THE TERNARY COMPLEX FORMED BETWEEN ACTIN, MYOSIN SUBFRAGMENT 1 AND ATP (β, γ-NH)

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1. Introduction

ATP analogs have been extensively used in muscle research, particularly in two areas.

1. Their application in studying the mechanism of ATP hydrolysis by myosin (e.g., [1,2]).
2. Their influence on the structural and mechanical properties of glycerinated muscle fibres (e.g., [3–5]).

Important information linking these two areas together, namely the interaction of the analogs with actomyosin in solution, is not available in detail. For this reason, the interaction of a non-hydrolysable analog of ATP, ATP (β, γ-NH), with acto-S1 in solution was investigated. With the results of experiments on permeable muscle fibres, it was hoped that this study would provide quantitative information on the interaction between the actin and nucleotide binding sites of myosin.

2. Materials and methods

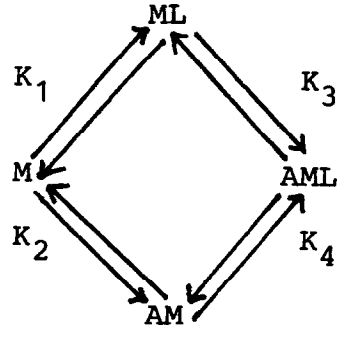
Myosin subfragment 1 (S1) from rabbit was prepared as in [6]. Actin was prepared as in [12] with the inclusion of a further depolymerisation–polymerisation step. ATP, NADH, phosphoenolpyruvate, pyruvate kinase and lactate dehydrogenase were obtained from Boehringer, Mannheim. ATPase measurements were made by measuring the rate of NADH disappearance at 340 nm in an Aminco DW-2 spectrophotometer in a cuvette containing the following: NADH (0.2 mM), phosphoenolpyruvate (1.0 mM), $MgCl_2$ (5 mM), lactate dehydrogenase (5 μl 10 mg/ml solution), pyruvate kinase (5 μl 10 mg/ml solution), S1, ATP and Tris–HCl (100 mM, pH 8.0). Light scattering was measured at 90° to the incident beam in a Perkin-Elmer-Hitachi MP4 fluorescence spectrophotometer using a wavelength of 350 nm.

3. Results and discussion

3.1. *Rate of ATP (β, γ-NH) release from S1 and acto. S1*

The rate of release of ATP (β, γ-NH) from its complex with S1 was obtained from the lag-phase in the development of the steady-state ATPase on adding an excess of ATP to the complex obtained by mixing S1 (10^{-6}–10^{-5} M) and ATP (β, γ-NH) (3.3×10^{-5} M) in the ATPase assay mixture above. The lag-phase, which was absent when ATP (β, γ-NH) was omitted from the reaction mixture, was exponential, and from this the rate constant for dissociation of the analog from S1 was estimated (k_{-1}, scheme 1).

Scheme I



Equilibria involved in formation of the ternary complex between actin (A), myosin or its protolytic subfragments (M) and nucleotide (L).

Table 1
Kinetic and thermodynamic parameters for ATP (β, γ-NH) dissociation from S1 and for the S1-ATPase

Release from ATPase of	k_{-1} s^{-1} (21°C)	ΔH kcal/mol (ATP (β, γ-NH) release)	ΔH kcal/mol (ATPase)
S1	7.5×10^{-3}	25.9	12.7
S1 + actin	2.6×10^{-2}	20.0	16.4

Conditions: S1, 2.2×10^{-6} M; actin, 7.8×10^{-7} M; Tris, 50 mM, pH 8.0; $MgCl_2$ 5 mM; temp. range, 2–26°C

This was determined over a range of temperatures (13–26°C) to obtain ΔH, the enthalpy of activation (table 1). At 21°C, a value of 7.5×10^{-3} s^{-1} was obtained, in reasonable agreement with that obtained at this temperature [1]. The high enthalpy of activation, ~ 26 kcal.mol^{-1}, is similar to that obtained for ADP [8].

When actin was added together with ATP to start an experiment as described above, the lag-phase in the approach to the steady-state was shorter. The effect of actin on the rate constant for ATP (β, γ-NH) release is shown in fig.1. The coupled-enzyme method is not suitable for measurement of more rapid rates, but in the range measured there is an approximately linear relationship between the observed rate constant for release of analog and actin concentration. These results indicate that actin and nucleotide bind to distinct sites on S1 which interact with each other. In the presence of actin, the enthalpy of activation for ATP (β, γ-NH) release is reduced (table 1).

The interaction between the two types of binding sites on S1 was investigated in a more direct manner by measuring the effect of ATP (β, γ-NH) on the degree of association of acto. S1. Light scattering at 90° to the incident beam was used as an indicator of dissociation. The results of such a titration are shown in fig.2. It can be seen that, at the actin concentration used, complete dissociation of acto. S1 does not occur, even at high ATP (β, γ-NH) concentrations, which confirms formation of a ternary complex, as seen in studies with muscle fibres [5]. Based on scheme 1, it can be shown that:

$$\frac{[A.M.] + [A.M.L]}{[M.L]\ [A]} = \frac{K_2}{K_1\ [L]} + K_3$$

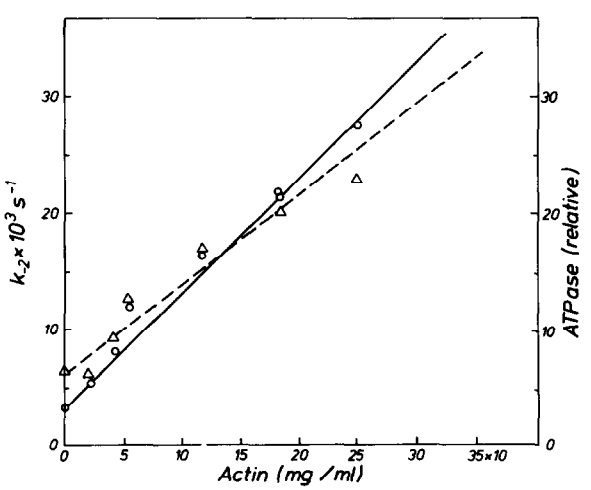


Fig.1. The influence of actin on the rate of release of ATP (β, γ-NH) from its complex with S1 (o——o) and on the S1-ATPase (△- - -△). Conditions as in section 2, 15°C, no added KCl.

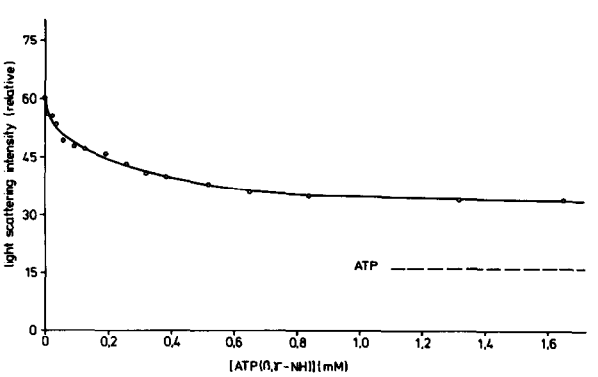


Fig.2. Light scattering titration of ATP (β, γ-NH) to acto. S1 [Actin] = 8.0×10^{-6} M; [S1] = 1.45×10^{-5} M. Conditions as in table 2.

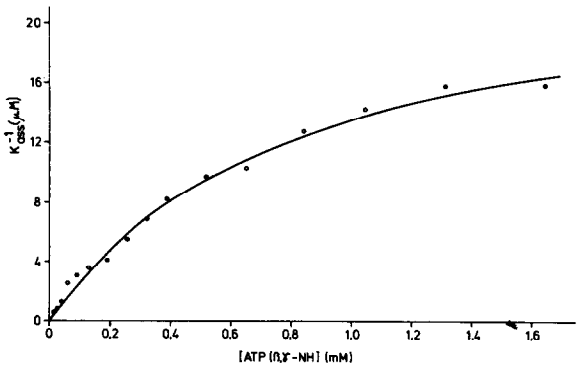


Fig.3. The dependence of the apparent dissociation constant of acto. S1 (K_{ass}^{-1}) on [ATP (β, γ-NH)]. Solid line shows best fit to experimental points. Conditions as in table 2.

and

$$\frac{[A.M] + [A.M.L]}{[M.L]\,[A]} \qquad (1)$$

can be regarded as an effective binding constant (K_{ass}) between actin and S1 at a particular concentration of ATP (β, γ-NH). Values for the constants K_3 and K_4 obtained by computer fitting of the best hyperbola to a plot of K_{ass}^{-1} against ATP (β, γ-NH) concentration (fig.3) are given in table 2. By comparison of K_4 with the known value of K_1, it can be seen that ATP (β, γ-NH) binds about 2×10^3 (K_1/K_4 or K_{int}) more weakly to acto. S1 than to S1, corresponding to a free energy of interaction between the sites, G°_{int}–4.5 kcal.mol^{-1}. The same ratio, K_{int}, also applies to actin binding to S1 and S1.ATP (β, γ-NH), respectively, so that K_2, the binding constant of actin to S1, can be estimated to be 1.1×10^8 M^{-1}. This is in reasonable agreement with the value of $\sim 10^8$ M^{-1} obtained [9] under similar conditions of ionic strength and temperature, although not at the same pH (they used pH 7.0). Results indicating formation of a ternary complex between actin, S1 and ATP (β, γ-NH) have also recently been obtained using an ultracentrifuge method. The results agree qualitatively with those above [13].

The ADP binding constant to acto. S1 is 5×10^3 M^{-1}, or somewhat greater than the value for ATP (β, γ-NH) obtained in this work [10]. Taking the known value for the ADP binding constant to S1,

Table 2
Equilibrium constants for scheme 1

	Equilibrium constant (M^{-1})	
K_1	3.3×10^6	[1]
K_2	1.1×10^8	(this work)
K_3	4.1×10^4	(this work)
K_4	1.24×10^3	(this work)

Conditions: KCl, 50 mM; Tris–HCl, 100 mM, pH 8.0; $MgCl_2$, 5 mM; temp., 23°C

Table 3

Method	Conditions		K_{diss} (M)	Ref.
Direct assay of binding	8°C	pH 7.0	1.11×10^{-4}	[5]
	8°C	pH 7.0	3×10^{-5}	[5][a]
Titration of mechanical change	8°C	pH 7.0	1.04×10^{-4}	[5]
X-ray titration	14°C	pH 6.9	9×10^{-5}	[4]
	23°C	pH 6.9	2.3×10^{-4}	(R. S. G. and W. F., unpublished)
Binding to acto. S1	23°C	pH 8.0	7×10^{-4}	(this work)

[a] Using rabbit psoas muscle; all other fibre experiments with *Lethocerus cordofanus* flight muscle

a value of 200 for K_{int} or 3.14 kcal.mol^{-1} for ΔG°_{int} can be calculated. These values are considerably smaller than those for ATP (β, γ-NH), indicating that the free-energy of interaction between the sites is dependent on nucleotide structure. This difference between ATP (β, γ-NH) and ADP quantitatively confirms the qualitative result [11] that the imido-analog dissociates actomyosin more effectively than ADP.

There is no good quantitative agreement between the dissociation constant of ATP (β, γ-NH) from acto. S1 in solution and from glycerinated muscle fibres. Values which have been obtained for fibres are shown in table 3.

Direct comparison of these results is made difficult by the variation in conditions, which were chosen partly for practical reasons (insect muscle is not stable above pH 7.0) and partly from convention (until recently, pH 8.0 and room temperature were normally used in kinetic experiments on myosin and actomyosin). However, large pH and temperature dependencies would be required to explain the differences, especially between binding to rabbit acto. S1 and rabbit fibres. Two alternative explanations are feasible.

1. The organized structure in the intact actomyosin matrix could influence the interaction of the myosin-active site with nucleotide.

2. If significant cross-bridge dissociation occurs (the strongest evidence against this is retention of dynamic stiffness of the fibres [5], the effective dissociation constant, K'_{diss}, of ATP (β, γ-NH) will be lower.

$$K'_{diss} = \frac{K_{diss}}{1 + \frac{1}{K_3[A]}}$$

where

$$K_{diss} = \frac{1}{K_4}$$

[A] represents the effective concentration of actin relative to myosin in the actin–myosin lattice, and it can be seen that $K'_{diss} = 1/K_4$ only if $K_3[A] \gg 1$. More detailed comparative studies are needed to determine whether this factor is important in muscle fibres. Such studies are of importance in interpreting the effects of ATP (β, γ-NH) on structural and mechanical properties of muscle; these changes are assumed to be related to events occurring in the normal contraction cycle.

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