micrographs show microtubules ×1400. Inset: microtubule at ×125000 magnification.

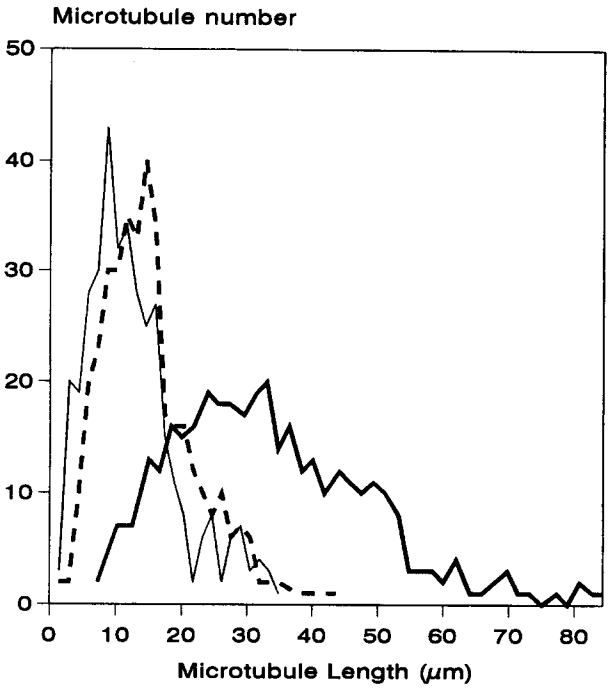

Fig. 3. Microtubule length distribution. Micrographs (see Fig. 2) were used to determine the lengths of individual microtubules. The number of microtubules with determined lengths were then plotted as a function of length. (Thin unbroken line) MAP1A microtubules, (broken line) MAP2-microtubules, (thick unbroken line) MAP1B microtubules.

(Fig. 3). MAP1B microtubules appeared to be longer when compared with either MAP2 or MAP1A microtubules. The difference in length of MAP1B and MAP2 microtubules probably reflects the lower level of nucleation induced by MAP1B as the final level of polymerisation is similar to that observed with MAP2 (see also [24]). On the other hand, MAP1A microtubules are shorter than MAP2 microtubules, the level of nucleation is similar for both proteins, but MAP1A exhibits a higher critical concentration for assembly [18].

Several microscopic fields, containing between 400 and 450 microtubules, were also analysed to determine the number of bendy or straight microtubules formed in the presence of each different MAP (Fig. 4). The measurement were performed on the entire range of distribution of microtubule lengths to eliminate the possibility that shorter microtubules may tend to be straight while longer microtubules may tend to be more bendy. The analysis showed that 90% or more of MAP1B microtubules were bendy and below 10% were straight whilst the opposite was true for MAP1A. MAP2 microtubules tended to be largely straight although up to 25% of the microtubules exhibited some bendiness.

4. Discussion

MAPs are associated with microtubules in vitro and in vivo and have been shown to alter microtubule assembly dynamics [3–5,10,25] and microtubule flexibility, although their effect on flexibility remains controversial [6,20,21]. These effects coupled with the spatial and temporal distribution of MAPs are generally considered to be important for the development and maintenance of cellular morphology.

The different microtubule shapes observed upon tubulin polymerisation in the presence of different MAPs suggest that MAPs may play a role in defining microtubule shape. Structural distortions as a consequence of fixation with glutaraldehyde are unlikely as glutaraldehyde has been reported to cause structural distortions at high concentrations but not at low concentrations such as those used in the present study ([26]; and unpublished observation). It is interesting to note that the morphology of the microtubules observed with each MAP appears to closely resemble the description of microtubules in the neuronal regions in which these MAPs are concentrated [1,2,8,27,28]. MAP1A and MAP2, which bind to distinct sites and can co-localise on the same microtubules both in vitro and in vivo, give rise to straight microtubules of the type found in MAP2-rich dendrites and MAP1A-rich axons. Furthermore, transfection studies with MAP1A constructs in HeLa cells result in rearrangement of microtubules into straight, short microtubules [19].

On the other hand, MAP1B microtubules are generally long and bendy and the protein is highly concentrated in growth cones [11] where splayed, looped, and bendy microtubules have been observed [1,2]. This morphology coupled with the fact that MAP1B is (a) less efficient at stabilising microtubules against depolymerising drugs [16], (b) promotes tubulin polymerisation in vitro more efficiently when compared with MAP2 and MAP1A [18,24], and (c) has a lower binding affinity to microtubules than that of other MAPs [12–14], suggests that MAP1B may play a specialised role in permitting the formation of dynamic and bendy microtubules in regions characterised by events requiring rapid morphological reorganisation [1,2].

The more bendy morphology of MAP1B microtubules during early stages of development may be gradually replaced by a more defined morphology as suggested by the straight mi-

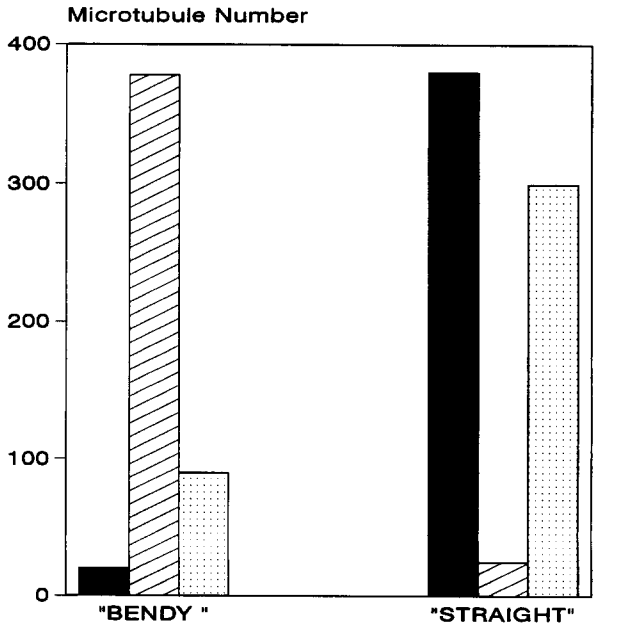

Fig. 4. Determination of microtubule shape. The shape of microtubules reassembled with MAP1A (filled bars), MAP2 (stippled bars), or MAP1B (hatched bars) was determined from the micrographs (see Fig. 2). The total number of microtubules which were 'bendy' or 'straight' (see section 3) for each MAP is shown.

crotubules formed in the presence of MAP1A. This may be expression of a more mature cytoskeletal architecture requiring additional stability [4]. This change in morphology is in agreement with the pattern of expression of these two proteins, MAP1A gradually replaces MAP1B during development [10]. It would be interesting to determine if the different microtubule shapes induced by these different MAPs also reflect alterations in microtubule 'flexibility'.

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References

[1] Lin, C.H. and Forscher, P. (1993) J. Cell Biol. 121, 1369–1383.
[2] Tanaka, E.M. and Kirschner, M. (1991) 115, 345–363.
[3] Wiche, G. (1989) Biochem. J. 259, 1–12.
[4] Matus, A. (1988) Annu. Rev. Neurosci. 11, 29–44.
[5] Chapin, S. and Bulinski, J.C. (1992) Cell Motil. Cytoskel. 23, 236–243.
[6] Matus, A. (1994) Trends Neurosci. 17, 19–22.
[7] Ferralli, J., Doll, T. and Matus, A. (1994) J. Cell Sci. 107, 3115–3125.
[8] Lewis, S.A, Ivanov, I.E., Lee, G. and Cowan, N.J. (1989) Nature 342, 498–505.
[9] Butner, K.A. and Kirschner, M.W. (1991) J. Cell Biol. 115, 717–730.
[10] Muller, R., Kinder, S. and Garner, C.C. (1994) in: Microtubules (Lloyd, C. and Hyams, J.S. eds.) pp. 141–154, Wiley-Liss, New York.
[11] Black, M.M., Slaughter, T. and Fischer, I. (1994) J. Neurosci. 14, 857–870.
[12] Bloom, G.S., Luca, F.C. and Vallee, R.B. (1985) Proc. Natl. Acad. Sci. USA 82, 5404–5408.
[13] Joly, C. and Purich, D.L. (1990) Biochemistry 29, 8916–8920.
[14] Noble, M., Lewis, S.A. and Cowan, N.J. (1989) J. Cell Biol. 109, 3367–3376.
[15] Brugg, B., Reddy, D. and Matus, A. (1993) Neuroscience 52, 489–496.
[16] Takemura, R., Okabe, S., Umeyama, T., Kanai, Y. Cowan, N.J. and Hirokawa, N. (1992) J. Cell Sci. 103, 953–964.
[17] Shiomura, Y. and Hirokawa, N. (1987) J. Cell Biol. 104, 1575–1578.
[18] Pedrotti, B. and Islam, K. (1994) Biochemistry 33, 12463–12470.
[19] Cravchik, A., Reddy, D. and Matus, A. (1994) J. Cell Sci. 107, 661–677.
[20] Dye, R.B., Fink, S.P. and Williams, R.C. (1993) J. Biol. Chem. 268, 6847–6850.
[21] Kurz, J.C. and Williams, R.C. (1995) Biochemistry 34, 13374–13380.
[22] Pedrotti, B. and Islam, K. (1995) Cell Motil. Cytoskel. 30, 301–309.
[23] Burns, R.G. and Islam, K. (1984) Eur. J. Biochem. 141, 599–608.
[24] Pedrotti, B. and Islam, K. (1995) FEBS Lett. 371, 29–31.
[25] Avila, J. (1990) FASEB J. 4, 3284–3290.
[26] Himes, R.H., Jordan, M.L. and Wilson, L. (1982) Cell Biol. Int. Rep. 6, 697–704.
[27] Yu, W. and Baas, P.W. (1993) J. Neurosci. 14, 2818–2829.
[28] Harada, A., Oguchi, K., Okabe, S., Kuno, J., Terada, S., Ohshima, T., Sato-Yoshitake, R., Takei, Y., Noda, T. and Hirokawa, N. (1994) Nature 369, 488–491.