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Peptide competition of actin activation of myosin-subfragment 1 ATPase by an amino terminal actin fragment

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The amino-terminal region of actin participates in the binding of myosin subfragment 1 (S1) during cross-bridge cycling, thereby assisting in the activation of the magnesium-dependent myosin ATPase. Effects of three actin fragments on the magnesium-dependent S1 and acto-S1 ATPase activities in solution were studied. One of the peptides, containing residues actin 1–44, mimicked the S1 ATPase-activating properties of actin and in turn inhibited acto-S1 ATPase both in a concentration-dependent manner. This suggests peptide competition for the actin binding site on myosin. The other fragments, residues actin 1–18 and 82–119, respectively, had no detectable effect on S1- and acto-S1 ATPase activity.

Actin; S1-ATPase; Acto-S1-ATPase; Actin peptide

1. INTRODUCTION

Identification of the myosin binding site on actin is an important step towards an understanding of the cyclical actin–myosin interaction and thus of muscle contraction. Chemical cross-linking experiments [1–3] as well as immunological studies [4,5] have given evidence that the amino-terminal region of actin is capable of binding to myosin. Chemical cross-linking of actin to S1 resulted in a marked activation of S1 Mg^{2+}-ATPase activity [6,7]. These results led to the notion that S1 and the smaller, 'outermost' N-terminal domain of actin [8,9] dock at multiple sites. Interestingly, it was reported very recently that a synthetic actin fragment, containing residues 1–28, slightly activated the S1 Mg^{2+}-ATPase [10]. Thus, more detailed information is to be expected from studies examining the functional properties of actin fragments containing the suggested segments to which myosin may bind.

In this report the influence of three actin peptides containing residues 1–44, 1–18 and 82–119, respectively, on S1 and acto-S1 Mg^{2+}-ATPase activities in solution is described. The peptide 1–44 activated the S1 Mg^{2+}-ATPase and inhibited the acto-S1 Mg^{2+}-ATPase. Both effects were concentration-dependent.

2. MATERIALS AND METHODS

ATPase activities were determined by use of the oil-well method for time resolved microfluorescence assays described earlier [11]. The method relies on the measurement of NADH breakdown which is coupled to the ATP turnover in the sample by means of an enzyme system containing pyruvate kinase (PK) and lactic dehydrogenase (LDH) as well as ATP, NADH and phosphoenolpyruvate (PEP) as substrates [12].

The published procedure [11] was modified as follows. The whole sample is illuminated by a light beam (wave-length about 340 nm) which causes the NADH contained in the solution to emit fluorescence light (at 470 nm). Illumination is intermittent, short intervals (3 s) of light exposure being followed by larger periods (90 s) with no light. This is necessary to minimize photo-bleaching of NADH which, under continuous stimulation by light, completely masks fluorescence changes as small as those detected in the experiments reported here. Using intermittent illumination allows the ATPase reaction to proceed without photo-bleaching of NADH during the 'dark' periods. The intensity of NADH-fluorescence is measured and its time course is simultaneously plotted on a chart recorder. The peak values of fluorescence in the short illuminating intervals show the time course of ATPase is linear (Fig. 1).

Two solutions are prepared for the ATPase measurements, solution A containing the substrates and enzymes required for the enzyme-coupled assay (ATP, NADH, PEP, PK, LDH), solution B containing S1 and, if required, actin and/or one of the actin fragments. One 0.5-μl-microdroplet of each of the two solutions is fixed within the light beam and the reaction is initiated by causing the two droplets to coalesce. Thus one droplet of 1.0 μl is formed which is instantaneously mixed with a piezo crystal-driven microstirrer.

The experimental solution contains (mM): Imidazole 9.5, dithiothreitol (DTT) 0.4, NaN_3 0.7, KCl 5.0, $MgCl_2$, 0.7, ATP 0.3, PEP 1.6, NADH 0.5; the content of enzymes is LDH 64.6 U/ml, PK 31.3 U/ml; pH is 7.0 in all experiments; temperature is kept constantly at 25°C.

S1 was prepared from rabbit skeletal muscle as described earlier [13]. In all experiments S1 was used at a concentration of 0.1 μM (i.e. 12.5 ng S1 in the probe volume of 1 μl).

Actin was extracted from rabbit skeletal muscle and purified according to [14]. For measurement of acto-S1 ATPase activity actin concentrations ranged from 2.0 to 10.2 μM.

The peptides 1–44 and 82–119 were isolated from a CNBr digest of actin by gel filtration on Sephadex G-50 followed by ion-exchange chromatography using SP Trisacryl. Peptide 1–18 was synthesized by

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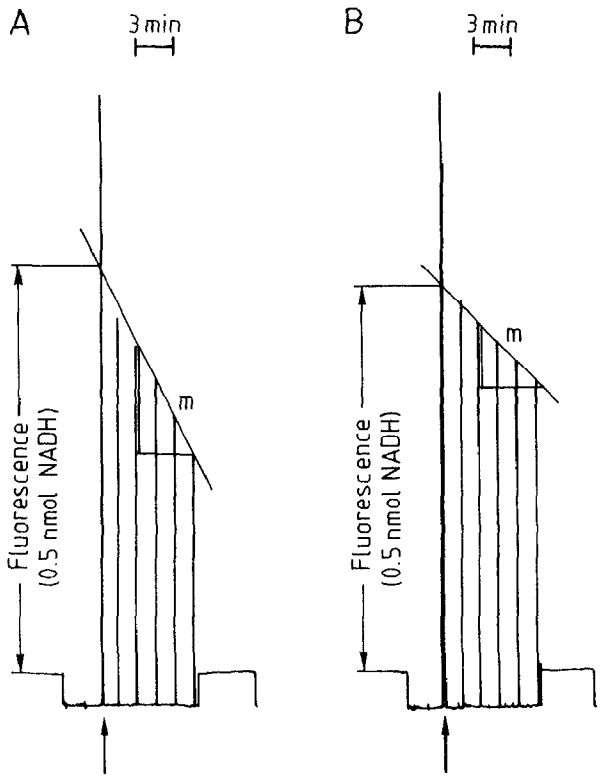

Fig. 1. Effect of the actin peptide 1–44 on acto-S1 Mg^{2+}-ATPase. Time course of NADH-fluorescence (see section 2). Concentrations are: S1, 0.1 μM; actin, 6.0 μM. A, without peptide; B, with 16.6 μM of peptide 1–44.

the solid-state method using FMOC-technology by Alta Bioscience (University of Birmingham). It was α-N-terminally acetylated prior to deblocking and cleavage from the resin and purified to homogeneity on a Vydac C-18 HPLC column. Concentrations of all peptides were determined by amino acid analysis after acid hydrolysis.

For each point of data means and standards errors of the means are given (n=6). Values given in the text and in Figs. 2 and 4 are original experimental data, values given in Fig. 3 are corrected for basal ATPase activity values of S1 alone and of peptide-activated S1, which are subtracted from acto-S1 ATPase values.

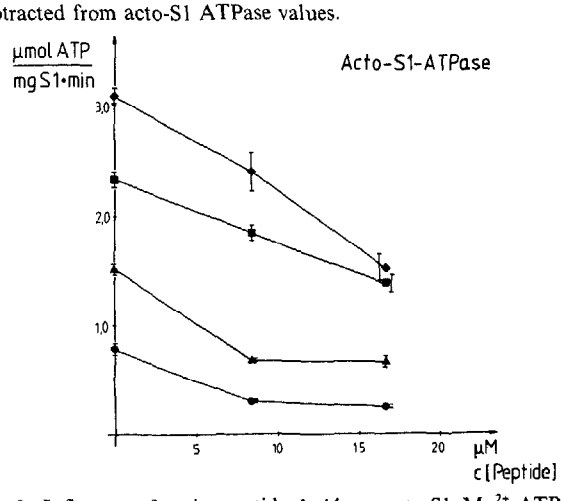

Fig. 2. Influence of actin peptide 1–44 on acto-S1 Mg^{2+}-ATPase. Concentrations are: S1, 0.1 μM; actin, (●) 2.0 μM, (▲) 4.0 μM, (■) 6.0 μM, (◆) 10.2 μM. The values given in the figure are original experimental data which were not corrected for the basal S1- and peptide-S1 ATPase activity.

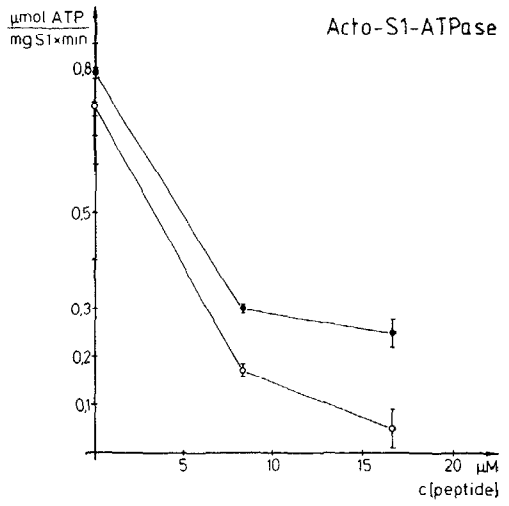

Fig. 3. Effect of actin peptide 1–44 on acto-S1 ATPase activity for c[actin] = 2.0 μM (also included in Fig. 2). Original experimental data are compared with values corrected for S1- and peptide-S1 ATPase activities. Actin activation is almost completely inhibited at c[peptide] = 16.6 μM. Half-maximal inhibition can be estimated to occur at about 6 μM of peptide 1–44. (●) Original data; (○) corrected data.

3. RESULTS

3.1. *Effect of the actin fragments on acto-S1 Mg^{2+}-ATPase activity (Figs. 1–3)*

Acto-S1 Mg^{2+}-ATPase activity was measured at different actin concentrations. The experiments were repeated with the actin peptide 1–44 at concentrations of 8.3 μM and 16.6 μM. The peptide showed a marked inhibition of the acto-S1 ATPase, the effect being concentration-dependent. Higher actin concentrations required higher concentrations of peptide 1–44 for inhibition. At the lowest actin concentration used in these experiments (2.0 μM) acto-S1 Mg^{2+}-ATPase was 0.78 ± 0.06 μmol ATP·mgS1^{-1}·min^{-1}. 8.3 μM of peptide caused the ATPase activity to drop to 0.30 ± 0.01 μmol ATP·mgS1^{-1}·min^{-1}, 16.6 μM of peptide led to a further decrease to 0.25 ± 0.03 μmol ATP·mgS1^{-1}·min^{-1}. Thus peptide inhibition was 61.5% and 68%, respectively.

At 10.2 μM of actin acto-S1 Mg^{2+}-ATPase was 3.10 ± 0.08 μmol ATP·mgS1^{-1}·min^{-1}. Addition of 8.3 μM and 16.6 μM of peptide 1–44 resulted in a decrease of ATPase activity to 2.41 ± 0.18 μmol ATP·mgS1^{-1}·min^{-1} and 1.52 ± 0.12 μmol ATP·mgS1^{-1}·min^{-1}, corresponding to an inhibition of 22% and 51%, respectively. For low actin concentration (2 μM) half-maximal inhibition of acto-S1 Mg^{2+}-ATPase was estimated to occur at about 6 μM peptide 1–44.

The actin fragment 1–18 had no detectable effect on the acto-S1 Mg^{2+}-ATPase under the same conditions. Also peptide 82–119 had no influence (data not shown).

3.2. *Effect on S1 Mg^{2+}-ATPase activity (Fig. 4)*

S1 Mg^{2+}-ATPase activity was measured at 0.1 μM S1. The actin fragment 1–44 activated the Mg^{2+}-ATPase of

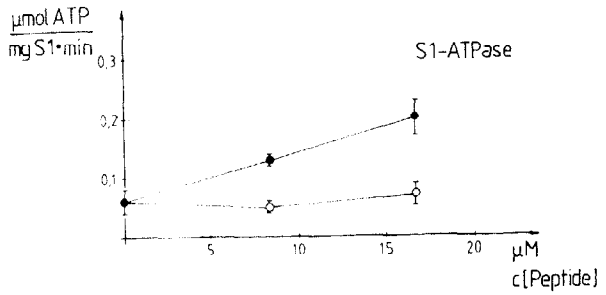

Fig. 4. Influence of actin peptides 1–44 and 1–18 on S1 Mg^{2+}-ATPase. Concentration of S1: 0.1 μM. Filled circles, actin residues 1–44; open circles, actin residues 1–18.

S1 in a concentration-dependent manner. ATPase of S1 alone was 60 ± 20 nmol ATP·mgS1^{-1}·min^{-1}. At 8.3 μM peptide S1 ATPase was increased to 130 ± 10 nmol ATP·mg S1^{-1}·min^{-1}, at 16.6 μM of peptide ATPase was 200 ± 30 nmol ATP·mgS1^{-1}·min^{-1}. Thus peptide activation was 2.2-fold and 3.3-fold, respectively. The peptide 1–18 on the other hand had no effect on the S1 Mg^{2+}-ATPase (Fig. 4), and neither did peptide 82–119 (data not shown).

4. DISCUSSION

In this paper we report that a short actin fragment containing the amino terminal residues 1–44 of the native actin sequence is able to both mimic the activating effect of actin on S1 Mg^{2+}-ATPase and to inhibit acto-S1 Mg^{2+}-ATPase in a concentration-dependent manner, confirming the specificity of its interaction with S1. The activating effect of peptide 1–44 on S1 Mg^{2+}-ATPase is weak in comparison to the activating properties of native actin which is able to potentiate S1 Mg^{2+}-ATPase by at least a factor of 200 [15]. This strong activating ability of actin however depends on its presence in the filamentous F-form. Monomeric G-actin was shown to activate S1 Mg^{2+}-ATPase by only a factor of about 4 (extrapolation of kinetic data to infinite concentrations of G-actin) [16]. As the peptide 1–44 does not contain the residues that by now have been identified as possible actin–actin recognition sites [17,18], the properties of the peptide should be compared to those of monomeric G-actin rather than of F-actin. Thus, activation of S1 Mg^{2+}-ATPase by peptide 1–44 is about as strong as that caused by G-actin.

A shorter amino terminal actin fragment (res. 1–18) and another peptide from subdomain 1 of actin (res. 82–119) have no effect on S1 Mg^{2+}-ATPase nor on acto-S1 Mg^{2+}-ATPase.

These results demonstrate that the peptide 1–44 not only binds to but also functionally interacts with S1 (which of course requires binding first), thus competing with actin for its binding site on myosin. This suggests that actin activation of S1 Mg^{2+}-ATPase must involve at least a portion of actin residues 1–44. The shorter fragment 1–18 of actin, which contains the site to which S1 can be covalently crosslinked [1,2], is not sufficient to functionally interact with S1.

This is not necessarily in contradiction to earlier suggestions [1,3,19] that the extreme amino terminal segment of actin is important for actin-myosin interaction. Binding of actin 1–18 to S1 may well occur, but this would only be detected in our experimental set-up, if its effect were sufficient, over the concentration range used, to effect either the S1- or acto-S1 Mg^{2+}-ATPase activity. It may well be that several S1-binding segments of actin have to act cooperatively to achieve this.

An immunological study showed [4] that antibodies directed against actin residues 18–28 are able to dissociate acto-S1. This finding supports the idea that the actin peptide 1–44 contains at least one additional site of interaction with myosin that is not contained by the shorter actin peptide 1–18. This additional site may be located in the segment 18–28 since it was found that an actin peptide 1–28 slightly activated the S1 Mg^{2+}-ATPase [10].

The effect of the actin peptide 1–44 in our study was, however, far stronger. There might be two possible explanations for this behaviour, either there is another site of actin myosin interaction contained in the segment 29–44 of actin, or the conformation of the longer fragment (actin 1–44) is more stable and fits better to the actin binding site on myosin than the shorter one (actin 1–28) in ref. [10], thus presenting the more adequate interface for interaction with myosin.

Residues 1–44 of actin contain all three segments of beta-pleated sheet contributed to subdomain 1 of actin by the N-terminal part of the protein, according to [8]. Residues 1–32 belong to subdomain 1, 33–44 to subdomain 2 of actin. Further experiments will have to show if residues 1–32 are as effective as 1–44 in inhibiting the actin activation of S1 ATPase or if the short subdomain 2-segment 33–44 is necessary for the effects reported here.

In any event, the data reported here and elsewhere support the notion that actin and myosin dock at multiple sites and that contact with more than one of these is necessary to activate the S1 Mg^{2+}-ATPase.

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