PRE-STEADY STATE KINETICS OF RABBIT MYOSIN- AND ACTOMYOSIN-ATPASE

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1. Introduction

The early phase of the myosin- and actomyosin ATPase has been studied by rapid reaction techniques [1–3]. The rates for binding and hydrolysis of the first ATP molecule are reported to be the same for myosin and actomyosin [2]. The rate-limiting step is the breakdown of an enzyme-product complex [1,3]. Actin functions as an activator for the myosin-ATPase as it combines with this complex and displaces the products at a much faster rate [2]. The nucleotide-free actomyosin complex is then dissociated by the binding of ATP [2].

Two findings remain, however, unaccounted for on the basis of the kinetic scheme suggested by Lymn and Taylor [2]. First, the deduction by these authors that the products of the ATP-splitting step, ADP and P_i, dissociate both with the same rate of either 0.05 sec^{-1} from the myosin or 20 sec^{-1} from the actomyosin fails to explain the occurrence of an 'early burst' in P_i liberation which is over in 50 msec. Further, this early burst did not show a simple dependence on ATP concentration [4], which led to the suggestion that the two heads on the myosin may not be equivalent [2].

As it has been shown for myosin ATPase that P_i is liberated very much faster than ADP [3], a finding which is supported from studies on the effect of pH, temperature and ionic strength on product release [1,4], the kinetics of the transient phase have been reinvestigated at 0.12 ionic strength and pH 6.9, conditions likely to prevail in living muscle. An early burst of P_i release was equally noted. In presence of 25–100 μM ATP the P_i was liberated from the myosin and actomyosin at a similar rate of 70–200 sec^{-1}, while ADP liberation was much slower, the rate constant being 0.15–0.5 sec^{-1} for myosin and 13–36 sec^{-1} for actomyosin. The dependence of these rate constants upon the ATP level suggests an interaction between the myosin heads which is controlled by ATP-binding.

2. Materials and methods

The proteins were isolated from rabbit psoas muscle by procedures previously described: myosin and actin–tropomyosin–troponin [5]; the heavy meromyosin (HMM) portion of the myosin containing the two myosin heads (M.W. 340 000) and subfragment-1(M.W. 170 000) were prepared according to Lowey et al. [6]. The actin in the medium is given in terms of the actin monomer content (M.W. 43 000 [5]).

To facilitate comparison with previous work the same type of rapid mixing apparatus was constructed and similar experimental conditions chosen as in the earlier studies [1,2,4]. The reaction at 20°C was started by mixing the ATPase system (2.78 μM HMM or S-1 and, if indicated, 13.4 μM actin incubated in 0.35 ml of 20 mM Tris-maleate, pH 6.9, and 5.10^{-5} M $CaCl_2$) with 0.35 ml 25 μM–10 mM Mg-ATP; the KCl content of the reaction mixtures was adjusted so as to give a final ionic strength of 0.12 upon mixing. The substrate was either [γ-^{32}P]ATP, synthesized according to Glynn and Chappell [7] or commercial [2-^{3}H]ATP. The reaction was terminated after intervals of 10-300 msec by forcing the mixture through

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a piston onto the top of a G-25 fine Sephadex bed (1 × 6 cm column), which rapidly separates the ATPase from the unreacted ATP [1,2]. For this purpose when the terminating drive stroke commenced the reaction medium was intermixed with 0.3 ml of a third solution (pre-cooled to 0°C), containing 500 μM each of unlabelled ATP, ADP and P_i as carrier. Aliquots of the protein-free filtrate were applied to a polyethylene-imine cellulose thin-layer chromatogram which was developed in 0.75 M KH_2PO_4 (pH 3.4). The eluted [2-^{3}H]ADP and $^{32}P_i$ (the latter runs at the front) were then estimated in an IDL scintillation counter. Internal standards for ATP, ADP and P_i were used. The largest source of error is the zero time blank.

3. Results and data evaluation in terms of a kinetic model

The rates of P_i liberation in the transient hydrolysis of ATP for both HMM and acto-HMM proved to be similar to those recorded by previous authors [2–4]. However, the difference between the rate constants for P_i release was somewhat smaller than that previously obtained at pH 8.0 [2].

The main result was the finding of a significantly slower release of the second product ADP from HMM and acto–HMM. Consistent with the general findings of Lymn and Taylor [2] ADP was released up to 87 times faster in presence of actin, but this was not observed for the P_i release (fig. 1).

Increasing the ATP concentration from 25 μM to only 100 μM shortened the release times for P_i and ADP by as much as one-third for HMM and acto-HMM, which indicates that this ATP effect is localized on the myosin and not the result of a faster dissociation of the acto-HMM complex [2]. When the ATP concentration was increased to as high as 2–10 mM, the ADP release in the acto–HMM system became very slow, with r_{ADP} the overall rate constant for ADP release, decreasing again to 0.45 sec^{-1}.

When a preparation of subfragment-1 (i.e. only one of the two myosin, heads) was investigated under the same conditions, ADP was released at a rate intermediate between that of HMM and acto-HMM. Further, r_{ADP} changed only from 4 sec^{-1} to 7 sec^{-1}, while the kinetics of P_i release remained essentially unaltered.

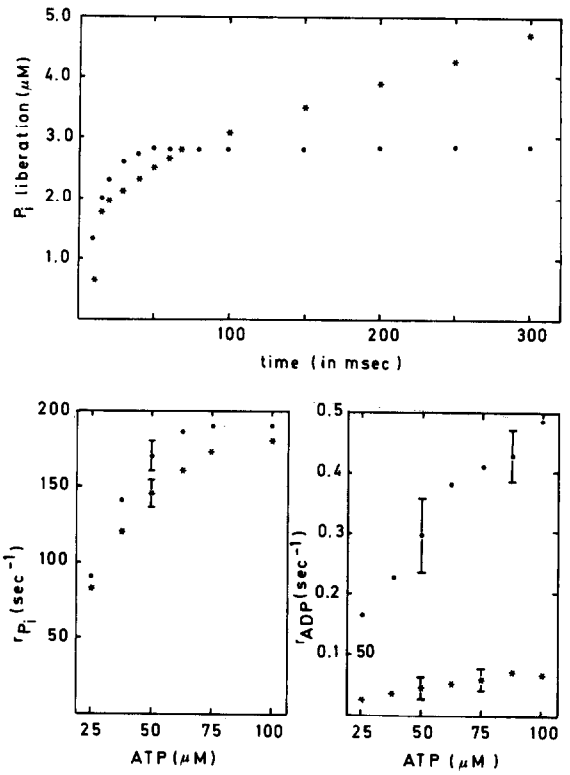

Fig 1. Product release in the pre-steady state hydrolysis of ATP by HMM and acto-HMM. All data referring to the HMM preparations are indicated by the solid circles, while those obtained with the acto–HMM complex are denoted by stars. Information about the time course of either P_i or ADP liberation was obtained by sampling the released products at the various time intervals. The over-all rate constants for P_i release, r_{P_i}, and ADP liberation, r_{ADP}, were evaluated from the initial rate as described by Lymn and Taylor [4]. To plot r_{ADP} for HMM and acto-HMM in the same diagram the scale for the latter had to be reduced, varying from 0–50 sec^{-1} for acto–HMM over the same range which covers 0–0.1 sec^{-1} for r_{ADP} from myosin. All data points represent the mean of 12 experiments with standard deviations given for the mean values of the rate constants.

In view of the fact that the kinetic model of Lymn and Taylor [2] describes many results of myosin and actomyosin ATPase, the attempt was made to find a parameter combination which can fit the present data to the basic kinetic scheme of these authors. For this purpose a computer-based procedure for parameter fitting [8] was employed. As weighting factors in the search strategy the general validity of the fol-

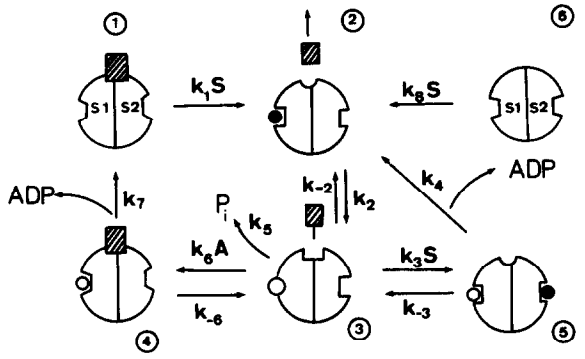

Fig. 2. Kinetic scheme for myosin and actomyosin ATPase. The two heads on the myosin [6] have been denoted by S1 and S2. The actin is indicated by the shaded squares, being bound only in states 1 and 4. Binding of ATP to the myosin is shown by the shaded circles (states 2 and 5), while that of the product ADP is reflected by the open circles (states 3,4 and 5). The dissociation of ADP (k_4 and k_7) has been considered as essentially irreversible at the ADP concentrations likely to occur under the experimental conditions used in fig. 1.

lowing kinetic constants verified by various authors [2,3,9,10] was accepted from the outset: for ATP-binding to myosin 2×10^6 M^{-1} sec^{-1} (k_8), for the rate constant of the hydrolytic step 200 sec^{-1} (k_2), and for the rate constant of ADP release from the free myosin 0.05 sec^{-1} (k_4).

Two additional assumptions were made: First, to account for the initial burst in P_i release (fig. 1) a very fast reaction step (k_5) was introduced at the level of the [myosin–ADP·P_i]* complex. The second assumption is based on the ability of each of the two myosin heads (when isolated as subfragment-1) to split its bound ATP, although the rate of ADP release is much slower in the intact HMM system. The hypothesis was therefore tested that negative cooperativity between the two heads on the myosin may limit the ATP-splitting rate and in this way prevent the undesirable dissipation of chemical energy.

The resulting kinetic scheme which had to be modified from that of Lymn and Taylor [2] is given in fig. 2. The basic model assumptions are: When the two myosin heads interact in the nucleotide-free complex with the actin (state 1), both S-1 subunits of the myosin head have still the same affinity for the substrate. As one of the myosin heads binds ATP with the rate constant k_1, the actomyosin complex dissociates, leading to state 2. This transition results in a negative cooperativity between the two heads so that the affinity for ATP in the second S-1 subunit (denoted in fig. 2 by S2) decreases. The bound ATP is split very fast, $k_2 \geqslant 200$ sec^{-1}. The myosin–product complex (state 3) liberates the P_i at a much faster rate (k_5) than the ADP, which is only released at a significantly fast rate after actin has become rebound (k_6), giving rise to the actomyosin-product complex (state 4) from which ADP can now readily dissociate (k_7) and state 1 is re-attained. As the ATP level increases the second S-1 subunit (i.e. S2 in fig. 2) can also bind an ATP molecule (state 5) which is contrast to the ATP on the first S-1 subunit is not hydrolysed and hence only suppresses the interaction of the myosin with the actin. ADP dissociates then from myosin in state 5 only with the slow rate constant k_4 characteristic for the myosin–product complex [2,3].

The kinetic constants evaluated by the parameter optimization procedure [8], as summarized in table 1, are rather similar to values reported in the literature. If the assay system contains pure myosin only states 2, 3, 5 and 6 and the kinetic constants k_2,k_3, k_4 and k_8 will have to be considered; the higher value of the ATP binding constant is probably the results of the improved access of ATP to its binding site in the absence of actin. The average deviation between the predicted curves from the optimized model parameters (table 1) and the experimental results in fig. 1 was 5.98%.

The kinetic scheme in fig. 2 offers the advantage that it makes use of the basic ideas advanced by Lymn and Taylor [2] but allows a quantitative prediction of the initial transients of both myosin and actomyosin ATPase. Alone the choice of the value for the actin-binding constant as made by Lymn and Taylor [2], which in their formulations is the rate-limiting step for the release of both P_i plus ADP, would at the actin concentration used in their experiments, 13.34 μM, have given a time occur. of 250 msec, much too long for an early burst of P_i release to occur. The kinetic model in fig. 2 predicts correctly a number of findings for actomyosin ATPase hitherto unexplained by the kinetic equations of Lymn and Taylor, such as the size of the early burst of P_i liberation with 1.1 mole P_i/mole myosin, the onset of the steady state after about 85 msec, the higher pre-steady state velo-

Table 1
Kinetic constants determining the pre-steady state of myosin- and actomyosin-ATPase

Constant	Dimension	Evaluated parameter	Comparative value in the literature
k_1	$mM^{-1}\ sec^{-1}$	550	1000 [2]; 21 [10]
k_5	sec^{-1}	140	> 10 [3]
k_6	$mM^{-1}\ sec^{-1}$	215	300 [2]
k_{-6}	sec^{-1}	2	< 10 [2]
k_7	sec^{-1}	40	10–20 [2]
k_3/k_{-3}	mM^{-1}	0.124	

The other kinetic constants k_2, k_4 and k_8 required for the model calculations were used as invariant parameters in the search strategy [8] for parameter estimation.

city of the myosin ATPase compared with that of the actomyosin ATPase, and that in view of the higher ATP binding constant k_8 the myosin ATPase is unaffected by ATP levels above 75 μM (fig. 1). The model postulate that the two myosin heads are not equivalent as concerns ATP hydrolysis and that cooperativity exists between them is also supported from the work on ADP-loaded myosin [11].

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