Biochimica et Biophysica Acta, 658 (1981) 308—317

BBA 69233

STUDIES ON THE SPECIAL PROPERTIES OF ACTOMYOSIN IN THE GEL FORM

I. UNUSUAL FEATURES OF THE Mg^{2+}-ATPase ACTIVITY

HARVEY M LEVY and WAI WON MOY

Department of Physiology and Biophysics, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, NY 11790 (U S A)

(Received December 10th, 1980)

Key words Actomyosin, Mg^{2+}-ATPase activity

Summary

The hydrolysis of MgATP by actomyosin gel at low ionic strength is known to show two unusual features (1) an Arrhenius plot with a shallow slope in the higher temperature range (35—16°C) and a steep slope in the lower temperature range (16—0°C), (2) a rate curve of hydrolysis that begins with a 'burst' and falls to a lower steady-state level. Both of these can now be interpreted in terms of a specific, relatively slow transformation in the gel ($t_{1/2}$ = 9 s at 25°C), induced by the binding of MgATP to the active sites of the myosin filaments.

In the rate curves, this transformation is reflected in the transition from the burst rate (catalyzed by the original gel) to the steady-state rate (catalyzed by the modified gel) Importantly, this transition does not occur to a significant extent at low temperatures. Thus, in the typical nonlinear Arrhenius plot, where steady-state rates are used, the shallow slope in the high temperature range is a property of the modified gel, whereas the steep slope at low temperatures is a property of the original gel. Consistent with this interpretation, when the burst rates (presumably due to the original gel) were used in the high temperature range (and when substrate inhibition of hydrolysis by high levels of MgATP was avoided), the Arrhenius plot was linear over the entire temperature range (40—0°C), the steep slope of this plot gives a high apparent heat of activation (25—30 kcal), similar to that reported for actin-activated hydrolysis by the soluble subfragment, heavy meromyosin. It is the steady-state form of the gel at high temperatures that gives a low apparent heat of activation (6—10 kcal).

Abbreviation EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid

It was found that the regulatory proteins with calcium activate hydrolysis by the original form but have no effect on the steady-state form of the gel. Oxygen exchange measurements made during the burst and steady state at 25°C indicate that the mechanism of hydrolysis is essentially the same for both, but that there is a higher effective actin concentration around the myosin sites in the original form.

Introduction

The nonlinear Arrhenius plot of actomyosin Mg^{2+}-ATPase [1] and also of myosin under certain conditions led to the hypothesis some years ago [2] that myosin undergoes a temperature-dependent conformational change. According to this concept, the stable form of actomyosin depends on temperature; above a narrow transitional range near 16°C, there is one stable form that catalyzes hydrolysis through a rate-limiting step with a low heat of activation (6—10 kcal) and below this nominal transition temperature, there is a different stable form that catalyzes hydrolysis through a rate-limiting step with a much higher heat of activation (25—30 kcal), thus explaining the markedly greater slope of the Arrhenius plot at low temperatures.

Actomyosin gel also consistently shows another unusual enzymatic feature. Hydrolysis, when it is started by the addition of MgATP, begins at a relatively high rate and then, over a period of half a minute or so, falls to a lower steady-state level. This phenomenon of the actomyosin 'burst' was discovered by Hasselbach and Weber in 1954 [3] It now appears, as we will discuss, that this burst is a manifestation of the same transformation that causes the nonlinear Arrhenius plot. Both phenomena can be explained by a relatively slow substrate-induced conformational change in the myosin filaments of the gel that only occurs to a significant extent at higher temperatures (i e. above about 16°C).

Experimental Procedures

Rabbit back and leg muscle were used for all the protein preparations. Actin was extracted from acetone-dried muscle powder by the method of Spudich and Watt [4]. Myosin was prepared essentially as described by Mommaerts and Parish [5], a step involving ammonium sulfate fractionation was added to obtain actin-free myosin. The purified myosin was precipitated between 40—60% saturation. Natural actomyosin with the regulatory system intact was made as described by Levy and Fleischer [6,7]. To prepare reconstituted actomyosin free of regulatory proteins, myosin was added to actin in a ratio of 4 . 1, slowly while stirring in a solution of 2 mM Tris-HCl (pH 7.4), 0.1 mM dithiothreitol and 0.5 M KCl. The gel was formed by a 10-fold dilution with the same solution containing no KCl. To remove the residual calcium sensitivity, which was always present, the gel was then washed repeatedly with the salt-free solution (usually 4—5 times with 10-times the volume of gel) until the Mg^{2+}-ATPase at 25°C was completely insensitive to either added EGTA or Ca^{2+}. Myofibrils were prepared by the method of Perry and Zydowo [8].

Tropomyosin and the troponin complex were prepared by the methods of Greaser and Gergely [9]. Protein concentrations were determined by the biuret reaction using bovine serum albumin as a standard.

The turbidity changes in the actomyosin gel suspension were measured at 545 nm. The mixture was stirred continuously with a magnetic stirrer and maintained at constant temperature in the sample cell (2 cm light path) of a Zeiss PMQ II spectrophotometer. The reaction was started by adding a small volume (e.g. 0.1 ml) of an ATP solution to 12 ml of the gel suspension through a polyethylene tube attached to a syringe dispenser. The method, routinely used in this laboratory, has been described in detail [6].

ATPase assays using the isobutanol extraction method [10] were done in a medium containing 25 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$, and ATP as specified, with either 1 mM MgEGTA (virtually no free Ca^{2+}) or 1 mM CaEGTA (about 10^{-5} M free Ca^{2+}). The regulated system, in most experiments, contained reconstituted actomyosin with optimal levels of tropomyosin and troponin, this required a ratio of actin/tropomyosin of 7 · 1 and an actin/troponin ratio of 5 : 1. In some cases, as indicated, natural actomyosin was used as the regulated system, with closely similar results.

In experiments where the substrate concentration was in the micromolar range, [^{32}P]ATP was used for measuring the rate of hydrolysis, as described previously [10], with creatine phosphate and creatine phosphokinase added to recycle ADP and maintain a constant level of ATP. [^{32}P]ATP was prepared by the method of Glynn and Chappell [11]. Intermediate oxygen exchange during ATP hydrolysis was measured as previously described [12]. To determine this exchange during the burst, the reaction was quenched with trichloroacetic acid 15 s after the addition of substrate, using a high concentration of actomyosin gel. For exchange during the steady state, the hydrolysis was allowed to go to completion over a reaction time of 1 h, using a low concentration of actomyosin. For measuring the oxygen exchange by superprecipitated actomyosin, the protein was first superprecipitated with unlabelled ATP at 10^{-4} M under standard conditions, washed free of substrate with reaction solution and then used for the labelled experiments.

Results and Discussion

The burst rate of hydrolysis

Fig. 1 shows the characteristic nonlinear rate curve for hydrolysis of MgATP by actomyosin gel at 25° C. There is an initial relatively rapid rate (the burst) that falls within half a minute or so to a slower steady-state level.

The curve shown in Fig 1 is actually a composite of five curves run at different concentrations of ATP from 1 to 20 μM. Each of the curves was normalized to a relative rate of 1.0 at 20 s. The points for all the curves fell randomly within the bars shown at each time point. Other curves obtained at ATP concentrations as high as 5 mM showed the same characteristic change of slope with time. Thus, over a wide range of ATP concentration, the ratio of the initial burst rate to the steady-state rate was constant, and the time for the transition did not change

The rate curve was also the same when the reaction was stopped by rapid fil-

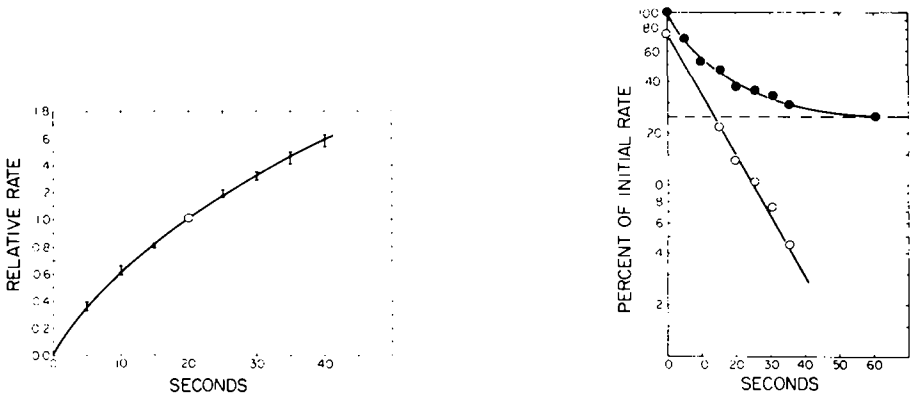

Fig 1. Composite rate curve of hydrolysis by actomyosin gel showing curvature independent of MgATP concentration Conditions 25 mM Tris-HCl (pH 7 4)/25 mM KCl/5 mM $MgCl_2$ The rate was measured at five different ATP concentrations 1, 2, 5, 10 and 20 μM The curves at each substrate level were normalized to a relative rate of 1 0 at 20 s. Values at other times then fell within the indicated bars The concentration of actomyosin gel was 0 1 mg/ml

Fig 2 Apparent first-order rate constant for the gel transformation indicated by the burst (●) Rate of hydrolysis taken from the slope of the composite curve in Fig 1 (○) Corresponding values in upper curve minus the final steady-state value indicated by the dashed line This curve gives an apparent half-time of 9 s for the transition (k = 0 08 s^{-1})

tration (to remove gel from the reaction solution), or when the hydrolysis of [^{32}P]ATP was quenched with unlabelled ATP. The results, in agreement with similar findings by Bowen et al. [13], show that this burst is not due to any protein-bound phosphate released by the acid-quenching normally used.

Assuming that the transition from the initial to the steady state is a first-order reaction, the half-time was estimated as shown in Fig. 2. The upper curve shows how the rate of hydrolysis (the slope of the composite rate curve in Fig. 1) changed with time. For the lower line, points were obtained by subtracting the final steady-state rate from each of the transitional rates, the slope of this line gives 0.08 s^{-1} as the apparent rate constant for the transition ($t_{1/2}$ = 9 s). This is many times slower than any step in the pathway of actin-activated hydrolysis by myosin [22]. In the work reported here, the apparent rate constant for hydrolysis by actomyosin gel at 25°C was 2 s^{-1} during the steady state and 5 s^{-1} or more during the burst.

Thus, neither the rate of MgATP binding to the active site nor the rate of hydrolysis limits the rate of transition from burst to steady state. Instead, as we have suggested [6,7], this appears to depend on a slow conformational change in the gel that is initiated by the binding of substrate. Other observations on the burst phenomenon are all consistent with this interpretation.

While hydrolysis followed its own course, the actomyosin gel, depending on the concentration of ATP, temperature and other conditions, usually changed its form in some obvious way. The gel suspension might for example have cleared (shown a fall in tubidity) or superprecipitated (shown a rise in turbidity) or, in some cases, first cleared and then superprecipitated, or vice versa. (The details of such turbidity changes will be presented and analyzed in the fol-

lowing paper [23].) In any case, the shape of the composite rate curve (in Fig. 1) was not affected by these transformations. For example, in Fig. 1, the gel at the lowest concentration of ATP (1 μM) superprecipitated so slowly that virtually all of it was in the starting form during the ATPase measurements. At intermediate concentrations of ATP, the gel superprecipitated completely in about 1 s so that the protein was in the superprecipitated form for most of the measurements, and at high physiological levels of ATP (1—5 mM), a superprecipitate formed and then appeared to be cleared by the high substrate. The independence of the shape of the rate curve of hydrolysis from superprecipitation was further demonstrated by the presence of a burst rate of hydrolysis immediately after the addition of MgATP even when the protein had been superprecipitated and washed free of substrate prior to the ATPase measurement

Fig. 3 shows that MgATP binding without hydrolysis induced the transformation from burst to steady state. In the bottom curve, ATP was added before calcium and 15 s later the system was activated by the addition of calcium The subsequent hydrolysis showed no burst, from the start, the rate was equal to the usual steady-state rate. As we interpret it, the transformation was complete before the addition of Ca^{2+}. The same linear rate was also obtained when actin-activated hydrolysis was initiated by the addition of actin to a mixture containing myosin and MgATP; and a loss of reduction in the size of the burst can also be obtained by prior addition of inorganic pyrophosphate (unpublished results), or by the addition of ITP prior to measuring the hydrolysis of [^{32}P]ATP [7]. Again, as we interpret it, the transformation in these cases has been completed before the rate measurements begin.

Above 16°C, the burst became more prominent as the temperature was raised, that is, the initial rate increased proportionately more than the steady-state rate On the other hand, below 16°C much less burst was evident. For example at 2°C the rate curve showed no significant fall-off from the starting rate (Fig. 4).

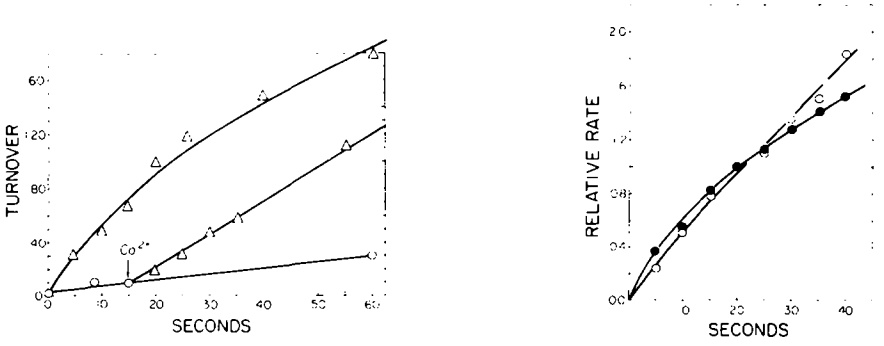

Fig. 3 A comparison of hydrolysis by regulated gel when the reaction is started by the addition of ATP or by Ca^{2+} Conditions were the same as for Fig 1 with 1 mM ATP (Upper curve) With Ca^{2+} present, the reaction was started by the addition of ATP at zero time (Lower curves) With Ca^{2+} absent, the reaction was started by the addition of ATP at zero time No Ca^{2+} added subsequently, Ca^{2+} added at indicated time

Fig 4 Normalized rate curves of hydrolysis at high (25°C) compared to low (2°C) temperature the absence of a burst at low temperature Conditions same as for Fig 1, with 20 μM ATP (○) 2°C, (●) (25°C)

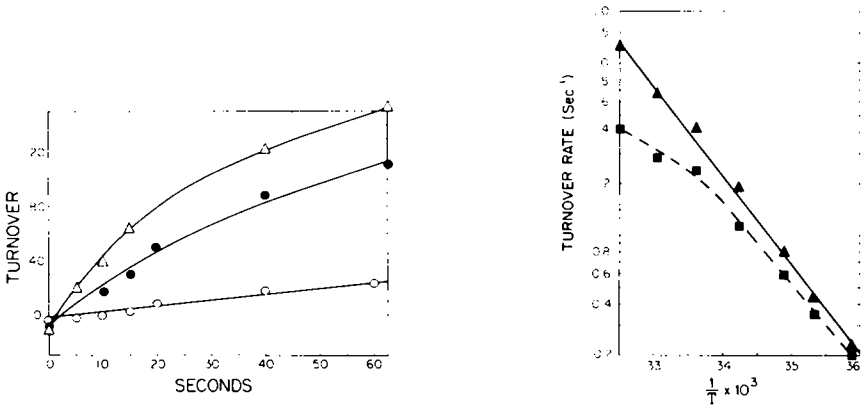

Fig 5 The effect of the regulatory proteins on the rate of actomyosin Mg^{2+}-ATPase at 25°C Conditions same as for Fig 1, with 1 mM ATP (△) With the regulatory proteins and calcium, (●) without the regulatory proteins, with or without calcium, (○) with the regulatory proteins in the absence of calcium

Fig. 6. Arrhenius plots of regulated actomyosin Mg^{2+}-ATPase measured during the burst and steady state General conditions same as for Fig 1 The values for V at each temperature are extrapolated from measurements at substrate levels at or below K_m Upper curve (▲), the initial burst rate of hydrolysis, lower curve (■), the steady-state rate

Above 16°C, the regulatory proteins with calcium increased the burst rate of hydrolysis, but had no significant effect on the steady-state rate (Fig. 5). Although activation of hydrolysis by the tropomyosin-troponin-Ca^{2+} complex has been observed in a number of studies, it has not been evident that this is limited to the burst phase. Below 16°C, the tropomyosin-troponin-Ca^{2+} complex had different effects depending on the concentration of MgATP. At relatively low concentrations of MgATP, just sufficient to saturate the high-affinity active sites for hydrolysis, the tropomyosin-troponin-Ca^{2+} complex activated hydrolysis; at much higher concentrations of MgATP, the tropomyosin-troponin-Ca^{2+} complex inhibited hydrolysis. It is known that the Mg^{2+}-ATPase of actomyosin is inhibited by high levels of MgATP. The rate of hydrolysis becomes progressively lower as MgATP concentration is raised above an optimal concentration into a much higher range. At each temperature, the level of substrate required for this effect is an order of magnitude higher than the level required to saturate the active sites for hydrolysis and, at a constant fixed level of MgATP, the inhibition increases as the temperature is lowered. The tropomyosin-troponin-Ca^{2+} complex apparently potentiates this effect. Thus, for example, at a low temperature with a high level of MgATP, the addition of the tropomyosin-troponin-Ca^{2+} complex (or tropomyosin alone) inhibited hydrolysis. We assume that substrate inhibition of this type is caused by swelling or dissolution of the gel, induced by the binding of MgATP to a hypothetical low-affinity site on myosin. The effect of such swelling is to decrease the effective concentration of actin around the myosin active sites within the gel. When this concentration falls into a range where it is rate-limiting, the hydrolysis is inhibited. Accordingly, the effect does not occur at moderate to low levels of substrate simply because binding to the low-affinity sites is negligible, and it does not occur in non-gel systems of actin-activated hydrolysis, for

example where soluble fragments of myosin are used, because in these cases the effective actin concentration is fixed at the actin concentration in solution

The nonlinear Arrhenius plot related to the burst

In the past, when steady-state rates were used for the high temperature points, Arrhenius plots for the Mg^{2+}-ATPase of natural actomyosin have been non-linear, resembling the bottom curve of Fig. 6 [1,14]. When it became apparent that the rate curves of hydrolysis below 16°C showed little or no burst, we reasoned that the constant rates at low temperature might correspond to burst rates, rather than steady-state rates, at high temperatures. If so, on an Arrhenius plot, these burst rates should fall in line with the low temperature points. The top plot in Fig. 6 shows that this is the case. When the burst rates were used, the Arrhenius plot was linear. Thus, the low-temperature form of actomyosin exists for only a short time at high temperature after the addition of MgATP, and it is responsible for the burst.

To obtain a linear Arrhenius plot with regulated actomyosin, in addition to using the burst rates above 16°C, it was also necessary to avoid the use of MgATP at levels that cause substrate inhibition. For example, the V values used for the linear (upper) plot of Fig. 6 were obtained by extrapolation from measurements made at levels of MgATP no higher than the apparent K_m at each temperature. When this was not done and the rates were directly measured at, e.g., 1 mM ATP, the Arrhenius plot curved downward at low temperatures (Fig. 7, open triangles). Barany [15], in a comparative study on the temperature dependence of actomyosin Mg^{2+}-ATPase, observed a similar effect and also

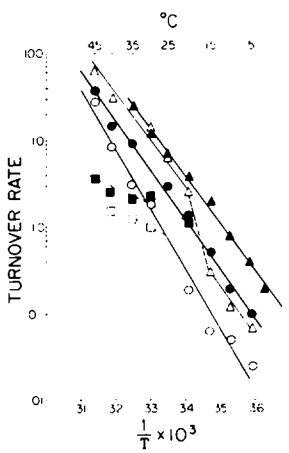

Fig. 7 Arrhenius plots of actomyosin Mg^{2+}-ATPase in the burst and steady-state phases of hydrolysis with and without the regulatory proteins and calcium. (———) Initial burst rates (within the first 5 s of reaction) (- - - - - -) Steady-state rates (measured between 30 and 60 s of the reaction) General conditions as given in Fig. 1 ATP concentration was 1 mM, except when indicated (●) Non-regulated gel, with or without calcium (○) Regulated gel without calcium (△) Regulated gel with calcium, note the substrate inhibition at low temperatures, where the values fall below those for nonregulated gel (●) (▲) Regulated gel with calcium, with ATP at each temperature kept below K_m and the plotted values for V obtained by extrapolation Note that the V holds the line at low temperatures without the substrate inhibition evident at 1 mM ATP (△) (■) Steady-state values for regulated gel with calcium or non-regulated gel (□) Steady-state values for regulated gel in the absence of calcium

ascribed it to dissociation of actin from myosin. He was able to minimize this kind of inhibition with certain muscle proteins by using a very low salt concentration.

In general, when the burst rates were used at higher temperatures and when substrate inhibition was avoided, the Arrhenius plots for actomyosin ATPase, were all linear and all had the same relatively steep slope, giving the same high apparent heat of activation (about 25 kcal). The line for regulated actomyosin in the presence of calcium lies above the line for the nonregulated system, reflecting the activation of the burst-type hydrolysis by the tropomyosin-troponin-Ca^{2+} complex. On the other hand, the steady-state rates of hydrolysis that form the flat upper portion of the usual Arrhenius plot (shown by the dashed lines in Fig. 7) were not significantly different with or without regulatory protein. The line for regulated gel in the absence of calcium lies below the others, indicating the inhibitory effect of the regulatory proteins under these conditions. In this work and in other published studies, however, this inhibition is not as complete as it must be in relaxed muscle. The relatively high residual activity in the absence of calcium, which has the same heat of activation as the fully activated system, is partly due to the low salt concentration It may also indicate a certain amount of calcium-insensitive actin and/or myosin in these in vitro preparations. Beyond this, however, we believe it indicates that not all conditions of the natural control system are fully met in reconstituted actomyosin models of this kind.

It is now evident that the degree of curvature in the Arrhenius plots of actomyosin ATPase will depend on factors such as (a) The integrity of the original gel that supports burst hydrolysis. (b) The time period for the measurement of the rates at higher temperatures, the longer this time the more the estimated rate represents the slow steady-state value and the flatter the upper part of the curve. (c) The concentration of MgATP; the higher this concentration, beyond a certain point, the greater is the extent of substrate inhibition, and at a fixed concentration of MgATP, this kind of inhibition increases as temperature is lowered, thereby causing a concave downward curvature in the plot. (d) The presence or absence of the regulatory proteins, these proteins with calcium potentiate substrate inhibition and also specifically activate burst hydrolysis without affecting the steady-state, both of which effects exaggerate curvature in the usual Arrhenius plot. These considerations are directly related to the findings of Hartshorne et al. [14] that Arrhenius plots for nonregulated actomyosin are linear but characteristically nonlinear for regulated actomyosin or fibrils. As a first approximation, this would be expected under the conditions of their experiments (a fixed high level of MgATP of 5 mM and rates estimated from a single long time point of 5 min).

Oxygen exchange and the mechanism of hydrolysis during the burst and steady-state

During the hydrolysis of MgATP by myosin, there is an exchange of oxygen between water and enzyme-bound nucleotide of certain intermediates that form along the enzymatic phathway [16,17]. Oxygen exchange is believed to occur by a repeated cycle of cleavage and reverse cleavage of the bound nucleotide. During cleavage, an oxygen atom from water is added to the P_γ of bound

MgATP, during reverse cleavage an oxygen atom returns to the water. It is assumed that at some stage the oxygens of P_γ can change position in space, i.e. effectively rotate about the phosphorus atom. Because of this scrambling, the oxygen lost by reverse cleavage can, by chance, be different from the one that enters during cleavage and each time this happens, an oxygen atom from water (labelled with ^{18}O) is incorporated into the bound phosphate that is ultimately released as P_i. Actin activation decreases the extent of oxygen exchange by decreasing the lifetime of the exchanging intermediates. A fuller description of the effects of actin on oxygen exchange has appeared in recent papers on this subject [12,17–20]

Our main interest here in measuring this reaction during the initial phase of hydrolysis (within the first 15 s after the addition of MgATP) was to test whether there is any intrinsic qualitative difference in the mechanism of hydrolysis during the burst compared to the steady state. The results show that this is not the case. There was no significant difference in the estimated rate of oxygen exchange catalyzed during the burst and steady-state periods, taking into account the shorter turnover time in the burst phase Specifically, the phosphate produced during the burst phase (the first 15 s of reaction) contained an average of 1.9 ^{18}O-labelled oxygen atoms (from water) per P_i molecule, and that produced during the steady state contained 2.3 ^{18}O-labelled oxygens per P_i The greater extent of exchange in the steady state probably relates to the longer turnover time for hydrolysis, about 0.5 s, compared to the turnover time of 0.2 s during the burst. These same values for exchange were obtained when hydrolysis was catalyzed by actomyosin in the original gel form or by protein that had been superprecipitated prior to the exchange measurements. Thus, superprecipitation per se had no effect on the oxygen exchange mechanism.

The results are consistent with the interpretation that the mechanism for actin-activated hydrolysis of MgATP by myosin is essentially the same for both phases but that during the burst, because of the original conformation of the myosin filaments, there is a higher average effective actin concentration around the myosin active sites in the protein matrix. For example, in the burst form, the myosin heads may on average be closer to actin, be better oriented for interaction, or have a greater affinity for the actin. In any case, a higher effective actin concentration would allow a faster average rate of actin activation and, therefore, a shorter average lifetime for the exchanging intermediates in the pathway of hydrolysis.

In a study by Barouch and Moos [21], the V values for actin-activated hydrolysis of heavy meromyosin in solution were obtained at different temperatures by extrapolation to infinite actin concentration using double-reciprocal plots of the actin concentration vs. rate. With the V values estimated this way, the Arrhenius plot for the Mg^{2+}-ATPase of acto-heavy meromyosin was linear and steep through the entire temperature range between 5 and 30°C, giving a high apparent heat of activation of about 25 kcal. In these measurements with the soluble fragment, heavy meromyosin, the extrapolation to infinite actin determines that actin concentration will not be rate-limiting.

The high apparent heat of activation in this case, then must be the property of some step in the hydrolytic pathway [22] that does not involve the association of actin with myosin. Since hydrolysis by gel in the original form shows

the same high heat of activation as acto-heavy meromyosin, it may be limited by this same step. However, hydrolysis by gel in the steady-state form appears to be limited by a different step, one with a low heat of activation. In this case, the rate-limiting step could involve the association of actin with myosin, i.e. it could be limited by the effective actin concentration around the myosin heads in the gel.

Acknowledgements

The work was supported in part by Grant No. PCM 78-03259 from the National Science Foundation and Grant No. 1451 from the Muscular Dystrophy Association.

References

1 Levy, H M , Sharon, N and Koshland, D E (1959) Proc Natl. Acad Sci U S A 45, 785—791
2 Levy, H.M , Sharon, N and Koshland, D E (1962) Biochim Biophys Acta 56, 118—126
3 Hasselbach, W. and Weber, A (1954) Biochim Biophys Acta 15, 237—245
4 Spudich, J A and Watt, S (1971) J Biol Chem 246, 4866—4871
5 Mommaerts, W F H M and Parrish, R.G (1951) J Biol Chem 188, 545—552
6 Levy, H M and Fleischer, M. (1965) Biochim. Biophys Acta 100, 479—490
7 Levy, H M and Fleischer, N (1965) Biochim Biophys Acta 100, 491—502
8 Perry, S V and Zydowo, M. (1959) Biochem J 71, 220—228
9 Greaser, M L and Gergely, J (1971) J Biol Chem 246, 4226—4233
10 Neufeld, A H and Levy, H M (1969) J Biol Chem, 244, 6493—6497
11 Glynn, I M and Chappel, J B (1964) Biochem J 90, 147—149
12 Shukla, K K and Levy, H M (1977) Biochemistry 16, 132—136
13 Bowen, W J , Stewart, L C. and Martin, H L (1963) J Biol. Chem 238, 2926—2931
14 Hartshorne, D J , Barns, E M , Packer, L and Fuchs, F (1972) Biochim Biophys Acta 267, 190—202
15 Barany, M (1967) J Gen Physiol 50, 197—218
16 Levy, H M and Koshland, D E. (1959) J Biol Chem 234, 1102—1107
17 Bagshaw, C R., Trentham, D.R., Walcott, R.G and Boyer, P D , (1975) Proc. Natl Acad Sci U S A 72, 2592—2596
18 Shukla, K.K and Levy, H M. (1977) Biochemistry 16, 5199—5206
19 Shukla, K K and Levy, H M (1978) J Biol Chem 253, 8362—8365
20 Sleep, J A , Hackney, D D and Boyer, P D (1980) J Biol. Chem 255, 1094—4099
21 Barouch, W.W and Moos, C (1971) Biochim Biophys. 234, 183—189
22 Bagshaw, C R. and Trentham, D R (1974) Biochem J 141, 331—349
23 Levy, H M and Moy, W.W (1981) Biochim Biophys Acta 658, 318—326