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The effect of myosin light chain phosphorylation on the actin-stimulated ATPase activity of myosin minifilaments

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It has been shown that in the absence of KCl, the actin-stimulated Mg^{2+}-ATPase activity of rabbit skeletal myosin minifilaments with phosphorylated regulatory lights chains (LC_2) exceeds 3–4-fold that of myosin minifilaments with dephosphorylated LC_2. Addition of KCl leads to a decrease in the difference between the two ATPase activities. LC_2 phosphorylation considerably increases the rate of ATPase reaction and only slightly decreases the affinity of myosin minifilaments for F-actin. It is suggested that the unusual effect of LC_2 phosphorylation on the kinetic parameters of the actin-stimulated ATPase reaction of myosin minifilaments can be accounted for by its influence on the interaction between myosin heads which results in the ordered self-assembly of minifilaments.

Myosin; Phosphorylation; Actin-myosin interaction; ATP hydrolysis; (Rabbit skeletal muscle)

1. INTRODUCTION

It is well known that myosin regulatory light chains (LC_2) in fast skeletal muscle undergo reversible phosphorylation [1]. Phosphorylation of LC_2 is not necessary for actin-stimulated ATPase activity of skeletal muscle myosin, however it modulates this activity [2–4]. The effect of LC_2 phosphorylation may be dependent on the conditions used for measuring the ATPase activity (e.g. salt concentration) [4]. At high salt concentrations (more than 100 mM KCl), LC_2 phosphorylation slightly decreased actin-stimulated ATPase activity [4], whereas at a low KCl concentration (25–50 mM) LC_2 phosphorylation resulted in a 1.5–2-fold activation of ATPase [2–4]. These data may indicate that in the absence of KCl, the activating effect of LC_2 phosphorylation should be very pronounced. However, under these conditions myosin tends to form large insoluble aggregates, and this makes the correct measurement of actin-stimulated ATPase very difficult.

Recently, Reisler et al. [5] described the formation of short bipolar myosin filaments (minifilaments) which are composed of only 16–18 myosin molecules. Unlike normal myosin filaments, these minifilaments are stable and soluble in the absence of KCl [5]. Thus, minifilaments are a very useful model for investigating the interaction of actin and myosin at low ionic strength [6,7]. Therefore, we used minifilaments for investigating the effect of LC_2 phosphorylation on actin-stimulated ATPase of myosin in the absence of KCl.

2. MATERIALS AND METHODS

Myosins with completely phosphorylated or dephosphorylated LC_2 were prepared from rabbit skeletal muscle according to Stepkowski et al. [4].

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Abbreviation: LC_2, myosin light chain 2

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The extent of phosphorylation of LC_2 was determined by polyacrylamide gel electrophoresis in the presence of 8 M urea [8]. F-Actin was prepared by the method of Spudich and Watt [9].

Minifilaments were prepared according to Reisler et al. [5] by two-step dialysis of myosin into a 10 mM citrate-35 mM Tris buffer (pH 8.0). The concentration of myosin in minifilament solutions was determined spectrophotometrically using an extinction coefficient of $E^{1\%}_{280\ nm} = 5.6\ cm^{-1}$ [10] after dissociation of the minifilaments to monomeric myosin by dilution with 0.6 M KCl. All preparations of myosin minifilaments were examined for homogeneity by sedimentation velocity experiments in a Spinco model E analytical ultracentrifuge with a photoelectric scanning system at rotor speeds of 40 000 rpm. The sedimentation coefficients of myosin minifilaments with phosphorylated and dephosphorylated LC_2 were virtually the same (about 27 S at 20°C in 10 mM citrate-Tris buffer, pH 8.0, at a myosin concentration of 1 mg/ml).

The actin-stimulated ATPase activities were determined at 25°C in a medium designed for maintaining the myosin minifilament structure (10 mM citrate-35 mM Tris, pH 8.0, 1 mM ATP, 4 mM $MgCl_2$). Myosin was present at 0.1 μM; actin concentrations ranged from 0.1 to 4.0 μM. The released P_i was measured colorimetrically as in [11].

3. RESULTS

Under our assay conditions (in the absence of KCl), the actin-stimulated Mg^{2+}-ATPase activity of myosin minifilaments with phosphorylated LC_2 exceeded that of minifilaments with dephosphorylated LC_2 nearly 4-fold (figs 1,2). These differences between the two forms of myosin minifilaments were most pronounced at 4 mM $MgCl_2$ (fig.2).

The addition of KCl to solutions of minifilaments is known to initiate their growth to normal myosin filaments [12]. From the very beginning of this process the actin-stimulated Mg^{2+}-ATPase activity of both minifilament preparations decreased rapidly, as shown in fig.3. After addition of KCl (15–50 mM) the difference in activity between the two types of minifilaments showed a dramatic decrease; in the presence of 50 mM KCl, this difference did not exceed 50%, whereas it amounted to 250% in the absence of KCl. As shown by sedimentation velocity experiments at 50 mM KCl, no filament formation was detected, but minifilaments were significantly less homogenous than in the absence of KCl [13]. Thus, preservation of an intact minifilament structure is necessary for a significant effect of LC_2 phosphorylation on the actin-stimulated ATPase activity of minifilaments.

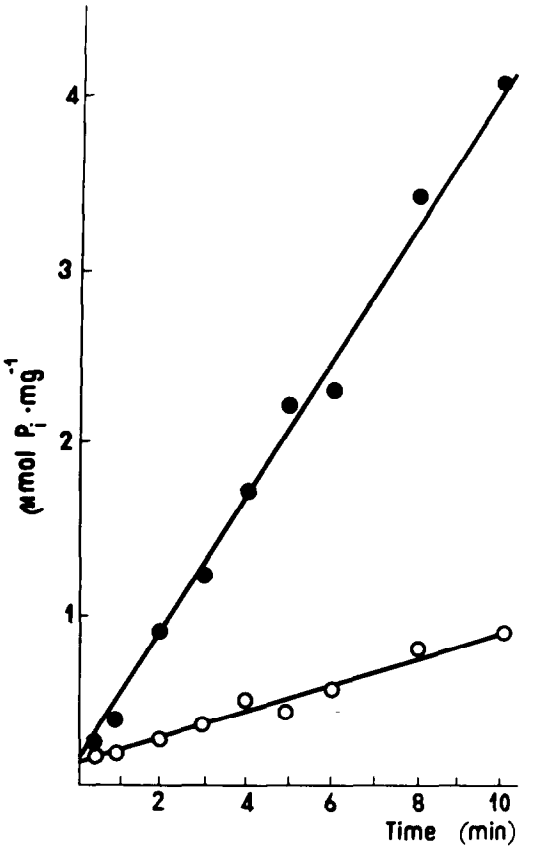

Fig.1. Time courses of ATP hydrolysis by myosin minifilaments with phosphorylated LC_2 (●) and dephosphorylated LC_2 (○) in the presence of F-actin. Conditions: 10 mM citrate-35 mM Tris (pH 8.0), 4 mM $MgCl_2$, 1 mM ATP, 0.1 μM myosin, 1.0 μM actin.

The dependences of phosphorylated and dephosphorylated minifilaments ATPase activities on actin concentration are presented in fig.4. The extrapolated values for the maximum velocity, V_{max}, and the apparent dissociation constant of actin from myosin, K_{app}, were as follows: $V_{max} = 0.23$

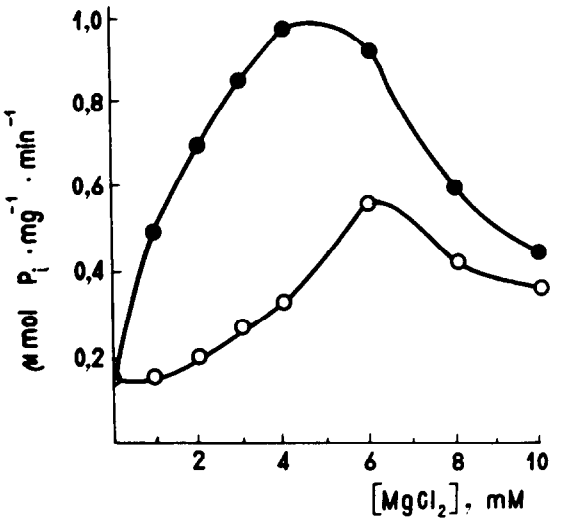

Fig.2. Influence of added $MgCl_2$ concentration on the actin-stimulated ATPase activity of myosin minifilaments with phosphorylated LC_2 (●) and dephosphorylated LC_2 (○). Conditions as in fig.1 except for $MgCl_2$ concentration.

μmol/min per mg myosin, $K_{app} = 0.28\ \mu$M actin for minifilaments with dephosphorylated LC_2; $V_{max} = 0.83\ \mu$mol/min per mg myosin, $K_{app} = 0.21$

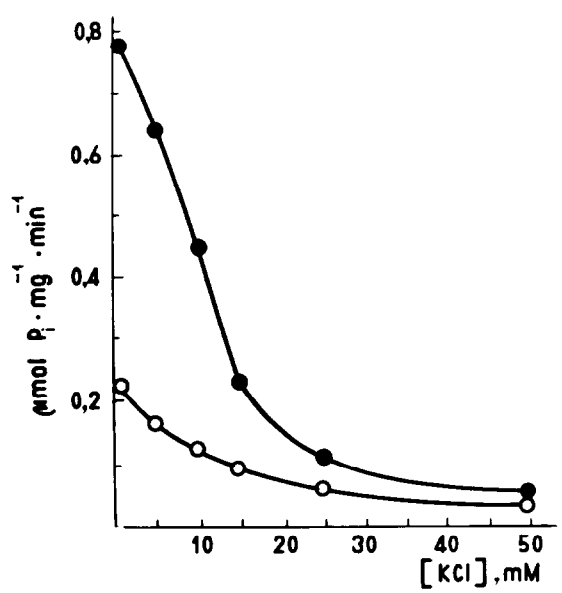

Fig.3. Effect of different concentrations of KCl on the actin-stimulated Mg^{2+}-ATPase activity of myosin minifilaments with phosphorylated LC_2 (●) and dephosphorylated LC_2 (○). Other conditions as in fig.1.

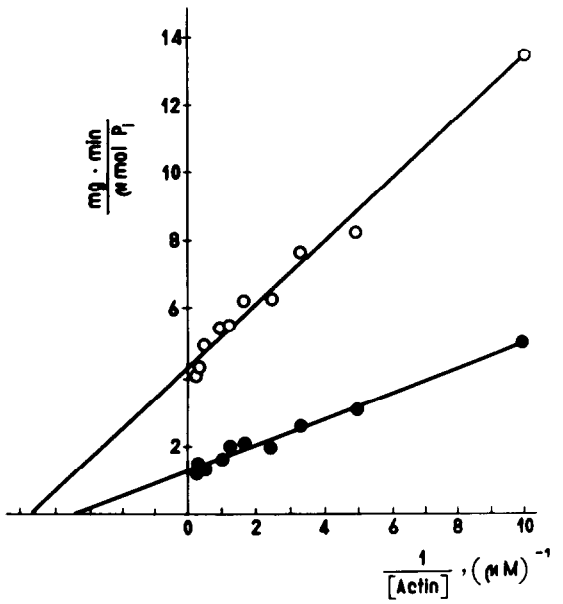

Fig.4. Actin dependence of Mg^{2+}-ATPase activities of myosin minifilaments with phosphorylated LC_2 (●) and dephosphorylated LC_2 (○). Double-reciprocal plot. Actin concentrations, 0.1–4.0 μM. Other conditions as in fig.1.

μM actin for minifilaments with phosphorylated LC_2.

4. DISCUSSION

Our data suggest that LC_2 phosphorylation increases the V_{max} of actin-stimulated ATPase of minifilaments 3–4-fold, and only slightly decreases the affinity of myosin minifilaments for F-actin. Thus, LC_2 phosphorylation acts differently on the kinetic parameters of actin-stimulated Mg^{2+}-ATPase reactions of myosin minifilaments and filaments. In the case of filaments, independent of the ionic strength, LC_2 phosphorylation significantly alters the affinity of myosin for F-actin but does not affect the V_{max} value [2–4]. In contrast, in the case of myosin minifilaments LC_2 phosphorylation strongly increases the V_{max} value and slightly decreases the affinity for F-actin.

We suggest that the observed unusual effect of LC_2 phosphorylation on the kinetic parameters of the actin-stimulated ATPase reaction of myosin minifilaments is due to some unique properties of the minifilaments, such as the ability to form ordered assemblies [14,15]. Under conditions

similar to those employed here (10 mM citrate-35 mM Tris, pH 8.0, 4 mM $MgCl_2$, 1 mM ATP, 0.1 μM myosin), these assemblies are represented by threads of minifilaments [15]. These structures are formed by interaction between the myosin heads arranged at the ends of minifilaments; such an interaction becomes stronger after LC_2 phosphorylation [14,15]. There are some indications that isolated myosin heads or subfragment 1 (S1) can interact under similar conditions and can form dimers [16,17]. The Mg^{2+}-ATPase activity of S1 dimers is higher than that of S1 monomers [18]. It seems probable that the unusual effect of LC_2 phosphorylation on the V_{max} value of the actin-stimulated Mg^{2+}-ATPase reaction of myosin minifilaments is due to its influence on the interaction between myosin heads resulting in the self-assembly of minifilaments.

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