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STRONGLY BASIC POLYPEPTIDES AMONG MICROTUBULE ASSOCIATED PROTEINS

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Isoelectrofocussing between pH 3.5 and 9.5, or 9 and 11 in the first direction of two-dimensional gel electrophoreses reveal that the bands of microtubule associated proteins,e.g. the high molecular weight proteins and the τ factor, contain several polypeptides. A distinct group of these associated proteins possesses an isoelectric point of 9.5, and molecular weights between 72000 and 280000 daltons. This family of basic proteins is not the result of proteolytic cleavage of one high molecular weight precursor, as could be shown by fingerprint methods. A separation of native MAPS is briefly described.

INTRODUCTION

Tubulins from porcine brain (1) and other sources (2), purified by several cycles of repolymerization, contain a group of associated proteins, whose composition and amounts seem to vary widely among species and tissues (3). We have recently published a method for the purification of native microtubules from porcine brain without any depolymerization step (4). Of the 7 groups of MAPS therein, only the so-called τ factor and the high molecular weight proteins 1 and 2 (5,6) possess a well defined influence upon the polymerization of tubulin.

In this paper we analyzed MAPS by two-dimensional electrophoreses, some purified groups of MAPS that contain distinctly basic proteins enhance tubulin polymerization.

Abbreviations: MAPS: Microtubule associated proteins, MES: 2(N-Morpholino-ethane)-sulfonic acid. PAGE: Polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

MES was purchased from Sigma Chemicals Co.,Munich. Hydroxiapatite, crystalline, was a product of Boehringer, Mannheim. [^{3}H], [^{14}C]-labeled acetic anhydride at specific activities of 40 and 5 mCi/mMole specific activity was obtained from Amersham-Buchler Co., Frankfurt.

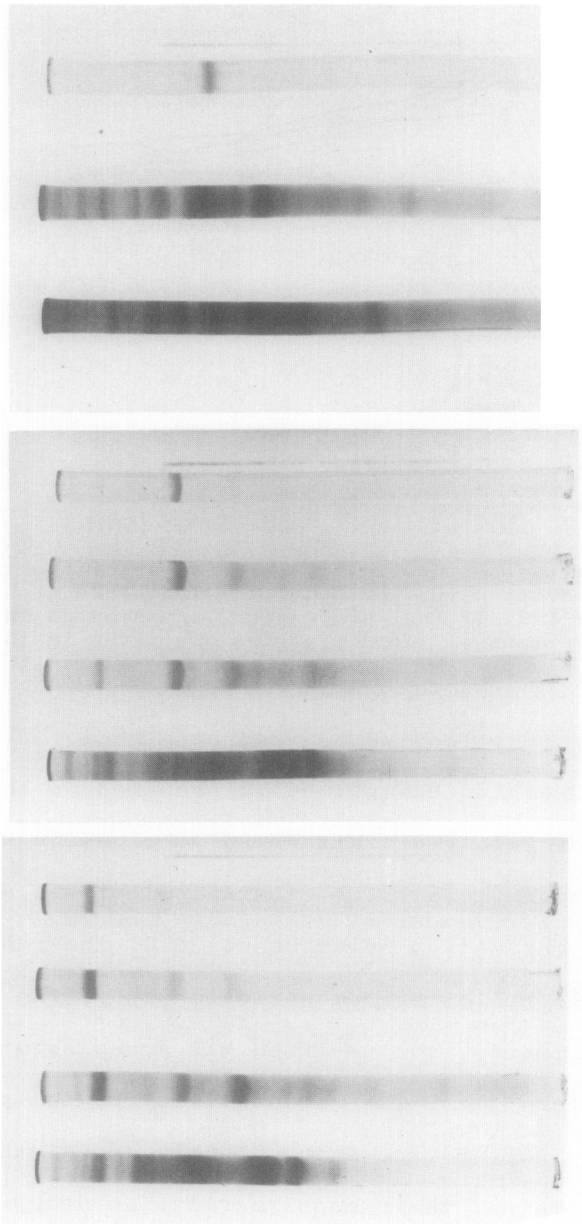
Polymerization of tubulin was measured in an Ostwald type capillary viscosimeter at 37°C. One-dimensional PAGE was run after conventional methods (7). For two-dimensional electrophoreses, the proteins were dialyzed against 0.02 M ammonium carbonate and lyophylized. They were dissolved in 2% ampholyte (LKB), 9.5 M urea, 20 mM dithioerythrol and 5% Nonidet P 40. Focussing was between 3.5 and 9.5, or in Servalyte 9-11, in 3% polyacrylamide, containing 9 M urea, 2% ampholyte, and 2% of nonidet. A current 0.5 mA per gel was applied, the electrode solutions were 0.02 M NaOH at the cathode and 0.01 M phosphoric acid or 0.02 M glycylglycine at the anode. The gels were then soaked in 0.05 M Tris-HCl-buffer, pH 6.8, containing 5% SDS, 1% mercaptoethanol, and 10% glycerol for 30 min. They were fixed to the top of a slab gel with the aid of 0.5% agarose suspension. The second dimension was run at 20 mA per gel until the extrusion of a bromophenol blue marker. pH gradients in the first dimensions were measured with the aid of an Ingold two-tip electrode of 1 cm distance.

RESULTS AND DISCUSSION

Purification of Native MAPS from Porcine Brain. MAPS were prepared from 8000 g batches of fresh porcine brain after homogenizing in 0.1 M MES buffer, pH 6.5, containing 1 mM EGTA, at 37°C. The purification involved a differential centrifugation (8000 and 100000g) at 37°C, inactivation of polymerization by N-ethyl maleimide (4) and chromatographic separations in 0.05 M MES buffer, pH 6.5. After a Sephadex G 100 filtration the first peak was applied to a phosphocellulose column, and eluted with a gradient to 1 M NaCl. A gradient to 0.45 M sodium phosphate was used on small hydroxiapatite columns, the final step was a Sephacryl S-200 filtration.

Fig. 1 shows the purification of three MAP fractions (4). In view of the results described below, however, these bands contain probably more than one protein.

Labeling and Copolymerization of Purified MAPS. To ascertain their native structure, these proteins were labeled with ^{3}H-acetic anhydride to a specific activity of 5×10^{5} cpm/mg in de-



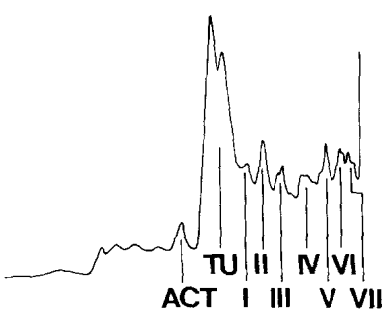


Fig. 1 Left: Gel electrophoresis densitometer scans from native microtubules, as described in ref. (4). ACT: Actin. TV: Tubulin double band. Fraction I is identical to the protein family, VI and VII correspond to HMW 2 and 1, respectively. Middle and Right: Purification of MAP fractions V, III and II. Gels were run (from left to right) after the phosphocellulose column, the first, and in the case of V and III, second hydroxiapatite columns, and after the final Sephacryl column (see Text).

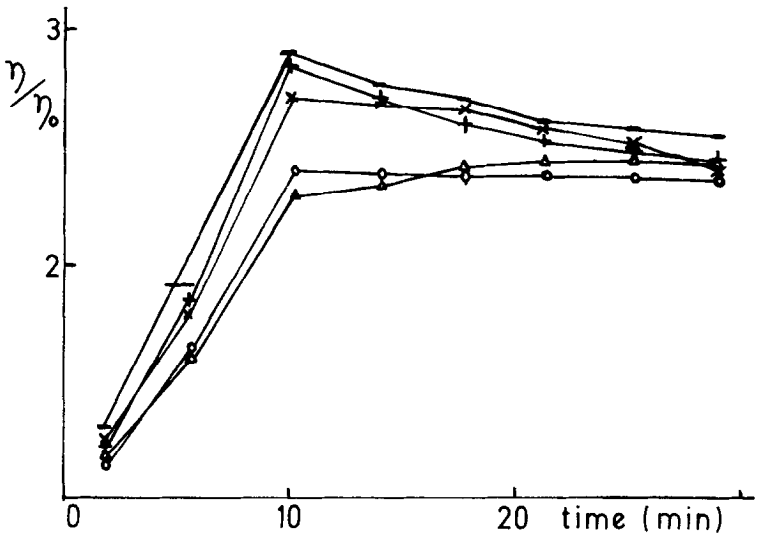


Fig. 2 Polymerization of tubulin, measured as described in Methods, without addition (△), or under the influence of fraction II (○), I (+), IV (×), or τ factor, prepared as in ref. (9). Concentrations of added proteins are given in the text.

polymerization buffer (8). They were added at a ratio of 1:60 w/w to depolymerized porcine brain tubulin prepared on the same day by three cycles of repolymerization. Polymerization was induced in the usual manner (8). The tubulin pellet was gently rehomogenized in polymerization solution at 30°C. The radioactivity in the tubule pellet now amounted to 68% of the total. In control experiments, the purified MAP group solutions were heated to 90°C for 5 min. The radioactivity in the pellet then amounted to only 26%. Bovine serum albumin and histones served as control proteins, their copolymerization dropped to 8 and 11%, respectively.

Initiation of Polymerization. Fig. 2 describes the influence of purified MAP fractions as well as of τ factor (9) upon polymerization. An overshoot shows that the nucleation process is primarily influenced (10). This effect is not simply caused by a denaturation of the purified protein fractions, because proteins denatured by heating as described above gave no polymerization enhancing effect.

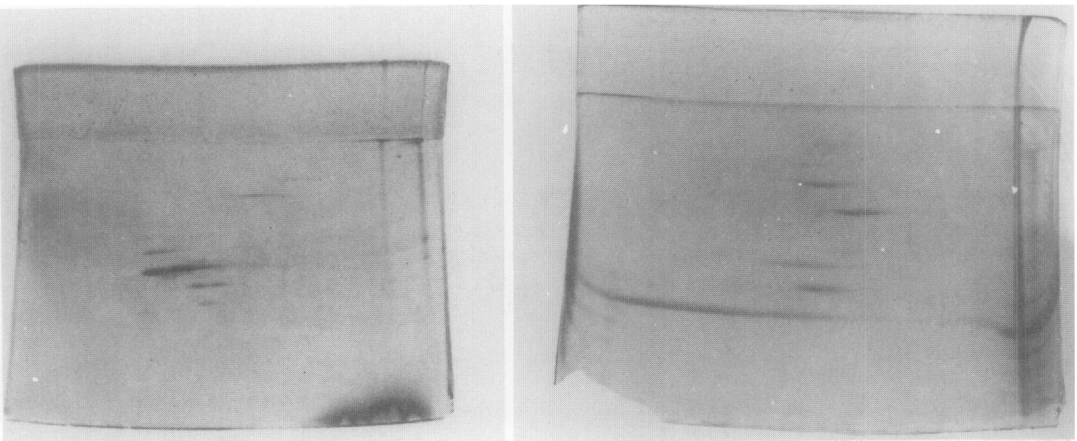


Fig. 3 2D gel electrophoreses by isoelectrofocussing in the first dimension (basic gradient edge to the right) between pH 3.5 and 9.5 (left), or between pH 9 and 11 (right), and SDS-PAGE in the second dimension. A once repolymerized tubulin marker gel was run on the right-hand edge in the second dimension.

Two Dimensional Electrophoresis Patterns. When native tubules from porcine brain (4) or tubulin preparations after four repolymerization cycles were subjected to isoelectrofocussing between pH 3.5 and 9.5, the pattern of MAPS was further resolved, as indicated in Fig. 3 (see also ref. 11). Proteins at the alkaline end of the gradient were dissolved however with the aid of a gradient between pH 8 and 11 (Fig. 3). The more acidic proteins now appeared at the anodic rim of the gel, while a series of 6 basic polypeptides formed a pattern between isoelectric points of 8.3 and 8.8. Their molecular weights were found in the approximate regions of 280000, 260000, 200000, 150000, 72000 and 40000 daltons from second dimension marker proteins. Their function can at present only be speculated upon. It should be remembered, however, that the polymerization of tubulin can be induced by strongly basic agents (12). We also found that the two purified MAP groups, described in Fig. 2, that do induce polymerization, and the τ factor proteins contain at least one basic protein each, while the MAP group referred to as IV does not. Furthermore, porcine brain tubulin

containing MAPS binds acidic components, especially nucleic acids, or, with the aid of the τ protein, calmodulin (15). Short treatment with trypsin abolishes the "arms" protruding from the tubule, and also to a large part the binding of DNA (13).

Fingerprinting of Basic MAPS. The similarity in isoelectric points, documented in Fig. 3, raised the suspicion that all of these basic proteins might be proteolytic fragments of one high molecular weight protein. We have recently published a method for the fingerprinting of amounts of protein down to 1 µg directly from polyacrylamide gels (14). No characteristically similar groups of peptide spots could be discerned in any of the six fingerprint patterns. Therefore we conclude that the basic MAPS are protein individuals within the porcine brain microtubular protein complex. We are currently engaged in investigating their distribution in the microtubules of various species and organs.

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REFERENCES

1. Herzog, W. and Weber, K. (1978) Eur.J.Biochem. 92,1.
2. Cleveland, D.W., Spiegelman, B.M. and Kirschner,M.W. (1979) J.Biol.Chem. 254, 12670.
3. Stearns, M.E. and Brown, D.L. (1979) Proc.Natl.Acad.Sci. USA 76, 5745.
4. Mann, K., Giesel, M., Fasold, H. and Haase, W. (1978) FEBS Lett. 92, 45.
5. Cleveland, D.W., Hwo, S.Y. and Kirschner, M.W. (1977) J.Mol.Biol. 116, 207.
6. Sloboda, R.D., Deutler, W.L. and Rosenbaum, J.L. (1976) Biochemistry 15, 4487.
7. Lepke, S., Fasold, H., Pring, M. and Passow, H. (1976) J.Membrane Biol. 29, 147.
8. Shelanski, M.L., Gaskin, F. and Cant r, C.R. (1973) Proc.Natl.Acad.Sci. USA 70, 765.
9. Witman, G.B., Cleveland, D.W., Weingarten, M.D. and Kirschner, M.W. (1976) Proc.Natl.Acad.Sci. USA 73, 4070.
10. Engel, J., Fasold, H., Hulla, F.W., Waechter, F. and Wegner, A. (1977) Mol.Cell.Biochem. 18,3.
11. Berkowitz, S.A., Katagiri, J., Binder, H.K. and Williams, R.C. (1977) Biochemistry 16, 5610.
12. Burton, P.R. and Frost, L.C. (1979) Abstr. 19th Ann. Meeting Am.Soc.Cell Biol., MT 1801, J.Cell Biol. 83.
13. Vallee, R. (1980) Proc.Natl.Acad.Sci.USA 77, 3206.
14. Brust, J. and Fasold, H. (1981) Anal.Biochem.116, 268.
15. Sobue, K., Fujita, M., Muramoto, Y. and Kakinchi, S. (1981) FEBS Lett. 132, 137.