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18 kDa microtubule-associated protein: identification as a new light chain (LC-3) of microtubule-associated protein 1 (MAP-1)

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SDS gel electrophoresis of microtubule proteins obtained from bovine brain by polymerization cycles revealed a new protein of 18 kDa. This protein was copolymerized with tubulin and its stoichiometry to tubulin remained constant for at least 5 cycles of assembly. Moreover, this protein remained bound to microtubules stabilized with 10 μM taxol and pelleted through a 4 M glycerol cushion. The same 18 kDa protein was found in a purified preparation of the high molecular mass microtubule-associated protein 1 (MAP-1). The 18 kDa protein copurified with the MAP-1 heavy chains during column chromatography on phosphocellulose, DEAE-cellulose, hydroxyapatite and Bio-Gel A-15m. Incubation of the MAP-1 preparation with a mouse monoclonal antibody to the light chain 1 (LC-1) of MAP-1 and with a second precipitating antibody (a rabbit antibody to mouse IgG) immunoprecipitated from the solution all the known components of MAP-1 (heavy chains, LC-1, LC-2), as well as the 18 kDa protein. Immunoblotting showed, however, that this antibody does not interact directly with the 18 kDa protein. These results indicate that the 18 kDa protein forms a complex with all other components of MAP-1. This polypeptide, therefore, is a new light chain (LC-3) of MAP-1.

Microtubule; Microtubule-associated protein 1; Immunoprecipitation

1. INTRODUCTION

Microtubules in many types of cells are composed of tubulin and a number of minor non-tubulin components, the so-called microtubule-associated proteins (MAPs). These proteins are also copolymerized with tubulin in vitro and therefore can be identified in the microtubule preparations obtained by polymerization cycles [1]. It was shown (and later used as a definition of microtubule-associated proteins) that in many cycles of polymerization the weight ratio of tubulin to these proteins remained constant [1,2].

MAPs from mammalian brain have been most extensively characterized. They are represented mainly by two groups of high molecular mass polypeptides (MAP-1 and MAP-2: about 350 and 300 kDa, respectively) and a group of tau proteins (60–70 kDa). Each of these proteins seems to be represented by several isoforms (review [3]). Furthermore, Berkowitz et al. [2] identified 34 and 31 kDa MAPs in a bovine brain microtubule preparation. These proteins were later shown to form a complex with MAP-1 [4,5] and were, therefore, called light chain 1 and light chain 2 (LC-1 and LC-2) of MAP-1.

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Abbreviations: MAP, microtubule-associated protein; LC-1, LC-2 and LC-3, light chains 1, 2 and 3 of MAP-1

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We describe here a new 18 kDa protein which is copurified with bovine brain microtubules. We have also found that this protein, like LC-1 and LC-2, forms a complex with MAP-1 heavy chains, and therefore is a new light chain (LC-3) of MAP-1.

2. MATERIALS AND METHODS

Microtubules, MAP-1 and tubulin were obtained from bovine brain in 50 mM imidazole-HCl buffer (pH_{20} 6.7) containing 50 mM KCL, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM 2-mercaptoethanol (buffer A), and supplemented with 1 mM phenylmethylsulphonyl fluoride. Microtubule proteins were obtained by two assembly-disassembly cycles [6] modified as in [7]. Tubulin and MAPs were separated by column chromatography on phosphocellulose (Whatman P-11) [8] in buffer A. MAP-1 was purified as in [9]. Immunoprecipitation was performed by using monoclonal antibody E-12 to LC-1 of MAP-1, as described in [5].

SDS-polyacrylamide gel electrophoresis was carried out in the discontinuous buffer system of Laemmli [10] on slab gels with a 100:1 (w/w) acrylamide: *N,N*-methylenebisacrylamide ratio. Gels were stained with Coomassie blue R-250 and the content of individual polypeptides was determined by densitometric scanning. Protein concentration was determined with the folin phenol reagent [11] using bovine serum albumin as a standard. Taxol was kindly provided by Dr M. Suffness (Natural Products Branch, National Cancer Institute, Bethesda, MD).

3. RESULTS AND DISCUSSION

The polypeptide composition of microtubule proteins purified from bovine brain by two polymerization-depolymerization cycles is shown in fig.1. Among these proteins, tubulin is the most prominent component. Many other proteins are also seen on the gel, including a relatively minor component of 18 kDa. Extensive purification of the microtubule preparation by three additional polymerization cycles (five cycles overall) did not remove the 18 kDa protein from the microtubule protein preparation. Moreover, the content of this protein in the preparation was constant in all the preparations starting with the third cycle (fig.2).

Fig.1. Protein composition of bovine brain microtubules obtained by two polymerization-depolymerization cycles. Electrophoresis in 12% polyacrylamide gel in the presence of SDS. Positions of tubulin (T), high molecular mass MAPs (MAP-1 + MAP-2) and the 18 kDa protein are indicated to the right of the gel.

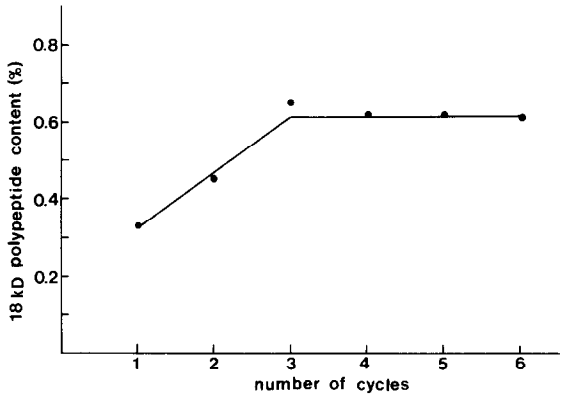

Fig.2. Content of the 18 kDa protein in the microtubule protein preparations obtained by 1–6 cycles of polymerization. For the sixth cycle microtubules were stabilized with 10 μM taxol and pelleted through a layer of 4 M glycerol in buffer A with 1 mM EGTA.

To purify microtubules most thoroughly, they were stabilized with 10 μM taxol and pelleted through a layer of 4 M glycerol in buffer A with 1 mM EGTA. Under these non-equilibrium conditions any protein dissociating from the microtubule wall would be removed from the preparation during centrifugation. However, even this procedure did not change the 18 kDa polypeptide content (fig.2). These results show that the 18 kDa component is one of the MAPs that copurifies with microtubules in the stoichiometric quantities.

Vallee and Davis [4] mentioned that purified preparations of MAP-1 contained a minor polypeptide of 17–19 kDa [4]. We have confirmed this finding. Fig.3A (lane a) shows a purified preparation of MAP-1 analyzed by electrophoresis in an 8% polyacrylamide gel. Electrophoresis of this preparation in a 12% polyacrylamide gel (fig.3A, lane b) shows that in addition to MAP-1 heavy chains, it also contains LC-1, LC-2 and a 18 kDa polypeptide. This polypeptide has the same electrophoretic mobility as the 18 kDa polypeptide of the microtubule preparation. Therefore, it would be reasonable to suppose that the 18 kDa MAP is a component of the MAP-1 molecule.

To study this possibility we first determined whether the 18 kDa component of the MAP-1 preparation can copolymerize with tubulin. Column-purified tubulin (2 mg/ml) was mixed with a MAP-1 preparation (0.4 mg/ml) and polymerized for 30 min at 37°C by 10 μM taxol, 1 mM EGTA and 1 mM GTP. Microtubules were pelleted by centrifugation for 30 min at 150000 × g through a layer of 4 M glycerol in buffer A with 1 mM EGTA. The pellet was analysed by SDS gel electrophoresis (fig.3B, lane b) and found to contain tubulin, MAP-1 heavy chains, LC-1, LC-2

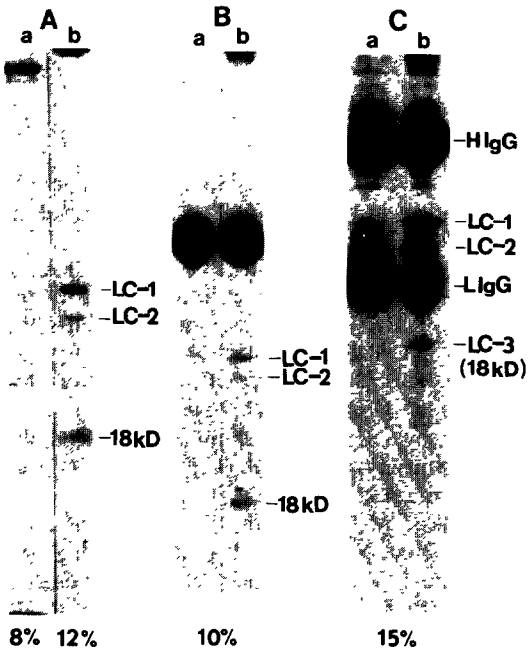

Fig.3. Identification of the 18 kDa polypeptide in the MAP-1 preparation. Electrophoresis in SDS-polyacrylamide gels. Acrylamide content in the gels is indicated under the lanes. A, purified preparation on MAP-1; B, microtubules containing only tubulin (lane a) or tubulin and MAP-1 (lane b); C, immunoprecipitation of MAP-1 polypeptides by monoclonal antibody E-12 to LC-1 of MAP-1: lanes: (a) a control precipitate formed in the absence of MAP-1 and containing only E-12 antibody and a second precipitating antibody (rabbit antibody to mouse immunoglobulins); (b) a precipitate formed in the presence of MAP-1. Positions of LC-1, LC-2, LC-3 (18 kDa polypeptide) and immunoglobulin polypeptides (H IgG and L IgG) are indicated.

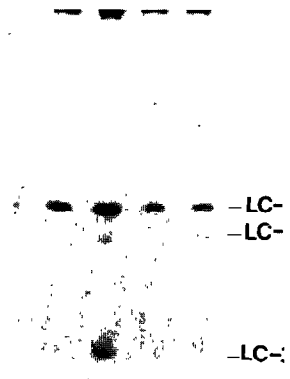

Fig.4. Chromatography of the MAP-1 polypeptides on a phosphocellulose column. A 1 × 3 cm column was loaded with 0.5 mg purified MAP-1 and eluted with a 0.05–0.5 M linear KCl gradient. Fractions were analysed by SDS-polyacrylamide gel electrophoresis. Only the protein-containing fractions are shown. Positions of LC-1, LC-2 and LC-3 are indicated to the right of the gel.

and the 18 kDa polypeptide. Both in the pellet and in the MAP-1 preparation the molar ratio of MAP-1 heavy chains to 18 kDa polypeptide was 0.7 ± 0.2 (molecular mass of MAP-1 heavy chains was considered to be 350 kDa).

We also showed that the 18 kDa polypeptide coeluted with MAP-1 heavy chains during gel filtration on Bio-Gel A-15m, hydroxyapatite chromatography, and ion-exchange chromatography on DEAE- and phosphocellulose. An example of elution from the phosphocellulose column is shown in fig.4. Electrophoretic analysis of the fractions eluted from the column by a 0.05–0.5 M linear KCl gradient showed that both MAP-1 heavy chains and the 18 kDa protein were found in the same fractions. The data from column chromatography confirmed the suggestion that the 18 kDa component forms a stable complex with the other polypeptides of MAP-1.

To show directly that this protein is really bound to the MAP-1 molecule, we used immunoprecipitation of the molecule with a monoclonal antibody against LC-1 of MAP-1 [5]. Immunoblotting experiments showed that this antibody, called E-12, did not bind directly to the 18 kDa polypeptide (not shown). The only protein that reacted with E-12 antibody on immunoblots was LC-1. Incubation of the MAP-1 preparation with E-12 antibody and then with a second precipitating antibody (a monospecific rabbit antibody against mouse immunoglobulins) resulted in precipitate formation. SDS gel electrophoresis showed that in addition to immunoglobulins, this precipitate contained MAP-1 heavy chains, LC-1, LC-2 and the 18 kDa polypeptide (fig.3C, lane b). None of these components were found in the precipitate formed in the absence of MAP-1 (fig.3C, lane a). When the E-12 antibody was replaced with monoclonal antibody Tu-01 against tubulin (kindly provided by Drs P. Draber and V. Vicklicky, Institute of Molecular Genetics, Prague, Czechoslovakia) none of the MAP-1 polypeptides were found in the precipitate. These results show that the 18 kDa polypeptide is a component of the MAP-1 molecule. We propose to call it light chain 3 (LC-3) of MAP-1.

Our results, as well as data from other papers [4,5], show that the MAP-1 molecule contains the heavy chain and three types of light chains. It was shown earlier that the heavy chains of MAP-1 could be resolved into at least three components with very similar electrophoretic mobilities, MAP-1A, MAP-1B and MAP-1C [12]. It remains to be determined whether all three light chains are associated with all the types of heavy chains or if there is some specificity in the binding of the light chains to any particular heavy chain.

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