Pressure-relaxation studies of pyrene-labelled actin and myosin subfragment 1 from rabbit skeletal muscle

Evidence for two states of acto-subfragment 1

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We have used actin labelled at Cys-374 with *N*-(1-pyrenyl)iodoacetamide [Kouyama & Mihashi (1981) Eur. J. Biochem. **114**, 33–38] to monitor pressure-induced relaxations of acto–myosin subfragment 1. This label greatly increases the sensitivity of measurement of dissociated actin and reveals the presence of two relaxations. The experimental data can be fitted by a model in which actin binds subfragment 1 relatively weakly ($K = 5.9 \times 10^4$ M^{-1}) and then isomerizes to a more tightly bound complex ($K = 1.7 \times 10^7$ M^{-1}). This directly observed isomerization supports the model of Geeves, Goody & Gutfreund [(1984) J. Muscle Res. Cell. Motil. **5**, 351–361]. The rate of the isomerization is too high to be observed in the pressure-jump apparatus (< 200 μs), but analysis of the amplitudes allows estimation of the equilibrium constant of the isomerization as 280 (20 °C, 0.1 M-KCl, pH 7). The equilibrium is sensitive to temperature, pressure, ionic strength and the presence of ethylene glycol. The pressure-sensitivity of the isomerization suggests a significant conformational change of the acto–myosin subfragment 1 complex.

INTRODUCTION

Muscle contraction is believed to be the result of a dynamic interaction of actin with myosin modulated by ATP. In order to understand the role of this interaction in force generation we have been studying the dynamics of the interaction between actin and S1 in solution. Geeves & Gutfreund (1982) reported that pressure relaxation could be used as a tool to study the mechanism of actin–S1 interactions. Increased pressure causes a small fraction of the bound S1 to dissociate from acto·S1; the rapid release of pressure back to 101 kPa (200 μs) allows the reassociation reaction to be monitored. The interaction was monitored by following the changes in light-scattering produced as S1 binds to the actin filament. The sensitivity of this method limited the study to conditions where the association constant of S1 to actin was in the range 3×10^4–10^6 M^{-1}. This allowed the interaction to be studied in the presence of ADP and AMP·PNP under a wide range of experimental conditions (Geeves & Gutfreund, 1982; Konrad & Geeves, 1982). In the absence of nucleotide, the interaction could only be studied in the presence of 0.5 M-KCl, under which condition the affinity of S1 for actin was decreased.

We report here the use of actin labelled at Cys-374 with *N*-(1-pyrenyl)iodoacetamide to monitor the interaction of actin with S1. This highly fluorescent actin responds to S1 binding by a 70% quenching of the pyrene fluorescence (Kouyama & Mihashi, 1981). Evidence was presented in the preceding paper (Criddle *et al.*, 1985) that the pyrene label has only a relatively small specific effect on the interaction of actin with S1. The use of this fluorescently labelled actin increases the sensitivity of detection by a factor of 10 and so allows the interaction of actin with S1 to be monitored at physiological ionic strength. This label also reveals the presence of additional acto·S1 intermediates.

MATERIALS AND METHODS

Proteins

F-actin was prepared as described by Lehrer & Kerwar (1972). Actin labelled at Cys-374 by *N*-(1-pyrenyl) iodoacetamide was prepared as described by Criddle *et al.* (1985) in the accompanying paper. Labelling was more than 90% for all the experiments reported here.

S1 was prepared by the method of Weeds & Taylor (1975). The isoenzymes A1 and A2 were not separated for use in these experiments as they appear to be identical in the way in which they interact with actin at any ionic strength above 0.05 M.

Instrumentation

The pressure-relaxation equipment has been described by Davis & Gutfreund (1976) and its use for measuring turbidity changes of acto·S1 solutions described by Geeves & Gutfreund (1982). For experiments on pyrene fluorescence, a high-pressure mercury lamp (Wotan HBO, 100 W) was used. Excitation at 368 nm was obtained by a Farrand monochromator and the light was passed through a Schott UG 11 filter. Emission was monitored through a Schott KV 393 filter. Kinetic data were collected on a Data Lab DL905 transient recorder and then transferred to an ITT 2020 microcomputer for

Abbreviations used: S1, S (in equations), myosin subfragment 1; pyr-actin, actin labelled at Cys-374 by *N*-(1-pyrenyl)iodoacetamide; A, D (mainly in equations), actin and adenosine diphosphate respectively; τ, relaxation time; overbars indicate 'free' concentrations.

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storage and analysis. The data was analysed by using a non-linear least-squares fitting routine as described by Edsall & Gutfreund (1983).

RESULTS AND DISCUSSION

The change in turbidity produced by a rapid reduction in hydrostatic pressure from 15.2 MPa (150 atm, 2196 lbf·in^{-2}) to 101 kPa (1 atm) on a solution of acto·S1 in the presence of 0.5 M-KCl is shown in Fig. 1. As shown by Geeves & Gutfreund (1982) for acto·S1 in the presence of 2 mM-ADP (0.1 M-KCl, pH 8, 20 °C) the data fit a single exponential, the amplitude is directly proportional to the pressure change and the observed rate is linearly related to the sum of the free actin and S1 concentrations. Geeves & Gutfreund (1982) interpreted their data in terms of a single-step binding model:

$$A+S\cdot D \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} A\cdot S\cdot D \qquad (1)$$

and

$$\frac{1}{\tau} = k_{+1}([\overline{A}]+[\overline{S\cdot D}])+k_{-1} \qquad (1a)$$

The data were fitted to this model by assuming a value of K_1 to calculate the free concentration of actin and S1, and using these in eqn. (1a) to obtain estimates of k_{+1} and k_{-1}. An iteration procedure was then carried out until K_1 and k_{+1}/k_{-1} agreed to within 10%. However, the value of $k_{+1}(5.4\times10^4$ M$^{-1}\cdot$s$^{-1})$ and the high activation energy of this process [$E = 107.5$ kJ/mol] suggested to these authors that formation of an encounter complex preceded A·S·D.

$$A+S\cdot D \overset{K_0}{\rightleftharpoons} A\cdot S\cdot D_0 \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} A\cdot S\cdot D_1 \qquad (2)$$

where step 0 is a rapid equilibrium and pressure perturbation occurs on step 1. In this case the observed relaxation is given by:

$$\frac{1}{\tau} = \frac{K_0k_{+1}([\overline{A}]+[\overline{SD}])}{1+K_0([\overline{A}]+[\overline{SD}])}+k_{-1} \qquad (2a)$$

and when $K_0([\overline{A}]+[\overline{S\cdot D}]) < 1$, then the slope of the plot $1/\tau$ against total free protein concentration gives K_0k_{+1} and the intercept gives k_{-1}.

The turbidity data shown in Fig. 1(*a*) can be treated in the same way. A plot of $1/\tau$ against total free protein concentration is shown in Fig. 1(*b*). In this case the activation energy is also too high for the process observed to be diffusion controlled [$E = 92$ kJ/mol] and a model similar to that of eqn. (2) is proposed where $K_0k_{+1} = 1.37\times10^5$ M$^{-1}\cdot$s^{-1}, $k_{+1} > 10$ s^{-1} and $k_{-1} = 0.38$ s^{-1}. This is in good agreement with stopped-flow data, which gives $K_0k_{+1} = 1.6\times10^5$ M$^{-1}\cdot$S^{-1} and $k_{+1} > 30$ s^{-1} (Criddle *et al.*, 1985).

Pyr-actin was used in a similar experiment, but the reaction was monitored by following the changes in pyrene fluorescence. The results are shown in Fig. 2. The turbidity changes occurring in the pyr-acto·S1 case cannot be successfully monitored owing to interference from the absorbance changes occurring in pyrene in the range 330–400 nm. These pressure perturbations produced two relaxations: a fast relaxation, which was complete within the pressure-release time (200 μs), and a slow exponential relaxation. The slow phase was fitted to a single

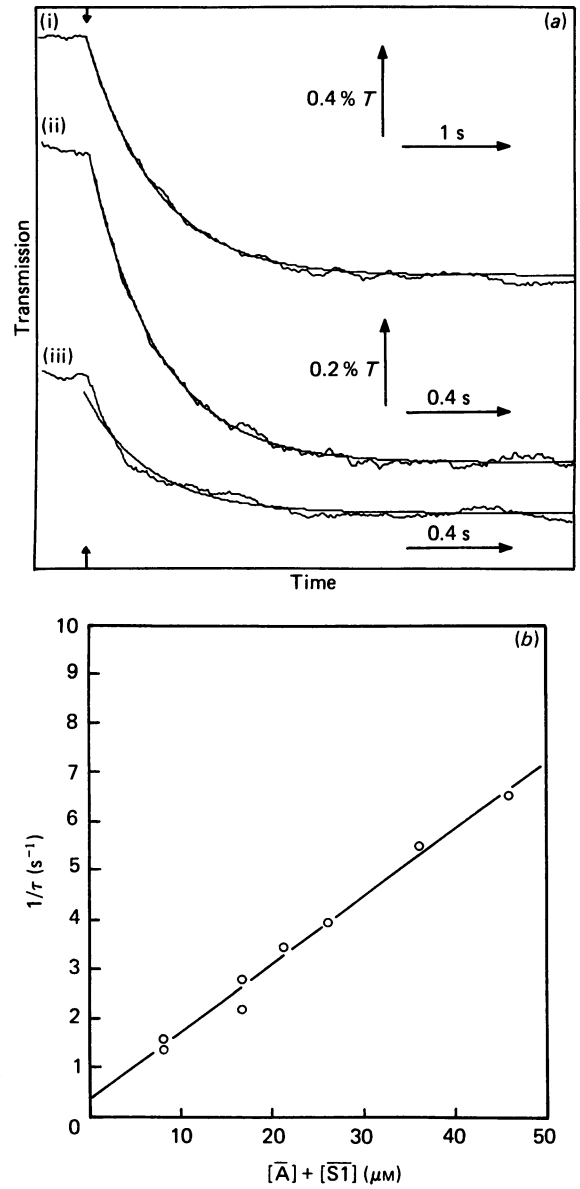


Fig. 1. (*a*) Pressure-induced changes in the transmission of a solution of acto·S1 and (*b*) plot of the reciprocal relaxation time ($1/\tau$) against the free concentrations of actin and S1

(*a*) The arrows indicate the time of pressure release, the pressure release in each case being a decrease from 15.2 MPa (150 atm) to 101 kPa (1 atm). Reaction conditions: 0.5 M-KCl/5mM-$MgCl_2$/20 mM-imidazole, pH 7 at 20 °C. Each trace represents the average for five relaxations on the same solution, and the best-fit single exponential is superimposed. (i) 4.9 μM-Actin, 10.0 μM-S1; $1/\tau = 1.48$ s^{-1}; (ii) 4.9 μM-actin, 30.1 μM-S1; $1/\tau = 3.98$ s^{-1}; (iii) 4.9 μM-actin, 50.2 μM-S1; $1/\tau = 6.67$ s^{-1}. (*b*) The line represents the best fit to the data and gives a gradient of 1.37×10^5 M$^{-1}\cdot$s^{-1} and an intercept of 0.38 s^{-1}.

exponential from a point where the fast phase was clearly complete, normally 10–50 ms after the release time; the fitted curves shown in Fig. 2(*a*) were then extrapolated back to zero time. The amplitudes of the fast phase increased and the slow phase decreased with increasing

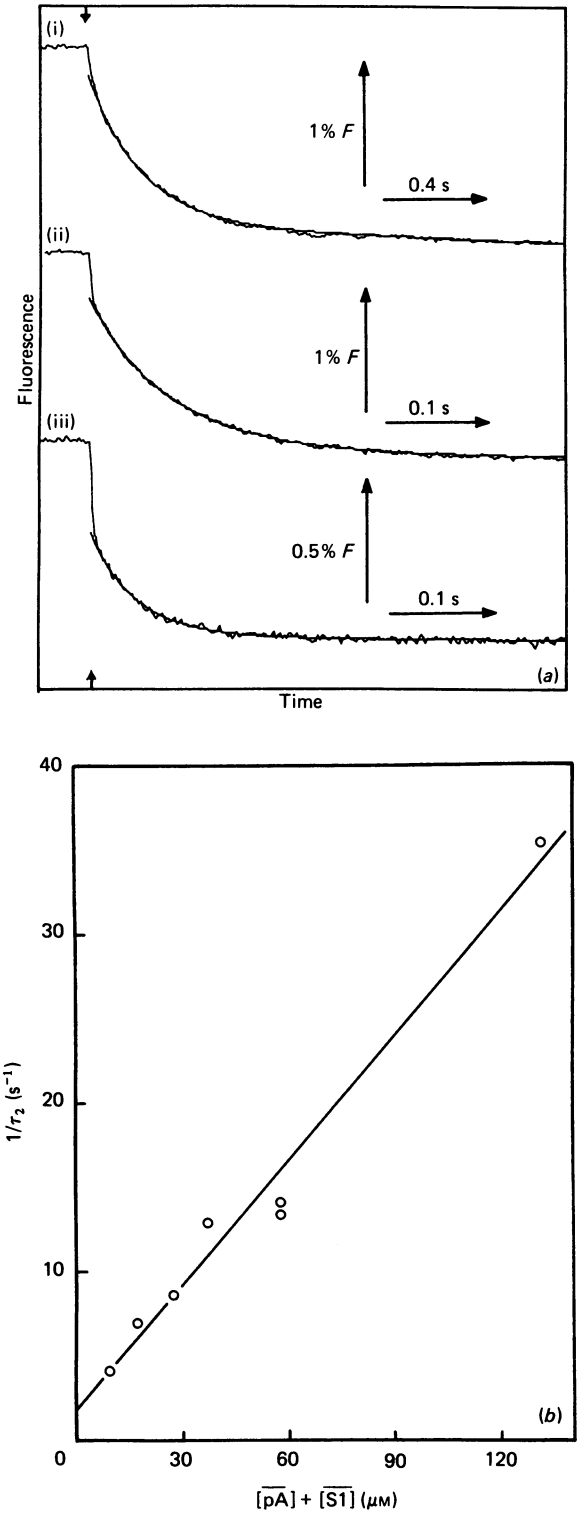


Fig. 2. (*a*) Pressure-induced changes in the fluorescence of a solution of pyrene-labelled acto·S1 and (*b*) plot of the observed rate of the slow phase of the relaxation against the free concentration of pyr-actin and S1

(*a*) The arrows indicate the time of pressure release, the pressure release in each case being a decrease from 10.1 MPa (100 atm) to 101 kPa (1 atm). Reaction conditions: 0.5 M-KCl/5 mM-$MgCl_2$/20 mM-imidazole, pH 7 at 20 °C. Each trace represents the average for five relaxations on the same solution, and the best-fit single exponential to the slow phase is superimposed. (i) 5.5 μM-Pyr-actin (pA), 10.5 μM-S1; $1/\tau = 4.1\ s^{-1}$; ratio of the amplitudes (amp_1/amp_2) = 0.172; (ii) 5.5 μM-pyr-actin, 62.8 μM-S1; $1/\tau = 13.5\ s^{-1}$; $amp_1/amp_2 = 0.34$; (iii) 5.5 μM-pyr-actin, 94.1 μM-S1; $1/\tau = 23.9\ s^{-1}$; $amp_1/amp_2 = 0.91$. (*b*) The line represents the best fit to the data assuming a value of 0.9 s^{-1} for the intercept. The fitted data give gradient of $2.5 \times 10^5\ M^{-1} \cdot s^{-1}$.

protein concentration. The relationship between the observed rates and the free protein concentration for pyr-actin are shown in Fig. 2(*b*). The data were fitted by assuming that the intercept was equal to the dissociation rate constant obtained by displacement (Criddle *et al.*, 1985) and then treated as described above. The estimates of K_0k_{+1} and K_0K_1 obtained from these plots agree with those obtained by stopped-flow and direct-titration measurements in the preceding paper (Criddle *et al.*, 1985).

The greater sensitivity of this fluorescent probe allowed the interaction to be studied at 0.1 M-KCl, where the induced turbidity changes were too small to be observed with unlabelled actin. These results are shown in Fig. 3. The same phenomena were observed as at higher ionic strength. The pressure perturbation produces two relaxations: the fast one was too fast to be measured. Analysis of the slow relaxation in the same way as for the example above $K_0k_{+1} = 3.63 \times 10^6\ M^{-1} \cdot s^{-1}$, $k_{-1} = 0.22\ s^{-1}$ and $K_0K_1 = 1.7 \times 10^7\ M^{-1}$, which agree with the values in the preceding paper (Criddle *et al.*, 1985).

Pressure perturbations of the fluorescently labelled actin and S1 produce two relaxations. These must represent two events in the interaction of actin with S1, since the fluorescence probe is attached to actin and cannot report directly events occurring on dissociated S1. A solution of actin alone does show a fast perturbation with small amplitude on the same time scale as the fast relaxation observed here. This varies with different actin preparations and can have a positive or negative amplitude. The fast relaxation observed here is very reproducible and the amplitude increases with increasing concentration of S1. We conclude, therefore, that it reflects an event occurring on the associated acto·S1.

The two-step model of eqn. (2) requires that step 0 is fast compared with step 1, that step 1 is perturbed by the pressure change, and that either the turbidity of $A \cdot S_0$ is equal to the turbidity of A or that the concentration of $A \cdot S_0$ is always very small. The assignment of the fluorescence change to either step 0 or to step 1 cannot produce the observed fluorescence relaxations. We therefore conclude that the fluorescence change must be reporting an additional event in the association reaction. In deriving a model to account for the observed relaxation we assume, for simplicity, that the change in fluorescence and the pressure-sensitivity occur primarily on one step. In order that the fast event is seen with fluorescence and not with turbidity the fluorescence signal must be monitoring the fast, pressure-sensitive step, which has no significant turbidity change. We therefore propose the following model:

$$A + S \overset{K_0}{\rightleftharpoons} AS_0 \underset{k_{-a}}{\overset{k_{+a}}{\rightleftharpoons}} AS_1 \underset{k_{-b}}{\overset{k_{+b}}{\rightleftharpoons}} AS_2 \qquad (3)$$

where $([\overline{A}]+[\overline{S}])k_0$, $k_{-0} \gg k_{+a}$, k_{-a} and $([\overline{A}]+[\overline{S}])K_0 \cdot k_{+a}$, $k_{-a} \ll k_{+b}+k_{-b}$. Step b is pressure-sensitive and

fluorescence reports this transition. Under these conditions:

$$\frac{1}{\tau_1} = k_{+b} + k_{-b} \tag{3a}$$

$$\frac{1}{\tau_2} = \frac{K_0 k_{+a}([\bar{A}]+[\bar{S}])}{1+K_0([\bar{A}]+[\bar{S}])} + \frac{k_{-a}\cdot k_{-b}}{k_{+b}+k_{-b}} \tag{3b}$$

again a plot of $1/\tau_2$ against $([\bar{A}]+[\bar{S}])$ will be linear and gradient/intercept =

$$K_0 k_{+a} \Big/ \frac{k_{-a}\cdot k_{-b}}{k_{+b}+k_{-b}} = K_0 \cdot K_a(1+K_b)$$

as is observed experimentally. In this model only the slow relaxation will be observed when studied using turbidity, which is primarily monitoring step a.

One further mechanism may be considered in which S1 can bind to actin in two different ways:

$$A+S \overset{K_0}{\rightleftharpoons} AS_0 \begin{matrix} \overset{k_{+1}}{\rightleftharpoons} AS_1 \\ \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} \end{matrix} AS_2 \tag{4}$$

This model may be eliminated by considering the signal change that comes from each of the species involved. If we assign the fast phase to step 1, then the fluorescence of AS_1 must be less than the fluorescence of A in order to produce the observed fast phase. If the fluorescence of A and AS_2 are equal, then the slow phase would represent the transition $AS_2 \rightarrow AS_0 \rightarrow AS_1$. In this case, monitoring the reaction by light-scattering would observe the fast phase and not the slow phase, the opposite of what is observed. There is no assignment of the fluorescent signal from the three states that would give the observed results for both light-scattering and fluorescence unless a large difference is assumed in the light-scattering of AS_1 and AS_2.

Models in which there is a change in both K_a and K_b with pressure and in which both steps produce a fluorescence signal cannot be ruled out, but the data can be fitted by the simpler model of eqn. (3).

Analysis of the amplitudes of the two phases also support the model of eqn. (3). The amplitude of the fast phase increases with increasing protein concentration, reflecting the increase in concentration of AS_2. The amplitude of the slow phase has a more complex behaviour, reflecting the degree of saturation of the equilibrium defined by K_0K_a after perturbation of step b. If we assume that the formation of the encounter complex is rapid compared with step a, then replacing K_0K_a with K'_a allows the use of the approach of Bernasconi (1976) to analyse the model described by eqn. (3). The change in concentration of AS_2 ($\Delta[AS_2]$) is then described by:

$$\Delta[AS_2] = \frac{\Delta K'_a \cdot K_b \cdot [\bar{A}][\bar{S}] + [\overline{AS}_1]\Delta K_b[1+K'_a([\bar{A}]+[\bar{S}])]}{1+K'_a([\bar{A}]+[\bar{S}])(1+K_b)} \tag{3c}$$

where $\Delta K'_a$ and ΔK_b are the changes in K'_a and K_b induced by a pressure change, ΔP. If perturbation only occurs on step b, then $\Delta K'_a = 0$ and:

$$\Delta[AS_2] = \frac{[\overline{AS}_1]\Delta K_b[1+K'_a([\bar{A}]+[\bar{S}])]}{1+K'_a([\bar{A}]+[\bar{S}])(1+K_b)} \tag{3d}$$

Furthermore, if step b is very much faster than step a then $\Delta[A\cdot S_2]$ will have two phases:

$$\Delta[AS_2] = \Delta^1[AS_2] + \Delta^2[AS_2]$$

the fast phase will be given by:

$$\Delta^1[AS_2] = \frac{[\overline{AS}_1]\Delta K_b}{1+K_b} \tag{3e}$$

and the slow phase by:

$$\Delta^2[AS_2] = \frac{[\overline{AS}_1]K_b\Delta K_b}{(K_b+1)[1+K'_a([\bar{A}]+[\bar{S}])(1+K_b)]} \tag{3f}$$

The ratio of the two amplitudes (Amp) is then:

$$\frac{Amp_1}{Amp_2} = \frac{\Delta^1[AS_2]}{\Delta^2[AS_2]} = \frac{1}{K_b} + K'_a \cdot \frac{(1+K_b)}{K_b} \cdot ([\bar{A}]+[\bar{S}]) \tag{3g}$$

A plot of amp_1/amp_2 against concentration of free protein will be linear and gives an intercept of $1/K_b$ and a gradient of $K'_a(1+K_b)/K_b$. Plots of this type are shown in Fig. 4 for the data already presented; $1/K_b$ is poorly defined by these data as it is small, but as this means that $(1+K_b)/K_b$ is approximately equal to 1, then K'_a can be obtained from the gradient. K_b can then be estimated from the independently obtained value of the association constant $[K'_a(1+K_b)]$.

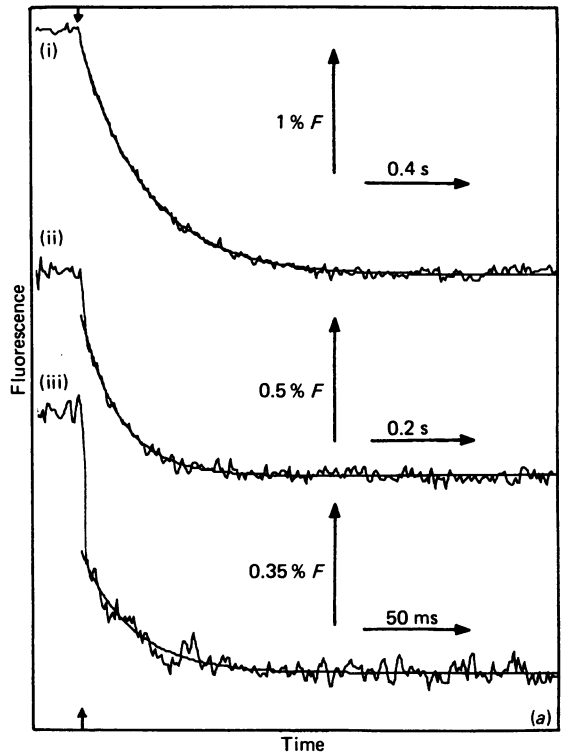
The values of K_0K_a and K_b obtained by this approach are shown in Table 1. This Table also shows the rate constants obtained from the analysis of the slower relaxation time and the effect of temperature, ionic strength and ethylene glycol on these constants. Under all conditions the first relaxation was too fast to be measured, i.e. the reaction was complete within 200 μs.

The results presented in Table 1 shows that K_0k_{+a} (and hence K_0K_a) is sensitive to changes in ionic strength, temperature and to solvent composition, as has been shown previously by stopped-flow studies. The dissociation rate shows smaller sensitivity to the same parameters. K_b, which reflects the proportion of acto·S1 in the two states $A\cdot S_1$ and $A\cdot S_2$, is also sensitive to each of these parameters. K_b changes from 280 to 37 on increasing the KCl concentration from 0.1 M to 0.5 M and from 280 to 22 in the presence of 40% ethylene glycol. A decrease in temperature from 20 °C to 2 °C produces a smaller change in K_b to 200. In addition to these quantified effects on K_b, we have shown here that K_b is sensitive to changes in hydrostatic pressure. In principle it is possible to use the amplitude data to obtain the volume change for the transition between the two acto·S1 states from the relationship:

$$\Delta \ln K = -\frac{\Delta V \cdot \Delta P}{RT}$$

where ΔP is the induced pressure change and ΔV is the molar volume change of the reaction.

In practice, however, the calculation is imprecise for fluorescence data and the change in concentration of $A\cdot S_2$ is very small (estimated to be about 1%). However, as K_b is much greater than 1 in all cases, a 1% change in $A\cdot S_2$ can mean a substantial change in the concentration of $A\cdot S_1$, so that K_b may change by as much as 50%. This would involve a volume change of the order of 100 cm³/mol of complex. This compares to a volume change of 24 cm³/mol for the ionization of phosphate (pK 7), 23 cm³/mol for the transfer of methane from a hydrophobic solvent to water and 280 cm³/mol for myosin filament propagation (Davis & Gutfreund, 1976). A volume change of this size implies a substantial rearrangement of the hydration sphere around the acto·S1 complex and would require either a substantial



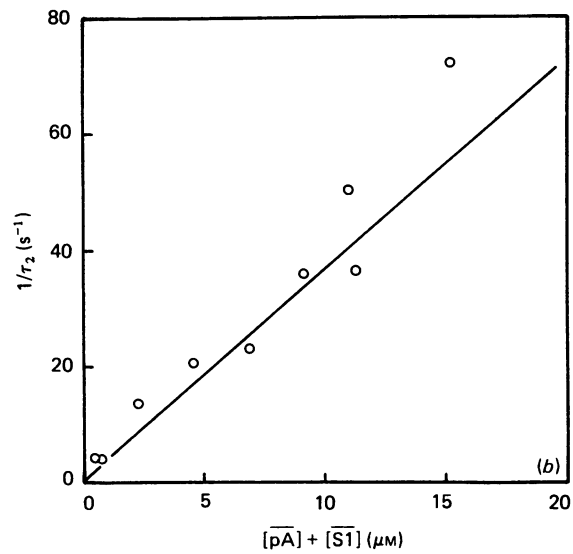


Fig. 3. (*a*) Pressure induced changes in the fluorescence of a solution of pyrene labelled acto·S1, and (*b*) dependence of the reciprocal relaxation time on the free concentrations of pyr-actin and S1

Reaction conditions: 0.1 M-KCl/5 mM-$MgCl_2$/20 mM-imidazole, pH 7 at 20 °C. (i) 2.7 μM-pyr-actin, 2.4 μM-S1; $1/\tau = 4.1\ s^{-1}$; $amp_1/amp_2 = 0.04$; (ii) 2.6 μM-pyr-actin, 4.8 μM-S1; $1/\tau = 13.6\ s^{-1}$; $amp_1/amp_2 = 0.26$; (iii) 5.0 μM-pyr-actin, 15.9 μM-S1; $1/\tau = 50.5\ s^{-1}$; $amp_1/amp_2 = 0.91$. All other conditions were as for Fig. 2. (*b*) The best-fit line assuming an intercept of 0.2 s^{-1} gives the line shown with a gradient of $3.6 \times 10^6\ M^{-1} \cdot s^{-1}$.

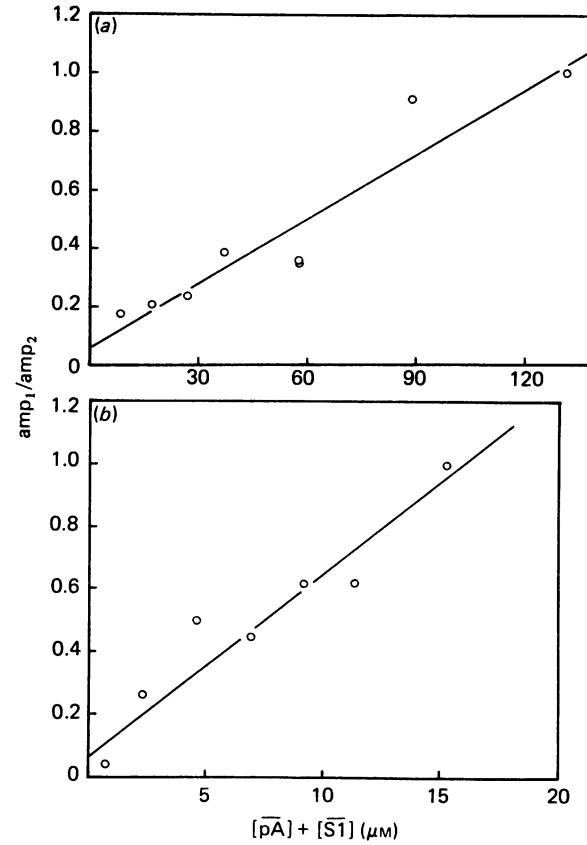


Fig. 4. Plots of the ratio of the observed amplitudes (amp_1/amp_2) against the concentration of free pyr-actin and S1

(*a*) Reaction conditions were as for Fig. 2. The line represents the best-fit line to the data and gives a gradient of $7.4 \times 10^3\ M^{-1}$ and an intercept of 0.054. (*b*) Reaction conditions were as for Fig. 3. The best-fit line is shown with a gradient of $5.9 \times 10^4\ M^{-1}$ and an intercept of 0.063.

conformational change in at least one of the two proteins or a change in the contact area between the two.

The two acto·S1 states, observed here for the first time, are of interest in terms of the cross-bridge model of muscle contraction. This model, as normally described, requires two types of attachment of myosin to actin (referred to as the '90°' and '45°' states) and the transition between the two states is intimately associated with the force generation. Geeves *et al.* (1984) discussed how studies on the kinetics of actin–S1 interactions can be interpreted in terms of two-state binding of S1 to actin. In their model, S1 binds to actin in two steps subsequent to the formation of the encounter complex. The first step is relatively independent of the nature of occupancy of the S1 nucleotide-binding site and a weak-binding state is formed. The isomerization to the strong-binding state is dependent on the nature of the nucleotide bound to S1. In the absence of nucleotide and in the presence of ADP, $K_b > 1$ and the strong-binding state predominates. With ATP or ADP·P_i bound to S1, $K_b < 1$ and the weak-binding state predominates. Geeves *et al.* (1984) provided estimates of K_b for the various nucleotides on the basis of several indirect pieces of evidence. We report here the first direct evidence to support this model in the case of acto·S1 in the absence of nucleotide and provide

Table 1. Rate and equilibrium constants evaluated for the interaction of pyr-actin and S1

Conditions	Association constant (M^{-1})	K_0K_a (M^{-1})	K_b	K_0k_{+a} ($M^{-1}\cdot s^{-1}$)	Dissociation rate* constant (s^{-1})	k_{-a}† (s^{-1})
Standard‡	1.7×10^7	5.9×10^4	280	3.63×10^6	0.22	61
2 °C	1.5×10^6	7.6×10^3	200	1.48×10^5	0.10	19.5
0.5 M-KCl	2.7×10^5	7.4×10^3	37	2.47×10^5	0.9	33
40% (v/v) Et.Gly.‖	2.5×10^6	1.1×10^5	22	3.7×10^5	0.50§	3.4

* Obtained by displacement, rate $= \frac{k_{-a}k_{-b}}{k_{+b}+k_{-b}}$.
† Obtained from $k_{-a} = K_0k_{+a}/K_0K_a$.
‡ 0.1 M-KCl/5 mM-$MgCl_2$/20 mM-imidazole, pH 7.0 at 20 °C.
§ Obtained from intercept of plot of $1/\tau_2$ against ([A]+[S]).
‖ Abbreviation used: Et.Gly., ethylene glycol.

a direct measure of the equilibrium between the weakly and strongly attached states.

Geeves *et al.* (1984) discussed how the transition between the weak and strongly attached states could be coupled to the force generation, either directly as in the model described by Huxley & Simmons (1971) and by Eisenberg *et al.* (1980) or indirectly as in the model described by Harrington (1971).

The results presented here suggest that the transition between the weak and strongly attached states involve a substantial change in the hydration shell of either one of the proteins or in the interface between them. The first implies a change in the conformation of one of the two proteins, the second a change in the nature of the contact between the two. Either interpretation is compatible with the above force-generation models.

We have also demonstrated that the transition from the weakly to the strongly attached state is inhibited by low temperature, high pressure, high ionic strength and the presence of ethylene glycol. This shows an interesting correlation with the effects of these same parameters on tension generation in muscle fibres.

Geeves & Ranatunga (1985) have shown that pressures over the range used in the present study will cause a 10–20% decrease in both the active and rigor isometric tension of a glycerine-treated rabbit psoas-muscle fibre.

Clarke *et al.* (1980) have shown that 50% (v/v) ethylene glycol will cause a decrease in the rigor tension of glycerine-treated muscle fibres. Both increasing KCl and decreasing temperature will cause a decrease in the tension generated by skinned fibres (Kuhn *et al.*, 1973).

All of the parameters used are non-specific, and may have many effects on muscle fibres in addition to the effect on K_b demonstrated here. Nonetheless, the correlation between the effect on K_b and tension generation is intriguing.

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