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ON THE USE OF HEAT STABILITY AS A CRITERION FOR THE IDENTIFICATION OF MICROTUBULE ASSOCIATED PROTEINS (MAPS)

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Summary: Solubility at elevated temperature is a striking biochemical property exhibited by a restricted number of the known microtubule associated proteins. This property has been extremely useful in the identification of these proteins and in their purification as well. It is reported here that heat stability is a function of the composition of proteins present during exposure to elevated temperature. All non-tubulin proteins in bovine microtubule preparations were found to remain soluble when tubulin was removed prior to heating. Addition of purified tubulin or bovine serum albumin to the preparation restored the selective heat stability normally seen in microtubule protein preparations.

Microtubules consist of tubulin and a variety of accessory proteins known as microtubule associated proteins (MAPs[1]). In brain tissue, the principal source of microtubules used for biochemical studies, the MAPs consist of a group of high molecular weight proteins (M_r~300,000) known as MAP 1 and MAP 2, a group of proteins of M_r~55,000-62,000 known collectively as tau, and a variety of less prominent species (for review, see references 1 and 2). The basis for the biochemical diversity of the MAPs is largely unknown.

A useful criterion for the classification of the MAPs has been the resistance of certain of these proteins to denaturation at elevated temperature (3-8). In particular, the high molecular weight MAPs, MAP 1 and MAP 2, can be distinguished on this basis since MAP 1 precipitates at elevated temperature, while MAP 2 remains soluble (5,6).

I report here that the differential response of MAP 1 and MAP 2 to elevated temperature is strongly influenced by protein environment. In the

[1] Abbreviations: MAP, microtubule associated protein; SDS, sodium dodecyl sulfate.

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absence of tubulin or other proteins that themselves denature at elevated temperature, both proteins, as well as other brain MAPs, remained soluble.

MATERIALS AND METHODS

Protein Purification and Fractionation: Microtubules were purified by two cycles of assembly/disassembly purification (9), frozen in liquid nitrogen, and stored at -80°C. All subsequent centrifugation steps were at 30,000xg. The microtubules were thawed, resuspended to 5 mg/ml in 0.1 Pipes, pH 6.6, containing 1.0 mM $MgSO_4$ and 1.0 mM EGTA (PEM buffer), plus 1.0 mM GTP, allowed to depolymerize on ice for 15 min, and centrifuged for 20 min at 2°C. Subsequent steps were based on the method described in reference 10. Taxol was added to 20 uM and the microtubules were centrifuged at 37°C. The microtubules were resuspended to 5 mg/ml in the same buffer with taxol, and NaCl was added to 0.35 M to dissociate the MAPs. The preparation was centrifuged at 37°C as before, yielding MAPs in the supernate and tubulin in the pellet. For some experiments MAPs and tubulin were separated by DEAE-Sephadex chromatography (11). The preparation of MAPs obtained by salt extraction of taxol-stabilized microtubules was used for all experiments, unless otherwise noted. Bovine serum albumin was cat. # A 7638 (Sigma Chemical Co., St. Louis, MO).

Exposure of proteins to elevated temperature: Protein preparations were brought to 0.75 M NaCl and 10 mM DTT in PEM buffer, incubated in a boiling water bath for 5 min, chilled in an ice/water slurry for 5 min, and centrifuged at 30,000xg for 30 min at 2°C (4-6).

Analytical Methods: SDS gel electrophoresis was performed according to Laemmli (12). Gels were stained with Coomassie Brilliant Blue R250 (13). Quantitative densitometry of electrophoretic bands was performed using a Quik-scan, Junior (Helena Instruments) as described previously (9,14). Protein was determined using the method of Schacterle and Pollack (15).

RESULTS AND DISCUSSION

When unfractionated brain microtubules are exposed to elevated temperature, MAP 2 and tau remain soluble, while tubulin and the remaining MAPs precipitate (4-6). This phenomenon has been exploited routinely by this and other laboratories as a means for identifying and isolating MAP 2 and tau from the other MAPs. I have noticed, however, that if similar conditions are applied to MAPs that have been separated from tubulin, all of the detectable polypeptides in the preparation remain soluble (Fig. 1). The difference in the behavior of MAP 1 (actually MAP 1A[2]) in the presence and absence of

[2] MAP 1 has been found to consist of three polypeptide species, MAP 1A, MAP 1B, and MAP 1C (1,16,17). In microtubules purified from bovine cerebral cortex, as used in the present study, MAP 1A constitutes almost all of the MAP 1 protein detectable by Coomassie Blue staining (17). In the present study, using immunoblotting with an anti-MAP 1B antibody, I found that MAP 1B, like MAP 1A, remained completely soluble when MAPs were exposed to elevated temperature in the absence of tubulin.

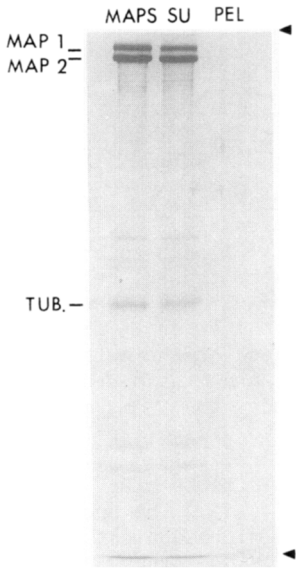

Fig. 1. Stability of MAPs to elevated temperature. MAPs (1 mg/ml) were incubated in a boiling water bath and centrifuged, and the resulting fractions were analysed by SDS gel electrophoresis using 9% polyacrylamide. MAPs) starting unfractionated MAPs; SU) supernate containing soluble MAPs; PEL) pellet containing insoluble MAPs. MAP 1 designates the position of MAP 1A; MAP 2 designates the position of MAP 2A and MAP 2B, seen as a single band in this gel system; TUB. designates the position of tubulin. In all figures, arrowheads designate top and dye front of gel.

tubulin was of particular interest because of the importance of heat stability in classifying the MAP 1 and MAP 2 polypeptides.

To determine whether it was the presence of tubulin itself that resulted in the normally observed precipitation of MAP 1, MAPs were exposed to elevated temperature at a series of added tubulin concentrations (Fig. 2 and 3). In the absence of added tubulin, very little of the MAP 1 or of the other non-tubulin proteins present in the preparation precipitated. In the presence of increasing concentrations of added tubulin, MAP 1 progressively disappeared from solution, while MAP 2 remained fully soluble. Complete depletion of MAP 1 occurred at 1.1 mg/ml tubulin.

It may be noted in Fig. 3 that the level of soluble MAP 1 in the sample lacking added tubulin was only about 70% of that of the starting MAPs sample. This probably reflected precipitation of a portion of the MAP 1 due to a small amount of residual tubulin present in the MAPs sample. I found,

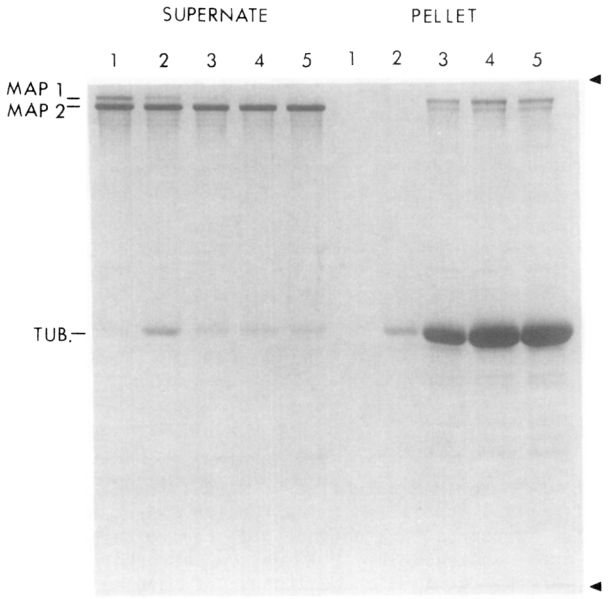

Fig. 2. Effect of tubulin on MAP solubility. MAPs (1 mg/ml) were incubated in a boiling water bath in the presence of increasing concentrations of tubulin obtained by salt-extraction of taxol stabilized microtubules. The samples were centrifuged and the supernates and pellets were subjected to electrophoresis (9% polyacrylamide). Tubulin concentrations were 1) 0; 2) 0.4; 3) 0.8; 4) 1.1; and 5) 1.5 mg/ml.

in fact, that MAPs preparations contained variable levels of tubulin, and the yield of soluble MAP 1 was correspondingly variable. However, in all experiments in which tubulin had been separated from the MAPs, appreciable levels of soluble MAP 1 were obtained.

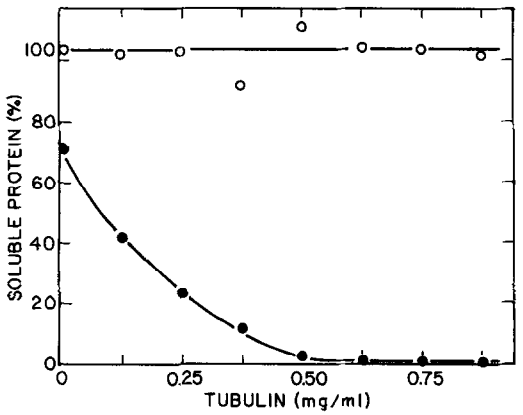

Fig. 3. Quantitative analysis of the effect of tubulin on solubility of MAP 1 and MAP 2. MAPs (1 mg/ml) were exposed to increasing concentrations of ion-exchange purified tubulin and incubated in a boiling water bath. Following centrifugation, the supernates were analysed by gel electrophoresis and the amount of MAP 1 and MAP 2 determined by quantitative densitometry.

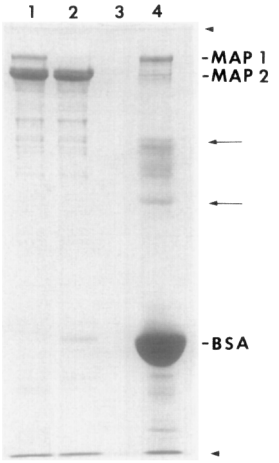

Fig. 4. Effect of BSA on MAP solubility. MAPs (1 mg/ml) prepared by salt extraction of taxol-stabilized microtubules were exposed to elevated temperature in the presence or absence of bovine serum albumin (3 mg/ml), centrifuged, and analysed by SDS gel electrophoresis using 5.5% polyacrylamide. Arrows designate positions of contaminants in commercial serum albumin preparation. Lanes represent: 1) supernate, MAPs alone; 2) supernate, MAPs + BSA; 3) pellet, MAPs alone; 4) pellet MAPs + BSA.

It may also be noted that a small amount of soluble tubulin was always seen in the reconstituted preparations after boiling, and that soluble tubulin showed a maximum at low levels of added tubulin (Fig. 2, Supernate, lane 2). This may reflect a threshhold concentration for the formation of a sedimentable tubulin precipitate.

To determine whether the effect of tubulin on MAP 1 heat stability was merely due to the additional protein concentration contributed by the tubulin, MAPs were prepared at high concentration by DEAE-Sephadex chromatography (11). Even at concentrations as high as 8 mg/ml, which is higher than that in any of the protein mixtures used in Fig. 1-4, most of the MAP 1 in the preparation remained in solution.

To determine whether the ability to induce the precipitation of MAP 1A was a unique property of tubulin, the effect of BSA on MAP solubility was also examined (Fig. 4). As with tubulin, the presence of BSA during exposure of the MAPs to elevated temperature resulted in the virtually complete precipitation of MAP 1.

The difference in the stability of MAP 1 and MAP 2 polypeptides to precipitation at elevated temperature has been useful both in classifying

these proteins and in the purification of MAP 2. Similarly, the solubility of other MAPs, including tau (3,5,6), a 200,000 dalton MAP (7,18), and a 150,000 dalton sea urchin species (8), has served to distinguish these proteins from other MAPs. It is now clear that this distinction is not absolute, and caution must be exercised in applying the criterion of heat stability in the identification of MAPs.

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