Two-State Equilibria of Myosin Subfragment 1 and Its Complexes with ADP and Actin†

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ABSTRACT: We previously reported that the nucleotide complex of myosin subfragment 1, S1·εADP, exists in two states on the basis of the temperature dependence of the fluorescence decay of bound 1,N^6-ethenoadenosine diphosphate (εADP) [Aguirre, R., Lin, S.-H., Gonsoulin, F., Wang, C.-K., & Cheung, H. C. (1989) *Biochemistry 28*, 799–809]. We have extended the previous study of the equilibrium between the two states, $S1_L$·ADP ⇌ $S1_H$·ADP, by using a fluorescently labeled myosin S1 (S1-AF). In S1 alkylated with IAF [5-(iodoacetamido)fluorescein], the decay of the label emission was biexponential both in the presence and absence of ADP and/or actin. In the presence of ADP, the two decay times were 4.30 (α_1 = 0.55) and 0.80 ns (α_2 = 0.45) at 12.4 °C, in a medium containing 60 mM KCl, 30 mM TES (pH 7.5), and 2 mM $MgCl_2$. The steady-state fluorescence intensities of S1-AF, (S1-AF)·ADP, acto·(S1-AF), and acto·(S1-AF)·ADP were dependent on temperature over the range of 5–30 °C. By combining lifetime and steady-state intensity data, we obtained for the two-state transition $(S1\text{-}AF)_L$·ADP ⇌ $(S1\text{-}AF)_H$·ADP the following parameters: $\Delta H°$ = 16.1 kcal/mol (67.3 kJ/mol) and $\Delta S°$ = 55.8 cal/(deg·mol) [233.5 J/(deg·mol)], in agreement with previous results obtained with εADP. The $\Delta H°$ values for the two-state transition of S1-AF, acto·(S1-AF), and acto·(S1-AF)·ADP are 13.0, 21.6, and 5.2 kcal/mol, respectively. The corresponding $\Delta S°$ values are 46.9, 79.5, and 17.4 cal/(deg·mol). Release of ADP from myosin is known to be considerably slower than from actomyosin. This kinetic difference may be related to the large difference in enthalpy for the two-state transition between (S1-AF)·ADP and acto·(S1-AF)·ADP.

Muscle contraction results from cyclic interactions between myosin molecules of the thick filaments and actin molecules of the thin filaments. These interactions are driven by the hydrolysis of ATP[1] by actomyosin ATPase and regulated by binding of intracellular Ca^{2+} to troponin. Elucidation of the mechanism of these cyclic interactions requires detailed knowledge of the conformation of myosin subfragment 1, where the nucleotide- and actin-binding sites are located. This knowledge must also include information on the change of S1 conformation induced by complex formation with nucleotides and actin as well as the free energy changes for the conformational transitions.

There are two reactive thiols, SH_1 (Cys-707) and SH_2 (Cys-697), on myosin heavy chain. Modification of Cys-707 with *N*-ethylmaleimide results in an increase of Ca^{2+} ATPase activity (Sekine & Kielley, 1964). These results suggest a possible relationship between SH_1 and the nucleotide-binding site on S1, although a direct involvement of SH_1 in nucleotide binding may be excluded (Windner et al., 1978; Botts et al., 1979; Perkins et al., 1984). SH_1 can be cross-linked to a segment of the heavy chain located about 12–16 kDa from the N terminus via the thiol-specific photoactivatable reagent 4-(2-iodoacetamide)benzophenone (Sutoh & Lu, 1987). This cross-linked segment appears to overlap the putative nucleotide-binding region, and this finding suggests that SH_1 is close to the nucleotide-binding site in the tertiary structure. Additional evidence indicates that S1 loses its actin-binding ability when SH_1 and SH_2 are cross-linked to each other (Katoh & Morita, 1984). Katoh et al. (1985) showed that, in addition to an actin-binding site located on the region between the 50- and 20-kDa segments, there may be a second actin-binding site located in the region around SH_1 and SH_2. Possible involvement of the SH_1 region in actin binding has been demonstrated with oligopeptides that have sequences around SH_1 and SH_2 (Suzuki et al., 1987, 1990) and from NMR studies with S1 labeled at SH_1 with a ^{19}F probe (Barden et al., 1989). Actin and nucleotides can induce changes in the environment of SH_1, and the modification of SH_1 affects some properties of the two binding sites in myosin S1. Thus SH_1, the actin-binding sites, and the nucleotide-binding site are reciprocally affected. The close relationship between SH_1, the actin-binding sites, and the ATPase site suggests that this thiol may occupy a key position in the path of intersite signal transmission.

Several lines of evidence suggest two states of S1·nucleotide from UV difference spectra (Morita, 1977), fluorescence spectroscopy (Bêchet et al., 1979), oxygen-exchange (Sleep & Hutton, 1980), ^{31}P NMR (Shriver & Sykes, 1981a,b), and fluorescence lifetime decay studies (Aguirre et al., 1989). Transient kinetic experiments have also provided evidence for two states of S1·ADP from S1 intrinsic fluorescence (Trybus & Taylor, 1980, 1982) and the emission of bound εADP and εATP (Garland & Cheung, 1979). ^{19}F NMR results from fluorine-labeled S1 indicate that there are two states of S1 in the absence of nucleotide with a highly temperature-dependent equilibrium (Shriver & Sykes, 1982). Pressure-relaxation experiments suggest that acto·S1 is present in two kinetic

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[1] Abbreviations: ADP, adenosine diphosphate; εADP, 1,N^6-ethenoadenosine diphosphate; A_2p^5, diadenosine 5′-pentaphosphate; ATP, adenosine triphosphate; DTT, dithiothreitol; IAF, 5-(iodoacetamido)-fluorescein; S1, chymotryptic myosin subfragment 1; S1-AF, myosin subfragment 1 labeled at Cys-707 by 5-(iodoacetamido)fluorescein; AF, acetamidofluorescein moiety; TES, 2-[[tris(hydroxymethyl)methyl]-amino]ethanesulfonic acid.

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Table I: Fluorescence-Decay Parameters of Labeled Myosin Subfragment 1 (S1-AF) and Its Complexes with ADP and Actin[a]

		lifetime (ns)		fractional intensity		
	temp (°C)	τ_1	τ_2	f_1	f_2	K_{eq} (f_1/f_2)
S1-AF	18.0	4.20 ± 0.03	0.63 ± 0.05	0.75	0.25	2.6
	24.0	4.30 ± 0.02	0.78 ± 0.09	0.85	0.15	5.5
(S1-AF)·ADP	12.4	4.30 ± 0.04	0.80 ± 0.11	0.84	0.16	5.1
	21.2	4.25 ± 0.02	0.68 ± 0.06	0.92	0.08	11.8
acto·(S1-AF)	11.0	4.26 ± 0.02	0.84 ± 0.03	0.90	0.10	8.9
	20.6	4.17 ± 0.05	1.08 ± 0.17	0.95	0.05	19.4
acto·(S1-AF)·ADP	12.4	4.03 ± 0.02	0.82 ± 0.07	0.79	0.21	3.7
	20.6	4.08 ± 0.04	1.05 ± 0.14	0.83	0.17	4.9

[a] Conditions: 3 μM S1-AF, 60 mM KCl, 30 mM TES (pH 7.5), and 2 mM $MgCl_2$. When present, actin was 15 μM, ADP was 100 μM, and A_2p^5 was 50 μM. The parameters for each temperature were the averages from three to six different experiments carried out with three different protein preparations; f_i (fractional intensities) are defined by eqs 1 and 2.

species (Coates et al., 1985), and other transient kinetic results suggest the existence of two acto·S1·ADP states (Geeves, 1989). In the present paper we report equilibrium-binding studies that establish two-state temperature-dependent transitions for S1, acto·S1, S1·ADP, and acto·(S1·ADP) on the basis of the emission of IAF covalently linked to S1 at Cys-707.

Materials and Methods

Reagents and Chemicals. A_2p^5, ADP, and ATP were obtained from Sigma Chemical Co. (St. Louis, MO) and were used without further purification. Chymotrypsin was obtained from Worthington Diagnostic Systems (Freehold, NJ). IAF was purchased from Molecular Probes (Junction City, OR), and all other chemicals were of reagent grade.

Protein Preparations. Myosin was prepared from rabbit skeletal muscle by the method of Flamig and Cusanovich (1981). Freshly prepared myosin was used to prepare S1 by chymotryptic digestion as described by Weeds and Taylor (1975). The two isozymes S1(A1) and S1(A2) isolated on a DE-52 column were pooled, dialyzed against 0.05 M ammonium acetate and 0.1 mM DTT, and lyophilized in the presence of 0.1 M sucrose. Actin was prepared from an acetone powder according to the method of Spudich and Watts (1971). A molecular weight of 115 000 was used to estimate the concentration of S1 from an absorbance of 0.75 $g^{-1}\cdot cm^{-1}$ at 280 nm. Actin was assumed to have a monomeric molecular weight of 42 000 and an absorbance of 0.63 $g^{-1}\cdot cm^{-1}$ at 290 nm (Houk & Ue, 1974).

The sulfhydryl group SH_1 (Cys-707) of myosin S1 was modified by the fluorescent reagent IAF by incubation of the protein with a 1.2-fold excess of IAF in a medium containing 60 mM KCl and 30 mM TES at pH 7.5 (buffer A) for 18 h at 4 °C. The concentration of bound dye was estimated from its absorbance ($\epsilon_{496\,nm}$ = 77 000 $M^{-1}\cdot cm^{-1}$) in buffer A containing 5 M urea (Takashi, 1979). The protein concentration of labeled S1 was estimated by the method of Lowry et al. (1951) from a calibration curve with unlabeled S1 whose concentrations had been independently determined by absorbance at 280 nm. The degree of SH_1 labeling by IAF was found to be in the range of 0.90–0.95.

Fluorescence Measurement. Fluorescence lifetimes were determined on a thermostated photon-counting PRA 2000 pulsed nanosecond fluorescence spectrometer. A Ditric three-cavity 490-nm interference filter was used for excitation of S1-AF with light polarized at 54.7° from the horizontal (magic angle) (Spencer & Weber, 1969), and a Corning 3-69 cut-off filter (cut-off wavelength 520 nm) was used to isolate the emission. Time-resolved fluorescence decay data were analyzed by a nonlinear least-squares iterative reconvolution procedure based on the Marquadt search algorithm (Grinvald & Steinberg, 1974). The decay curves were fitted to a linear combination of exponential terms. As fitting criteria, we used the Durbin–Watson parameter, the reduced chi-square ratio (χ_R^2), the weighted residuals, the autocorrelation function of the weighted residuals, and the runs test to judge the goodness of fit. A fit was considered acceptable when the Durbin–Watson parameter satisfied theoretical estimates (for a two-exponential fit, the DW value is larger than 1.75), plots of the weighted residuals, and the autocorrelation function showed random deviations about zero with a reduced χ_R^2 value of about 1 and a runs test between −2 and +2 (Lampart et al., 1983).

Steady-state fluorescence measurements were carried out over the range of 5–30 °C on a Perkin-Elmer MPF-66 spectrofluorometer equipped with a thermal electric temperature control holder and interfaced to a PE-7300 computer. The magic angle was also used for steady-state intensity measurements.

Results

Fluorescence Decay of S1-AF. The emission decay of AF covalently attached to S1 (S1-AF) was studied in the absence and presence of ADP [S1-AF and (S1-AF)·ADP] and in the presence of actin with and without ADP [acto·(S1-AF) and acto·(S1-AF)·ADP]. When the ternary complex acto·(S1-AF)·ADP was studied, A_2p^5 was added to inhibit contaminating myokinase in order to prevent regeneration of ATP from the added ADP. A typical emission decay curve for S1-AF is shown in Figure 1. The decay data could not be fitted to a single-exponential function but were satisfactorily fitted to a sum of two exponential terms yielding two decay times. The decay curves for the other three S1-AF species likewise could not be fitted to a monoexponential function but could be fitted to a biexponential function. The decay parameters for the four S1 species are given in Table I. The two fractional intensities (f_1 and f_2) associated with the two decay components are given by

$$f_1 = \alpha\cdot\tau_1/(\alpha_1\cdot\tau_1 + \alpha_2\cdot\tau_2) \quad (1)$$

$$f_2 = \alpha_2\cdot\tau_2/(\alpha_1\cdot\tau_1 + \alpha_2\cdot\tau_2) \quad (2)$$

where τ_1 and τ_2 are the two lifetimes with two associated fractional amplitudes α_1 and α_2. The two fractional intensities provide a measure of the relative populations of the two decay components. Since the decay of IAF in the adduct β-mercaptoethanol-AF is monoexponential (Aguirre et al., 1986), the two lifetimes may reflect the presence of two states of isolated S1-AF or S1-AF liganded to nucleotide or actin. The conclusion is supported by the monoexponential decay of S1-AF in 6 M guanidine hydrochloride: τ = 4.65 ± 0.01 ns at 10 °C, and τ = 4.51 ± 0.01 ns at 20 °C. If the two states are in equilibrium, the ratio f_1/f_2 is an equilibrium constant

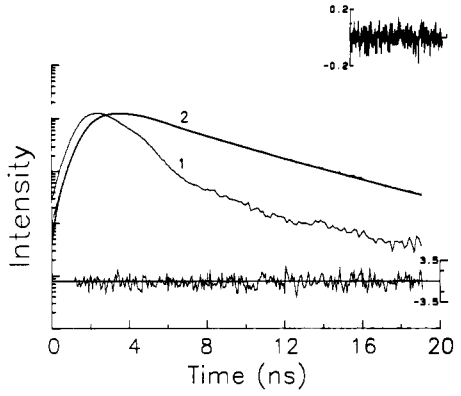

FIGURE 1: Fluorescence decay of IAF in the complex acto·(S1-AF)·ADP. The sample contained 3 μM S1-AF, 15 μM actin, 100 μM ADP, 50 μM A_2P^5, 60 mM KCl, 30 mM TES and 2 mM $MgCl_2$, at pH 7.5 and 20.6 °C. Curve 1 is the lamp profile, and curve 2 (solid curve) is the best fit of the data to a biexponential function yielding two lifetimes, τ_1 = 4.25 ns and τ_2 = 0.68 ns, with corresponding fractional amplitudes α_1 = 0.65 and α_2 = 0.35. The tracing across the plot shows the deviation between the calculated and experimental data. The upper right-hand panel shows the autocorrelation function of the weighted residuals between observed data and the chosen function, with χ_R^2 = 1.05, DW parameter = 1.95, and runs test Z = –0.677. In this experiment the peak channel accumulated 10^4 photon counts.

(K_{eq}) for the transition $(state)_2 \rightleftharpoons (state)_1$. The ratio f_1/f_2 for each of the S1 species is larger at a higher temperature (Table I) suggesting that $(state)_1$ is favored at high temperature and $(state)_2$ at low temperature regardless of whether S1 is liganded or unliganded.

We previously used the temperature dependence of f_1/f_2 of bound ϵADP obtained from decay measurements to establish a two-state transition of S1·ϵADP over a range of temperatures. In the present work we used a modified procedure to determine f_1/f_2 at different temperatures. Because the pulse/single-photon-counting method was used to measure fluorescence decay, a sample had to be maintained at a given temperature for a period of time for a single measurement. We were concerned about possible thermal effects on the proteins at higher temperatures, and in addition we would like to reduce the length of time required to complete a single set of measurements of a large number of samples. To circumvent these problems, we combined decay measurements with measurements of steady-state intensity to determine f_1/f_2 as a function of temperature at small temperature intervals as described in the next section.

Steady-State Fluorescence of S1-AF. Addition of ADP to S1-AF in the absence of actin induced a decrease in the fluorescence intensity of the probe by about 20%. Addition of actin to this (S1-AF)·ADP had no effect on the AF fluorescence. On the other hand, addition of actin to S1-AF in the absence of nucleotide produced a 2-fold increase in fluorescence. This enhancement was abolished upon addition of nucleotide. These results are in agreement with Aguirre et al. (1986). The final fluorescence level in the system containing actin, S1-AF, and MgADP was identical with that observed with (S1-AF)·ADP. The intensities of S1-AF and acto·(S1-AF) increased with increasing temperature, and in the presence of nucleotides the intensities of (S1-AF)·ADP and acto·(S1-AF)·ADP decreased with increasing temperature (Figure 2). If S1-AF exists in two states, its steady-state fluorescence intensity is the summation of the intensities of the two states

$$F(T) = f_1 \cdot F_H + f_2 \cdot F_L \quad (3)$$

where $F(T)$ is the observed fluorescence intensity at temperature T, f_1 and f_2 are the fractions of the high-temperature state and the low-temperature state, and F_H and F_L are the molar fluorescence intensities of the high-temperature and low-temperature state, respectively. At a given temperature the fractional intensities f_1 and f_2 determined from measurement of decay times (eqs 1 and 2) should be the same as the values determined from steady-state intensity (eq 3). From the f_1 and f_2 values derived from decay measurements at two temperatures, F_H and F_L in eq 3 are readily calculated. We found that F_H and F_L were reasonably independent of the temperatures at which the measurements were made. From these values of F_H and F_L, f_1 and f_2 at other temperatures can be determined from the measured steady-state intensity, $F(T)$. This procedure was used to obtain f_1/f_2 (K_{eq}) at 14–18 different temperatures over the range of 5–30 °C. These data were analyzed by the van't Hoff equation as shown in Figure 3. We found that in these samples the pH was essentially constant between 9 and 28 °C but sensitive to temperature changes outside this narrow range. The measured pH was as much as 0.3 unit off at the extreme temperatures. The intensity of S1-AF and acto·(S1-AF) increased with increasing pH, and this increase was smaller for the corresponding ADP complexes. Restoration of the pH to 7.5 was difficult, although attempts were made in the experiments reported in Figure 3. The pH of S1-AF at the two highest temperatures was still

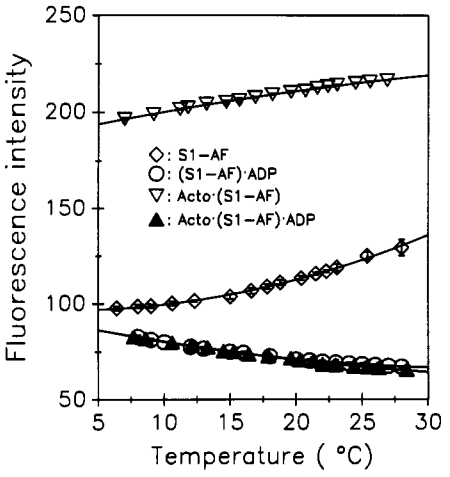

FIGURE 2: The effect of temperature on the steady-state fluorescence intensity of S1-AF in the absence and presence of actin and ADP.

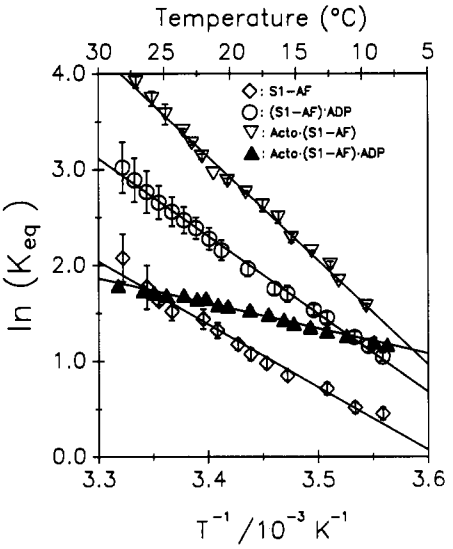

FIGURE 3: van't Hoff plots of S1-AF and its complexes with actin and ADP.

Table II: Thermodynamic Parameters for the Two-State Transition of Fluorescently Labeled Myosin Subfragment 1

S1 species[a]	$\Delta H°$ (kcal/mol)	$\Delta S°$ [cal/(deg·mol)]
S1	13.0 (54.4)[b]	46.9 (196.2)[c]
S1·ADP	16.1 (67.3)	55.8 (233.5)
acto·S1	21.6 (90.4)	79.5 (332.6)
acto·S1·ADP	5.2 (21.7)	17.4 (72.8)

[a] S1 in every species was IAF-labeled S1 (S1-AF). [b] The numbers in parentheses are in units of kJ/mol. [c] The numbers in parentheses are in units of J/(deg·mol).

slightly off after adjustment, and this small pH difference was likely the reason for the apparent deviation from linearity shown in the figure. These results indicate that the four labeled S1 species each can be described by a two-state equilibrium between a low-temperature state and a high-temperature state: $S1_L \rightleftharpoons S1_H$. For the transition of (S1-AF)·ADP, $\Delta H° = 16.1$ kcal/mol (67.3 kJ/mol) and $\Delta S° = 55.8$ cal/(deg·mol) [233.5 J/(deg·mol)], in good agreement with the values [$\Delta H° = 13.3$ kcal/mol (55.6 kJ/mol) and $\Delta S° = 49$ cal/(deg·mol) [205.0 J/(deg·mol)] previously obtained for the transition of S1·ϵADP (Aguirre et al., 1989). The thermodynamic parameters for this and the other S1-AF species are listed in Table II.

Discussion

The major findings of this work are that S1 and its complexes with ADP and actin can each exist in two equilibrium states. These results extend our previous report of a two-state equilibrium of unmodified S1 complexed to ϵADP. We also have established the existence of a two-state equilibrium of the same fluorescent S1 in its binary complex with actin (acto·S1) and the ternary complex with actin and ADP (acto·S1·ADP). Since the fluorescent probe is on S1, it must report on conformational states of S1 and the shift of the two-state equilibrium induced by complexation of S1 with ligands. These transitions are temperature dependent and can be characterized as two-state equilibria between a low-temperature and a high-temperature state. The two states of each S1 species differ energetically by large differences in enthalpy and entropy. The $\Delta H°$ and $\Delta S°$ values for the transition of S1 (S1-AF) from the low-temperature to the high-temperature state, $S1_L \rightleftharpoons S1_H$, are both positive and similar to the values previously established for S1·ϵADP (Aguirre et al., 1989). This agreement provides confidence that S1 labeled with IAF has similar conformational properties to native S1.

Many previous studies have suggested the existence of two states of S1·nucleotide in the myosin ATPase pathway, including NMR studies of S1 with [^{31}P]nucleotides (Shriver & Sykes, 1982) and the early transient kinetic studies of the interaction of S1 with ϵADP and ϵATP (Garland & Cheung, 1979) and natural nucleotides (Trybus & Taylor, 1982). Garland et al. (1988) more recently showed that the kinetics of the ADP-induced change of fluorescence resonance energy transfer between SH_1 and SH_2 are compatible with their previously proposed three-step kinetic binding mechanism of ADP to S1 in which two isomerizations of S1·ADP occur. These results suggest that S1 itself may also have two conformational states. This two-state equilibrium of S1 is demonstrated here from equilibrium measurements of a labeled S1.

The changes of enthalpy and entropy are very similar for the two-state transitions of S1 and S1·ADP. These results suggest that the conformation of S1 in these species may be similar and that similar conformational changes may be responsible for the observed temperature-dependent transitions. However, the enthalpy and entropy changes are considerably different for the transition of acto·S1 and the ternary complex acto·S1·ADP. These differences would suggest that one or both states of S1 in the actin complex may have different conformations from those of S1 or S1 in the ADP complex.

Recent evidence has suggested that the complex actin·myosin (A·M) may exist in two states: one state in which myosin is weakly bound to actin and the other state in which myosin is strongly bound to actin. The formation of actomyosin (A·M) in the absence and presence of ADP (D) may be depicted by (Geeves et al., 1984)

$$A + M \xrightarrow{K_1} A\text{–}M \xrightarrow{K_2} A\cdot M \quad (4)$$

$$A + M\cdot D \xrightarrow{K_1^D} A\text{–}M\cdot D \xrightarrow{K_2^D} A\cdot M\cdot D \quad (5)$$

where A–M and A–M·D denote weakly bound actomyosin and A·M and A·M·D strongly bound actomyosin. The first evidence for two states of acto·S1 came from pressure-relaxation kinetic experiments in which the transient kinetics of the interaction between native S1 and a fluorescently labeled actin were monitored by light scattering and the fluorescence of the pyrene label covalently attached to Cys-374 (Coates et al., 1985). While light scattering detected monophasic kinetics for this reaction, fluorescence showed a rapid kinetic phase followed by a slow phase with a time constant that is the same as that detected by light scattering. The rapid phase was not resolved because pressure release was not sufficiently fast for its resolution. The authors interpreted the results in terms of a three-step mechanism for formation of acto·S1 in which the encounter complex AS_0 undergoes two isomerizations to two acto·S1 states. In a recent pressure-relaxation study using the same fluorescent actin, Greeves (1989) has proposed a kinetic scheme consistent with eq 5 for the binding of actin to S1·ADP. The two-state transitions reported here provide evidence, for the first time, of two states of acto·S1 and acto·S1·ADP on the basis of equilibrium studies. At room temperature the high-temperature states predominate. We (Aguirre et al., 1989) recently showed that the equilibrium constant of the transition of S1·ϵADP from the low-temperature to the high-temperature state is reduced by a factor of 7 in the presence of orthovanadate. Since the power stroke in muscle is accompanied by release of phosphate (Eisenberg & Greene, 1980), this result led us to suggest that the two-state transition from the low-temperature to the high-temperature state is related to the force-generating event. The original model of actomyosin interaction of Geeves et al. (1984) proposed that the A–M·D to A·M·D transition is related to the power stroke. Thus our two-state transitions of acto·S1 and acto·S1·ADP may be equivalent to step 2 in eqs 4 and 5, respectively. Our equilibrium constant of the acto·S1·ADP transition (corresponding to K_2^D) is 18 at 20 °C in 60 mM KCl, 2 mM $MgCl_2$, and pH 7.5. This value is in reasonable agreement with the value of ~10 for K_2^D recently deduced by Geeves (1989) from pressure-relaxation kinetic measurements. Our equilibrium constant of the transition in the absence of actin (K_2) is about 10, which is one order of magnitude smaller than the K_2 value (200) previously deduced from pressure-relaxation measurements carried out in 0.1 M KCl (Coates et al., 1985). In spite of the apparent discrepancy, we are in qualitative agreement with Coates et al. and Geeves in that the equilibrium constant of the transition of acto·S1 is smaller in the presence of ADP than in its absence.

Other investigators have identified two acto·S1·ADP states from kinetic studies (Sleep & Hutton, 1980; Siemankowski & White, 1984). It was recently suggested by Geeves (1989) that the AM'·ADP state identified by Sleep and Hutton may

be compatible with A–M·D. Our previous result on the transition S1·ϵADP·vanadate suggests that phosphate may prefer the low-temperature state of S1·ADP, and if A–M·D is compatible with the low-temperature state $(acto\cdot S1\cdot ADP)_L$ identified here, A–M·D can be expected to bind phosphate more tightly than A·M·D, a possibility suggested by Geeves (1989).

The work presented here shows that the state of S1 in the ternary complex acto·S1·ADP is shifted in favor of the low-temperature state relative to S1 in the binary complex S1·ADP. A role of actin in the ternary complex is to facilitate release of ADP during contraction. Kinetic evidence suggests that ADP is likely released from A·M·D rather than A–M·D since the breakdown of A–M·D is slow and limited to a rate of ≤ 4 s^{-1} regardless of whether actin or ADP dissociates (Geeves, 1989). In our simple model this is equivalent to release of ADP from the high-temperature state of the ternary complex, $(acto\cdot S1\cdot ADP)_H$, and this release is preceded by a two-state transition:

$$(S1\cdot ADP)_L \overset{a}{\rightleftharpoons} (S1\cdot ADP)_H \overset{b}{\longrightarrow} S1 + ADP \quad (6)$$

$$(acto\cdot S1\cdot ADP)_L \overset{a'}{\rightleftharpoons} (acto\cdot S1\cdot ADP)_H \overset{b'}{\longrightarrow} acto\cdot S1 + ADP \quad (7)$$

If we assume that the activation enthalpy is about the same for the two forward reactions of the two-state transitions (steps *a* and *a'*) in eqs 6 and 7, then the activation enthalpy for the reverse reaction (transition from the high-temperature to the low-temperature state) must be considerably smaller for the binary complex (eq 6) than the ternary complex because the enthalpy change of the transition of the binary complex is 16.1 kcal compared to 5.2 kcal for the ternary complex. Thus the net rate of transition to form the high-temperature complex would be larger with the ternary complex than the binary complex. If we further assume that step *a* is rate limiting in both eq 6 and 7, then the rate of ADP release must be faster from acto·S1·ADP than from S1·ADP. This simple model assumes that activation enthalpy is the principal factor governing the overall transition rate and ignores possible entropic effects.

The fluorescence of the probe in S1-AF is significantly quenched relative to that of β-mercaptoethanol-AF (Ando, 1984; Aguirre et al., 1986), and the quenching is increased in the presence of ADP. This quenching was ascribed to a static interaction between the fluorescein moiety of the probe and the groups surrounding SH_1. Potential candidates for these groups include the side chains of Trp, Tyr, and Met since they have been shown to quench the emission of free fluorescein effectively (Watt & Voss, 1977). Cross-linking studies of S1 suggested that the ATPase site of S1 is near the region where the three 25-, 50-, and 20-kDa proteolytic segments of the heavy chain are contiguous (Hiratsuka, 1987). The C-terminal 20-kDa segment contains SH_1, and the N-terminal 25-kDa segment contains two Trp, eight Tyr, and four Met in the stretch from Asn-80 to Tyr-142 (Tong & Elzinga, 1983). Lu and Wang (1989) recently showed that Glu-88 is the major site and Asp-89 and Met-92 are the minor sites involved in the nucleotide-independent cross-linking of the 25-kDa segment to SH_1 via photoactivatable benzophenone iodoacetamide (BPIA). The close proximity of Met-92 to SH_1 in tertiary structure makes static quenching of the emission of AF attached to SH_1 a distinct possibility. The aromatic residues in the putative nucleotide pocket (Tyr-109 to Tyr-142) may be too far removed to contribute significantly to static quenching since this region does not appear to be cross-linked by BPIA. We previously showed that the 2-fold increase in the steady-state intensity of S1-AF observed in the presence of actin likely resulted from a decrease of static quenching. This conclusion was based on a 2-fold decrease of the fractional amplitude of the short-decay component of the biexponential emission decay of S1-AF. We suggest that formation of acto·S1 disrupts the static interaction that is responsible for the quenching of the AF fluorescence intensity in S1-AF. Thus actin binding induces a region of the 25-kDa segment to move away from the SH_1 region of the 20-kDa segment. This movement appears to be reversed when ADP binds to acto·S1-AF since the actin-enhanced intensity is now abolished and decreased to a level below that observed in S1-AF alone. This quenching by ADP could result from additional static quenching contributed by aromatic residues within the nucleotide pocket. Okamoto and Yount (1985) reported that Trp-130 is the major site labeled by the nucleotide analogue *N*-(4-azido-2-nitrophenyl)-2-aminoethyl triphosphate. ADP binding could result in exposure or movement of other tryptophans within the nucleotide pocket toward SH_1 and form static quenching with the fluorescein moiety in S1-AF. This additional quenching could also result from an induced movement of SH_1 toward the nucleotide pocket since ADP binding was shown to induce a relative movement of SH_1 and SH_2 toward each other by fluorescence resonance energy transfer (Dalbey et al., 1983; Cheung et al., 1985; Garland et al., 1988). The quenching and enhancement of the AF fluorescence previously observed and demonstrated here are compatible with the notion that the SH_1 region senses relative movements of the 20- and 25-kDa segments induced by actin and nucleotide binding to the S1 heavy chain and strengthen the idea that segmental movements within the S1 heavy chain form a molecular basis for intersite communication in actomyosin.

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