Correlation of ActoS1, Myofibrillar, and Muscle Fiber ATPases†

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ABSTRACT: Our objective was to determine a good *in vitro* model for muscle fiber ATPase, and we compared the kinetics of Ca^{2+}-activated myofibrils and cross-linked actoS1 in a buffer of physiological ionic strength. The myofibrils were cross-linked chemically to mimic the isometric condition of fibers or were un-cross-linked (the isotonic condition), and temperature perturbation was used to probe their ATPase mechanisms. At 4 °C, we have already shown that the kinetics of cross-linked actoS1 and myofibrils (cross-linked or not) are similar: there were large P_i bursts and k_{cat} values of about 1 s^{-1}, close to that obtained with fibers [Herrmann, C., Sleep, J., Chaussepied, P., Travers, F. & Barman, T. (1993) *Biochemistry 32,* 7255–7263]. So, at 4 °C cross-linked actoS1 and myofibrils are equally good as models for fiber ATPase. At 20 °C, this similarity vanishes: progress curves with the myofibrils (cross-linked or not) had large P_i bursts, but with cross-linked actoS1, bursts could not be discerned. This shows that at 20 °C the predominant steady-state intermediates are ATP complexes with actoS1 but are products complexes with the myofibrils, as with fibers [Ferenczi, M. A. (1986) *Biophys. J. 50,* 471–477]. Further, the k_{cat} values were different: 15.5 s^{-1} with cross-linked actoS1, 8.3 s^{-1} for myofibrils, and 3.5 s^{-1} for cross-linked myofibrils. With fibers, k_{cat} = 3.3 s^{-1}. These results show that cross-linked myofibrillar ATPase is a good model for muscle fibers contracting isometrically. Our results may also help to explain the Fenn effect, namely, that as the load on the system is increased so k_{cat} decreases: cross-linked actoS1 → myofibrils → cross-linked myofibrils.

Muscle contraction depends on the cyclic interaction of actin with myosin, the energy for which is supplied by the hydrolysis of adenosine 5′-triphosphate (ATP)[1] by the myosin heads. The obvious problem is to determine the relation between the different steps of ATP hydrolysis and the physiological events involved in contraction. It is very difficult to study directly the ATPase reaction pathway in muscle fibers. The use of caged compounds is an elegant way of attacking this problem [e.g., see Goldman et al. (1982), Hibberd and Trentham (1986), and Homsher and Millar (1990)]. This technique is much used in transient mechanical studies, but its use in the chemical transient analysis of fiber ATPase remains difficult, and solution studies remain the main source of precise kinetic information on actomyosin ATPase [e.g., see Goldman (1987)].

Most muscle kineticists have directed their attention toward the properties of soluble muscle systems (i.e., involving dispersed molecules), especially actoS1 [for reviews, see Trentham et al. (1976), Taylor (1979), Geeves et al. (1984), and Geeves (1991)]. These several works have provided a large amount of data which form part of the large pool of information concerning the physicochemical properties of the dispersed myosin, actin, and regulatory proteins, under a variety of conditions. The problem, then, is to select from this pool what is relevant to muscle contraction. A particular problem is that to study the actoS1–ATP system one must work at very low ionic strengths [it has been shown that certain steps of the ATPase are highly sensitive to ionic strength; e.g., see Taylor (1979)], and much data have been collected under these unphysiological and unorganized conditions. A way of overcoming this problem is to chemically cross-link actoS1 (Mornet et al., 1981).

Consequently, there are questions as to the suitability to actoS1 ATPase as a model for muscle fiber ATPase. First, the maximum activity of actin-activated S1 ATPase is 10–30 times higher than the ATPase rate during isometric contraction of muscle fibers [e.g., see Goldman (1987)]. Second, consider "P_i burst" experiments. In these, progress curves are obtained by quenching ATP plus, e.g., actoS1 reaction mixtures in acid and then determining the bound plus free P_i. These experiments are important in that they give information on the predominant intermediates of the ATPase in the steady state. Thus, with muscle fibers Ferenczi et al. (1984) obtained a large P_i burst at 13 °C which shows that intermediates containing products predominate. However, with actoS1 at 20 °C (weak ionic strength buffer; Rosenfeld & Taylor, 1984; Tesi et al., 1990) or with cross-linked actoS1 (moderate ionic strength buffer; Biosca et al., 1985), the P_i bursts were insignificant. This suggests that with actoS1, intermediates containing ATP predominate.

Another *in vitro* model for fiber ATPase is myofibrillar ATPase. Myofibrils are the functional contractile units of muscle, and yet they are small enough for study by rapid reaction techniques. Kinetic studies on the myofibrillar ATPases (Ca^{2+}-activated or not) have appeared (White, 1985; Miyata et al., 1989; Taylor, 1990; Ohno & Kodama, 1991; Houadjeto et al., 1991, 1992; Herrmann et al., 1992; Ma & Taylor, 1993). Experiments have been carried out on myofibrils prevented from shortening by chemical cross-linking (Glyn & Sleep, 1985; Duong & Reisler, 1989; Herrmann et al., 1993). Here we present a comparison of the ATPases of cross-linked actoS1 and Ca^{2+}-activated myofibrils at physiological ionic strength. To aid us in this, we used temperature perturbation as a tool. We conclude that at 4 °C, the ATPases of actoS1 and myofibrils appear to be good models for fiber ATPase. However, at 20 °C and above, whereas the kinetic

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[1] Abbreviations: ATP, adenosine 5′-triphosphate; S1, myosin subfragment 1; Tris, tris(hydroxymethyl)aminomethane; P_i, inorganic orthophosphate.

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Scheme 1

$$M + ATP \underset{}{\overset{K_1}{\rightleftharpoons}} M{\bullet}ATP \xrightarrow{k_2} M^{\bullet *} ATP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$$

$$M^{\bullet **} ADP{\bullet}P_i \xrightarrow{k_4} M + ADP + P_i$$

characteristics of myofibrils and muscle fibers appear to be similar, those of actoS1 are significantly different.

MATERIALS AND METHODS

Proteins and Reagents. Myofibrils, cross-linked myofibrils, and actoS1 were prepared as in Herrmann et al. (1993). The concentration of protein in the myofibrils was determined by dissolving them in 2% SDS and measuring the absorbance at 280 nm. The extinction coefficient was determined from a calibration curve using a 1:3 mole ratio of myosin to actin and the bicinchoninic acid biuret reagent of Pierce Chemical Co. This gave an $E^{1\%}_{1cm}$ of 7, in agreement with Sutoh and Harrington (1977). The proportion of myosin in the myofibrils was determined from SDS–PAGE stained with Coomassie blue and found to be 50% of the myofibrillar proteins. ATPase sites were determined by the cold ATP chase method (Houadjeto et al., 1992). [γ-^{32}P]ATP was from Amersham International.

Kinetic Experiments. These were carried out by the rapid flow quench method (Barman & Travers, 1985). Two apparatuses were used, depending upon the time scale required and also on the material studied (see Results). Both apparatuses were thermostatically controlled.

For experiments in the 4–350-ms time range, the apparatus used was of the continuous-flow type. Work with this apparatus is laborious, and for a complete time course, 1.5–2 h is required. For experiments in the 0.35-s to tens of seconds time scale, a time delay apparatus was used. With this, it takes about 15 min to obtain a full time course.

There were two types of experiments: (A) In *cold ATP chase* experiments, myofibrils or actoS1 plus [γ-^{32}P]ATP reaction mixtures are first quenched in a large molar excess of unlabeled ATP–Mg^{2+} (25 mM) in the rapid flow quench apparatus. The quenched reaction mixtures are incubated on ice for 2 min and then finally stopped by addition of 22% trichloroacetic acid, and [^{32}P]P_i is determined (Reimann & Umfleet, 1978). In these experiments, one obtains a transient burst phase of tightly bound ATP, whose *kinetics* give $k = k_2[ATP]/([ATP] + K_1)$ (see Scheme 1) and *amplitude* gives the ATPase site concentration. The transient is followed by a steady-state rate, and by dividing this by the ATPase site concentration, k_{cat} is obtained. For a fuller discussion of the ATP chase technique, see Barman and Travers (1985).

(B) In *P_i burst experiments*, reaction mixtures are quenched directly in acid, and [^{32}P]P_i determined.

The data obtained were interpreted by Scheme 1 where M represents the myosin heads in myofibrils with or without actin interaction.

Experimental Conditions. The experiments were carried out in 0.1 M potassium acetate, 5 mM KCl, 2 mM magnesium acetate, 0.1 mM $CaCl_2$, and 50 mM Tris adjusted to pH 7.4 with acetic acid.

Treatment of Data. The kinetic data were treated according to Houadjeto et al. (1992).

RESULTS

Effects of Temperature on the Steady-State Parameters of Ca^{2+}-Activated Myofibrils and Cross-Linked ActoS1. The effect of temperature on the burst size and steady-state rates of activated native and cross-linked myofibrils is illustrated in Figure 1. As the main aim of these experiments was to determine the temperature dependencies of the burst sizes, the [γ-^{32}P]ATP to myosin head ratio was low (5:1). The time delay flow quench apparatus was used so the kinetics of the burst were not measured. These faster kinetics were obtained with the continuous flow apparatus, below.

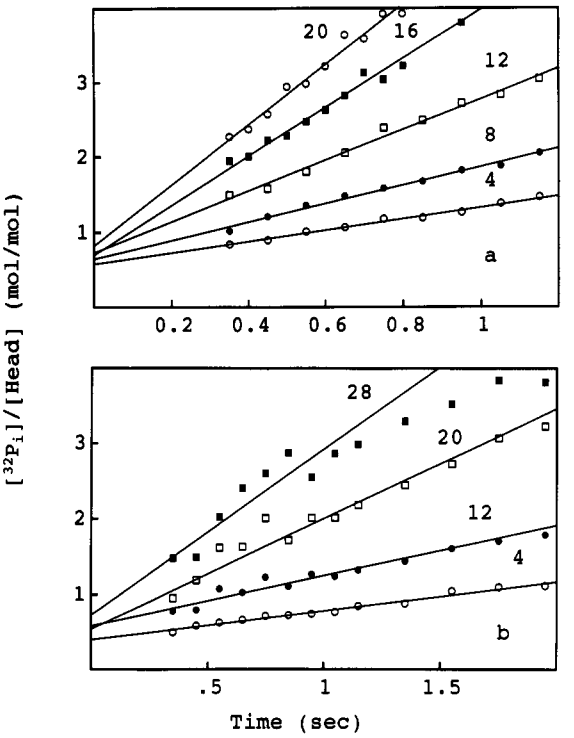

FIGURE 1: Effect of temperature on the steady-state parameters of activated native (a) and cross-linked (b) myofibrils. The reaction mixtures (6 μM in myosin heads + 30 μM [γ-^{32}P]ATP) were quenched in acid, and [^{32}P]P_i was determined. The temperatures (degrees centrigrade) used are shown above the time courses.

The temperature dependence of the steady-state parameters of activated, un-cross-linked myofibrils is shown in Figure 1a. This ATPase activity is difficult to study as it is squeezed between a P_i burst phase and a slow ATPase, presumably due to that of overcontracted myofibrils (Houadjeto et al., 1991). As the temperature is increased, the three phases become more difficult to distinguish, and our experiments were limited to the temperature range 4–24 °C. In Figure 1a, the time course is given up to 1 s only, and the slow steady state is not included.

P_i burst experiments with cross-linked myofibrils are shown in Figure 1b. With these myofibrils, overcontraction does not occur, and in P_i burst experiments, there should be only two phases: a rapid P_i burst and a steady state (Herrmann et al., 1993). Nevertheless, at the highest temperature used (28 °C), the progress curve was nonlinear. This is explained by the low ATP to myosin head ratio used and the rapidity at which the ATP is hydrolyzed; at times above 1 s, the reaction approaches single-turnover conditions, and consequently there is a reduction in the overall rate of hydrolysis.

There was some variation in P_i burst sizes in these experiments. We do not believe that this is significant—there is a large error introduced by extrapolating to zero time since the first time point is at 0.35 s. At shorter times, the kinetics of the transient phase interfere. Accurate estimates for burst sizes require sampling in the millisecond time range (see

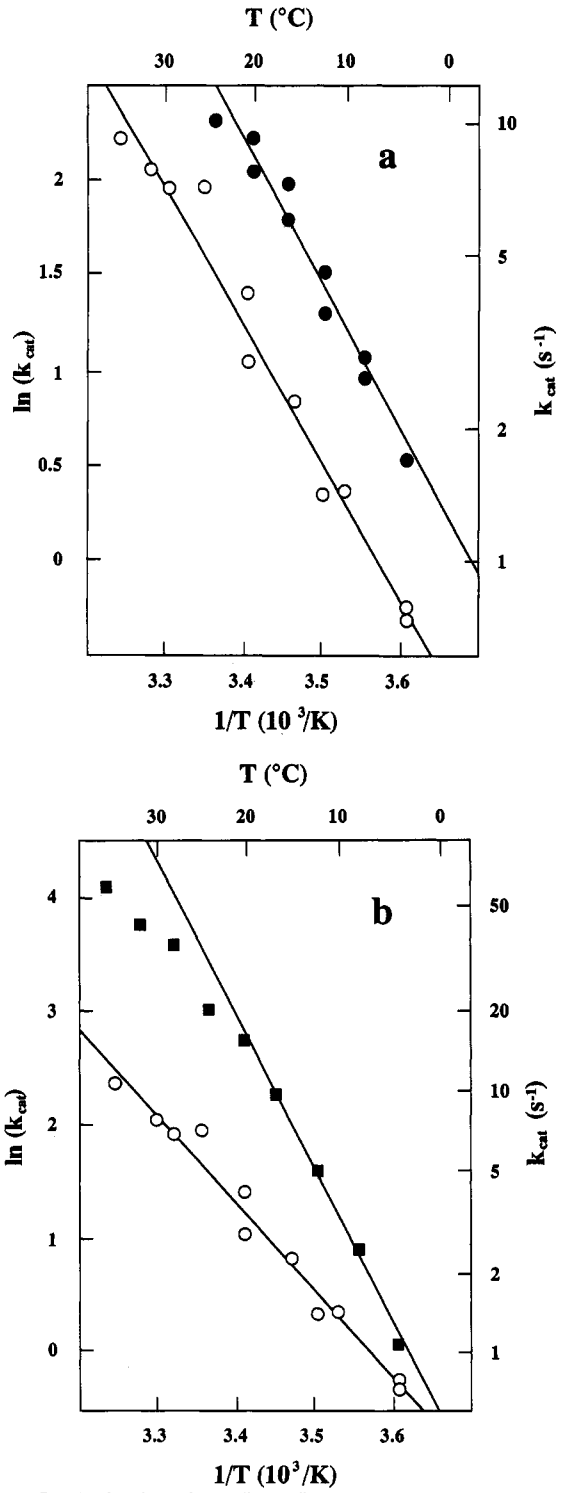

FIGURE 2: Arrhenius plots of k_{cat} for activated native and cross-linked myofibrils (a) and for cross-linked actoS1 and cross-linked myofibrils (b). Native and cross-linked myofibrils are indicated as (●) and (○), respectively, and actoS1 as (■). For further details, see Results.

below). What the experiments in Figure 1 do show is that with both native and cross-linked myofibrils the burst sizes remain high at the higher temperatures. This is different for the situation with cross-linked actoS1 where the P_i burst size decreases with the temperature (see below).

The temperature dependencies of k_{cat} with the myofibrillar and actoS1 ATPases are illustrated in Figure 2. Here it was the precision of the steady state that was important, and with cross-linked myofibrils and actoS1, the experiments were carried out for several tens of seconds at relatively high ATP

Table 1: Temperature Dependencies of the Steady-State ATPases of Certain Muscle Systems[a]

system	$\Delta H^{\ddagger}$ (kJ mol^{-1})	k_{cat} (s^{-1}) at 4 °C	20 °C	39 °C[b]
dispersed molecules				
S1[c]	58	0.02	0.08	0.35
actoS1[d]	103	0.8	8.5	~100
cross-linked actoS1	109	1.1	15	~70
myofibrils				
$-Ca^{2+}$ [c]	57	0.02	0.07	0.30
$+Ca^{2+}$	62	1.7	8.3	~40
cross-linked + Ca^{2+}	60	0.8	3.5	14

[a] For full experimental details, see the text. [b] Extrapolated from Arrhenius plots (Figure 2). [c] From Herrmann et al. (1992). [d] Low ionic strength buffer (Tesi et al., 1991).

Table 2: Comparison of the Mg^{2+}-ATPase Activities of Certain Muscle Systems at Different Temperatures

	ATPase[a] (s^{-1})			
		myofibrils + Ca^{2+}		
temp (°C)	muscle fibers[b]	cross-linked	native	actoS1
5	1 (Brenner, 1988)	0.85	1.7	1.3
10	0.84 (Pate et al., 1993)	1.3	3.4	3.2
13	1.9 (Ferenczi et al., 1984)	1.8	4.2	5.1
15	1.8 (Glyn & Sleep, 1985)	2.2	5.1	7
20	3.2 (Webb et al., 1986)	3.5	8.2	15

[a] The values for the myofibrils and actoS1 are k_{cat} as they are expressed in terms of active-site concentration as determined from cold ATP chase experiments. [b] Isometric.

to myosin head ratios: with cross-linked myofibrils, 30 μM ATP plus 1 μM in heads; with actoS1, 1 mM ATP and 2 μM in heads. With the native myofibrils, because of overcontraction, the concentrations were 30 μM ATP plus 3 μM in myosin heads. Also included in Figure 2 are the steady-state rates obtained from Figure 1a; these were carried out at 30 μM ATP plus 6 μM myosin heads. The two sets of experiment agree well.

In the temperature range studied (4–24 °C for native and 4–36 °C for cross-linked myofibrils), the plots were linear, and the energies of activation were similar (Table 1). This similarity is further evidence that the cross-linking of myofibrils does not affect their overall ATPase mechanism (Herrmann et al., 1993).

As illustrated in Figure 2b, the temperature dependencies of cross-linked actoS1 and cross-linked myofibrils are significantly different with $\Delta H^{\ddagger}$ = 109 and 60 kJ mol^{-1}, respectively (Table 1). With cross-linked actoS1, there was a break at about 20 °C. This could be evidence for a change in the rate-limiting step, but a stability problem with the cross-linked actoS1 in the higher temperature range cannot be excluded. With non-cross-linked actoS1 ATPase (at weak ionic strengths), Arrhenius plots are linear and also give high $\Delta H^{\ddagger}$: 150 kJ mol^{-1} (White & Taylor, 1976) and 103 kJ mol^{-1} (Tesi et al., 1991).

The difference in the $\Delta H^{\ddagger}$ between cross-linked myofibrils and actoS1 ATPases is noteworthy. Thus, whereas at 4 °C the k_{cat} values for the two systems are similar, at 20 °C they differ by a factor of about 4.5. The $\Delta H^{\ddagger}$ values for the myofibrillar system are similar and close to that with S1. We note that at 20 °C the k_{cat} for cross-linked myofibrils is similar to that with fibers contracting isometrically (Table 2).

In Table 1 are summarized the $\Delta H^{\ddagger}$ values for several muscle systems for both dispersed molecules and organized systems. Also included are the k_{cat} values for the different systems at

4 °C (temperature of much previous myofibrillar work), 20 °C (temperature of many mechanical studies), and 39 °C (the body temperature of the rabbit). With the un-cross-linked myofibrils, the highest experimentally obtained k_{cat} was at 24 °C. Therefore, the extrapolation to 39 °C must be taken with caution as a "break" in the 24–39 °C range cannot be excluded.

We now continued our comparison of the myofibrillar and actoS1 ATPases by studying the ATP binding kinetics (steps 1 and 2, Scheme 1), the cleavage step (step 3), and the products release step (step 4).

ATP Binding Kinetics. The ATP binding kinetics were obtained from cold ATP chase experiments. To ensure high precision and to facilitate the measurements, the experiments were carried out under single-turnover conditions at low reagent concentrations and using the time delay flow quench apparatus. Under these conditions, it is the concentration of the myosin ATPase sites that determines the kinetics, and since $k_{-2} \ll k_{cat} \ll k_2$ (Herrmann et al., 1993), the obtained kinetics of tight ATP binding are given by $k = k_2[M]/([M] + K_1)$ where K_1 and k_2 refer to Scheme 1 and M is the myosin head ATPase site concentration. Since the reagent concentrations are low, $K_1 \gg [M]$. Therefore, $k = (k_2/K_1)[M]$. Thus, a plot of k versus [M] is linear with slope k_2/K_1, the second-order binding constant for ATP. The method is illustrated in Figure 3. Figure 3a shows a typical ATP chase experiment at 4 °C with activated un-cross-linked myofibrils (0.05 μM in myosin heads). Since the myofibrillar preparation used titrated 0.43 mol of ATPase site/mol of myosin heads, the active-site concentration used was 0.0215 μM. The data were fitted to a single exponential, giving $k = 0.013\ s^{-1}$. Figure 3b shows the dependence of k on the ATPase site concentration; the plot is linear, and from the slope, $k_2/K_1 = 0.68\ \mu M^{-1}\ s^{-1}$. This is in reasonable agreement with the 1 $\mu M^{-1}\ s^{-1}$ found under multiturnover conditions (Houadjeto et al., 1992). In those experiments, the reagent concentrations were much higher than here, and the values of k obtained were more than 1000 times higher.

The temperature dependence of k_2/K_1 for native myofibrils is shown in Figure 4 and gives an apparent $\Delta H^{\ddagger}$ of 33 kJ mol^{-1}.

Cleavage and Release of Products Steps. The kinetics of the cleavage and release of products steps were obtained from P_i burst experiments. These are carried out in the millisecond time range and require the continuous flow quench apparatus. Because of problems with cross-linked myofibrils settling out during the length of time needed with this apparatus, especially at temperatures >20 °C, the experiments were restricted to a comparison of Ca^{2+}-activated native myofibrils and cross-linked actoS1.

Typical time courses for the hydrolysis of ATP by myofibrils and cross-linked actoS1 at 4 °C are shown in Figure 5. By the cold ATP chase method, the myofibrils titrated 0.4 and the actoS1 0.6 mol of ATPase site/mol of myosin head. The P_i burst sizes and steady-state ATPase activities appear to be similar, in agreement with our first impression (Houadjeto et al., 1991). Each time course consists of three phases: a lag, a burst, and finally a steady-state phase. However, kinetic analysis revealed a significant difference in the kinetics of the cleavage step. In the analysis, the lag phases were taken to be a manifestation of the initial ATP binding kinetics (k) and the kinetics of the burst, of k, $k_3 + k_{-3}$, and k_4 (Scheme 1). The presence of the lag phases shows that under the conditions used, the ATP binding and P_i burst kinetics are similar and taken together with the steady-state rates and active-site

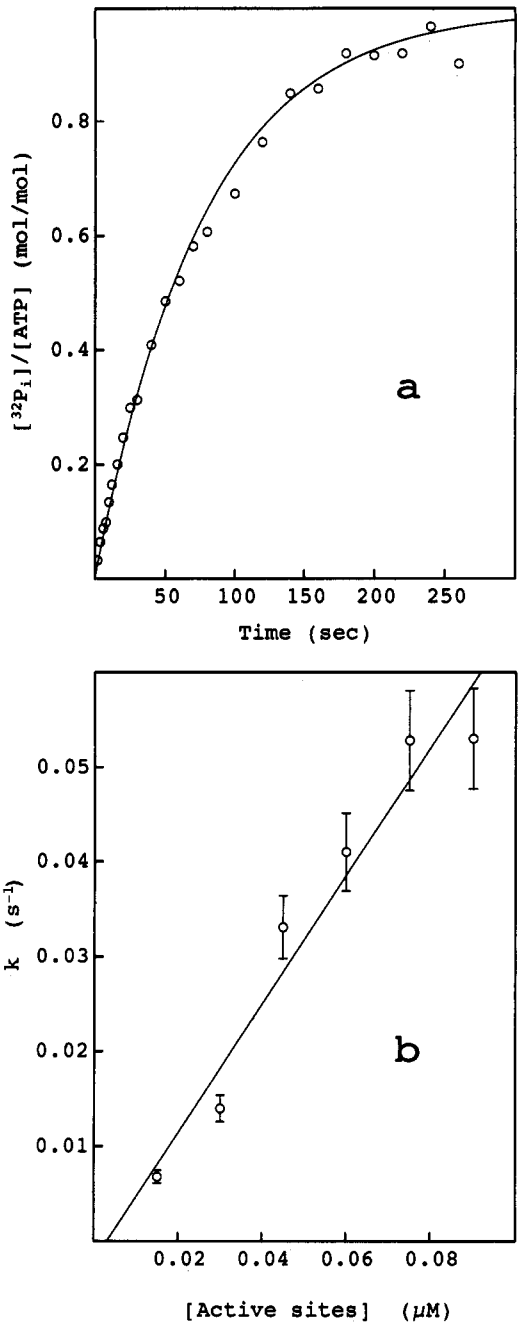

FIGURE 3: Cold ATP chase experiments with activated native myofibrils under single-turnover conditions. (a) Typical experiment. The reaction mixtures (0.05 μM in myosin heads + 0.005 μM [γ-^{32}P]-ATP) were quenched in 20 mM cold ATP, incubated on ice for 2 min, and quenched in acid, and [^{32}P]P_i was determined. The points were fitted to a single exponential of $k = 0.013\ s^{-1}$. (b) Dependence of the kinetics of binding of ATP to myofibrils (k) on the myosin site concentration. The slope gives a second-order binding constant for ATP = 0.68 ± 0.05 $\mu M^{-1}\ s^{-1}$.

concentrations they allowed estimates for k_2/K_1, $k_3 + k_{-3}$, and k_4 for myofibrils and actoS1. These constants are summarized in Table 3.

With myofibrils, as found previously (Houadjeto et al., 1992), the size of the P_i burst is very similar to that found in a cold ATP chase experiment carried out under the same conditions (result not shown). With cross-linked myofibrils too, the two burst sizes were very similar (Herrmann et al., 1993). The similarity can be explained by a large equilibrium constant for the cleavage step, K_3.

With cross-linked actoS1, the P_i burst size is smaller than the amplitude of the burst phase in the chase experiment (data

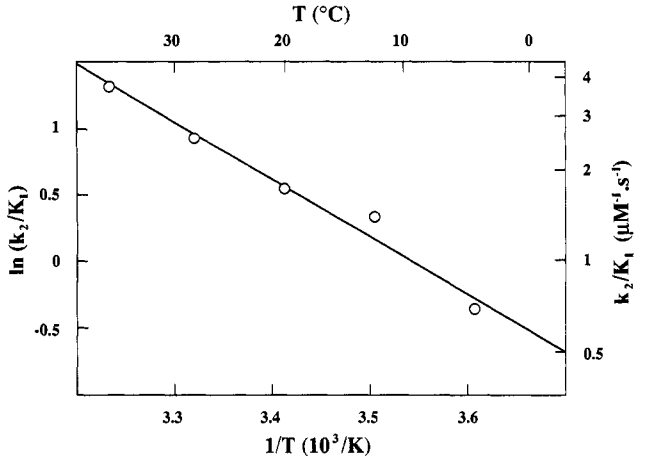

FIGURE 4: Arrhenius plot of the second-order binding constant (k_2/K_1) of activated native myofibrils. The slope gives an apparent ΔH^* of 33 kJ mol^{-1}.

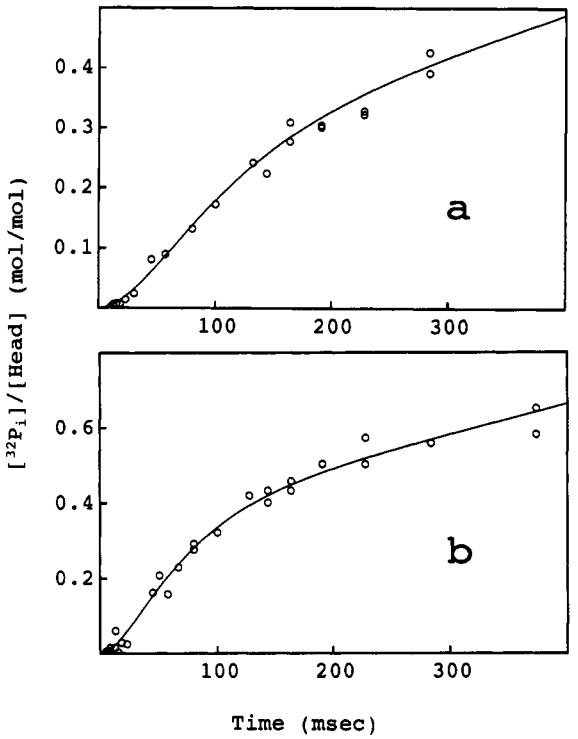

FIGURE 5: Time courses for P_i burst experiments with cross-linked actoS1 (a) and with activated native myofibrils (b) at 4 °C. The reaction mixtures [(a) 6 μM in S1 + 20 μM [γ-^{32}P]ATP; (b) 10 μM in myosin heads + 50 μM [γ-^{32}P]ATP] were quenched in acid, and [^{32}P]P_i was determined. The curves were obtained by computer simulation using Scheme 1 and the constants in Table 3.

Table 3: Certain Kinetic Constants for Ca^{2+}-Activated Myofibrillar and Cross-Linked ActoS1 ATPases at 4 °C[a]

constant	myofibrils	actoS1
k_2/K_1 (μM^{-1} s^{-1})	0.7	about 1[b]
k_3 (s^{-1})	15	8
K_3	>10	1.3
k_4 (s^{-1})	2	2.3
k_{cat} (s^{-1})	1.7	1.1

[a] For experimental conditions and further details, see the text. [b] Houadjeto et al. (1992).

not shown), which is explained by a smaller K_3 than with myofibrils (Table 3). It is noteworthy that the K_3 for cross-linked actoS1 is identical to that found with S1 under the same conditions (Houadjeto et al. 1992).

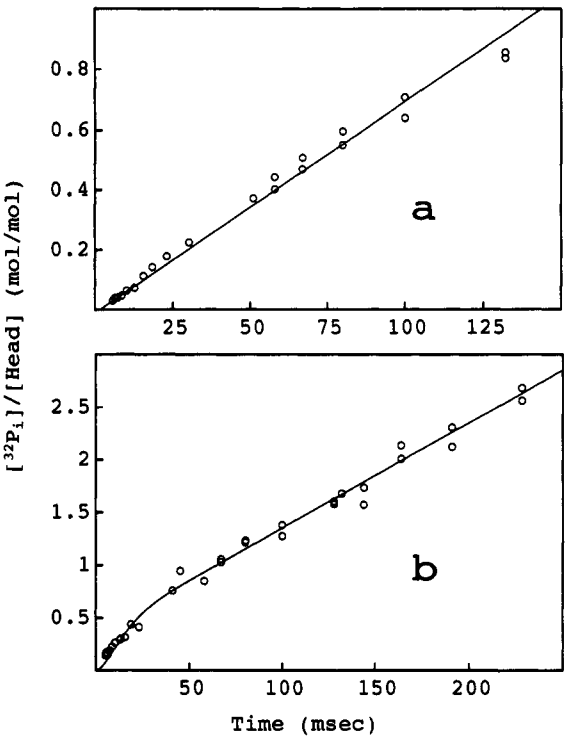

FIGURE 6: Time courses for P_i burst experiments with cross-linked actoS1 at 20 °C (a) and with activated myofibrils at 25 °C (b). The reaction mixtures [(a) 6 μM in S1 + 20 μM [γ-^{32}P]ATP; (b) 4.5 μM in myosin heads + 48 μM [γ-^{32}P]ATP] were quenched in acid, and [^{32}P]P_i was determined. The data for actoS1 were fitted to a straight line as described in the text. The curve with the myofibrils was obtained by computer simulation using Scheme 1 and the constants in Table 4.

At higher temperatures (25 °C for myofibrils, 20 °C for cross-linked actoS1), the two P_i burst progress curves were very different (Figure 6). With the myofibrils, there was a large P_i burst which confirms the steady-state experiment in Figure 1. With actoS1, the data were fitted to a straight line giving an intercept of –0.02 ± 0.01 mol of P_i/mol of S1. The steady-state rate obtained was 14 s^{-1}; this is somewhat larger than the 9 s^{-1} calculated from a k_{cat} of 15 s^{-1} (Figure 2 and Table 1) and an ATPase site titration of 0.6 mol of site/mol of S1 (from a cold ATP chase experiment, data not shown). A slightly better fit was obtained by fitting the data to a lag–burst–steady-state situation, but the burst size remained small (0.05 ± 0.03 mol of P_i/mol of S1). In this fit, the steady-state rate was reduced to 12 s^{-1}, and the curve intersects the origin. In any event, the burst size with actoS1 at 20 °C is clearly very small. This is in accord with Rosenfeld and Taylor (1984), who could not detect a P_i burst at 20 °C with un-cross-linked actoS1. At 15 °C too, a P_i burst was not detected with cross-linked actoS1 (Biosca et al., 1984a,b). Thus, at the higher temperatures, the predominant complexes in the steady-state ATPase with myofibrils involve the products, but with actoS1, they presumably contain ATP. The absence of a P_i burst with actoS1 can be explained by the kinetics of the products release step (k_4) being faster than those of the re-formation of ATP (k_3). This would be in agreement with Webb and Trentham (1982), who showed that with cross-linked actoS1 and [γ-$^{18}O_3$]ATP there was little exchange: only [$^{18}O_3$]P_i was produced.

We now determined the temperature dependencies of the forward constant for the cleavage step (k_3) and the release of products steps (k_4) with un-cross-linked myofibrils. In all cases, $K_3 > 10$ so k_{-3} could not be calculated with precision. In addition to the experiments at 4 °C (Figure 5b) and 25 °C

Table 4: Temperature Dependencies of the Kinetic Constants for ATP Binding and Cleavage and for Products Release with Ca^{2+}-Activated Myofibrils[a]

constant	$\Delta H^{\ddagger}$ (kJ M^{-1})	values at temp (°C)[b] of 4	10	20	25	39
k_2/K_1 (μM^{-1} s^{-1})	33.3	0.7	1.05	1.8	2.45	(4)
k_3 (s^{-1})	54	17	45	(80)	100	(330)
k_4 (s^{-1})	73	2	4.5	(12.5)	20	(80)
k_{cat} (s^{-1})	62	1.7	3.4	8.4	13.1	(40)

[a] The buffer was 50 mM Tris–acetate, 0.1 M potassium acetate, 5 mM KCl, and 0.1 mM $CaCl_2$, pH 7.4. [b] Values in parentheses are extrapolated; see the text.

(Figure 6b), a P_i burst experiment was carried out at 10 °C (result not illustrated). The results are summarized in Table 4 together with extrapolated values for k_3 and k_4 at 20 and 39 °C. Needless to say, the values at 39 °C must be taken with caution as curvatures in the dependencies cannot be excluded in the higher temperature range (as for actoS1, Figure 2b). Also included in Table 4 are values for k_2/K_1 (from Figure 4) and k_{cat} (Figure 2).

It is noteworthy that the $\Delta H^{\ddagger}$ values for k_3, k_4, and the composite k_{cat} are similar but only about half that of the k_{cat} for actoS1.

DISCUSSION

Our purpose here was to test actoS1 and Ca^{2+}-activated myofibrillar ATPases (cross-linked or not) as models for muscle fiber ATPase, and with this aim, we compared certain of their kinetic properties in a buffer of near-physiological ionic strength. Included in the comparison are literature data obtained at ionic strengths similar to that used here. The comparison is a challenge in that four materials are considered: actoS1, cross-linked actoS1, myofibrils, and cross-linked myofibrils. Obviously one cannot carry out all types of experiment with all of these materials, and the comparison must be considered within this limitation. In the following, "actoS1" and "myofibrils" refer to the un-cross-linked materials.

As previously [e.g., see Herrmann et al. (1993)], we interpret our data by Scheme 1 in which k_4 refers to the release of both products (P_i and ADP). This is, of course, a simplification as P_i and ADP are almost certainly released sequentially (as for myosin), but we have little information on these steps. We conclude by discussing which of the *in vitro* models most closely mimics muscle fiber ATPase.

ATP Binding Kinetics. At 4 °C, the second-order binding constants for ATP binding, k_2/K_1, with myofibrils and cross-linked actoS1 were similar (Table 3). With the myofibrils, the $\Delta H^{\ddagger}$ for k_2/K_1 is rather low at 33 kJ mol^{-1} (Figure 4). This could be explained by k_2 and K_1 increasing about equally with temperature. With S1, the apparent $\Delta H^{\ddagger}$ for k_2/K_1 was large since k_2 has a high $\Delta H^{\ddagger}$ (120 kJ mol^{-1}) and K_1 a low $\Delta H^{\ddagger}$ (45 kJ mol^{-1}; Biosca et al., 1983).

At 20 °C too, k_2/K_1 values with myofibrils and actoS1 appear to be similar. With myofibrils, k_2/K_1 (obtained from cold ATP chase experiments) was 1.8 μM^{-1} s^{-1}. With actoS1, the ATP binding kinetics were obtained from ATP-induced dissociation. By this method, White and Taylor (1976) and Millar and Geeves (1983) obtained a second-order constant for ATP binding of 1.5 μM^{-1} s^{-1} at 20 and 25 °C, respectively. Further, as with myofibrils, the $\Delta H^{\ddagger}$ for the ATP binding kinetics was low (Millar & Geeves, 1983). This similarity in the ATP binding kinetics with actoS1 and myofibrils is in accord with the observation of Goldman et al. (1984) that the rate of ATP binding and cross-bridge detachment is similar in the filament lattice (in their case, muscle fibers) to that in solution.

P_i Burst Experiments. P_i burst experiments are important in that they can tell us which are the predominant states on a reaction pathway. It is the interactions of the different states and the chronology of these interactions that are implicated in muscle contraction.

At 4 °C both with cross-linked actoS1 and, in particular, with myofibrils (whether cross-linked or not), there were large P_i bursts (by "large" we mean that the amplitudes of the bursts were similar to the active-site concentrations as determined in ATP chase experiments). This tells us that at 4 °C product states predominate on both pathways. Further, the steady-state activities of the two ATPases were similar and close to that found with fibers (Table 2). Therefore, had we restricted ourselves to experiments at this temperature, we would have concluded that actoS1 and myofibrils are equally good as models for muscle fiber ATPase, and we came to this conclusion in our preliminary work at 4 °C (Houadjeto et al., 1991).

However, at higher temperatures, this similarity vanished. For example, at about 20 °C—a temperature at which much kinetic work with muscle fibers has been carried out—the P_i progress curves with cross-linked actoS1 and myofibrils are very different (Figure 6).

With cross-linked actoS1 (Figure 6a), the size of the P_i burst is insignificant, which shows that either the ATP binding kinetics or the cleavage step is rate-limiting. It is unlikely that the ATP binding kinetics are rate-limiting. At 20 μM ATP and with k_2/K_1 = 1.5 μM^{-1} s^{-1} (see above), ATP binds with a constant of 30 s^{-1}. This is considerably higher than the k_{cat} of 15 s^{-1} (Table 1). Further, if the ATP binding kinetics were rate-limiting, k_{cat} would be very sensitive to the ATP concentration in the range used, and this is not the case (Biosca et al., 1985). It appears, therefore, that with cross-linked actoS1 at 20 °C ATP states predominate in the steady state. Rosenfeld and Taylor (1984) came to the same conclusion with actoS1 in a low ionic strength buffer.

With myofibrils (cross-linked or not), there were large P_i bursts at all the temperatures tested (Figures 1, 5b, and 6b). Sizable P_i bursts were also obtained (at 20 °C) by White (1985) with relaxed myofibrils and by Ohno and Kodama (1991) with activated myofibrils. A large P_i burst was obtained with muscle fibers contracting isometrically (at 13 °C) (Ferenczi et al., 1984; Ferenczi, 1986). With myofibrils, the rate constant of the burst is about 100 s^{-1} (Figure 5b), which is in agreement with the greater than 60 s^{-1} estimated for the muscle fibers by Ferenczi (1986). Taken together, these results show that myosin head products states predominate on the reaction pathways of both myofibrillar and fiber ATPases.

The different burst sizes with cross-linked actoS1 and myofibrils (cross-linked or not) at the higher temperatures show that the rate-limiting steps on the two pathways are different—with actoS1, it appears to be the cleavage step; with myofibrils, it is the products release step. The overall ATPases of the two systems are therefore controlled by different steps which could explain the different $\Delta H^{\ddagger}$ values for the two k_{cat} (Figure 2 and Table 1).

The different $\Delta H^{\ddagger}$ values for the kinetics of cleavage steps with cross-linked actoS1 and myofibrils are noteworthy. With cross-linked actoS1, the $\Delta H^{\ddagger}$ is large (109 kJ mol^{-1}, given by k_{cat}, Table 1) whereas with myofibrils it is small (about 55 kJ mol^{-1}, from P_i burst kinetics, Table 4). This is further evidence that the kinetics of the cleavage step with actoS1 and myofibrils are different (Table 3).

It is intriguing that whereas the kinetics of the cleavage step with actoS1 (cross-linked or not) and S1 appear to be similar [cross-linked actoS1, this study and Houadjeto et al. (1992); actoS1, Rosenfeld and Taylor (1984)], they are different from those with myofibrils, whether these are relaxed or activated [this study and also see Herrmann et al. (1992)]. This difference could be related to differences in the load in the different systems. Alternatively, it could be due to differences in the chemical kinetics of the dispersed S1 and actoS1 and the organized myofibrillar systems.

A Model for Muscle Fiber ATPase: ActoS1 or Myofibrils? Since there is so little detailed chemical kinetic data available in muscle fibers, we cannot make a detailed comparison with the myofibrillar and actoS1 ATPases. Further, we have little data on the kinetic details of the sequential release of the products, P_i or ADP, on any of these systems.

The most telling kinetic parameter is k_{cat}. At 20 °C, the k_{cat} of cross-linked myofibrils is very similar to that of fibers (Table 2). In both of these systems, the contraction is isometric; i.e., it occurs at maximum load. As the load is decreased, so k_{cat} increases: 3.5 s^{-1} with cross-linked myofibrils (external and internal load), 8.3 s^{-1} with unheld myofibrils (internal load only), and 15 s^{-1} with actoS1 (presumably no load). This may be a manifestation of the Fenn effect (Fenn, 1924), namely, that mechanical factors such as stress and strain on the force producing cross-bridges may limit the ATPase rate in the isometric cross-bridge cycle [for a discussion, see Huxley (1988)].

Another interesting kinetic parameter is the P_i burst size: at 20 °C, both myofibrils and fibers have large P_i bursts whereas at 25 °C the burst size with actoS1 was insignificant. Therefore, the chemical kinetic evidence is that the myofibrils are a better *in vitro* model than actoS1 for the muscle fiber, at least in the isometric condition. It goes without saying that the myofibril is a more complete system than actoS1: it contains not only the regulatory system and structural elements of the fiber but also the whole is highly organized.

The kinetic similarity of cross-linked myofibrils and muscle fibers contracting isometrically is especially noteworthy. There is also a structural similarity. Thus, from their studies on the rotational dynamics of the actin-bound myosin heads in myofibrils, Berger and Thomas (1993) concluded that "EDC cross-linked myofibrils are an excellent analogue of isometrically contracting muscle fibers". Very recently Friedman and Goldman (1993) achieved force measurements with myofibrils. This important advance means that it is now possible to carry out detailed mechanical and chemical kinetic experiments on the *same organized muscle system under identical experimental conditions*, an analysis which has until now been difficult.

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