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ATPase activity of the microvillar 110 kDa polypeptide-calmodulin complex is activated in Mg^{2+} and inhibited in K^+-EDTA by F-actin

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Highly purified microvillar 110 kDa polypeptide-calmodulin (110K-cam) complex was confirmed to have ATPase activities characteristic of a myosin. The effect of F-actin on these activities was investigated. The Mg^{2+}-ATPase is activated about 2-fold by F-actin in a dose-dependent fashion, whereas the K^+-EDTA-ATPase is inhibited by >90% by F-actin. These data provide evidence for a functional relationship between the ATPase activity of 110K-cam and its interaction with F-actin. They also extend the similarities between 110K-cam and myosin. The results suggest that higher cells contain in addition to myosin a second class of myosin-like molecules represented by 110K-cam.

Calmodulin; Myosin; Actin; Microvillus; Cytoskeleton; ATPase

1. INTRODUCTION

Regularly spaced lateral bridges attach the core bundle of actin filaments of intestinal microvilli to the plasma membrane [1,2]. Recently, evidence has been obtained to suggest that these bridges are comprised of a 110 kDa polypeptide-calmodulin complex (110K-cam) [3–8]. The purified complex shares some properties with muscle and non-muscle myosins, although these myosins typically consist of two heavy chains of ~200 kDa and four light chains of ~18 kDa. Similarities between 110K-cam and the myosins include: (i) high K^+-EDTA and Ca^{2+}-ATPase activities and low Mg^{2+}-ATPase activity [6,9]; (ii) saturable binding to F-actin in an ATP-reversible manner [7]; and (iii) the ability to 'decorate' F-actin to give rise to an arrowhead appearance [7]. Moreover, as with myosins, both the ATPase and F-actin binding activities reside on the heavy polypeptide chain and are independent of the calmodulin 'light chains' [8]. The Mg^{2+}-ATPase activity has been reported not to be activated by F-actin [6] or to be modestly activated [9]. 110K-cam has not yet been demonstrated to couple ATP hydrolysis with mechanical work.

We recently described an improved method for the purification of native 110K-cam and explored its interaction with F-actin [7]. Here, we investigate the ATPase activity of 110K-cam in relation to its interaction with F-actin. We show that its ATPase activity, like that of myosin, is enhanced by F-actin in the presence of Mg^{2+} and inhibited by F-actin in its absence.

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2. MATERIALS AND METHODS

Microvillar 110K-cam was purified as described [7]. Brush border myosin was purified according to

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Table 1

Specific ATPase activities of 110K-cam and brush border myosin

	1 mM EDTA, 1 mM EGTA[a]	1 mM Ca^{2+}	5 mM Mg^{2+}
110K-cam			
50 mM KCl	502	357	14
0.5 M KCl	211	105	19
Myosin			
0.5 M KCl	625	382	10

[a] All assays were performed in ATPase buffer with additions to give the final concentrations indicated. Values are expressed in nmol P_i/mg protein per min

a new procedure, adapted from [6]. Rabbit skeletal muscle actin was purified [10] and gel filtered [11] before use. ATPase assays were performed [12] in 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, pH 8.0, with appropriate additions as indicated, at 37°C. The assay was found to be linear with time up to 30 min (the longest time point used). All the assays were repeated on a number of different 110K-cam preparations and representative values are presented in section 3. Gel electrophoresis in the presence of SDS was as described [7,13]. Protein was determined colorimetrically [14] using bovine serum albumin as a standard.

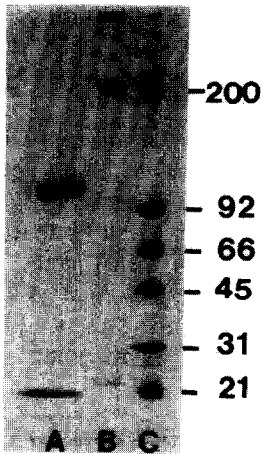

Fig.1. 7.5%/15% SDS-polyacrylamide gel of purified microvillar 110K-cam (A), and purified brush border myosin (B). Molecular mass standards (in kDa) are indicated in lane C.

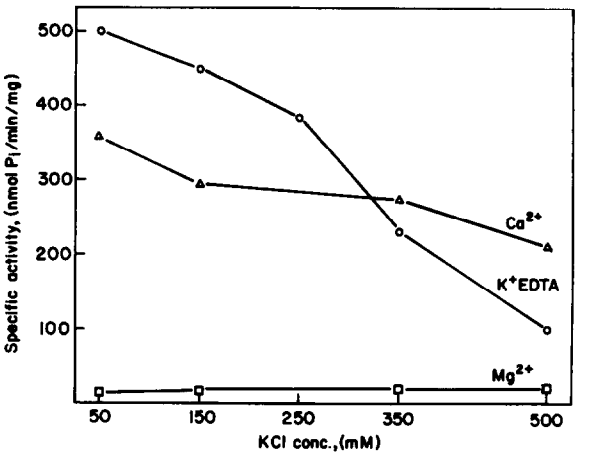

Fig.2. Effect of KCl concentration on the ATPase activities of 110K-cam. The curves represent the activities in the presence of 5 mM $MgCl_2$, 5 mM $CaCl_2$ and 1 mM EDTA, 1 mM EGTA.

3. RESULTS

The typical ATPase activities of purified 110K-cam are listed in table 1, together with results obtained using brush border myosin. The purity of a 110K-cam preparation and brush border myosin as assessed by SDS gel electrophoresis is shown in fig.1. Since the activities of myosins are typically measured in 0.6 M KCl to inhibit filament forma-

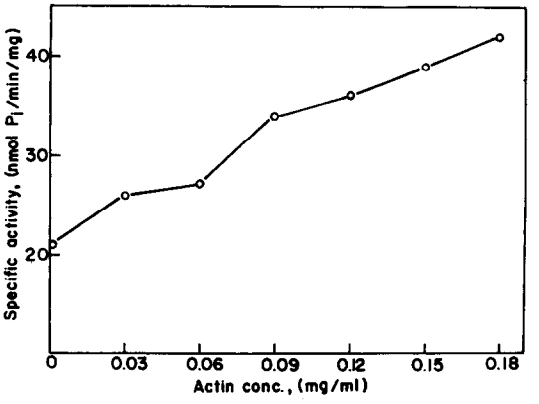

Fig.3. Effect of F-actin on the Mg^{2+}-ATPase activity of 110K-cam. The assays contained 100 μg/ml 110K-cam and the indicated amounts of actin. The very low ATPase activity of F-actin alone was subtracted from the values obtained.

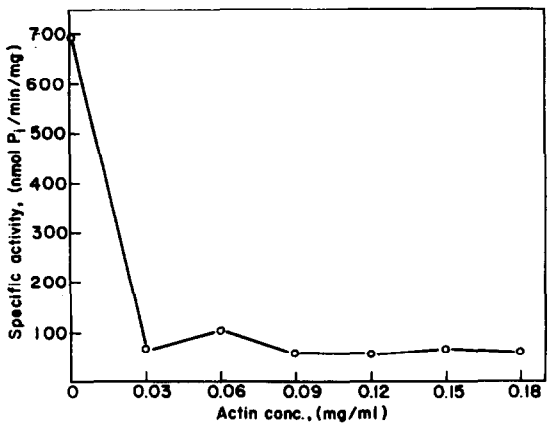

Fig.4. Effect of F-actin on the ATPase activity of 110K-cam in the presence of 1 mM EDTA, 1 mM EGTA. The assays contained 20 μg/ml 110K-cam and the indicated amounts of actin. The very low ATPase activity of F-actin alone was subtracted from the values obtained.

tion, we explored the effect of KCl concentration on the ATPase activities of 110K-cam and found that the K^+-EDTA/EGTA and Ca^{2+}-ATPases were inhibited by increasing KCl concentrations, whereas Mg^{2+}-ATPase was slightly enhanced (fig.2). Since 110K-cam does not aggregate in low salt [7] and these conditions are more representative of a normal physiological state, all further ATPase measurements on 110K-cam were made at relatively low KCl concentrations.

Fig.3 shows the effects of F-actin on Mg^{2+}-ATPase of 110K-cam. F-Actin activation was invariably found, although the degree of activation varied from about 2- to 3-fold from one 110K-cam preparation to another. The Ca^{2+}-ATPase activity was either unaffected by the presence of F-actin, or slightly reduced (not shown).

By contrast, the K^+-EDTA/EGTA activity was invariably found to be inhibited by ~90% in the presence of F-actin (fig.4). In a similar manner, the K^+-EDTA/EGTA ATPase of 0.1 mg/ml myosin was reduced from 625 to 14 nmol P_i/mg per min by the presence of 0.2 mg/ml F-actin.

4. DISCUSSION

In this report we confirm that microvillar 110K-cam is an ATPase with the enzymatic characteristics of a myosin. The specific activities are somewhat higher than those reported by others [6,9], but show the same ratio between the K^+-EDTA/EGTA, Ca^{2+}- and Mg^{2+}-ATPase activities. The higher activities may be due to the greater purity of the preparation and the lack of proteolytic products. We also show that 110K-cam exhibits two enzymatic characteristics of a myosin: an Mg^{2+}-ATPase that can be modestly activated by F-actin, and a K^+-EDTA/EGTA ATPase activity that is inhibited by F-actin. The reason for the variable activation of the Mg^{2+}-ATPase by F-actin is not yet clear. One possibility is that, like myosin, it depends on the degree of phosphorylation [15]. In this regard, it is of interest to note that the 110 kDa polypeptide is a substrate for a kinase present in microvillar preparations [5].

The present data together with earlier results [5], may give some insight into the interaction between 110K-cam and F-actin. Dissociation of 110K-cam from F-actin requires both ATP (or other nucleoside triphosphates) and a divalent cation [5]. Thus, in the presence of K^+-EDTA/EGTA and ATP, 110K-cam remains bound to F-actin, but dissociates in Mg^{2+} and ATP. The data are consistent with a model in which 110K-cam associates repeatedly with F-actin in Mg^{2+} and ATP, and thereby increases 110K-cam's Mg^{2+}-ATPase activity, whereas in K^+-EDTA/EGTA 110K-cam is bound to F-actin which inhibits this ATPase activity. These results provide further evidence for a functional correlation between the ATPase activity of 110K-cam and its ability to bind F-actin in an ATP-reversible manner. Further, they suggest that hydrolysis of Mg^{2+}-ATP may be required in the binding/dissociation cycle of 110K-cam and F-actin, in a manner similar to the cross-bridge cycle between myosin heads and F-actin.

The results add substantially to the emerging biochemical similarities between 110K-cam and bona fide myosins and suggest that there may be at least two distinct classes of myosin-like molecules in higher non-muscle cells.

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