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MAP2 COMPETES WITH MAP1 FOR BINDING TO MICROTUBULES

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SUMMARY: A question whether MAP1 and MAP2 (the major microtubule associated proteins from mammalian brain) bind to common or distinct sites on the microtubule surface was studied. Microtubules were assembled from tubulin and MAP1 and then centrifuged through a layer of MAP2 solution under conditions where no repolymerization of tubulin with MAP2 could occur. During centrifugation, MAP2 displaced most of MAP1 on the microtubules. This implies that MAP1 is reversibly bound to microtubules and that MAP2 binding interferes with MAP1 binding. The latter means that binding sites for MAP1 and MAP2 are identical or overlap.

Microtubules are composed of tubulin and so-called microtubule associated proteins (MAPs). The main mammalian brain MAPs are two high molecular weight proteins: MAP1 with molecular weight of about 350,000 and MAP2 of somewhat lower molecular weight (1,2). Both of the proteins have been purified and shown to promote tubulin polymerization and to form side projections on microtubules in vitro (3-9).

Although similar in some properties, MAP1 and MAP2 are definitely distinct proteins. Besides the difference in molecular weight, they show considerable differences in their one-dimensional peptide maps (7) and in thermosensitivity of their polymerization-promoting activity (8).

The existence of different MAPs raises a question whether they bind to identical sites on the microtubule wall, Here we demonstrate that MAP2 can displace MAP1 on the microtubules

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assembled from tubulin and MAP1, which suggests that binding sites for these MAPs are overlap.

MATERIALS AND METHODS

Tubulin, MAP1 and MAP2 were obtained from bovine brain in a buffer containing 50 mM imidazol-HCl (pH 6.7), 50 mM KCl, 0.5 mM $MgCl_2$, 0.1 mM EDTA and 1 mM 2-mercaptoethanol (buffer A). Unfractionated microtubule proteins were prepared by assembly-disassembly procedure (10), as modified in (11). Tubulin was then purified by phosphocellulose chromatography (12). MAP1 and MAP2 were purified as in (7) and (3) respectively. Just before an experiment MAP2 solution was clarified by centrifugation at 150,000 g.

MAP1-stimulated tubulin polymerization was performed at 37°C for 30 minutes in the buffer A with 1 mM GTP and 1 mM EGTA at tubulin and MAP1 concentrations of 0.2-0.4 and 0.7-0.9 mg/ml respectively. For MAP-free microtubule assembly, tubulin (2 mg/ml) was incubated for 30 min at 37°C in the buffer A supplemented with 1 mM GTP, 1 mM EGTA and 4 M glycerol.

For electron microscopy, microtubules were pelleted by centrifugation through a layer of buffer A containing 1 mM EGTA and 4 M glycerol at 20°C and 200,000 g. The pellet was fixed with 1% glutaraldehyde in 10 mM sodium phosphate buffer (pH 7.0) containing 1% tannic acid (13) and stained with OsO_4.

The centrifugation of MAP1-containing microtubules through MAP2 solution was carried out in Beckman SW 60 Ti rotor at 40,000 rpm for 40 min at 25°C. The microtubule suspension (0.1 ml) was layered on the cushion comprising three glycerol solutions in the buffer A supplemented with 1 mM EGTA: 3 ml of 4 M solution on the bottom of the centrifuge tube, then 0.5 ml of 2 M solution with MAP2 (0.4-0.8 mg/ml), and 0.6 ml of 1 M solution on the top.

SDS-gel electrophoresis was performed as in (14) in 10% polyacrylamide gels at an acrylamide/methylenebisacrylamide ratio of 100:1. Gels were stained with Coomassie R-250.

Protein concentration was determined by Lowry method (15) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Fig. 1B shows longitudinal and transverse sections through microtubules obtained in MAP1-driven tubulin assembly. Side projections ("arms") are clearly seen on the surfaces of the microtubules, which confirms a recent report (9). Such projections were not observed on MAP-free microtubules assembled from pure tubulin in glycerol solution (Fig. 1A). The arrangement of MAP1 arms on the microtubule surface was generally similar to that described earlier for MAP2 (4,5).

To answer the question about MAP1 and MAP2 binding sites identity we examined whether MAP2 would compete with MAP1 for

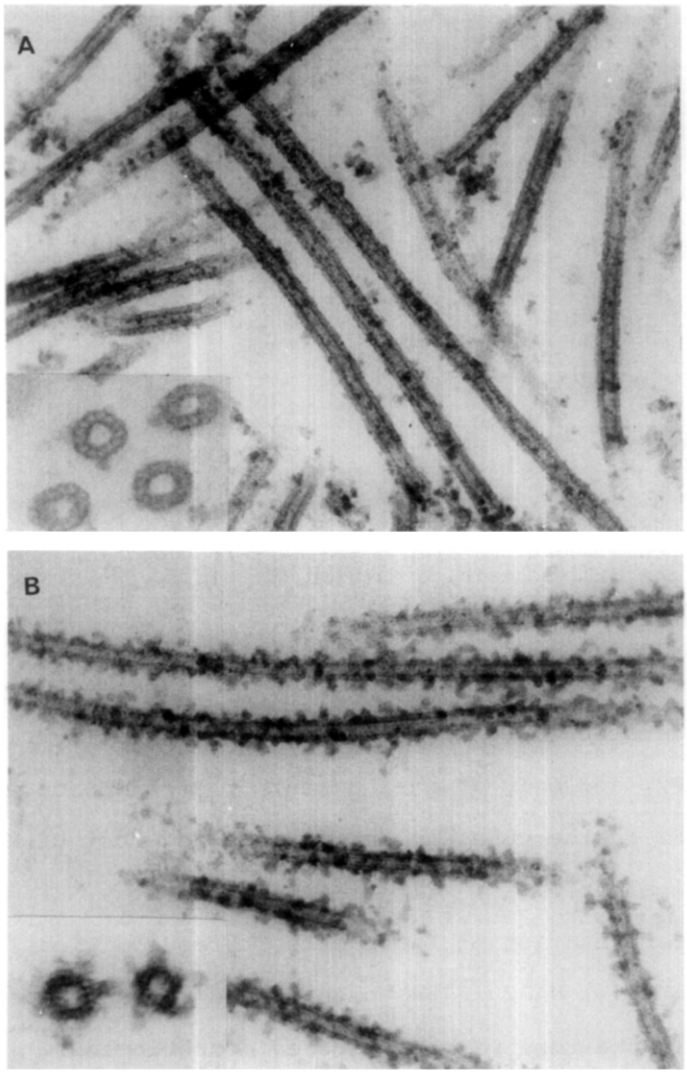

Fig. 1. Thin sections of the microtubules obtained by polymerisation of purified tubulin in the presence of glycerol (A) and MAP1 (B). x 150,000 (insets x 300,000).

binding to microtubules. For this, tubulin was polymerized with MAP1, the microtubules formed were centrifuged through a layer of MAP2 solution, and the protein composition of the pellet was determined by SDS-gel electrophoresis. Fig. 2a shows the composition of the control pellet, i.e. the microtubules pelleted through the MAP2-free cushion. They consisted of tubulin, MAP1, and traces of MAP2 contaminating the MAP1 preparation. In cont-

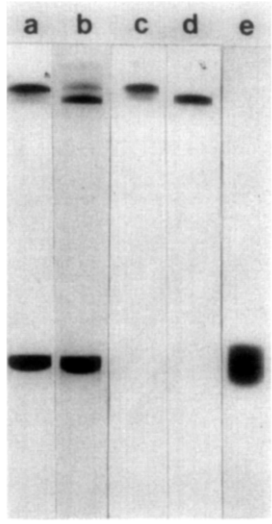

Fig. 2. SDS-gel electrophoresis of the microtubules pelleted by centrifugation through the MAP2-free cushion (a) and the MAP2-containing cushion (b). (c,d,e) - MAP1, MAP2 and tubulin, respectively, used in the experiment.

rast, the microtubules pelleted through the MAP2 layer contained tubulin, MAP2, and only a small amount of MAP1. Hence, the presence of MAP2 in the cushion led to replacing most of MAP1 by MAP2 in the pellet.

For interpretation of these data, three additional results are important. (1) MAP2 in the cushion did not sediment by itself (i.e. in the tube without layered microtubules). (2) MAP2 was not detected in the pellet after centrifugation, through the MAP2-containing cushion, of a GTP-free MAP1-tubulin polymerization mixture (in which microtubules do not assemble, for MAP1-promoted tubulin polymerization is GTP-dependent)(8). (3) Microtubule assembly from tubulin and MAP2 in the buffer A supplemented with 2 M glycerol and 1 mM EGTA was shown in separate experiments to be GTP-dependent. Hence, even if microtubules were partly depolymerizing during centrifugation, repolymerization of tubulin by MAP2 could not occur in the cushion because of the absence of GTP.

It is clear from these results that the appearance of MAP2 in the pellet was a consequence of MAP2 co-sedimentation with the microtubules that had been assembled from tubulin with MAP1 and then layered on the cushion. Moreover, since the appearance of MAP2 was accompanied with MAP1 disappearance, we may conclude that MAP2 displaced MAP1 on the microtubules during centrifugation.

It appears most likely that MAP1 can reversibly bind to and dissociate from microtubules and that in the MAP2 layer reassociation of dissociated MAP1 was competitively inhibited by MAP2 binding. Whether this means that MAP1 and MAP2 binding sites are identical or that they only have some common parts is unclear. Since a small fragment of MAP2 has been described (16) which, though able to displace MAP2, did not displace MAP1 on brain microtubules, the second possibility seems more probable. But in any event, it can be concluded that the binding sites for MAP1 and MAP2 are not independent of each other.

It has already been shown that MAP2 can compete, for binding to microtubules, with tau, a low molecular weight MAP (17). Now it is of interest whether MAP1 and tau will compete.

REFERENCES

1. Sloboda, R.D., Rudolph, S.A., Rosenbaum, J.L. and Greengard, P. (1975) Proc.Natl.Acad.Sci USA 72, 177-181.
2. Berkowitz, S.A., Katagiri, J., Binder, H.-K. and Williams, R.C. (1977) Biochemistry 16, 5610-5617.
3. Kuznetsov, S.A., Rodionov, V.I., Gelfand, V.I., and Rosenblat, V.A. (1978) FEBS Lett. 95, 339-342.
4. Herzog, W., and Weber, K. (1978) Eur.J.Biochem. 92, 1-8.
5. Kim, H., Binder, L., and Rosenbaum, J. (1979) J. Cell Biol. 80, 266-276.
6. Zingheim, H.-P., Herzog, W., and Weber, K. (1979) Eur.J.Cell Biol. 19, 175-183.
7. Kuznetsov, S.A., Rodionov, V.I., Gelfand, V.I. and Rosenblat, V.A. (1981) FEBS Lett. 135, 237-240.
8. Kuznetsov, S.A., Rodionov, V.I., Gelfand, V.I. and Rosenblat, V.A. (1981) FEBS Lett. 135, 241-244.
9. Vallee, R.B., and Davis, S.E. (1983) Proc.Natl.Acad.Sci USA, 80, 1342-1346.
10. Shelanski, M.L., Gaskin, F., and Cantor, C.R. (1973) Proc. Natl.Acad.Sci USA 70, 765-768.

11. Rodionov, V.I., Gelfand, V.I., and Rosenblat, V.A. (1978) Dokl.Acad.Nauk SSSR 239, 231-233.
12. Weingarten, M.D., Lockwood, A.H., Hwo, S.-Y., and Kirschner, M.W. (1975) Proc.Natl.Acad.Sci USA 72, 1858-1862.
13. Haimo, L.T., Telzer, B.R., and Rosenbaum, J.L. (1979) Proc. Natl.Acad.Sci USA 76, 5159-5163.
14. Laemmly, U.K. (1970) Nature 227, 680-685.
15. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J.Biol.Chem. 193, 265-275.
16. Vallee, R.B. (1980) Proc. Natl.Acad.Sci USA 77, 3206-3210.
17. Kim. H., and Rebhun, L.I. (1982) J.Cell Biol. 85, 349a.