ACTIN-INDUCED STIMULATION OF MICROTUBULE-ASSOCIATED ATPase ACTIVITY

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Received 23 December 1980

1. Introduction

Previous results from our laboratory indicate the presence of ATP hydrolase activity in a microtubule preparation, associated with a high molecular weight microtubule-associated protein (MAP) [1]. The fact that the enzyme exhibits low specific activity prompted a search for activation factors. We report here the stimulation of Mg^{2+}-dependent microtubule-associated ATPase activity by F-actin.

2. Materials and methods

2.1. *Preparation of proteins*

Microtubule protein obtained from bovine brain was purified by repeated cycles of assembly–disassembly according to [2] as modified [3]. Tubulin was separated from MAPs by phosphocellulose ion-exchange chromatography [4]. MAPs were eluted with 0.6 M NaCl in 20 mM PIPES (pH 6.8) and desalted by gel filtration on Sephadex G-25 equilibrated with 100 mM Tris–HCl (pH 8.0). Actin was prepared from rabbit skeletal muscle according to [5] as in [6]. Actin was extracted from acetone powder in 2 mM Tris (pH 7.5) essentially as in [7].

2.2. *Biochemical assays*

ATPase activity was assayed as in [1]. The incubation medium consisted of 100 mM Tris (pH 8.0), 2 mM $MgSO_4$ or 2 mM $CaCl_2$, 2 mM ATP and MAPs and actin in varying concentrations. P_i was measured according to [8,9], modified for the sample size used in the present experiments. Results are expressed as nmol P_i liberated . mg protein^{-1} . min^{-1} at 37°C. Experimental values were corrected for the low activity arising from F-actin as well as from the spontaneous hydrolysis of ATP. Protein concentration was determined according to [10], using bovine serum albumin as standard.

2.3. *Electron microscopy*

Samples of F-actin were negatively stained in 1% aqueous uranyl acetate and examined in a Philips EM 301 electron microscope.

2.4. *Gel electrophoresis*

SDS–polyacrylamide gel electrophoresis was performed using 7.5% gels according to [11]. Gels were stained in 0.25% Coomassie brilliant blue in methanol: acetic acid: water (5:1:5) and destained in 7% acetic acid and 5% methanol. Destained gels were scanned at 600 nm in a Gelman DCD-16 densitometer.

3. Results

Densitometer scans of the proteins used in the assays indicate that the actin preparation was free from contamination, as the protein migrated as a single band with est. M_r 42 000 (fig.1). Polymerized actin exhibited the normal morphology of actin filaments (fig.2). The MAPs fraction contained primarily the high molecular weight microtubule-associated proteins, but minor protein components were present as well.

The Mg^{2+}-ATPase activity of the MAPs fraction was increased up to 1.5-fold by the addition of F-actin (fig.3), reaching a maximum at ~15 nmol actin/mg MAPs (1 mg actin/mg MAPs). The optimal molar ratio of actin to MAPs was estimated to be 4 in the presence of 2 mM Mg^{2+} (calculated on the basis M_r 300 000 for MAP). This corresponded to spec. act. 5.44 ± 0.69 nmol P_i liberated/mg protein, min. Ca^{2+}-ATPase activity was somewhat inhibited, 67–84% of control values

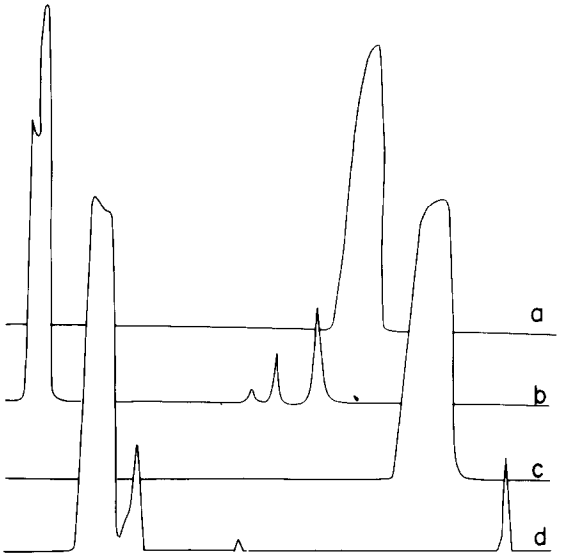

Fig.1. Densitometer scans of gels with the proteins used in the ATPase assays and, for comparison, muscle myosin: (a) tubulin; (b) MAPs fraction; (c) rabbit skeletal muscle actin; (d) myosin.

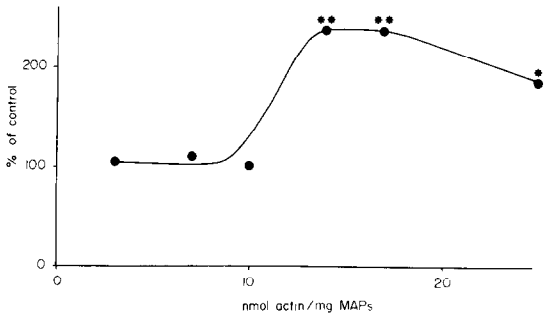

Fig.3. The effect of F-actin on microtubule-associated Mg^{2+}-ATPase activity. Assay conditions: Tris–HCl (pH 8.0), 2 mM Mg^{2+}, 2 mM ATP. For statistical analysis, the Mann-Whitney *U*-test was used. *indicates $p < 0.10$; **$p < 0.05$.

at 1–10 nmol actin/mg MAPs (0.06–0.04 mg actin/mg MAPs) (fig.4). With increasing concentrations of F-actin, activity returned to near-control values. Mg^{2+}- and Ca^{2+}-ATPase activities of tubulin were also measured, but found to be negligible and not subject to

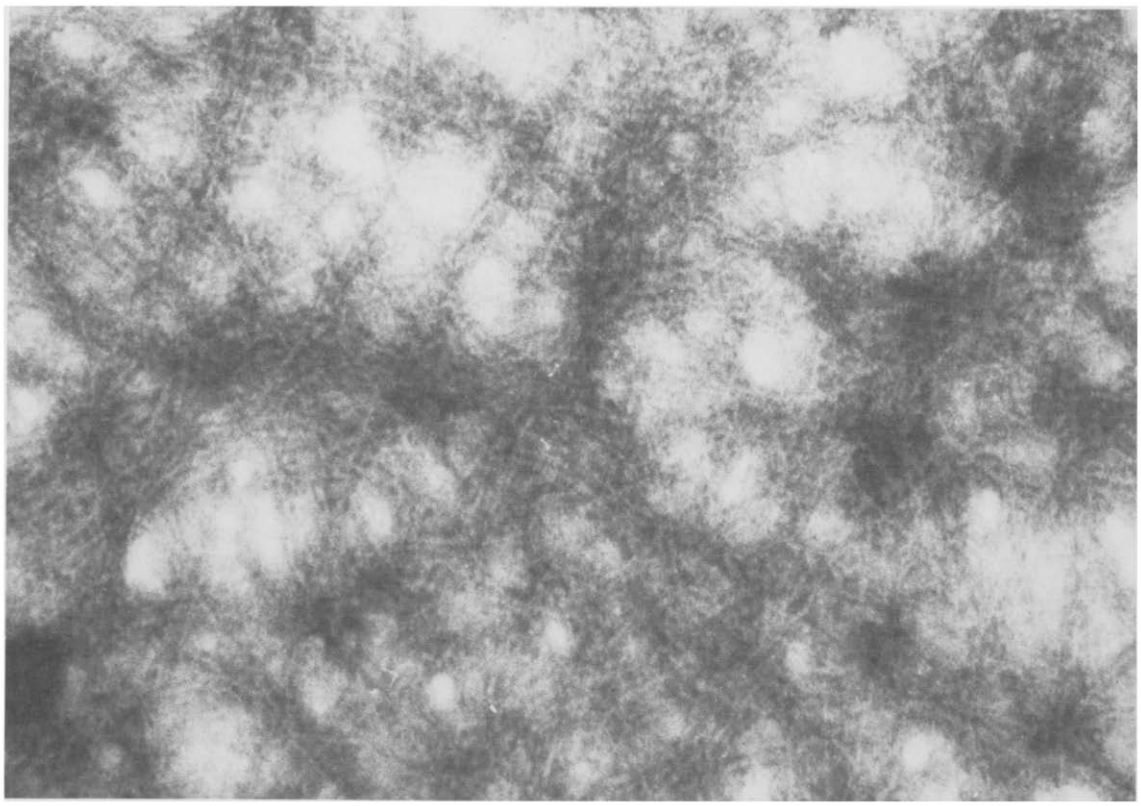

Fig.2. Electron micrograph of F-actin incubated at 37°C. Negative staining with 1% uranyl acetate. X108 000.

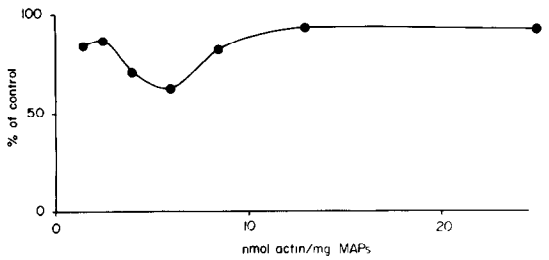

Fig.4. The effect of F-actin microtubule-associated Ca^{2+}-ATPase activity. Assay conditions: Tris–HCl (pH 8.0), 2mM Ca^{2+}, 2 mM ATP. For statistical analysis, the Mann-Whitney *U*-test was used.

stimulation by F-actin (not shown). G-actin had no effect on the ATPase activity of either of the proteins tested. In the presence of 0.6 M KCl or 0.6 M KCl + 0.1 mM EDTA, little or no activity could be detected in the MAPs preparation. A summary of the conditions tested is presented in table 1.

4. Discussion

Although the ATP hydrolase activity of dynein from a variety of sources is well characterized [12–16], little is known about the ATPase activity associated with cytoplasmic microtubules. In [17] both Mg^{2+}- and Ca^{2+}-dependent ATPase activity was present in vinblastine-induced microtubule paracrystals from murine fibroblasts. In [18,19] brain microtubules contained a Mg^{2+}-ATPase activity [18,19]. In [1] Mg^{2+}- and Ca^{2+}-ATPase activities were in a MAPs fraction separated from tubulin on phosphocellulose. Here, we show that under optimal conditions (pH 8.0, 2 mM Mg^{2+}, 2 mM ATP) the Mg^{2+}-dependent enzyme can be activated by skeletal muscle actin.

Table 1
Microtubule-associated ATPase activity – Summary of the conditions tested

Condition	Stimulate	Inhibit	No effect
10 mM Mg^{2+} + F-actin		X	
2 mM Mg^{2+} + F-actin	X		
2 mM Mg^{2+} + G-actin			X
2 mM Ca^{2+} + F-actin		X	
0.6 M KCl		X	
0.6 M KCl + 0.1 mM EDTA		X	
0.6 M KCl + F-actin		X	
0.6 M KCl + 0.1 mM EDTA + F-actin		X	

Actin has been demonstrated to stimulate the Mg^{2+}-ATPase activity of dynein and of its tryptic fragment, fragment A [12,13], although to a much lesser extent than the activation of myosin ATPase. Proteins resembling dynein have been found in extracts of microtubule protein from mammalian brain [20,21]. These proteins, often referred to as MAP_1 and MAP_2 (M_r 280 000–345 000) have been described as projections extending from the surfaces of cytoplasmic microtubules [22–26]. As such, they can be conceived as candidates for mechanochemical force generation in several of the functions in which cytoplasmic microtubules have been implicated, for example, axonal transport, particularly considering the reports of ATP hydrolase activity associated with these proteins. Investigations of the stimulation of microtubule assembly by MAPs actin [27] and the stimulation of MAP ATPase activity by actin reported here add support to the suggestion that there is a functional significance of the actin–MAPs interaction.

However, the enzyme activity reported here may be due to contaminants in the protein preparations. As determined by electrophoresis, myosin is not present in the MAPs preparation. It is not likely that one or more of the proteins in the MAPs fraction is of myosin type, as the present ATPase activity is inhibited by 0.6 M KCl and 0.6 M KCl + 0.1 mM EDTA. The actin used for the experiments was found to be pure, as judged by polyacrylamide gel electrophoresis. Phosphatases have been found in microtubule preparations [28], but optimal conditions for their activity differ from those of the present ATPase. Furthermore, the ATPase activity associated with bovine brain microtubules was demonstrated to be a unique phosphohydrolase, not resulting from a futile cycle of protein kinase and phosphoprotein phosphatase activity [29].

The MAPs fraction used is partially purified, consisting primarily of MAP_1 and MAP_2, but tau proteins and minor components are also present. This undoubtedly accounts for the difficulty in obtaining kinetic data as well as for the variation in specific activity observed among MAPs preparations. Therefore, to elucidate the significance of the actin–MAPs interaction with respect to ATPase activity, further investigations will require separation of MAP_1 and MAP_2 and a kinetic analysis of actin-induced stimulation of MAP ATPase activity.

Acknowledgements

This investigation was supported by grants from the Swedisch Natural Science Research Council (no. 2535–104), Hierta-Retzius' Stipendiefond, Längmanska Kulturfonden, Anna Ahrenbergs Fond and Kungliga Vetenskaps och Vitterhets-Samhället.

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