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Is a four-state model sufficient to describe actomyosin ATPase?

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Four- [(1984), J. Biol. Chem. 259, 11908] and six- [(1985) Science 227, 999] state models have been proposed for actomyosin ATPase. A key experiment in deciding between these is whether or not there is a transient P_i burst at high actin. In the first, the cleavage and release of products rates are similar and the P_i burst is low; in the second, there are additional product complexes and the P_i burst is large. We reinvestigated the problem by carrying out burst experiments under the conditions in [(1985) Science 227, 999]. Since we find that the P_i burst at high actin is low, we conclude that the four-state model is sufficient to describe actomyosin ATPase.

Actomyosin ATPase; Four-state; Six-state; P_i burst, transient

1. INTRODUCTION

Muscle contraction consists of the cyclic attachment and detachment of myosin heads to actin filaments. The energy needed for these processes is supplied by the hydrolysis of ATP by the myosin heads. The problem is to relate the different steps of this chemical reaction to the physiological events involved in muscle contraction.

It is very difficult to obtain the chemical kinetics of organised systems: it is from solution studies that models for actomyosin ATPase have been proposed [1-3]. We are then confronted with the problem of relating such mechanisms to the situation in the intact muscle. Further, most solution studies have been carried out at unphysiologically low ionic strengths, i.e. under conditions where the actomyosin interactions are strong and significant experiments possible.

Most workers agree that these problems are difficult to overcome. The use of caged compounds is an elegant way of attempting this [1], but solution studies remain the main source of kinetic information on actomyosin ATPase. However, despite extensive studies, there is disagreement as to a mechanism for actomyosin ATPase in solution, let alone in muscle. Here we address ourselves to a problem that has given rise to much debate, namely the number of intermediates on the actomyosin ATPase pathway.

It is generally agreed that myosin ATPase is described by the Bagshaw-Trentham scheme [1]:

Scheme 1:

$$\begin{array}{ccccccc} M+ATP & \overset{K_1}{\rightleftharpoons} & M.ATP & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & M.^*ATP & \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & M.^{**}ADP.P \\ & & & & & & \Big\| \; k_4 \\ & & & & & \overset{P_i}{\searrow} & \\ M+ADP & \underset{K_7}{\rightleftharpoons} & M.ADP & \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} & M.^*ADP & \underset{K_5}{\rightleftharpoons} & M.^*ADP.P \end{array}$$

A key feature of this scheme is that the rate-limiting step is clear-cut (the release of P_i, k_4). Therefore, in the steady-state myosin-product complexes accumulate and these give rise to a P_i 'burst' when reaction mixtures are quenched in acid [4]. Actin interacts with certain of the intermediates of this pathway and this modifies the rate constants concerned.

A simple scheme for actomyosin ATPase is based upon the early work of Lymn and Taylor [2]. At high ATP concentrations, there are essentially four intermediates containing ATP or ADP.P_i. This has been termed the four-state model which takes into account an interaction of M*.ATP with actin [5] and uses the nomenclature of Stein et al. [6-8]:

Scheme 2:

$$\begin{array}{ccccccc} & & M.ATP & \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} & M.ADP.P_i & & \\ & & K_3 \Big\| & & \Big\| K_{13} & & \\ AM & \rightleftharpoons & AM.ATP & \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} & AM.ADP.P_i & \overset{k_{10}}{\rightleftharpoons} & AM.ADP \rightleftharpoons AM \end{array}$$

Eisenberg and Kielley [9] discovered that at actin concentrations that fully activate myosin ATPase, only a small amount of myosin is bound to the actin, but on

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further increasing the actin concentration, it was possible to fully saturate the myosin without significantly changing the steady state. The difference in these saturation kinetics has been confirmed [10,11].

To explain their results with reference to the information available at the time, Eisenberg and Kielley [9] suggested that there is a state in the actomyosin pathway that interacts only very weakly with actin. They termed this the refractory state: more recently the terms refractory and non-refractory states were abandoned for $AM.ADP.P_i^I$ and $AM.ADP.P_i^{II}$, respectively [6]. Accordingly, Eisenberg and his coworkers [3,6–8] extended the four-state model of Taylor to a six-state scheme:

Scheme 3:

$$\begin{array}{ccccc} M.ATP & \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} & M.ADP.P_i^I & \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} & M.ADP.P_i^{II} \\ \Big\| K_3 & & \Big\| K_{13} & & \Big\| K_{14} \\ AM \rightleftharpoons AM.ATP & \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} & AM.ADP.P_i^I & \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} & AM.ADP.P_i^{II} \end{array}$$

$$\overset{k_{10}}{\rightleftharpoons} AM.ADP \rightleftharpoons AM$$

Now, it has been proposed that the four- and six-state models differ in one major experimental prediction: the size of the P_i burst (e.g. [12]). Thus, it is proposed that in the four-state model, the kinetics of the hydrolysis and release of P_i steps are similar and, therefore, that the P_i burst is small and in the steady-state, actomyosin-substrate complexes predominate. In the six-state model, the rate-limiting step follows hydrolysis which predicts a large P_i burst, i.e. that the steady-state complexes at full actin activation are actomyosin-product complexes. Stein et al. [7] put their six-state model to the test by carrying out P_i burst experiments with acto S1 at high actin concentrations. They found that the size of the P_i burst with S1 alone was not greatly reduced with actin. They confirmed the small effect of actin upon the sice of P_i burst with acto S1 [8] and crosslinked acto S1 [12].

But Rosenfeld and Taylor [11] obtained quite different results: they showed that the P_i burst size decreases to a very low level at high actin concentrations. Further, they obtained a low P_i burst with crosslinked acto S1. Biosca et al. [13] also obtained insignificant P_i bursts with crosslinked acto S1. Rosenfeld and Taylor [11] conclude that a four-state model is sufficient to explain the difference between the actin concentrations needed to saturate myosin in the presence of ATP and to obtain maximum actomyosin ATPase.

As other workers (e.g. [1]), we are puzzled by this difference, which is important, and we made an attempt at determining its cause.

A problem with the experiments so far is that each laboratory has its own set of experimental conditions. It is well known that actin-myosin interactions in the presence of ATP are very sensitive to these, especially the ionic strength. Further, P_i burst experiments are carried out by the rapid flow quench method. This is a point-by-point method and to convincingly distinguish an initial rapid rise of product (P_i burst) from a fast steady-state, as with actomyosin, one needs a large number of experimental points over a large time range.

Here we carried out P_i burst experiments with actomyosin under the experimental conditions of Stein et al. [6–8], i.e. in a low ionic strength buffer at 15°C. In our hands, the P_i burst decreased as the actin concentration was increased: at high concentrations, a P_i burst was difficult to discern because of the rapid steady-state. In agreement with Rosenfeld and Taylor [11], we conclude that a four-state model is sufficient to describe actomyosin ATPase in solution.

2. MATERIALS AND METHODS

2.1. *Proteins and reagents*

References to the preparation of myosin, S1 and actin are in [14]. [γ-^{32}P]ATP was from Amersham International.

2.2. *P_i burst experiments*

These were carried out in a rapid flow quench apparatus which was thermostatically controlled. The apparatus had been designed for viscous solutions and had been tested accordingly [15]. S1 or acto S1 plus [γ-^{32}P]ATP reaction mixtures were aged for the times shown in figs 1 and 2, quenched in 22% trichloroacetic acid and the $^{32}P_i$ determined. Zero time points and the total radioactivities in [γ-^{32}P]ATP solutions were obtained as described previously [16].

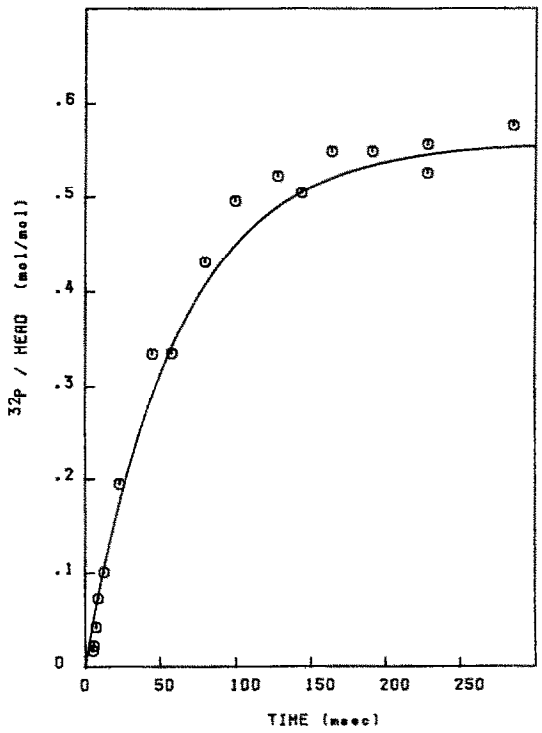

Fig.1. Time course for P_i burst with S1. The reaction mixture was 10 μM S1 and 100 μM [γ-^{32}P]ATP. For other details, see the text and for the kinetic constants obtained, see table 1.

Table 1

The effect of actin on the kinetic parameters of the P_i burst of S1

Actin (μM)	Amplitude of P_i burst P_i/S1 (mol/mol)	k_o s^{-1}	k_{ss} s^{-1}
0	0.56 (±0.01)	16.5 (± 1.5)	0.045 (±0.05)
10	0.49 (±0.05)	18.7 (± 3.8)	1.5 (±0.4)
17.5	0.24 (±0.01)	19.5 (±10.5)	2.6 (±0.4)
40	0.15 (±0.03)	24.5 (±15.5)	4.5 (±0.6)

For full details, see the text and legends to figs 1 and 2. The numbers in parentheses are standard deviations.

As the objective here was to obtain with precision the sizes of P_i bursts, accurate values for P_i at zero time was essential. Six zero time points were taken in each experiment (three before and three after each run). Whether or not there was a transient burst phase, back-extrapolation of the data to zero time gave a blank that agreed well with the experimentally obtained blanks.

2.3. *Experimental conditions*

The experiments were carried out under conditions which were as far as possible identical to those used by Stein et al. [6–8]. Thus, the buffer was 10 mM imidazole, 1.8 mM $MgCl_2$, 1 mM dithiothreitol, pH 7 (HCl) and the temperature 15°C. As did Stein et al., we included actin in both the S1 and [γ-^{32}P]ATP syringes of the rapid flow quench apparatus. With the high actin concentrations used, there was a low ATPase activity which was corrected for.

3. RESULTS AND DISCUSSION

P_i burst experiments with S1 alone (fig.1) and with acto S1 at increasing concentrations of actin (fig.2) were carried out under the conditions of Stein et al. [6–8]. As other workers, we interpreted the results in terms of the transient formation of P_i (described by a single exponential with a constant k_o) followed by a steady state, k_{ss}. The size of the P_i burst was taken to be the intercept on the Y-axis by the steady-state. The results obtained are summarized in table 1.

In our hands, there was a large decrease in the P_i burst size as the actin concentration was increased. This is at variance with Stein et al. [7,8], who conclude that at actin concentrations greater than 20 μM, there is a plateau in the P_i burst size of about 0.5 mol/mol S1. We cannot explain this discrepancy but we note that our results agree well with those of Rosenfeld and Taylor [11] obtained under different conditions.

We suggest that the four-state model (Scheme 2) is flexible enough to accommodate most of the results obtained with acto S1 to date. It could be even more accommodating if we were to be sure that the chemical step changes with actin. The kinetics of the burst appear to increase with the actin concentration but because of the low burst sizes and high steady-states at high actin concentrations it is difficult to be certain of this (table 1). Rosenfeld and Taylor [11], too, suggest that the burst kinetics increase with the actin concentration, but Stein et al. [8] conclude that they decrease.

It appears that, in common with a number of enzymes [17], there is no clear cut rate-limiting step on acto S1 ATPase. In particular, the constants involved in the formation and decomposition of enzyme-product complexes are similar. It follows that the values of P_i obtained from extrapolated P_i bursts are lower than the actual concentrations of enzyme-product complexes [18]. This does not help matters and kinetic data on the chemical step of actomyosin remains elusive. We are attempting to obtain these data by perturbing the system by cryoenzymology (Tesi et al., unpublished work).

In conclusion, we suggest that the four-state model of Taylor (Scheme 2) is adequate to describe actomyosin ATPase. Since the initial tight binding of ATP is much

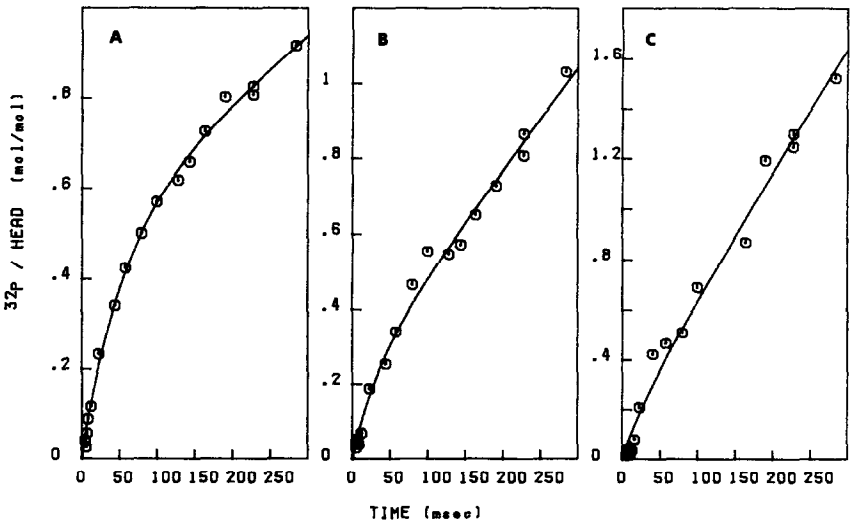

Fig.2. Time course for the P_i bursts with acto S1. The reaction mixtures were 10 μM S1 and: (A) 10 μM actin and 50 μM [γ-^{32}P]ATP; (B) 17.5 μM actin and 100 μM [γ-^{32}P]ATP; (C) 40 μM actin and 100 μM [γ-^{32}P]ATP. For further details, see the text and for the kinetic constants obtained, see table 1.

faster than the succeeding steps [14,19], the predominant intermediates are enzyme-substrate complexes.

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