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What is the true ATPase activity of contracting myofibrils?

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The ATPase activity of contracting myofibrils was obtained in a solvent of ionic contents that mimic in vivo conditions: pH 7.4 and 0.1 M potassium acetate. Contracting myofibrils are fleeting structures and their ATPase activity is of short duration: even at 4°C it was over after a reaction time of 1 s and rapid reaction equipment was needed for its study. The ATPase (1.1 s^{-1}) was very similar to that of crosslinked actoS1 (1 s^{-1}). It was 100 × that of relaxed myofibrils.

Myofibril; Contraction; Actomyosin; ATPase activity

1. INTRODUCTION

Muscle contraction depends on the cyclic interaction of actin with myosin the energy for which is supplied by the hydrolysis of ATP by the myosin heads. The problem is to determine the relation between the different steps of ATP hydrolysis and the physiological events involved in contraction. Most kinetic work has been carried out with actin and myosin in solution and under ionic conditions (type of ion, concentrations) which cannot even be considered to be an approximation of those obtained physiologically. The question is, can one extrapolate from these solution studies to more organised systems such as muscle? A way of approaching this problem is to use myofibrils.

Myofibrils retain the filament structure of muscle. However, as they are not anchored, experiments with them suffer from the difficulty that at excess concentrations of ATP they overcontract to shorter sarcomer lengths than occurs naturally with a corresponding loss of structure. Takashi et al. [1] report that upon contraction myofibrils 'deform into very dense spheres, like meatballs'. Very recently Harrington et al. [2] showed that at 21°C myofibrils contract in two steps. Upon the addition of ATP, there is a rapid contraction (rate about 5 s^{-1}), the sarcomer lengths shortening from 2.7 to about 1.3 μm. No loss of structure appeared to occur at this stage. This is followed by a slower process in which the myofibrils undergo further shortening which leads in a few seconds to a loss of structure.

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Abbreviation: S1, myosin subfragment 1

What is the ATPase activity of contracting myofibrils before they lose their structure? Several studies have been carried out on the ATPase of activated myofibrils under multi-turnover conditions (e.g. [3–6]). However, judging from the time scales used in these works, the activities obtained were of overcontracted myofibrils. Myofibrils in action are ephemeral species and it is only by the use of rapid kinetics techniques that one can attempt to obtain their kinetic parameters.

Here we studied, by the rapid flow quench method, the ATPase activity of Ca-activated and relaxed myofibrils under multi-turnover conditions in a solvent of pH and ionic contents which we suppose to mimic those in vivo: pH 7.4 and 0.1 M potassium acetate. Because of difficulties in interpreting the progress curves for ATP hydrolysis at high temperatures, the experiments were carried out at 4°C. We find that a k_{cat} can be obtained during the first second after mixing myofibrils with an excess of ATP. This k_{cat} is very similar to that obtained with crosslinked actoS1 under the same conditions.

2. MATERIALS AND METHODS

2.1. *Proteins and reagents*

Myofibrils were prepared from rabbit psoas muscle following Knight and Trinick [7] but with a different extraction buffer: 50 mM KCl, 50 mM Tris, 5 mM EDTA, 1 mM DTT, 0.1 mM PMSF, pH 7 (HCl). The concentration of myosin heads was determined according to [5]. References to the preparation of myosin, S1, actin and crosslinked actoS1 are in [8]. [γ-^{32}P]ATP was from Amersham International.

2.2. *P_i burst experiments*

These were carried out in a thermostatically controlled flow quench apparatus [9]. The buffer was 0.1 M potassium acetate, 5 mM KCl, 50 mM Tris, 2 mM magnesium acetate, 1 mM DTT, pH 7.4 (acetic

acid). With Ca-activated myofibrils 0.1 mM $CaCl_2$ was included. Experiments in the absence of Ca were carried out in the same buffer but without $CaCl_2$ and containing 2 mM EGTA and 5 mM magnesium acetate. In certain experiments ATPase activities were obtained by a colorimetric method based on that of Briggs [10]. These were carried out on the minutes time scale at 1 mM ATP in the buffer above.

2.3. *Microscopic studies*

These were carried out using a phase-contrast microscope (Nikon Microphot fxa with Normarski optics) at a magnification of 800 ×. The photomicrographs obtained are not illustrated here, first, because they are classical (e.g. [11]) and second, because the conclusions from each slide study were based on the examinations of several fields.

3. RESULTS

As observed under the microscope, the myofibrils used here contained 5–15 sarcomeres and they appeared to be fully overlapped. They were viable under our experimental conditions (4°C or 25°C, acetate buffer; see above). Thus, when Ca activated, they contracted rapidly upon the addition of excess ATP. When relaxed, they appeared not to be affected by ATP.

Initially we wanted to experiment at a temperature as nearly physiological as possible, but this proved to be difficult. Thus, at 25°C the P_i formation was multiphasic. Further, there was a rapid loss of activity and it was difficult to obtain a steady state rate. Therefore, we decided to work at 4°C.

A progress curve for the ATPase activity of Ca-myofibrils at 4°C is shown in Fig. 1. Here there were distinct phases: a P_i burst (0.53 mol P_i per mol myosin heads), an apparently rapid linear phase (1 s^{-1}) and then a low ATPase (0.15 s^{-1}) after about 1 s. This low ATPase is very similar to that obtained on the minutes time scale at high [ATP] (Table I).

Ca-myofibrils did not appear to lose their structural integrity upon being aged up to 1 s after rapid mixing with ATP. Thus reaction mixtures (myofibrils 3 μM in myosin heads plus 32 μM ATP) were aged for different times in the quench flow apparatus, quenched in 3 mM EDTA and then examined under the microscope. At times up to about 1 s the myofibrils appeared to be intact but at longer times there seemed to be a certain loss of structure. Myofibrils that had been mixed with buffer and aged for 1 s in the flow quench apparatus gave identical P_i burst curves to the unmixed material.

The rapid linear phase is of particular interest. It lasted only a short time (0.2–1 s): it was squeezed between a rapid transient and the decrease in ATPase activity. Because of its linearity and because the P_i formed is greater than 1 mol per myosin head, the phase is presumably not a transient. It was obtained with all calcium activated myofibrils tested.

The rapid phase appears to depend upon the myofibrils being structurally intact. Thus, when Ca-myofibrils were preincubated with an excess of ATP (3 mol ATP per myosin head) for 30 min, they lost

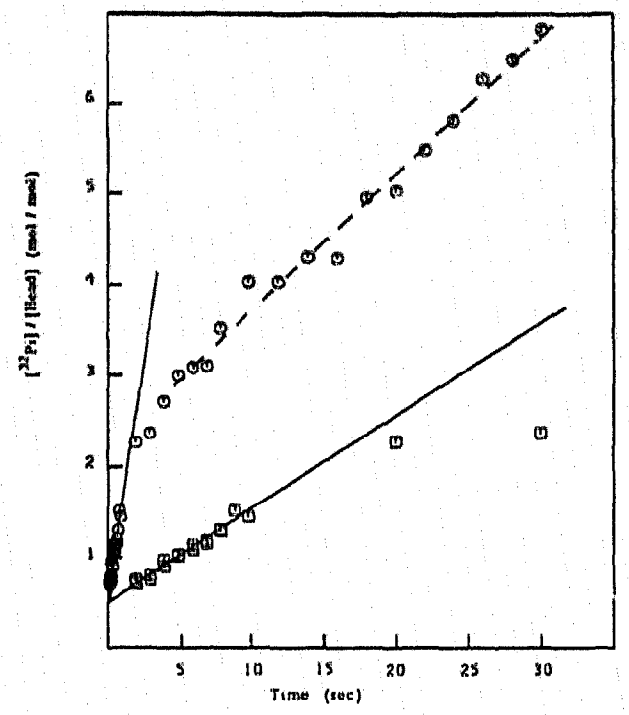

Fig. 1. Time course for Ca-activated myofibrillar ATPases at 4°C. The reaction mixtures (3 μM in myosin heads + 32 μM [γ-^{32}P]ATP) were quenched in 22% trichloroacetic acid and the $^{32}P_i$ determined [10]. For points (○) the time scale is as indicated, for (□) it is divided by 10. The continuous line was fitted to 1 s^{-1} and the dashed line to 0.15 s^{-1}.

their structures as judged from microscopic observations. These myofibrils did not give a short rapid linear phase in a P_i burst experiment: with them there was a rapid rise (0.6 mol P_i per mol myosin head) which was followed immediately by a low ATPase activity (about 0.2 s^{-1}).

Table I

Comparison of certain of the steady state parameters of myofibrils obtained on different time scales and of crosslinked actoS1 and S1 at 4°C

	Experimental time range			
	0.2–1 s[a]		Minutes[b]	
	Amplitude of P_i burst (mol P_i/mol head)	Steady state rate (s^{-1})	Steady state rate (s^{-1})	
Myofibrils (+Ca^{2+})	0.64 (±0.15)	1.1 (±0.4)	0.15	(±0.01)
Myofibrils (−Ca^{2+})	0.62 (±0.05)	0.011 (±0.001)	0.018	(±0.002)
Crosslinked actoS1	0.72 (±0.1)	1.0 (±0.2)	–	
S1	0.55 (±0.05)	0.013 (±0.001)	–	

[a] From experiments using [γ-^{32}P]ATP (32 μM) and the rapid flow quench apparatus. With activated myofibrils the steady state rate refers to the rapid linear phase (e.g. Fig. 1). The values given are the averages of several experiments

[b] [ATP]= 1 mM. P_i determined colorimetrically [10]

We suggest that the fast linear phase represents the ATPase activity of intact Ca-activated myofibrils. A way of confirming this is to show that the activity is the same as that with actoS1 at saturation in actin. With the high ionic strength buffer used here this could be not tested with actoS1 but we did obtain the P_i burst amplitude and ATPase activity of crosslinked actoS1. As shown in Table I activated myofibrils and crosslinked actoS1 have very similar steady state parameters.

A progress curve for the ATPase activity of relaxed myofibrils at 4°C was also obtained (not shown). There was a rapid rise of amplitude equal to that with Ca-myofibrils followed immediately by a slow steady state rate (0.011 s^{-1}). A very similar progress curve was obtained with S1 (Table I). Therefore, Ca activation leads to a 100 × increase in the activity of relaxed myofibrils.

4. DISCUSSION

When activated myofibrils are mixed with ATP, there is a rapid P_i burst followed by a short rapid steady state and then a loss of activity. The rapid steady state rate is very similar to that with crosslinked actoS1. From our results and the observations of Harrington et al. [2], we suggest that the rapid steady state rate is the ATPase activity of contracting myofibrils before they overcontract and lose their structures. Of course, we cannot wholly exclude the possibility that the similarity of the ATPases of activated myofibrils and crosslinked actoS1 is fortuitous. Because of the difficulties with the P_i burst experiments we were unable to determine whether or not the two ATPases remain similar at higher temperatures.

Our experiments were carried out at 4°C but this is probably not deleterious. First, Ca-activated myofibrils are viable at this temperature and second, it has been shown that at 5°C skinned psoas fibres are able to relax and contract normally [12].

There are values for the ATPase activities of myofibrils in the literature but these are presumably of overcontracted myofibrils [3–6]. This would explain the lower Ca activations found previously: 20–40 ×, here about 100 ×.

Attempts have been made to obtain the ATPase activity of intact myofibrils. Glyn and Sleep [13] prevented contraction by crosslinking chemically myofibrils. Under conditions similar to ours they obtained an ATPase activity of 0.85 s^{-1} at 15°C. This is lower than the activity at 4°C (1.1 s^{-1}, here) and further, it is much lower than that 14 s^{-1} with crosslinked actoS1 at 15°C [8]. Sleep [6] followed the hydrolysis of ATP by myofibrils under single turnover conditions at 0°C, i.e. under conditions in which overcontraction does not occur. The progress curve obtained was multiphasic and did not lead directly to the kinetics of the P_i release step. In any event, single turnover experiments only lead to k_{cat} if the P_i release step is rate limiting [14].

It has been suggested that the steady state rates of organized systems such as muscle fibres and myofibrils are lower than those for soluble systems, e.g. [6]. But under our conditions, activated myofibrils appear to hydrolyse ATP as rapidly as the soluble actoS1. Further, we note that the ATPase activity for myofibrils of 1.1 s^{-1} at 4°C is consistent with the 2 s^{-1} at 10°C obtained with calcium activated skinned fibres [15].

It is noteworthy that the P_i burst amplitude of activated myofibrils was large and further, that it was similar to those with relaxed myofibrils, crosslinked actoS1 and S1 (Table I). This suggests that the predominant steady state intermediate on activated myofibrillar ATPase contains bound P_i.

In conclusion, the steady state rate of the ATPase of contracting myofibrils is difficult to study: even at 4°C it has only a short existence and is over after 1 s. We are now studying the transient kinetics of myofibrillar ATPase.

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