Articles

Inhibition of ATP Binding to Myofibrils and Acto–Myosin Subfragment 1 by Caged ATP†

John Sleep,*,‡ Christian Herrmann,§,∥ Tom Barman,§ and Franck Travers§

INSERM U128, CNRS, BP 5051, 34033 Montpellier Cedex, France, and MRC, King's College London, 26-29 Drury Lane, London WC2B 5RL, U.K.

Received November 10, 1993; Revised Manuscript Received March 4, 1994®

ABSTRACT: The inhibitory effect of P^3-[1-(2-nitrophenyl)ethyl]adenosine 5′-triphosphate (caged ATP) on the binding of Mg^{2+}-ATP to myofibrils was investigated. The most sensitive method was found to be the monitoring of single turnovers of [γ-^{32}P]ATP hydrolysis using the quench flow technique. The method was tested using ADP, which was found to have an inhibition constant of 145 μM, in agreement with previous reports. Caged ATP behaved as a simple competitive inhibitor of ATP binding with an inhibition constant of 1.6 mM. The inhibitory effect of these ligands on the binding of ATP to acto–myosin subfragement 1 was investigated using the same method. The inhibition constants of caged ATP and ADP were found to be 0.35 mM and 50 μM, respectively. This inhibitory effect of caged ATP on ATP binding accounts for the lower rate of ATP binding to fibers, deduced from caged ATP [$(0.5–1) \times 10^6$ M^{-1} s^{-1}], than that reported for acto–S1 (3.5×10^6 M^{-1} s^{-1}) [Goldman, Y. E., Hibberd, M. G., & Trentham, D. R. (1984) *J. Physiol. (London) 354, 577*].

Caged compounds have been invaluable for the study of the transient kinetics of muscle fibers as their use overcomes the problem of the diffusion time from the periphery to the center of the fiber and allows spatially uniform changes in concentration (Goldman et al., 1984a,b; Dantzig et al., 1992). Ideally, the caging of a compound should not only prevent any chemical reaction or physiological response to ligand binding but also prevent binding in any form so as to avoid problems of competitive inhibition. There have been reports that the original form of caged ATP [P^3-[1-(2-nitrophenyl)ethyl]-adenosine 5′-triphosphate; Kaplan et al., 1978] binds to myosin with a constant in the region of 0.5–1 mM (Dantzig et al., 1989). Such binding would be expected to result in significant inhibition of the rate of adenosine 5′-triphosphate (ATP)[1] binding in skinned muscle fiber experiments involving the use of caged ATP. For example, in a typical experiment, about 1 mM ATP is released from a total concentration of 5 mM caged ATP. With an inhibition constant of 0.5–1 mM, the rate of ATP binding would be inhibited by a factor of 5–9.

There is considerable evidence that the second-order rate constant for ATP binding to myofibrils is the same as for acto–S1 in solution (Smith & White, 1985; Taylor, 1990; Houadjeto et al., 1992; White & Taylor, 1976). However, Goldman et al. (1984a,b) reported a second-order rate constant of 5×10^5 to 10^6 M^{-1} s^{-1} for the rate of ATP binding to fibers whereas they quoted a rate of 3.5×10^6 M^{-1} s^{-1} for the rate of binding to actomyosin. The rates are clearly broadly similar, but in this paper we suggest that the difference betweenthe measurements is caused by inhibition due to the binding of caged ATP.

Knowledge of the inhibition constant of caged ATP is important because when ATP is released in fibers in either the presence or the absence of Ca^{2+} the response is limited by a combination of the rate of release of ATP (80–100 s^{-1} at 20 °C), the second-order rate of ATP binding (3.5×10^6 M^{-1} s^{-1} for acto–S1), and a subsequent step, probably hydrolysis (80–100 s^{-1}). In order to correctly analyze the tension rise after release of ATP into these three components, it is essential to characterize any inhibition due to caged ATP.

In this study, inhibition of ATP binding by caged ATP was studied using myofibrils to facilitate the rapid mixing required for quenched flow experiments. The hydrolysis of [γ-^{32}P]-ATP was monitored, and the experiments were carried out under single-turnover conditions to maximize sensitivity. The concentration of the myofibrils was chosen so that the rate of hydrolysis was much faster than ATP binding and thus the rate of hydrolysis accurately reported on the latter.

MATERIALS AND METHODS

Proteins and Reagents. The method of Herrmann et al. (1993) was used for the preparation of myofibrils and the methods of Tesi et al. (1991) for myosin subfragment 1 (S1) and actin. Apyrase (grade VII, A6535) and P^1,P^5-di-(adenosine-5′)pentaphosphate (Ap5A) were from Sigma Chemical Co. [γ-^{32}P]ATP was from Amersham International. Caged ATP was prepared as described by Walker et al. (1988). Some experiments were done with caged ATP which had been purified only using the second DE52 column and omitting the preparative HPLC step. There are two optical isomers of caged ATP, and these were used basically in the ratio produced by the synthetic route. The isomers are resolvable by HPLC, and it is possible that there was a small

† This work was supported by a grant from the EEC [SCI 0327-C(J)R]. J.S. is grateful to the Royal Society for a travel grant and C.H. to INSERM for a fellowship.

‡ King's College London.

§ INSERM U128.

∥ Present address: Max-Planck Institut für Molecular Physiologie, Rheinlanddamn, 44026 Dortmund, Germany.

® Abstract published in *Advance ACS Abstracts,* April 15, 1994.

[1] Abbreviations: ATP, adenosine 5′-triphosphate; S1, myosin subfragment 1; P_i, inorganic orthophosphate; MOPS, morpholinopropanesulfonic acid.

0006-2960/94/0433-6038$04.50/0

change in the ratio of the HPLC-prepared batch. All the data in the figures of this paper resulted from experiments in which the caged ATP had not received the preparative HPLC step. However, results using HPLC-purified caged ATP showed no significant difference from those in the figures. Early studies (Dantzig et al., 1989) suggested that there was a difference in the inhibitory properties of the two isomers. However, Thirlwell et al. (1993) showed that the minor ADP contamination routinely occurring in caged ATP preparations is sufficient to markedly inhibit relaxation, and the experiments of Dantzig et al. need to be repeated using apyrase to eliminate ADP contamination before one can be confident of a difference in the inhibitory effects of the isomers.

HPLC Analysis. Samples were analyzed on an Ultrapac TSK DEAE-5PW column (7.5 × 75 mm), equilibrated with 20 mM MOPS, pH 7.0. The nucleotides were eluted using a linear NaCl gradient from 0 to 0.3 M at a flow rate of 1 mL/min. The following elution times were obtained: caged ATP, 15.4 min; ATP, 13.5 min; ADP, 12 min; and AMP, 9.5 min. Following chromatography of samples containing apyrase, the column was washed successively with 0.2 M NaOH and 1 M sodium acetate for 10 min. This treatment was necessary as it was found that apyrase contaminated the main HPLC column as well as the precolumn. This contamination resulted in the hydrolysis of any ATP or ADP in a succeeding sample.

Use of Apyrase. The caged ATP solutions used in the kinetic experiments were treated with apyrase at 3.5 μg/mL (Sleep & Burton, 1990). This enzyme hydrolyzes any contaminating ATP and ADP to AMP, which interacts very poorly with myosin. Control experiments showed that at this concentration 50 μM ATP or ADP was hydrolyzed almost completely in 2 min even in the presence of 2 mM caged ATP. Caged ATP was not hydrolyzed after incubation for 2 h.

Kinetic Experiments. (1) Procedure. The experiments were carried out in the thermostatically controlled quench flow apparatus described by Barman and Travers (1985). In a typical experiment with caged ATP as inhibitor, the caged ATP was first incubated with apyrase at 20 °C for 30 min. The myofibrils were then added, and this solution was mixed with a substoichiometric amount of [γ-^{32}P]ATP. After being aged for 0.3–200 s, the reaction mixtures were quenched in acid (22% trichloroacetic acid with 1 mM carrier phosphate), and [^{32}P]P_i was determined (Reimann & Umfleet, 1978). In experiments with ADP as inhibitor, the ADP was incubated with the myofibrils (or acto–S1) plus 50 μM Ap5A (to inhibit any myokinase) for 15 min at 4 °C to allow any ATP contamination in the ADP to be hydrolyzed and then mixed with [γ-^{32}P]ATP in the quenched flow apparatus as for the caged ATP experiments. The caged ATP was found to release ATP under normal laboratory lights only very slowly, but as a precaution, experiments were done under subdued lighting conditions with the syringes of the quench flow apparatus covered with aluminum foil.

(2) Experimental Conditions. The kinetic experiments were carried out at 4 °C in 50 mM imidazole, 0.1 M potassium acetate, 2 mM magnesium acetate, and 0.1 mM $CaCl_2$ adjusted to pH 7 with acetic acid. Inhibitors were added as a 1:1 ratio with magnesium acetate so that if anything the free [Mg^{2+}] increased with addition of inhibitor. In some sets of single-turnover experiments, 50 μM PP_i was included in the solutions in order to block the contribution of myosin heads which were not interacting with actin. This concentration is much greater than the binding constant of PP_i to myosin and much less than the binding constant to actomyosin. The effect of this addition of PP_i was not investigated in detail. In the first set of experiments in which it was used, the fraction of hydrolysis in the slow phase was reduced while there was no significant effect on the fast phase. The amplitude of the slow phase was somewhat variable from protein preparation to preparation: a more thorough investigation would be needed to prove its value.

(3) Treatment of Data. The data were interpreted by Scheme 1 in which M represents myosin heads with or without actin attachment.

Scheme 1

$$M + ATP \xrightarrow{k_1} M^{*}\cdot ATP \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} M^{**}\cdot ADP\cdot P_i \xrightarrow{k_3} M + ADP + P_i$$

Experiments were carried out under multi- and single-turnover conditions. Under multiturnover conditions, the ATP concentration was typically 3–5 times that of the myosin head concentration. Here progress curves consist of a rapid P_i burst phase (of $M^{**}\cdot ADP\cdot P_i$, Scheme 1) followed by the steady-state rate (release of P_i). At low ATP concentrations, as here, the kinetics of the P_i burst give the ATP binding kinetics (k_1) directly as they are slower than the kinetics of the cleavage step. Most of our experiments were carried out under single-turnover conditions in which the myosin head concentrations were typically 10 times that of the ATP. In these circumstances, it is the myosin head concentration that determines the rate of ATP binding, and the head concentrations were chosen to make ATP binding rate-limiting, that is, much slower than the kinetics of the cleavage step such that all of the ATP is hydrolyzed in a single-exponential process which reports the ATP binding process. To obtain K_i, the dissociation constant for the binding of a competitive inhibitor, I (here ADP or caged ATP), the data were treated by $k' = kK_i/(K_i + [I])$ where k' and k are the rates for ATP binding in the presence and absence of inhibitor, respectively. This treatment is only appropriate if the process of caged ATP binding is in rapid equilibrium relative to the rate of ATP binding in the experiment. One validation of this assumption is that the model fits. A second argument is that the weak binding probably represents a state akin to a collision complex in which case the rate of binding is likely to be diffusion-controlled ($>10^6$ M^{-1} s^{-1}), which with a 1 mM binding constant would give an off rate of >1000 s^{-1}.

As found previously [for acto–S1, see Biosca et al. (1985); for S1, see Tesi et al. (1989); for myofibrils, see Houadjeto et al. (1992)], some of the progress curves were biphasic (e.g., with acto–S1 in Figure 4). This kinetic heterogeneity can be explained by the interference of a second site that binds ATP loosely, possibly without hydrolysis (Tesi et al., 1989). In cases, e.g., Figure 4, where the data are clearly biphasic, two exponentials were used to fit the complete set of inhibition data. The dominant fast exponential was assumed to represent ATP binding, and it is the inhibition of this phase which was characterized. However, if the same data were fitted with single exponential, the resultant inhibition constant was only marginally affected.

RESULTS

Effect of Caged ATP upon Acto–S1 Dissociation. The dissociation of acto–S1 was assayed using the turbidity at 450 nm. The experiments were carried out in 20 mM MOPS, 0.1 M NaCl, and 2 mM magnesium acetate, pH 7 and 4 °C. A mixture of 4 μM actin and 4 μM S1 was dissociated completely

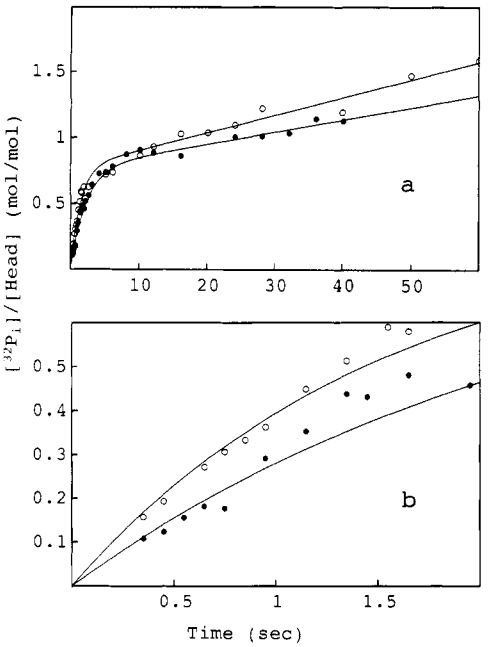

FIGURE 1: Time courses for P_i bursts with Ca^{2+}-activated myofibrils in the absence (○) or presence (●) of 1 mM caged ATP. The reaction mixtures (1 μM in myosin heads + 3.5 μM [γ-^{32}P]ATP) were quenched in acid at the times indicated, and [^{32}P]P_i was determined. Each set of data was fitted to an exponential (giving the kinetics of ATP binding) followed by a steady-state rate. (a) Complete time course; (b) time course of initial ATP binding kinetics.

by 150 μM ATP, but the turbidity remained unchanged when 2 mM Mg caged ATP was added. This observation that caged ATP does not dissociate acto–S1 is in accord with McCray et al. (1980). The lack of acto–S1 dissociation shows that the binding of ATP is not strongly coupled to actin binding and limits the maximum possible difference between caged ATP binding to S1 and acto–S1.

Effect of Caged ATP on the Binding of ATP to Myofibrils under Multiturnover Conditions. Preliminary multiturnover experiments were carried out at 3.5 μM ATP and 1 μM heads. The time course of ATP binding, as monitored by the degree of hydrolysis, in the presence and absence of 1 mM Mg caged ATP is shown in Figure 1. In the absence of caged ATP, the rate is 0.68 s^{-1}. In the presence of 1 mM caged ATP, the rate was reduced to 0.44 s^{-1}, which would correspond to an inhibition constant of 1.8 mM. The signal to noise ratio is not that good with this protocol, and for this reason, the inhibition was investigated in more detail under single-turnover conditions.

Effect of ADP and Caged ATP on the Binding of ATP to Myofibrils under Single-Turnover Conditions. Single-turnover experiments in the absence of ADP and at two ADP concentrations are shown in Figure 2a. The curves were fitted to single exponentials and the rate plotted as a function of the ADP concentration in Figure 2b. The fitted curve corresponds to an inhibition constant of 145 μM, a value in good agreement with those of Johnson and Adams (1984), Sleep and Glyn (1986), and Biosca et al. (1988). This agreement validates the method for investigating caged ATP.

Single turnovers in the absence and presence of caged ATP are shown in Figure 3a. Single exponentials were fitted to the data and the rates plotted as a function of caged ATP concentration in Figure 3b. The K_i was 1.6 mM.

Inhibition of ATP Binding to Acto–S1 by Caged ATP and ADP. The effect of caged ATP on the binding of ATP to acto–S1 is illustrated in Figure 4. Two phases of hydrolysis are evident. In this case, the slow phase is probably due to

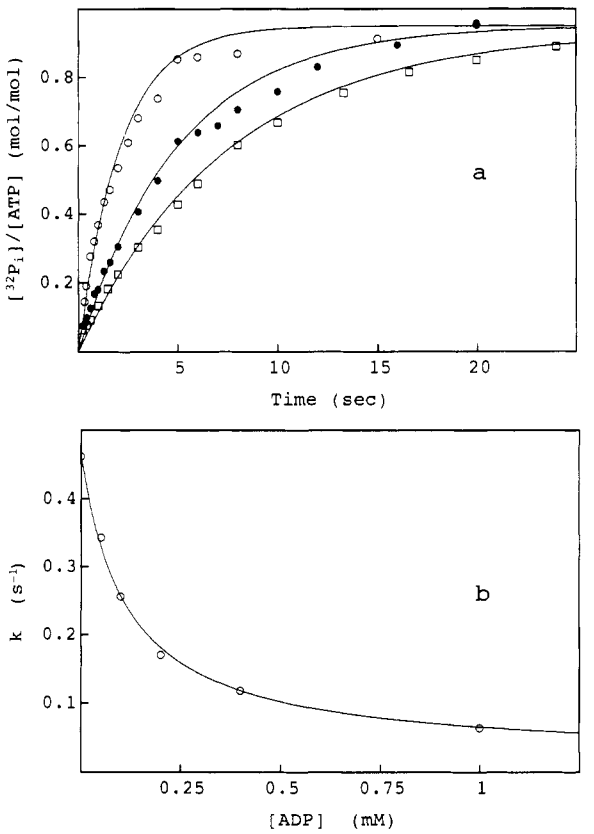

FIGURE 2: (a) P_i burst experiments with myofibrils under single-turnover conditions in the absence (○) and presence of ADP [(●) 0.2 mM; (□) 0.4 mM]. The reaction mixtures (3 μM in myosin heads + 0.6 μM [γ-^{32}P]ATP) were quenched in acid at the times indicated, and [^{32}P]P_i was determined. The data were fitted to single exponentials and gave k (in the absence of ADP) or k' (in the presence of ADP), the rate of ATP binding to myosin heads. For further details, see the text. (b) Dependence of k' upon the ADP concentration. The fitted curve corresponds to $k' = kK_i/(K_i + [I])$. The resultant K_i is 0.14 ± 0.01 mM (mean ± SD).

the hydrolysis which occurs as the cleavage step reequilibrates during product release. At physiological ionic strength and the low actin concentration used (1.5 μM), actin accelerates the myosin ATPase very little, and thus the slow rate of the second phase is compatible with this interpretation. In the absence of caged ATP, k = 0.78 s^{-1}, and in the presence of 1 mM caged ATP, k' = 0.21 s^{-1}. The K_i for caged ATP was calculated from these values and is given in Table 1 together with that obtained for ADP (P_i burst experiments not illustrated). Also given in Table 1 are the K_i values for caged ATP and ADP to myofibrils.

The binding constants to acto–S1 are significantly tighter than to myofibrils. McCray et al. (1980) reported the absence of any significant inhibition by 3 mM caged ATP on the rate of actomyosin dissociation by ATP. This discrepancy is surprising, but the full details of their experiments were not given.

DISCUSSION

The effects of caged ATP and ADP on the kinetics of the binding of ATP to myofibrils and acto–S1 have been investigated. Myofibrils were used to allow the application of rapid mixing transient kinetic methods because the diffusion time to the center makes the use of demembranated fibers unsatisfactory. Although spectral methods have been used to monitor the rate of ATP binding (Smith & White, 1985; Taylor, 1990), the attractions of the stopped-flow technique

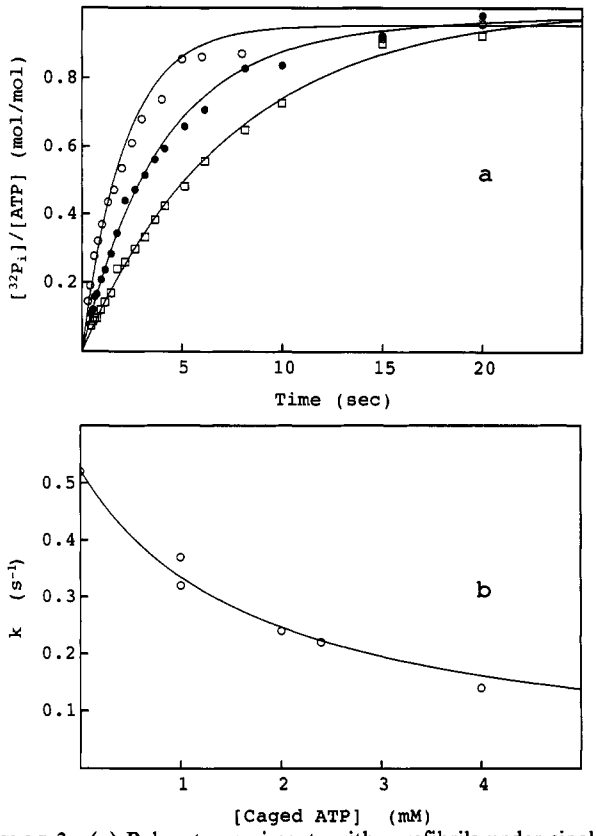

FIGURE 3: (a) P_i burst experiments with myofibrils under single-turnover conditions in the absence (○) and presence of caged ATP [(●) 2 mM; (□) 4 mM]. The fitted curves are single exponentials. The experiment and reaction mixtures are similar to those of Figure 2a. (b) Dependence of k' upon the caged ATP concentration. Fitted curve as for Figure 2b. The resultant K_i is 1.6 (±0.2) mM.

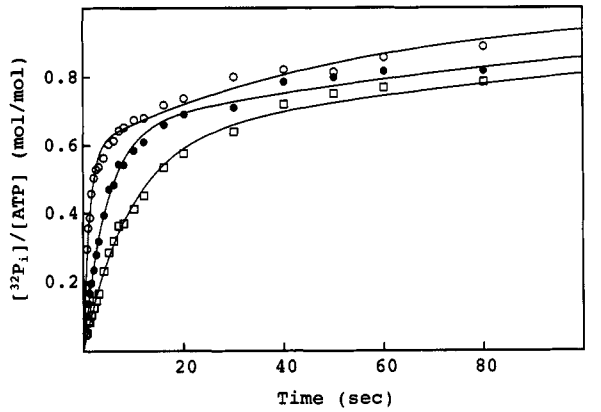

FIGURE 4: P_i burst experiments with acto–S1 under single-turnover conditions in the absence (○) and presence of caged ATP [(●) 1 mM; (□) 2 mM]. The reaction mixtures (1 μM S1, 1.5 μM actin + 0.1 μM [γ-^{32}P]ATP) were quenched in acid at the times indicated, and [^{32}P]P_i was determined. The data were fitted to two exponentials. The average amplitude of the fast phase was 62% of the total and was assumed to represent ATP binding, and the effect of the inhibitor on this phase was used to deduce the inhibition constant.

relative to the quenched flow are partially offset either by the need to use fluorescent nucleotides or by the need to label the myofibrils with an extrinsic fluorescent probe. We chose to monitor ATP binding by monitoring the rate of [γ-^{32}P]ATP splitting using the rapid flow quench method. To ensure maximum precision, the experiments were carried out with single turnovers, and under the conditions that we chose (low concentration of myosin heads), the P_i production was an accurate reflection of ATP binding. This approach was validated by experiments with ADP in which the inhibition

Table 1: Inhibition Constants for ADP and Caged ATP with Myofibrils and Acto–S1 at 4 °C[a]

system	ligand	K_i (mM)
myofibrils	ADP	0.14 (±0.01)
	caged ATP	1.6 (±0.2)
acto-S1	ADP	0.05 (±0.01)
	caged ATP	0.35 (±0.1)

[a] The data were from P_i burst experiments under single-turnover conditions. The experimental conditions were 50 mM imidazole, 0.1 M potassium acetate, and 0.1 mM $CaCl_2$, pH 7. For further details, see Figures 2–4.

constants obtained were in good agreement with the literature values.

Implication of the Binding of Caged ATP to Myofibrils. Our results show that caged ATP is a simple competitive inhibitor of ATP with a binding constant of 1.6 mM. Dantzig et al. (1989) measured the effect of caged ATP on the rate of relaxation of fibers and reported a somewhat tighter binding constant (0.6 mM). However, by using apyrase to eliminate ADP contamination, Thirlwell et al. (1993) found that ADP is a potent (10 μM) inhibitor of the relaxation process, and the experiments of Dantzig et al. need to be repeated on the two isomers of caged ATP using apyrase to eliminate ADP.

Knowledge of the inhibition constant for caged ATP allows the conditions for mechanical experiments to be optimized. For example, doubling the concentration of caged ATP from 5 to 10 mM doubles the amount of ATP released, and in the absence of inhibition, this would double the rate of ATP binding. However, with the caged ATP inhibition constant of 1.6 mM reported in this paper, 5 mM caged ATP reduces the rate of ATP binding by a factor of 4.1 and 10 mM by a factor of 7.25. Thus, doubling the caged ATP concentration only results in an increase in the rate of ATP binding by a factor of 1.1, and this small increase hardly justifies having an extra 5 mM Mg caged ATP in the solution.

A value of 1.6 mM for the K_i of caged ATP accounts for the reported slower binding of ATP to fibers (measured using caged ATP) than to acto–S1 (measured in the absence of caged ATP). It also accounts for the observation that the rate of shortening following the release of ATP from caged ATP is significantly slower than expected at the almost saturating [ATP] released (1 mM; Burton, Irving, and Sleep, unpublished results). This inhibition of the shortening velocity has been investigated in a more direct manner by Thirlwell et al. (1994), who reported a value for K_i of 1–2 mM.

Binding of Caged ATP and ADP to Acto–S1. Both ADP and caged ATP appear to bind more tightly to acto–S1 than to myofibrils. With ADP, the difference may be due to this ligand partially dissociating the acto–S1 at the physiological ionic strength and low protein concentrations used. S1 binds ADP much more tightly than acto–S1, and the possibility of dissociation means that the observed K_i is a lower limit. With caged ATP, we cannot think of an attractive explanation for the difference in binding constants for acto–S1 and myofibrils. As we observerd, the binding of caged ATP does not result in significant dissociation of acto–S1, and the explanation proposed for the ADP result is not appropriate.

How Does Caged ATP Bind to the Myosin Heads? Only in the case of caged GTP binding to Ha-Ras p21 is there anything significant known about the interaction of caged nucleotides and proteins. In solution, caged GTP binds very tightly to this protein (10^{10} M^{-1}), almost as tightly as GDP (10^{11} M^{-1}) (Schlichting et al., 1990). However, in the crystal, caged GTP binds differently from GTP, GPPNP, and GDP, and this is thought to be due to crystal packing forces. The

Ras example demonstrates clearly the possibility of inhibition, but it is clear that caged ATP binding to myosin is very different.

The binding constant of ATP to S1 is about 10^{11} M^{-1} whereas we report a binding constant 7 orders of magnitude weaker for caged ATP. As might be expected, caged ATP is not a significant dissociating agent for acto–S1. The importance of the triphosphate for tight binding is evidenced by the fact that the P_i binding constant is about 1 mM (Bagshaw & Trentham, 1974) whereas that for PP_i is about 0.1 μM (Greene & Eisenberg, 1980). By analogy with other P_i binding proteins, Rayment et al. (1993) deduced the mode of binding of ATP to the S1 crystal structure. The triphosphate group is pointing into the ATP pocket, and it is probable that the bulky cage of the γ-P_i is preventing the normal mode of binding and that this accounts for the massive reduction in the strength of binding and the lack of coupling of binding to actin dissociation. It seems likely that caged ATP binding to myosin bears only a limited relation to the binding of other nucleotides.

REFERENCES

Bagshaw, C., & Trentham, D. R. (1974) *Biochem. J. 141*, 331–349.

Barman, T. E., & Travers, F. (1985) *Methods Biochem. Anal. 31*, 1–59.

Biosca, J. A., Travers, F., Barman, T. E., Bertrand, R., Audemard, E., & Kassab, R. (1985) *Biochemistry 24*, 3814–3820.

Biosca, J. A., Greene, L. E., & Eisenberg, E. (1988) *J. Biol. Chem. 263*, 14231–14235.

Dantzig, J. A., Goldman, Y. E., Luttmann, M. L., Trentham, D. R., & Woodward, S. K. A. (1989) *J. Physiol. (London) 418*, 61P.

Dantzig, J. A., Goldman, Y. E., Millar, N. C., Lacktis, J., & Homsher, E. (1992) *J. Physiol. (London) 451*, 247–278.

Goldman, Y. E., Hibberd, M. G., & Trentham, D. R. (1984a) *J. Physiol. (London) 354*, 577–604.

Goldman, Y. E., Hibberd, M. G., & Trentham, D. R. (1984b) *J. Physiol. (London) 354*, 605–624.

Greene, L. E., & Eisenberg, E. (1980) *J. Biol. Chem. 255*, 543–548.

Herrmann, C., Sleep, J., Chausspied, P., Travers, F., & Barman, T. (1993) *Biochemistry 32*, 7255–7263.

Houadjeto, M., Travers, F., & Barman, T. (1992) *Biochemistry 31*, 1564–1569.

Johnson, R. E., & Adams, P. H. (1984) *FEBS Lett. 174*, 11–14.

Kaplan, J. H., Forbush, B., & Hoffman, J. F. (1978) *Biochemistry 17*, 1929–1935.

McCray, J. A., Herbette, L., Kihara, T., & Trentham, D. R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7237–7241.

Rayment, I., Rypniewski, W. R., Schmidt-Bäse, K., Smith, R., Tomchick, D. R., Benning, M. M., Winkelman, D. A., Wesenberg, G., & Holden, H. H. *Science 261*, 50–58.

Reimann, E. M., & Umfleet, R. A. (1978) *Biochim. Biophys. Acta 523*, 516–521.

Schlichting, I., Almo, S. C., Rapp, G., Wilson, K., Petratos, K., Lentfer, A., Wittinghofer, A., Kabsch, W., Pai, E. F., Petsko, G. A., & Goody, R. S. (1990) *Nature 345*, 309–315.

Sleep, J., & Glyn, H. (1986) *Biochemistry 25*, 1149–1154.

Sleep, J., & Burton, K. (1990) *Biophys. J. 57*, 542a.

Smith, S. J., & White, H. D. (1985) *J. Biol. Chem. 260*, 15156–15162.

Taylor, E. W. (1990) *Biophys. J. 57*, 336a.

Tesi, C., Bachouchi, N.; Barman, T. E., & Travers, F. (1989) *Biochimie 71*, 363–372.

Tesi, C., Kitagishi, K., Travers, F., & Barman, T. (1991) *Biochemistry 30*, 4061–4067.

Thirlwell, H., Bancel, F., & Ferenczi, M. A. (1993) *Biophys. J. 64*, 251a.

Thirlwell, H., Sleep, J., Corrie, J. E. T., Trentham, D. R., & Ferenczi, M. A. (1994) *Biophys. J. 66*, A302.

Walker, J. W., Reid, G. P., McCray, J. A., & Trentham, D. R. (1988) *J. Am. Chem. Soc. 110*, 7170–7177.

White, H. D., & Taylor, E. W. (1976) *Biochemistry 15*, 5818–5826.