MECHANISM OF MYOSIN AND ACTOMYOSIN ATPase IN CHICKEN GIZZARD SMOOTH MUSCLE

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Received 2 December 1977

1. Introduction

The reaction cycles of myosin and actomyosin ATPase have been investigated in detail [1–4], but with few exceptions [5] such studies have been confined to white skeletal muscle actomyosin from the rabbit and little is known about the ATPase mechanism in smooth muscle. As part of a comparative study of the four different types of vertebrate myosin (S.B.M. and E.W.T., in preparation) we have now prepared myosin and S-1 from gizzard smooth muscle and have investigated their behaviour by steady state and transient kinetic techniques.

2. Methods

Chicken gizzard myofibrils and myosin were prepared as in [6]. Subfragment-1 was prepared by direct digestion of the myofibrils with papain by a modification of [7]. Myosin had light chains at 20 000 and 17 000 daltons whilst the S-1 had a degraded 20 000 light chain (fig.1). Rabbit fast muscle actin was prepared as in [8].

Stop-flow measurements were made with an apparatus constructed in this laboratory, fully described in [3]. ATPase hydrolysis rates were measured by assaying P_i release [9].

All the experiments were done with 5 μM gizzard S-1 in 5 mM Tris, 5 mM morpholino-*N*,*N*-ethane sulphonic acid (MES), 1 mM $MgCl_2$, 10 mM KCl, pH 7.0, 25°C.

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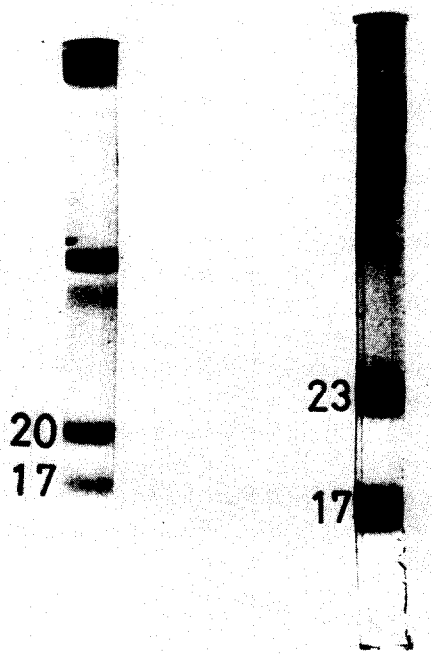

Fig.1. $7\frac{1}{2}$% Polyacrylamide–SDS gel electrophoresis of gizzard myosin and S-1, prepared by papain digestion.

3. Results and discussion

3.1. *Myosin and S-1 MgATPase mechanism*

The initial step of the reaction is a rapid binding of Mg.ATP to myosin. As with rabbit S-1 [2], the binding occurs in two steps – a rapidly reversible formation of S-1.ATP followed by a conformational change which is accompanied by a 10% increase in protein tryptophan fluorescence.

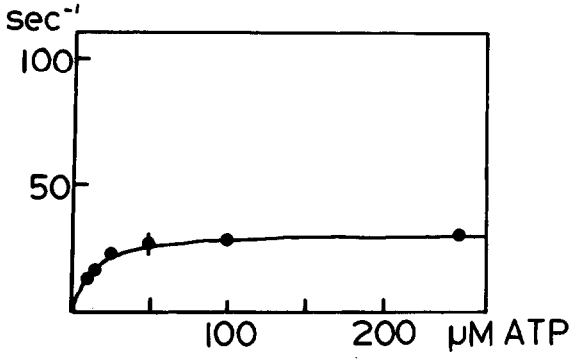

Fig.2. Dependence of the rate of protein fluorescence enhancement on ATP concentration. K_{app} 11 × 10^4 $M^{-1}.s^{-1}$; k_{+6} is 29 s^{-1}.

$$\text{ATP} + \text{S-1} \underset{}{\overset{a}{\rightleftharpoons}} \text{S1.ATP} \xrightarrow{b} \text{S-1.ATP*}$$

The rate of fluorescence enhancement thus increases hyperbolically with increasing ATP concentration from which K_{app} (which approximates to k_{+a}/k_{-a}) and k_{+b} may be determined (fig.2). The values under our conditions differ from fast skeletal S-1 by only a factor of 4.

S-1–ATP* is rapidly cleaved to S-1.ADP.P_i**. We found that the release of ADP from myosin was at least 100-times faster than the ATP turnover rate so the rate-limiting step is probably a conformational change:

$$\text{S-1.ADP.P}_i\text{**} \xrightarrow[d]{0.06\ s^{-1}} \text{S-1.ADP.P}_i^{II} \underset{e}{\overset{>10\ s^{-1}}{\rightleftharpoons}} \text{S-1} + \text{ADP} + \text{P}_i$$

The ATP turnover rate was about 0.06 s^{-1} for gizzard myosin and S-1; this rate is quite similar to the turnover rate in fast skeletal muscle.

3.2. *Actin-activated S-1 ATPase*

Rabbit skeletal actin activates the Mg.ATPase of gizzard S-1 by up to 10-fold despite the absence of an intact regulatory light chain [10]. The ATPase activity was usually not calcium sensitive though an inhibition of up to 60% in EGTA was sometimes observed.

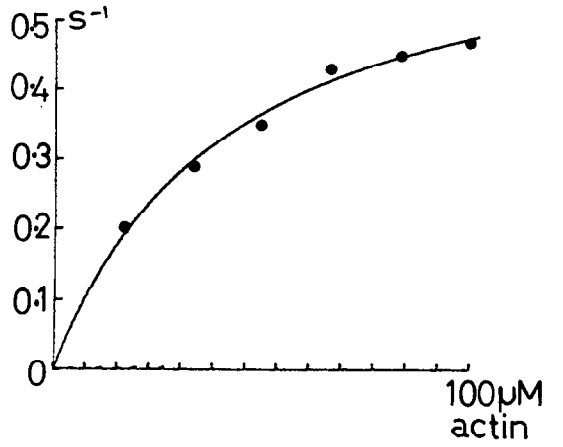

Fig.3. Actin activation of gizzard S-1 Mg.ATP hydrolysis rate. 5 μM gizzard S-1, 2 mM Mg.ATP, 0–100 μM, f.actin. K_m 50 μM; V_{max} 0.66 s^{-1}.

ATPase rate depends on the actin concentration in a hyperbolic manner (fig.3). The K_m is 60 μM and the V_{max} is 0.66 s^{-1}. This rate is similar to the rate estimated in intact muscle and is some 50-times slower than fast skeletal muscle [11].

The initial step of the ATPase is a rapid reversible binding of ATP to acto.S-1 followed by rapid dissociation of acto.S-1.ATP to actin and S-1.ATP. This is demonstrated by the hyperbolic dependence of the dissociation rate on ATP concentration (fig.4). If there are only 2 steps:

$$\text{A.S-1} + \text{ATP} \underset{1}{\rightleftharpoons} \text{A.S-1.ATP} \xrightarrow[2]{} \text{A} + \text{S-1.ATP}$$

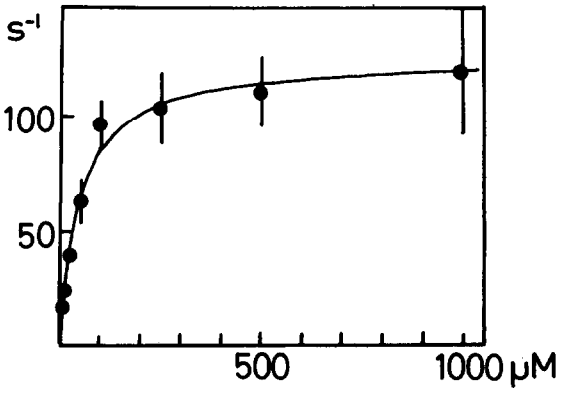

Fig.4. Dependence of the rate of gizzard acto.S-1 dissociation on Mg.ATP concentration. K_{app} 3 × 10^4 $M^{-1}.s^{-1}$; plateau rate 104 s^{-1}.

$K_{app} \simeq k_{+1}/k_{-1}$ and the plateau rate at high ATP concentrations is $k_{+2} + k_{-2}$. Thus k_{+2} is at least 10-times slower than the corresponding reaction of fast skeletal S-1 [1], where 2 step binding cannot be demonstrated. Note the similarity of K_{app} for S-1 and acto.S-1 ATPases.

S-1.ATP then undergoes a conformational change accompanied by fluorescence enhancement at about the same rate as k_{+b} (fig.2). This is followed by formation of S-1.ADP.P_i**. The recombination of S-1.ADP.P_i** with actin was measured by a double-mixing technique [4]. The observed rate was very slow and increased linearly with actin concentration in the range 0–15 μM actin. Specific rate constant is 5×10^3 (M actin)$^{-1}$.s^{-1}, compared with 5×10^5 (M actin)$^{-1}$.s^{-1} for fast skeletal S-1. In contrast the rate of combination of S-1 with actin was 10^7 M^{-1}.s^{-1} for both gizzard and fast skeletal S-1.

The minimum scheme for the association is:

$$\text{S-1.ADP.P}_i\text{**} + \text{A} \underset{5}{\rightleftharpoons} \text{A.S-1.ADP.P}_i\text{**}$$

$$\xrightarrow[6]{} \text{A.S-1} + \text{ADP} + \text{P}_i$$

The low apparent rate constant correlates with the low V_{max} of the ATPase and is simply explained if step 5 is a rapid equilibrium followed by a conformational change which determines V_{max}. In this scheme:

Specific recombination rate = 5×10^3 M^{-1} s^{-1}
$= K_5 k_{+6}$

V_{max} of ATPase = 0.6 s^{-1}
$= k_{+6}$

K_m of ATPase = 5×10^{-5} M
$= 1/K_5$

The 3 measured parameters fit this scheme for gizzard acto.S-1 as they do for fast and slow skeletal muscles. They do not fit a scheme involving a refractory state [12].

Since ADP release was found to be 2 orders of magnitude faster than V_{max}, step 6 should be subdivided into a slow conformational change which is rate-limiting followed by rapid product release.

We conclude that:

1. The mechanism of Mg.ATP hydrolysis by gizzard S-1 and acto.S-1 follows the same pathway as that determined in fast skeletal muscles.

2. The rate constants of the gizzard S-1 Mg.ATPase are not very different from rabbit S-1 (fig.5).

3. The gizzard acto.S-1 ATPase has a low V_{max} which correlates with the low rate of ATP turnover in vivo and slow rate of unloaded contraction [5,9].

4. Two processes, steps 4 and 6, are markedly slower in smooth muscle compared with skeletal muscle (fig.5). Step 6 is probably the rate-limiting step in both muscles at 25°C. The presence of a long-lived attached complex explains the high holding economy and slow maximum speed of contraction in smooth muscle.

Acknowledgements

Supported by USPHS NIH Grant GM10992 and the Muscular Dystrophy Association of America.

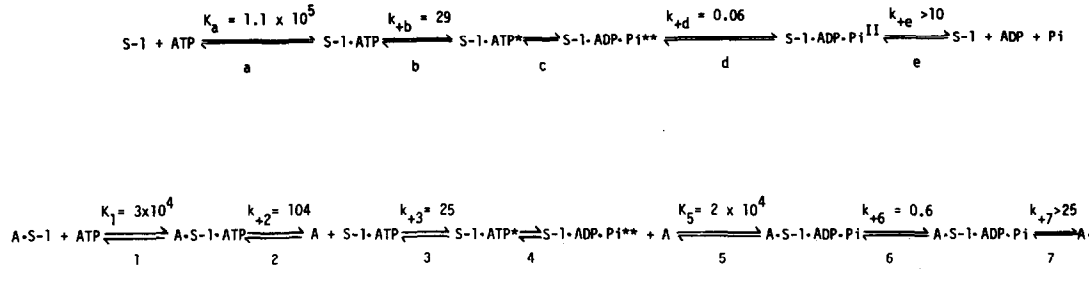

Fig.5. Reaction mechanism for gizzard S-1 and acto-S-1 ATPase at 25°C, pH 7.0, 10 mM KCl.

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