PURIFICATION OF HIGH-M_r MICROTUBULE PROTEINS MAP1 AND MAP2

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1. Introduction

Microtubules prepared from mammalian brain by polymerization and depolymerization cycles consist of tubulin and several minor components, so-called microtubule-associated proteins (MAPs). The most prominent MAPs are two high M_r weight proteins, MAP1 and MAP2, according to the nomenclature of [1].

MAP2 has an M_r of 280 000–350 000, promotes microtubule assembly in vitro and is associated with microtubules in cells [2–8]. Methods for MAP2 purification have been developed in several laboratories [2,3,5]. All these use, for removing contaminating proteins, a thermoprecipitation stage at which MAP2 is heated to 85–100°C. So far, purification of native, unheated MAP2 has not been reported.

The other proteins, MAP1, having a still higher M_r, has not been isolated and characterized at all.

Here we describe a procedure for purification of MAP1 from bovine brain and obtaining unheated purified MAP2. By the peptide mapping of isolated proteins we show also that MAP2 is not a product of proteolytic degradation of MAP1.

2. Materials and methods

The following buffers were used: buffer A 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, and buffer B containing 50 mM potassium phosphate (pH_{20} 6.7), 0.5 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and 0.1 mM PMSF.

Abbreviations: EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetate; EDTA, ethylenediaminetetraacetate; PMSF, phenylmethylsulphonylfluoride; SDS, sodium dodecylsulphate; MAP, microtubule-associated protein; M_r, relative molecular mass.

Microtubule proteins were prepared in buffer A from bovine brain by two cycles of polymerization and depolymerization [9]. Tubulin and MAPs were separated by chromatography on phosphocellulose (Whatman P-II) [10] in buffer A. Before the elution of MAPs with 0.5 mM KCl in buffer A, the column was washed overnight with buffer A supplemented with KCl to a final concentration of 0.1 M.

Hydroxyapatite powder (Bio-Gel HTP, Bio-Rad Labs) was hydrated in 50 mM sodium citrate solution and equilibrated with buffer B. DEAE-Sephadex A-50 (Pharmacia) was equilibrated with buffer A. Bio-Gel A-15m (Bio-Rad Labs) was equilibrated with buffer A containing 0.1 mM PMSF.

Protein concentration was determined as in [11], bovine serum albumin being used as a standard.

SDS-gel electrophoresis was performed according to [12] on 10% polyacrylamide slab gels at the acrylamide to *N,N'*-methylene-bisacrylamide ratio of 100:1 (w/w).

One-dimensional peptide mapping of proteins was carried out by the method of [13]. MAP1 or MAP2 was incubated with α-chymotrypsin (Sigma) or *Staphylococcus aureus* protease V8 (Miles) for 10 min at 37°C in a solution containing 0.0625 M Tris–HCl (pH_{20} 6.8) and 0.1% SDS. Final concentrations of proteases and MAPs were 0.1 and 0.4 mg/ml, respectively. Proteolytic products were analysed by SDS gel electrophoresis on 15% polyacrylamide slab gels at the acrylamide to *N,N'*-methylenebisacrylamide ratio of 30:0.8 (w/w).

3. Results

The starting material for MAP1 and MAP2 purification was the MAP fraction prepared by phosphocellulose chromatography from two-cycled micro-

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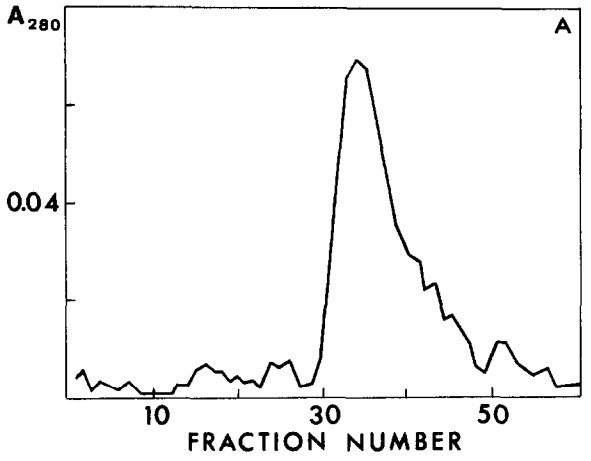
tubule proteins. From 3 kg brain we usually obtained 50 mg MAPs. All subsequent operations were done at 0–4°C.

MAPs (50 mg in 100 ml) were clarified by centrifugation (100 000 × *g*, 30 min), dialyzed against buffer A and applied to a DEAE-Sephadex A-50 column (bed vol. 50 ml) equilibrated with buffer A. The column was washed with 250 ml buffer A supplemented with KCl to 0.25 M then eluted with buffer A containing 0.5 M KCl and 0.1 mM PMSF. KCl (0.25 M) released from the column low-M_r proteins, and 0.5 M KCl eluted MAP1, MAP2 and some other proteins (fig.1B).

All the buffers used further were supplemented with 0.1 mM PMSF. KCl-eluate (0.5 M) from the DEAE-Sephadex column was dialyzed against buffer B, clarified by centrifugation (100 000 × *g*, 30 min) and applied to hydroxyapatite column (bed vol. 10 ml). The column was washed with 20 ml buffer B and step-eluted with 0.16 and 0.3 M potassium phosphate buffers (pH_{20} 6.7) containing 0.5 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 0.1 mM PMSF. The fraction eluted from the column with 0.16 M phosphate contained highly purified MAP2 (fig.1C). Only trace amounts of several proteins of lower M_r contaminated this MAP2 preparation. From 50 mg total MAPs we obtained usually 2–3 mg MAP2.

The fraction eluted from the hydroxyapatite col-



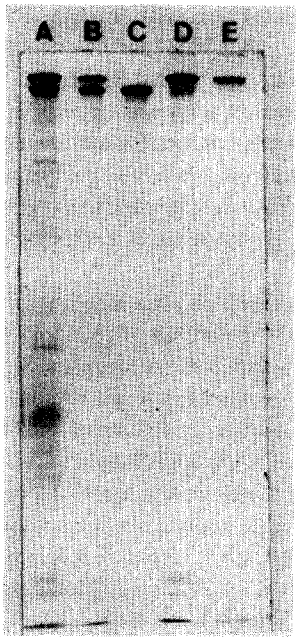


Fig.1. SDS electrophoretic analysis of the steps in MAP1 and MAP2 purification: (A) total MAPs; (B) MAP1 and MAP2 eluted from DEAE-Sephadex A50 with 0.5 M KCl; (C) MAP2 eluted from hydroxyapatite with 0.16 M phosphate; (D) MAP1-enriched material eluted from hydroxyapatite with 0.3 M phosphate buffer; (E) pooled MAP1 fraction after gel-filtration on Bio-Gel A-15m.

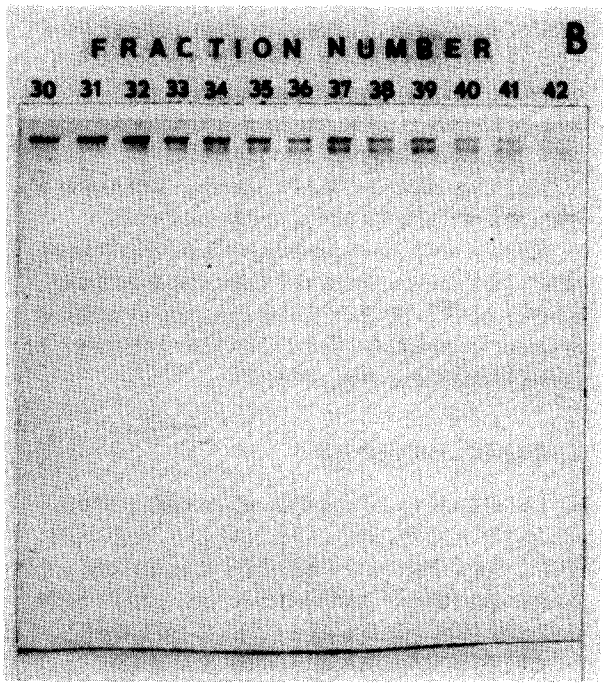


Fig.2. Purification of MAP1 by gel-filtration on Bio-Gel A-15m. The fraction enriched for MAP1 by hydroxyapatite chromatography (10 ml) was applied onto a 2.6 × 90 cm column with Bio-Gel A-15m and eluted with buffer A containing 0.1 mM PMSF at 24 ml/h; fraction vol. 10 ml: (A) elution profile; (B) SDS electrophoresis of some individual fractions.

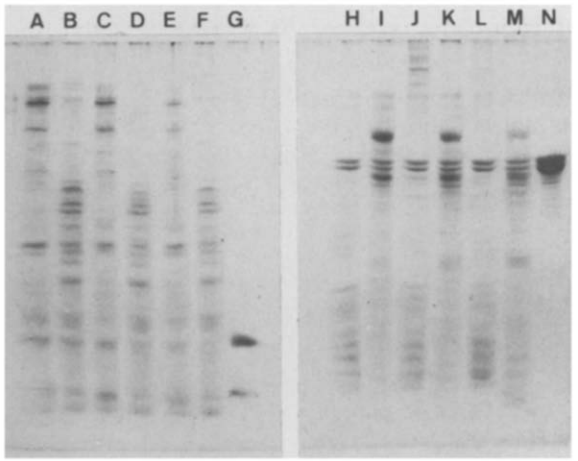


Fig.3. One-dimensional peptide maps of MAP1 and MAP2. Digestion of MAP1 (A,C,E,H,J,L) and MAP2 (B,D,F,I,K,M) with chymotrypsin (A–F) and *Staphylococcus aureus* V8 protease (H–M). Time of hydrolysis was 5 min (A,B,H,I), 10 min (C,D,J,K) or 20 min (E,F,L,M). In slots G and N chymotrypsin and protease V8, respectively, were applied.

umn with 0.3 M phosphate contained predominantly MAP1 (fig.1D). Further purification of MAP1 was achieved by gel filtration on a Bio-Gel A-15m column (2.6 × 90 cm). The elution profile and the polypeptide composition of some fractions, as revealed by SDS-gel electrophoresis, are shown in fig.2. The fractions that contained purified MAP1 (the left part of the main protein peak, fractions 30–32) were pooled and concentrated on a small phosphocellulose column (bed vol. 1–2 ml).

As shown in fig.1E, the pooled MAP1 preparation contained, besides MAP1, barely detectable amounts of MAP2 and of 2 polypeptides of $M_r < 40\,000$. The yield of MAP1 was 1–1.5 mg from 50 mg total MAPs.

High-M_r MAPs have been reported to be sensitive to brain proteases [14,15]. Our data agree well with these observations. As revealed by electrophoresis (not shown), purified MAP1 and MAP2 completely degraded in 4–6 days at 4°C in the absence of protease inhibitors. This circumstance made it difficult to isolate and store MAP1 and MAP2, and we attempted to select an appropriate inhibitor preventing MAP1 and MAP2 proteolysis. Among the inhibitors tested (EGTA, 0.1 mM; leupeptin, 5 μg/ml; PMSF, 0.1 mM) it was only PMSF that was effective.

In view of possible proteolysis of MAPs during their purification the question arose whether MAP2, having a lower M_r than MAP1, might be a product of MAP1 proteolytic cleavage. A component similar to MAP2 in its electrophoretic mobility was really observed sometimes among the products of MAP1 degradation by an endogenous protease in the absence of PMSF. So we compared one-dimensional peptide maps of the purified MAP1 and MAP2. The peptide patterns resulting from chymotrypsin or *S. aureus* protease V8 digestion of the 2 MAPs are shown in fig.3. The MAP2 patterns differed strongly from that of MAP1, which indicates that these proteins have different primary structures and that MAP2 is not a product of MAP1 degradation.

4. Conclusions

We have developed, for the first time, a method for purification of brain microtubule protein MAP1. The method permits us to isolate also the other high-M_r microtubule-associated protein, MAP2. Although MAP2 purification has been reported earlier, our procedure is the first one that does not include heating the protein to a high temperature.

The isolation of MAP1 makes possible a direct investigation of its physico-chemical and functional activities. We have begun such an investigation and report in [16] that purified MAP1 is able to promote microtubule assembly in vitro.

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