Biochem. J. (1982) **203**, 453–460
Printed in Great Britain

The rates of formation and dissociation of actin–myosin complexes

Effects of solvent, temperature, nucleotide binding and head–head interactions

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(*Received 17 November 1981/Accepted 8 January 1982*)

The rates of formation and dissociation of actin–subfragment 1 and actin–heavy meromyosin complexes were measured by using light-scatter and the change in fluorescence of *N*-iodoacetyl-*N'*-(5-sulpho-1-naphthyl)ethylenediamine (IAEDANS)-labelled actin as probes. Association rate measurements were made at low protein concentration, where the transients approximated to single exponentials with rate constants proportional to the concentration of reactant in excess. Dissociation rate measurements were made by displacing IAEDANS–actin from myosin with excess native actin and by a salt jump. The second-order rate constant of association for actin–subfragment 1 was $3 \times 10^6 M^{-1} \cdot s^{-1}$ in 60mM-KCl at 13°C. It was decreased 10-fold in 500mM-KCl and in 50% (v/v) glycol. It was decreased 6-fold when MgADP or Mg[$\beta\gamma$-imido]ATP bound to myosin. The dissociation rate constant was $0.012 s^{-1}$ in 60mM-KCl at 13°C. It was increased 4-fold by 500mM-KCl, 25-fold by 50% glycol, 8-fold by MgADP binding and 170-fold by Mg[$\beta\gamma$-imido]ATP binding. E_a for association was $70 kJ \cdot mol^{-1}$ and for dissociation $35 kJ \cdot mol^{-1}$. Heavy meromyosin associated at twice the rate observed for subfragment 1 and dissociated at less than one-twentieth of the rate for subfragment 1 (60mM-KCl, 25°C), but when Mg[$\beta\gamma$-imido]ATP bound actin–heavy meromyosin dissociated at one-half the rate for subfragment 1. There were significant correlations between increase in the dissociation rate constant, decrease in binding constant and increase in magnitude of conformational change. The association rate constant did not correlate with any property of the actin–myosin complex.

Actin and myosin, the major proteins involved in muscular contraction, bind very tightly to each other in the absence of substrates ($K \simeq 10^8 M^{-1}$; Marston & Weber, 1975) to form the so-called 'rigor' complex. When ADP, the product of actomyosin ATPase, or the substrate analogue [$\beta\gamma$-imido]ATP (5'-adenylyl imidodiphosphate) are bound to myosin's active site, its affinity for actin is decreased by 10–50-fold and 200–1000-fold respectively (Hofmann & Goody, 1978; Marston *et al.*, 1979; Greene & Eisenberg, 1980*a*; Inoue & Tonomura, 1980). The binding is also weakened by increasing salt concentrations or by changing the solvent; for instance, K is decreased by 200-fold in aq. 50% (v/v) glycol (Clarke *et al.*, 1980*b*).

The interaction between actin–myosin and the substrate MgATP is responsible for the generation of contractile force in muscle. Studies have shown that the weaker binding complexes (actin–myosin–Mg[$\beta\gamma$-imido]ATP and actin–myosin in 50% glycol) have conformations different from the rigor complex *in vitro* and in 'glycerinated' fibres (Marston *et al.*, 1976; Clarke *et al.*, 1980*a*; Marston, 1980) and that the transition of these complexes back to the aqueous rigor complex is coupled, via the conformational change, to work production (Kuhn, 1977; Marston *et al.*, 1979). The formation and dissociation of actin–myosin links is therefore one of the important determinants of the overall mechanical properties in muscle.

Although the equilibria between actin and the soluble subfragments of myosin, heavy meromyosin and subfragment 1 have been thoroughly investigated, only a little is known about the kinetics of formation and dissociation of the complexes (White & Taylor, 1976; Inoue & Tonomura, 1980; White, 1981), and no systematic studies have been made.

Abbreviation used: IAEDANS, *N*-iodoacetyl-*N'*-(5-sulpho-1-naphthyl)ethylenediamine.

0306-3275/82/050453-08$01.50/1

In the present paper I describe the determination of the rates of formation and dissociation of actin–subfragment 1, actin–subfragment 1–MgADP and actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP complexes and the effects of temperature, KCl concentration, 50% glycol and the interaction between two active sites (by using heavy meromyosin). From the results obtained I have been able to make several generalizations about the basis of the actin–myosin interaction and its relationship to contraction.

Materials and methods

Myosin and polymeric actin were prepared from rabbit back and leg muscles by standard methods (Perry, 1955; Rees & Young, 1967). Subfragment 1 and heavy meromyosin were prepared by chymotryptic digestion (Weeds & Taylor, 1975) and purified by $(NH_4)_2SO_4$ fractionation. F-actin was labelled with IAEDANS as described by Marston (1980). ADP and [$\beta\gamma$-imido]ATP were supplied by Sigma Chemical Co.; the [$\beta\gamma$-imido]ATP was further purified by chromatography on DEAE-cellulose. F-actin was treated with Dowex 1-X8 to remove nucleotides from the solution (see Marston & Taylor, 1980).

Reactions rates were measured in a stopped-flow fluorimeter similar to the one described by Sleep & Taylor (1976). IAEDANS–actin fluorescence was excited at 367nm and emission was measured at 90° through a 450–500nm filter combination (Barr & Stroud LP1 + GG400). The 90° light-scatter was measured at 367nm. Formation of actin–myosin bonds resulted in a 20–50% enhancement of IAEDANS fluorescence or light-scatter (White & Taylor, 1976; Porter & Weber, 1979).

The standard (rigor) buffer was 5mM-Pipes (1,4-piperazinediethanesulphonic acid) / KOH buffer, pH 7.1, containing 5mM-EGTA, 5mM-$MgCl_2$ and 60mM-KCl. Association rates were measured by fluorescence with about 0.5μM-IAEDANS–actin mixed with 1–12μM-subfragment 1 or -heavy meromyosin or by light-scatter with about 0.5μM-subfragment 1 mixed with 1–10μM-actin (unlabelled). The dissociation rate, k_-, of myosin–actin complexes was measured directly by mixing 1μM-subfragment 1 plus 1μM-IAEDANS–actin or 1μM-heavy meromyosin plus 1μM-IAEDANS–actin with a 10-fold excess of unlabelled actin, which displaced the labelled actin from myosin. For the actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP complex it was possible to measure dissociation by a salt jump with unlabelled actin. The complex in buffer containing 5mM-KCl was mixed with buffer containing 115mM-KCl. The binding constant decreased from 10^6 to 0.2×10^6 M^{-1} as a result of the salt jump, thus causing dissociation (Marston *et al.*, 1979; Marston, 1980).

Results

Kinetic analysis

Figs. 1 and 2 show representative transients obtained for the association and dissociation reac-

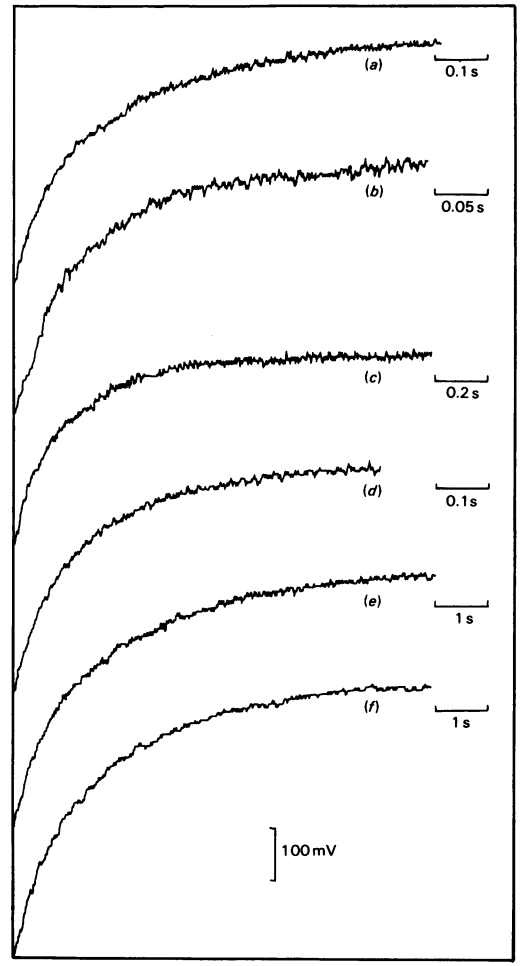

Fig. 1. *Representative association reaction transients measured by the fluorescence method*

For full experimental details see the text. The time scale in seconds and fluorescence change in millivolts relative to a 5V photomultiplier output at the end of the reaction are indicated. Actin* indicates IAEDANS-labelled actin. (*a*) 0.6μM-Actin* + 3μM-subfragment 1 at 13°C in rigor buffer; $\lambda = 5.9\,s^{-1}$. (*b*) 0.6μM-Actin* + 10μM-heavy meromyosin at 25°C in rigor buffer; $\lambda = 15.8\,s^{-1}$. (*c*) 0.6μM-Actin* + 7.5μM-subfragment 1 at 13°C in the presence of 0.5mM-MgADP; $\lambda = 4.3\,s^{-1}$. (*d*) 1μM-Actin* + 12μM-subfragment 1 at 13°C in the presence of 1.5mM-Mg[$\beta\gamma$-imido]ATP; $\lambda = 7.9\,s^{-1}$. (*e*) 0.4μM-Actin* + 12μM-subfragment 1 at 13°C in 50% glycol buffer; $\lambda = 0.74\,s^{-1}$. (*f*) 0.4μM-Actin* + 5μM-subfragment 1 at 13°C in rigor buffer, 0.5M-KCl; $\lambda = 0.80\,s^{-1}$.

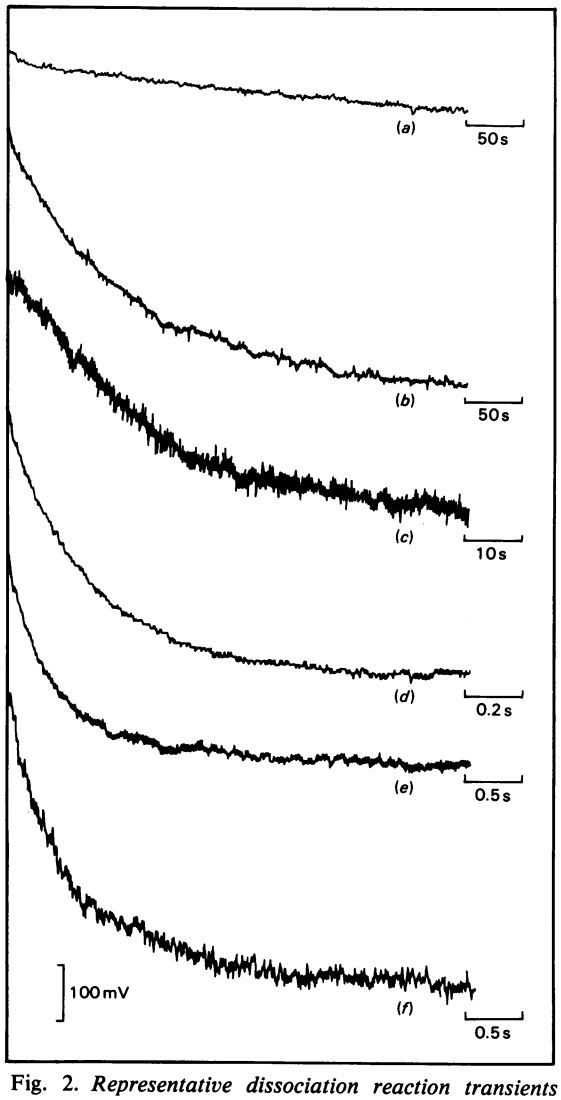

Fig. 2. *Representative dissociation reaction transients measured by the fluorescence method*

The time scale in seconds and fluorescence change scale in millivolts are indicated on the Figure. Actin* indicates IAEDANS-labelled actin. (*a*) 0.5 μM-Actin*, 0.6 μM-heavy meromyosin + 5 μM-actin in rigor buffer at 25°C (displacement method); $k_- = 0.0018\,s^{-1}$. (*b*) 0.5 μM-Actin*, 0.6 μM-subfragment 1 + 5 μM-actin in rigor buffer at 13°C; $k_- = 0.013\,s^{-1}$. (*c*) 0.5 μM-Actin*, 0.6 μM-subfragment 1 + 5 μM-actin in rigor buffer, 0.5 M-KCl; $k_- = 0.53\,s^{-1}$. (*d*) 0.75 μM-Actin*, 1.5 μM-subfragment 1, 5 mM-KCl + 8 μM-actin, 115 mM-KCl in the presence of 1.5 mM-Mg[$\beta\gamma$-imido]ATP at 25°C (salt jump combined with displacement); $k_- = 3.7\,s^{-1}$. (*e*) 0.75 μM-Actin*, 1.5 μM-subfragment 1, 5 mM-KCl + 115 mM-KCl (salt jump method) in the presence of 1.5 mM-Mg[$\beta\gamma$-imido]ATP at 13°C; $\lambda = 2.2\,s^{-1}$. (*f*) 0.75 μM-Actin*, 1.5 μM-heavy meromyosin, 5 mM-KCl + 8 μM-actin, 115 mM-KCl in the presence of 1.5 mM-Mg[$\beta\gamma$-imido]ATP at 25°C; $k_- = 1.76\,s^{-1}$.

tions under various conditions. These transients were analysed by means of semi-logarithmic plots; within the accuracy of the method there were no systematic deviations from a straight line by using either the light-scatter or the fluorescence signal provided that one reactant was in good excess. Plots of the apparent rate of association versus subfragment 1 concentration or actin concentration could be satisfactorily fitted to the relationship $\lambda = k_+[S] + k_-$, where [S] is the concentration of the species in excess (Fig. 3).

If the association reaction involved a single step:

$$A + S \underset{k_-}{\overset{k_+}{\rightleftharpoons}} AS$$

the results obtained in our simple analysis are predicted: $\lambda = k_+[S] + k_-$ and dissociation rate $= k_-$ (or $k_- + k_+[S]$ in the case of a salt-jump experiment, where the dissociation does not go to completion). k_+ may be obtained from the slope of λ-versus-[S] plots (Fig. 3), and the intercept at $[S] = 0$ gives an independent measure of k_- that was indeed found to be close to the directly measured k_- (see Table 1).

There is, however, some evidence that the binding reaction involves two steps (Trybus & Taylor, 1980; S. B. Marston, K. A. Johnson & E. W. Taylor, unpublished work):

$$A + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AS_1 \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} AS_2$$

Such a mechanism could result in biphasic transients or a non-linear relationship between λ and [S] or both, depending on the relative fluorescence or light-scatter changes in the two steps. This raises the question as to whether the analysis is adequate.

The measurements described in the present work were restricted to low concentrations of reactant, where transients have been found to be indistinguishable from a single-exponential process with a rate constant proportional to [S], despite two steps (Trybus & Taylor, 1980). It may be shown that, given the assumptions $K_2 \gg 1$ and $k_{+1}[S] \ll k_{+2}$, the kinetic equation for a two-step reaction reduces to a single exponential process with:

$$\lambda = \frac{k_{+1} \cdot k_{+2} \cdot [S] + k_{-1} \cdot k_{-2}}{k_{+1} \cdot [S] + k_{-1} + k_{+2}}$$

The kinetic behaviour would then be as I found in my simple analysis (see Figs. 1, 2 and 3) with:

$$\underset{[S]\to 0}{k_+} = \frac{k_{+1} \cdot k_{+2}}{k_{-1} + k_{+2}} \quad \text{and} \quad k_- = \frac{k_{-1} \cdot k_{-2}}{k_{-1} + k_{+2}}$$

Trybus & Taylor (1980) estimated $k_{+2} = 20\,s^{-1}$ at 4°C, and we (S. B. Marston, K. A. Johnson & E. W. Taylor, unpublished work) found $k_{+2} \simeq 70\,s^{-1}$ at 25°C; so the criterion $k_{+1}[S] \ll k_{+2}$ is satisfied. It is

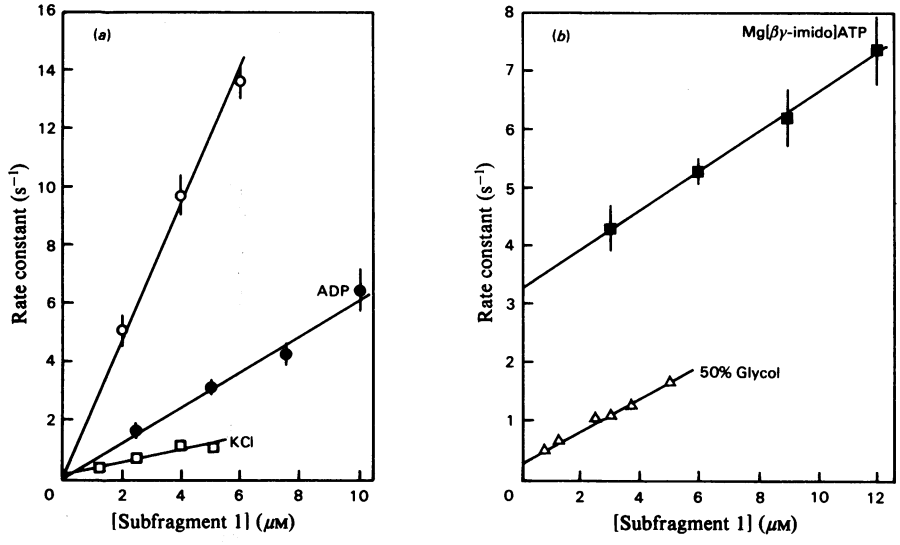

Fig. 3. *Relationship between the observed rate constant of association of 0.5–1 μM-IAEDANS–actin with subfragment 1 on the subfragment 1 concentration*

Measurements were made in rigor buffer at 13°C alone (○) or with the addition of 1 mM-MgADP (●), 0.5 M-KCl (□) or 1.5 mM-Mg[$\beta\gamma$-imido]ATP (■) and in 50% glycol (△) as indicated. Means ± S.D. for four determinations are given. The continuous lines are least-squares fit of the data set. Slope = k_+; intercept = k_-.

concluded that the analysis of kinetic data in terms of single-exponential transients and a linear relationship between λ and [S] is a reasonable approximation.

Comparison of rates measured by IAEDANS fluorescence and light-scatter

Two methods were used to measure the interaction of actin and subfragment 1 or heavy meromyosin in the present study. The increase in light-scatter due to association has been extensively used in kinetic studies and has the advantage that it may be used with native actin; however, the method cannot be used to measure k_- directly. The increase in IAEDANS–actin fluorescence may be used to measure both k_+ and k_- directly, and has the advantages of a more nearly linear relationship between fluorescence change and occupancy of actin and a better signal-to-noise ratio in our apparatus.

However, the kinetics might be altered by the covalent labelling of actin. It was observed that k_+ in rigor buffer and in 50%-glycol buffer was the same measured by the two methods (Table 1). Measurement of k_- for the actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP complex by the salt-jump technique was also the same for both methods and equal to k_- measured by displacement (Table 1). The kinetics of native and IAEDANS-labelled actin were therefore indistinguishable; the more versatile fluorescence technique was used for most of the experiments. Previous work has already shown that actin labelling does not alter actin–myosin affinity significantly (Porter & Weber, 1979; Marston, 1980).

Association rates of actin–subfragment 1 complexes

The values of k_+ at 13°C under various conditions are given in Table 1. In the rigor buffer k_+ was $3 \times 10^6\,M^{-1}\cdot s^{-1}$; this was decreased by about 10-fold if the solvent was changed by increasing the KCl concentration to 0.5 M or by replacing water by 50% glycol. The rates of association of subfragment 1–MgADP and subfragment 1–Mg[$\beta\gamma$-imido]ATP with actin were roughly equal and about one-sixth of the rate of association of subfragment 1 with actin. It is noteworthy that the decreases in k_+ are relatively small compared with the decreases in actin–subfragment 1 affinity (up to 1000-fold). There was no clear correlation between k_+ and the binding constant, K. k_+ was increased by 3-fold between 13°C and 25°C both for the formation of actin–subfragment 1 and for the formation of actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP (Table 2). The estimated activation energy was $70\,kJ\cdot mol^{-1}$.

Dissociation rate of actin–subfragment 1 complex

k_- was measured by the displacement method and

Table 1. *Rates of formation and dissociation of actin–subfragment 1 complexes at 13°C at pH 7.1*

Measurements were made in 5mM-Pipes buffer, pH 7.1, containing 5mM-EGTA, 60mM-KCl and 5mM-$MgCl_2$. Means and 95% confidence limits are given (6–30 measurements). Abbreviations: n.d., not determined; m.i., measurement impossible.

Conditions ...	No additions	1mM-ADP	0.5M-KCl	50% Glycol	2mM-Mg[$\beta\gamma$-imido]ATP
$10^{-6} \times k_+$ ($M^{-1} \cdot s^{-1}$)					
Measured by fluorescence	2.8 ± 0.9	0.64 ± 0.4	0.19 ± 0.01	0.45 ± 0.20	0.43 ± 0.08
Measured by light scatter	3.0 ± 1.1	n.d.	n.d.	0.50 ± 0.22	n.d.
k_- (s^{-1})					
Measured by displacement	0.012 ± 0.002	0.090 ± 0.010	0.052 ± 0.006	m.i.	1.8 ± 0.2
Measured by salt-jump	m.i.	m.i.	m.i.	m.i.	2.0 ± 0.2
Measured by extrapolation	m.i.	m.i.	m.i.	0.32 ± 0.1	3.1
$\left(\frac{k_+ \text{ (fluorescence)}}{k_- \text{ (displacement)}}\right)$ (M^{-1})	2.3×10^8	7.1×10^6	3.6×10^6	1.4×10^6	0.23×10^6
Binding constant (M^{-1})	$3.8 \times 10^8 \pm 1.0 \times 10^8$*	$1.2 \times 10^6 \pm 0.4 \times 10^6$†	2×10^6§	$0.5 \times 10^6 \pm 0.2 \times 10^6$‖	$0.19 \times 10^6 \pm 0.06 \times 10^6$†
	$0.5 \times 10^8 \pm 0.2 \times 10^8$†				
	$1 \times 10^8 \pm 0.2 \times 10^8$‡				

* S. B. Marston & E. W. Taylor (unpublished work); in 60mM-KCl at 25°C.
† Marston *et al.* (1979); in 60mM-KCl at 10°C.
‡ Marston & Weber (1975); in 60mM-KCl at 25°C.
§ Greene (1981); in 450mM-KCl at 22°C.
‖ Clarke *et al.* (1980); in 60mM-KCl at 10°C.

by the extrapolation of the observed rate of association to zero [S] (Fig. 3); the extrapolation method regularly gave a slightly higher value than did the displacement method. k_- was strongly dependent on solvent or nucleotide binding (Table 1). In the rigor buffer the rate of dissociation was very low (half-life 1min at 13°C), but it was increased 4-fold by 0.5M-KCl and 25-fold in 50% glycol. The ternary complex actin–subfragment 1–MgADP dissociated 8 times as fast and the complex actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP 170 times as fast as actin–subfragment 1. There was an approximate correlation between $1/k_-$ and the affinity of actin for subfragment 1 under the various conditions. There was a 1.8-fold increase in k_- between 13 and 25°C for both the actin–subfragment 1 and the actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP complex. Estimated E_a was $35 kJ \cdot mol^{-1}$. My values of k_- for actin–subfragment 1 and actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP are similar to determinations made by different techniques (White & Taylor, 1976; Inoue & Tonomura, 1980; White, 1981).

Effect of head–head interaction

The kinetics of the single-headed (subfragment 1) and double-headed derivatives (heavy meromyosin) of myosin were compared at 25°C for the actin–myosin and actin–myosin–Mg[$\beta\gamma$-imido]ATP complex. It is known that heavy meromyosin binds much more tightly to actin than does subfragment 1, since it has twice as many binding sites (Highsmith, 1978; Greene & Eisenberg, 1980*b*). The rates of association of heavy meromyosin were only 2-fold greater than those for subfragment 1 (measured on a molar basis), but the dissociation rates for heavy meromyosin were much lower than those for subfragment 1. Heavy meromyosin dissociation from the rigor complex was so slow that there is some doubt about the accuracy of the value given (Table 2), since there could have been photodecomposition during the prolonged measurement. The rate of $0.0013 s^{-1}$ (half-life of 13min) may be an overestimate; it is some 20 times slower than the rate of subfragment 1 dissociation under the same conditions. In the presence of saturating Mg[$\beta\gamma$-imido]ATP a different pattern of results was obtained. Heavy meromyosin–Mg[$\beta\gamma$-imido]ATP bound to actin at 3 times the rate of subfragment 1–Mg[$\beta\gamma$-imido]ATP, but k_- of the actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP and actin–heavy meromyosin–Mg[$\beta\gamma$-imido]ATP complexes differed by only a factor of 2.

Determination of binding constant from ratio k_+/k_-

If the formation of actin–myosin complexes is a single-step reaction, then by definition the ratio k_+/k_- equals the binding constant. It may be shown that even for a multiple-step binding this relationship

Table 2. *Effects of temperature and head–head interactions on the rates of formation and dissociation of the complexes actin–myosin (A–M) and actin–myosin–Mg[βγ-imido]ATP (A–M–Mg[βγ-imido]ATP)*

Measurements were made in 5 mM-Pipes buffer, pH 7.1, containing 5 mM-EGTA, 60 mM-KCl and 5 mM-$MgCl_2$. Abbreviation: m.i., measurement impossible.

	Heavy meromyosin, 25°C		Subfragment 1, 25°C		Subfragment 1, 13°C	
Complex ...	A–M	A–M–Mg[βγ-imido]ATP	A–M	A–M–Mg[βγ-imido]ATP	A–M	A–M–Mg[βγ-imido]ATP
$10^{-6} \times k_+$ ($M^{-1} \cdot s^{-1}$) per mol of heavy meromyosin or subfragment 1						
Measured by fluorescence	13	2.8	6.5	0.9	2.2	0.31
k_- (s^{-1})						
Measured by displacement	0.0013	1.7 ± 0.2	0.024 ± 0.002	3.4 ± 0.2	0.013	2.0 ± 0.1
Measured by extrapolation	m.i.	m.i.	m.i.	4.3	m.i.	3.1
$\frac{k_+}{k_-}$ (M^{-1})	10^{10}	1.6×10^6	2.7×10^8	0.26×10^6	1.7×10^8	0.15×10^6
Binding constant	4×10^{10}*	1.1×10^5*	$3.8 \times 10^8 \pm 1.0 \times 10^8$† $1 \times 10^8 \pm 0.2 \times 10^8$‡	—	$0.5 \times 10^8 \pm 0.2 \times 10^8$§	$0.19 \times 10^6 \pm 0.06 \times 10^6$§

* Greene (1981); in 100 mM-KCl at 22°C.
† S. B. Marston & E. W. Taylor (unpublished work); in 60 mM-KCl at 25°C.
‡ Marston & Weber (1975); in 60 mM-KCl at 25°C.
§ Marston *et al.* (1979); in 60 mM-KCl at 10°C.

should remain true (see above under 'Kinetic analysis').

Comparison of published values for K with my determination of k_+/k_- show quite good agreement under all the conditions studied (Tables 1 and 2). Exact agreement is not to be expected, since measurements were not made under identical conditions. The data therefore support the proposition that $k_+/k_- = K$.

Relative values of k_+/k_- for subfragment 1 at 13°C were 1 (rigor), 0.03 (ADP), 0.016 (0.5 M-KCl), 0.006 (50% glycol) and 0.001 (Mg[βγ-imido]ATP). k_+/k_- increased with increasing temperature, giving a ΔH value of $+28 kJ \cdot mol^{-1}$, in agreement with Highsmith (1977), for both actin–subfragment 1 and actin–subfragment 1–Mg[βγ-imido]ATP complexes. The kinetic experiments have enabled me to give a minimum value for the affinity of heavy meromyosin for actin of $10^{10} M^{-1}$ at 25°C in rigor solution, and of $1.6 \times 10^6 M^{-1}$ in the presence of Mg[βγ-imido]ATP. Previously direct measurements have been unable to measure binding constants in excess of $10^9 M^{-1}$ (Marston & Weber, 1975); my value agrees with indirectly measured values (Greene & Eisenberg, 1980*b*).

Discussion

Evaluation of the techniques used

The present study is concerned with the rates of formation and dissociation of actin–myosin bonds; it therefore depends critically on the probes used to measure bond formation. Light-scatter and IAEDANS–actin fluorescence are known to give reliable and reproducible changes when bonds are formed at equilibrium under many conditions (White & Taylor, 1976; Porter & Weber, 1979; Marston, 1980; Trybus & Taylor, 1980). To use these probes for a full kinetic analysis of binding and dissociation transients requires knowledge of whether the binding reaction seen by the probe involves one or several steps; there is already evidence that the reaction involves at least two steps when measured by the light-scatter method (Trybus & Taylor, 1980).

Since I was interested in determining the effect of a wide range of conditions on the kinetics rather than analysing in detail the behaviour under one set of conditions, I restricted measurements to low concentrations. It may be shown theoretically that the observed kinetic behaviour is then simple (transients are single exponentials and the observed association rate constant $\lambda = k_- + k_+[S]$) even for multiple-step reactions, and the data were analysed on this basis (Figs. 1, 2 and 3). The validity of this simplified analysis may be tested by the following predicted relationships: (*a*) $\lim_{[S] \to 0} \lambda = k_-$ measured

by the direct methods (Fig. 3 and Table 1); (*b*) $k_+/k_- = K$ measured by independent methods (Tables 1 and 2). These relationships were found to be true under all conditions examined, despite variations in k_+ by 35-fold and in k_- by 2000-fold; moreover, the same results were obtained with the use of two different probes. Any significant breakdown of the relationships or difference between the results with the two probes would have meant that the simple analysis was inadequate.

Evaluation of the results

Actin binds to subfragment 1 tightly ($K \simeq 10^8 \text{M}^{-1}$). This is because the observed rate of association is rapid ($3 \times 10^6 \text{M}^{-1} \cdot \text{s}^{-1}$) and dissociation is slow (0.012s^{-1}) (see Tables 1 and 2). When binding is weakened by changing the solvent or by binding nucleotides at the myosin active site, there is a relatively small change in the rate of association (maximum of 15-fold). Indeed, for the four measurements with 0.5 M-KCl, 50% glycol, subfragment 1–MgADP and subfragment 1–Mg[$\beta\gamma$-imido]ATP, k_+ differs only by a factor of 3. This suggests that the rate of association is determined by the same reaction step in all cases, perhaps the probability of collision of subfragment 1 with actin (k_{+1}). Binding becomes weaker in the presence of 50% glycol or bound nucleotide, primarily because the rate of dissociation of actin from subfragment 1 is increased by up to 170-fold.

The weakly bound complexes, especially actin–subfragment 1–Mg[$\beta\gamma$-imido]ATP and actin–subfragment 1 in 50% glycol, have conformations that are different from the actin–subfragment 1 complex *in vitro* and in 'glycerinated' muscle fibres (Marston *et al.*, 1976; Marston, 1980; Clarke *et al.*, 1980*a*). In 'glycerinated' muscle the transition from one complex to another can produce external work and thus provides an easy-to-study analogue of muscle contraction (Kuhn, 1977; Marston *et al.*, 1979). In general it has been found that the magnitude of the change in cross-bridge conformation correlates with the weakening of actin–subfragment 1 bonds. The results given in the present paper show that it also correlates with an increased rate of dissociation. Since a large increase in k_- can be achieved either by the specific binding of a substrate analogue at the myosin active site or by the extremely non-specific treatment of replacing aqueous solvent by 50% glycol, it is reasonable to propose that the increase in k_- is a primary requirement for altering the equilibrium cross-bridge conformation. The most extreme conformational change (contraction) is obtained with MgATP binding, which increases k_- to 1000s^{-1} (White & Taylor, 1976).

It is noteworthy that, under conditions where actin–myosin links exchange at the rate of up to 3s^{-1} (e.g. in 50% glycol solution or 2 mM-Mg[$\beta\gamma$-imido]ATP), 'glycerinated' muscle fibres do not relax and can maintain tension for many hours (Clarke & Tregear, 1982). This suggests either that exchange is dramatically slowed down in the myofibrillar matrix or, more probably, that the rapidly exchanging cross-bridges re-bind to the same actin that they came off, thereby maintaining the muscle at constant length (see Pate & Brokaw, 1980).

Comparison of single myosin active heads (subfragment 1) with the double-headed heavy meromyosin reveals that the association rate, k_+, of the latter is about twice that of the former (Table 2); this result might be due to there being two chances of a heavy meromyosin–actin collision forming a bond compared with one for subfragment 1. The dissociation rate of heavy meromyosin from actin in rigor solution is very low, at least 20 times slower than that for subfragment 1, presumably because the probability of both heads dissociating at the same time is low. The ratio k_+/k_- gives a rough value of K of at least 10^{10}M^{-1}, which is in reasonable agreement with the values given by Greene & Eisenberg (1980*b*). It will be noted that the principle of K being mainly correlated with $1k_-$ holds for heavy meromyosin as it did for subfragment 1. In contrast with the rigor case, in 2 mM-Mg[$\beta\gamma$-imido]ATP k_- was almost the same for heavy meromyosin and subfragment 1, and the estimated K for heavy meromyosin was only 6 times greater than that for subfragment 1 (compared with 400 times in rigor).

It has been pointed out by several workers that K_{HMM} should equal $[K_{\text{S1}}]^2$ (where K_{HMM} and K_{S1} are the K values for heavy meromyosin and subfragment 1 respectively) if both heads could bind without restriction. Since it does not, there must be a negative interaction opposing the simultaneous binding of the two heads of heavy meromyosin (Highsmith, 1978; Greene & Eisenberg, 1980*b*; Hill & Eisenberg, 1980). Greene (1981) has suggested, from equilibrium data, that in Mg[$\beta\gamma$-imido]-ATP this interaction may be sufficient to prevent binding of the second head altogether; in consequence the observed kinetics would be, essentially, those of just one head. The similarities of behaviour of subfragment 1 and heavy meromyosin in Mg[$\beta\gamma$-imido]ATP found in the present work (Figs. 2*e* and 2*f* and Table 2) are compatible with the hypothesis.

Finally, it is worth pointing out that the ratio k_+/k_- has been shown to equal the binding constant of actin to myosin over a range of conditions where K varies by five orders of magnitude. Kinetic measurements provide an easy and extremely versatile technique that should be useful in the future investigation of actin–myosin interactions.

I thank Dr. R. T. Tregear and M. L. Clarke for discussions on the effects of glycol and [$\beta\gamma$-imido]ATP on muscle fibres, and Professor E. W. Taylor for the derivation of the two-step rate equation.

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