Mornet, D., Pantel, P., Audemard, E., & Kassab, R. (1979) *Biochem. Biophys. Res. Commun. 89*, 925–932.
Mornet, D., Bertrand, R., Pantel, P., Audemard, E., & Kassab, R. (1981a) *Biochemistry 20*, 2110–2120.
Mornet, D., Bertrand, R., Pantel, P., Audemard, E., & Kassab, R. (1981b) *Nature (London) 292*, 301–306.
Oaklay, B. R., Kirsch, D. R., & Morris, N. R. (1980) *Anal. Biochem. 105*, 361–363.
O'Farrell, P. Z., Goodman, H. M., & O'Farrell, P. H. (1977) *Cell (Cambridge, Mass.) 12*, 1133–1142.
Prince, H. P., Trayer, H. R., Henry, G. D., Trayer, I. P., Dalgarno, D. C., Levine, B. A., Cary, P. D., & Turner, C. (1981) *Eur. J. Biochem. 121*, 213–219.
Sivaramakrishnan, M., & Burke, M. (1982) *J. Biol. Chem. 257*, 1102–1105.
Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.
Suck, D., Kabsch, W., & Mannherz, H. G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4319–4323.
Sutoh, K. (1981) *Biochemistry 20*, 3281–3285.
Taylor, K. A., & Amos, L. A. (1981) *J. Mol. Biol. 147*, 297–324.
Toyoshima, C., & Wakabayashi, T. (1979) *J. Biochem. (Tokyo) 86*, 1887–1890.
Vandekerckhove, J., & Weber, K. (1978a) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1106–1110.
Vandekerckhove, J., & Weber, K. (1978b) *Eur. J. Biochem. 90*, 451–462.
Vandekerckhove, J., & Weber, K. (1978c) *J. Mol. Biol. 126*, 783–802.
Wagner, P. D., & Weeds, A. G. (1977) *J. Mol. Biol. 109*, 455–473.
Wakabayashi, T. (1980) *Muscle Contraction* (Ebashi et al., Eds.) pp 79–97, Japan Scientific Societies Press, Tokyo.
Wakabayashi, T., & Toyoshima, C. (1981) *J. Biochem. (Tokyo) 90*, 683–701.
Weeds, A. G., & Taylor, R. S. (1975) *Nature (London) 257*, 54–56.
Winstanley, M. A., & Trayer, I. P. (1979) *Biochem. Soc. Trans. 7*, 703–704.
Yamamoto, K., & Sekine, T. (1979) *J. Biochem. (Tokyo) 86*, 1869–1881.
Yamamoto, K., Okamoto, Y., & Sekine, T. (1978) *Anal. Biochem. 84*, 313–316.

Fluorescence Anisotropy of Labeled F-Actin: Influence of Divalent Cations on the Interaction between F-Actin and Myosin Heads†

Masao Miki, Philippe Wahl,* and Jean-Claude Auchet

ABSTRACT: The interaction between F-actin and soluble proteolytic fragments of myosin, heavy meromyosin and myosin subfragment 1 without ATP, has been studied by measuring the static anisotropy and the transient anisotropy decay of the fluorescent chromophore *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine bound to F-actin. In the presence of Ca^{2+} ions, the mobility of the chromophore was strongly decreased by adding heavy meromyosin or myosin subfragment 1, and this conformation change of F-actin showed a strong cooperativity; that is, a very small amount of myosin heads induced the maximum anisotropy change. On the other hand, in the presence of Mg^{2+} ions, the addition of a small amount of myosin subfragment 1 or of heavy meromyosin increased the mobility of labeled F-actin that reached a maximum at a molar ratio of about 1/25 or 1/50, respectively. With further addition of myosin heads, the mobility of the labeled actin decreased. From these studies, one concludes that F-actin undergoes a conformation change by interacting with myosin heads, which depends on the nature of the divalent cations present in the solution.

The cyclic interaction of myosin and actin coupled with the ATP hydrolysis generates the mechanical force in the muscle contraction. During this process, the system passes through several states; one of these is thought to be identical with the stable complex formed by myosin and actin in the absence of ATP (the rigor complex). The presence of Mg^{2+} ions is required for the mechanochemical transduction of the ATP hydrolysis. The Mg–ATPase of myosin alone is small and is strongly enhanced by the myosin–actin interaction.

Until now it has not been established whether Mg^{2+} has a structural role in the actin–myosin interactions. The rigor complex has been studied by physical measurements in the presence and in the absence of Mg^{2+} (Thomas et al., 1979; Oosawa et al., 1973; Tawada, 1969; Fujime & Ishiwata, 1971; Los Calzo et al., 1975). But the influence of this ion on the conformation of the complex has not been systematically studied.

In the present work, we measured the average fluorescence anisotropy and the fluorescence anisotropy decay of F-actin labeled with *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine, in solutions of which various amounts of the active subfragments of myosin, heavy meromyosin (HMM) and subfragment 1 (S_1), were added. These kinds of measurements brought information on the mobility of this fluorescent label in the time range between 1 ns and 1 μs (Wahl et al., 1975; Tawada et al., 1978; Ikkai et al., 1979). When the myosin subfragments were added to the F-actin solutions, we found that the label mobility changed in a different way according to whether Ca^{2+} or Mg^{2+} was present in the solution. These results suggested that the conformation of the rigor complex was dependent on the nature of the divalent cations that were present in the solution.

† From the Centre de Biophysique Moléculaire, C.N.R.S., 45045 Orleans Cedex, France. *Received November 10, 1981; revised manuscript received March 29, 1982.*

0006-2960/82/0421-3661$01.25/0

Materials and Methods

Reagents. All solutions were prepared with doubly distilled water. *N*-(Iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine was purchased from Aldrich-Europe, ATP was from Sigma, and other chemicals were of analytical grade from Merck.

Preparation of Muscle Proteins. (*1*) *Actin.* Acetone-dried muscle powder was prepared from rabbit back and leg striated muscles by the method of Straub (1943) modified as follows: for removal of the regulatory proteins, the minced muscles deprived of myosin were incubated in doubly distilled water overnight at 4 °C before acetone treatment. G-Actin was extracted from acetone-dried powder at 4 °C with 20 mL of 1 mM Tris-HCl[1] (pH 8.0)/g of dried muscle powder. Actin was purified by ultracentrifugation and by repeating the cycle of polymerization and depolymerization (Mommaerts, 1952). G-Actin was polymerized with 30 mM KCl in order to avoid the contamination of tropomyosin. $NaDodSO_4$–polyacrylamide gel electrophoresis showed that there were no other proteins present.

(*2*) *Heavy Meromyosin.* Heavy meromyosin was prepared from myosin by digestion with trypsin according to the method of Lowey & Cohen (1962) and purified by ammonium sulfate fractionation between 45 and 55% saturation. It was lyophilized in solutions containing 0.1 M sucrose and stored at −20 °C.

(*3*) *Myosin Subfragment 1.* Myosin subfragment 1 was prepared from myosin by digestion with chymotrypsin according to the method of Weeds & Pope (1977) and purified by gel chromatography on AcA 34 (28 × 1000 mm), equilibrated with 0.1 M KCl and 20 mM Tris-HCl (pH 8.0) with a flow rate of 40 mL/h. It was lyophilized in solutions containing 0.1 M sucrose and stored at −20 °C.

Fluorescence Labeling of Actin. Actin was labeled with *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine and purified by chromatography on Sephacryl S-200 as previously described (Ikkai et al., 1979).

We measured the molar ratio of dye to actin by absorption, using the molar coefficient 6100 M^{-1} cm^{-1} at 336 nm given by Hudson & Weber (1973). This molar ratio varied from 0.4 to 0.2 according to the preparations.

ATPase Activity of Myosin Fragments. The ATPase activity of heavy meromyosin was measured by the method of Tausky & Shorr (1953) at 25 °C under the conditions described by Eisenberg & Moos (1968). The specific activity of our heavy meromyosin preparation was 0.02 mol min^{-1} mg^{-1} without actin (heavy meromyosin concentration 3 mg/mL) and 0.18 mol min^{-1} mg^{-1} with 1 mg/mL actin (HMM concentration 0.2 mg/mL) in 0.08 M KCl, 1 mM $MgCl_2$, 2 mM ATP, and 50 mM imidazole (pH 7.0).

The ATPase activity of myosin subfragment 1 was measured at 25 °C under the solvent conditions described by Mornet et al. (1979). The specific activity of our myosin subfragment 1 preparation was 1.0 mol min^{-1} mg^{-1} for Ca^{2+}–ATPase in 0.25 M KCl, 5 mM $CaCl_2$, 2.5 mM ATP, and 50 mM Tris-HCl (pH 7.5) and 3.6 mol min^{-1} mg^{-1} for K^+-EDTA–ATPase in 1 M KCl, 5 mM EDTA, 2.5 mM ATP, and 50 mM Tris-HCl (pH 7.5). The subfragment 1 Mg^{2+}–ATPase was also measured. Its specific activity was 0.04 mol min^{-1} mg^{-1} without actin and 3.1 mol min^{-1} mg^{-1} with 1 mg/mL actin for 0.05 mg/mL myosin subfragment 1 in 6 mM KCl, 1.5 mM $MgCl_2$, 2.5 mM ATP, and 50 mM Tris-HCl (pH 8.0). The ATPase of myosin subfragment 1 was activated almost as well by labeled actin as by unlabeled actin, in agreement with the previous study by Takashi (1979).

Preparation of Protein Solutions. Protein concentrations of heavy meromyosin and myosin subfragment 1 were determined from ultraviolet absorbance, the absorption coefficients being $A^{1\%}_{280nm}$ = 6.0 cm^{-1} (Margossian & Lowey, 1973) and $A^{1\%}_{280nm}$ 7.5 cm^{-1} (Weeds & Pope, 1977), respectively. The concentration of actin was determined by biuret reaction with an absorbance value of 0.070 for 1 mg/mL protein at 540 nm. The molecular weights of actin, heavy meromyosin, and myosin subfragment 1 were taken to be 42 000 (Elzinga et al., 1973), 340 000 (Lowey et al., 1969), and 110 000 (Onodera & Yagi, 1971), respectively.

A stock solution of labeled F-actin has been prepared by polymerizing a solution of G-actin at room temperature and at a concentration of about 1 mg/mL in buffer F containing 0.1 M KCl, 20 mM Tris-HCl (pH 7.6), 1 mM sodium azide, and 0.5 mM 2-mercaptoethanol for Ca–F-actin and in buffer F plus 1 mM $MgCl_2$ for Mg–F-actin. Ca^{2+} ion and ATP concentrations were 0.1 mM in this stock solution since the G-actin solution contained 0.2 mM $CaCl_2$ and 0.2 mM ATP. This stock solution was kept at 4 °C.

For fluorescence anisotropy measurements, samples were prepared by diluting the F-actin stock solution and mixing this diluted solution with HMM or S_1 solutions. The dilution solvent and the myosin subfragment solutions contained no ATP. The solutions were kept at room temperature for at least 6 h before the anisotropy decay measurements.

Absorption and Steady-State Fluorescence Measurements. Absorption spectra and optical density were measured with a Beckman Acta III spectrophotometer. Steady-state fluorescence measurements were performed with a Jobin Yvon spectrophotometer JY 3C connected to a Tektronix desk computer, 4051. For the average anisotropy measurements ($\bar{r}$), each polarized fluorescence component, I_{vv}, I_{vh}, I_{hh}, and I_{hv}, was automatically measured 20 times and averaged by the desk computer; $\bar{r}$ was given by

$$\bar{r} = (I_{vv} - \beta I_{vh})/(I_{vv} + 2\beta I_{vh}) \quad (1)$$

where $\beta = I_{hv}/I_{hh}$. The excitation wavelength was 370 nm ($\Delta\lambda$ = 6 nm), and the emission wavelength was 480 nm ($\Delta\lambda$ = 10 nm).

Pulse Fluorometry. The fluorescence decay experiments were carried out by the single-photoelectron counting method. The measurement conditions were the same as previously described (Tawada et al., 1978; Ikkai et al., 1979).

The fluorescence decay $S(t)$ and the anisotropy decay $R(t)$ were assumed to be sums of exponential functions, $\sum_i a_i \exp(-t/\tau_i)$ and $\sum_i \alpha_i \exp(-t/\theta_i)$, respectively. The parameters of these functions were determined by the least-squares method (Grinvald & Steinberg, 1974; Wahl, 1979).

In the present study, the experimental data were fitted with sums of two exponential terms for $S(t)$ and for $R(t)$. In the case of $S(t)$, both fluorescence lifetimes were very close to each other and could not be accurately determined. More significant was the average fluorescence lifetime, defined as

$$\bar{\tau} = \sum a_i \tau_i / \sum a_i \quad (2)$$

The values of the anisotropy decay parameters obtained by computation were appreciated by examining the shape of the deviation function and the residual χ^2, which were defined as

$$DV_k = (r_k^{ex} - r_k^c)/\sigma_k \quad (3)$$

$$\chi^2 = (1/n)\sum_i^n (DV_k)^2 \quad (4)$$

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; ATP, adenosine 5'-triphosphate.

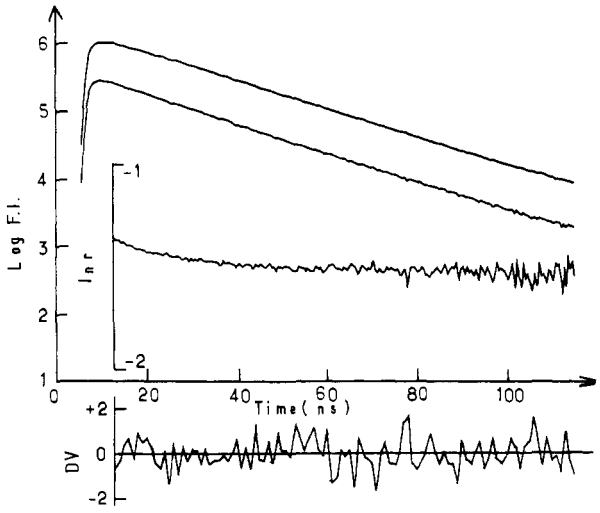

FIGURE 1: Transient fluorescence of labeled F-actin (0.2 mg/mL) and heavy meromyosin complex (25/1 molar ratio) in buffer F plus 0.1 mM $CaCl_2$ at 20 °C. From the top one finds successively $s^{ex}(t)$, $d^{ex}(t)$, $r^{ex}(t)$, and the deviation function relative to $r^{ex}(t)$ with the following parameters of $R(t)$: $\alpha_1 = 0.054$, $\theta_1 = 9.9$ ns, $\alpha_2 = 0.226$, and $\theta_2 = 1129$ ns.

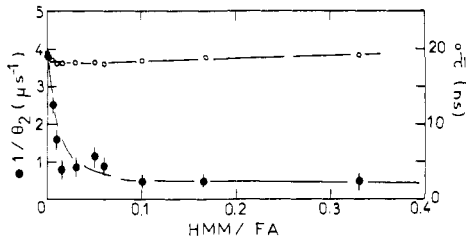

FIGURE 2: Inverse of correlation time θ_2 (●) and average lifetime (○) of F-actin as a function of the molar ratio of heavy meromyosin and F-actin in buffer F plus 0.1 mM $CaCl_2$ at 20 °C.

where r_k^{ex} and r_k^c were the experimental and computed values of $r(t)$ at channel k, σ_k was the variance of r_k^{ex}, and n was the channel number. For a good fit, DV_k oscillated randomly around 0 with an average amplitude of 1, and χ^2 was close to unity.

Results

Interaction of F-Actin with HMM and S_1 in the Presence of Ca^{2+} Ions. At first we studied the interaction of F-actin and myosin heads in the presence of Ca^{2+} ions. The transient polarized fluorescence of labeled F-actin in buffer F plus 0.1 mM $CaCl_2$ at 20 °C was measured. The concentration of actin was 0.2 mg/mL. The fluorescence anisotropy decay could be analyzed with two exponential functions as previously described (Tawada et al., 1978). The decay parameters of $R(t)$ were $\alpha_1 = 0.065$, $\theta_1 = 13.5$ ns, $\alpha_2 = 0.125$, $\theta_2 = 260$ ns, and $\chi^2 = 0.92$, respectively. Under these conditions, the contribution of G-actin to θ_2 was neglibile as shown in a work that will be published elsewhere.

When HMM or S_1 was added to the F-actin solutions, θ_2 increased. Figure 1 shows the transient polarized fluorescence of the labeled F-actin–HMM complex (molar ratio 25/1) in buffer F plus 0.1 mM $CaCl_2$. The decay parameters were $\alpha_1 = 0.054$, $\theta_1 = 9.9$ ns, $\alpha_2 = 0.226$, $\theta_2 = 1129$ ns, and $\chi^2 = 1.1$. The corresponding deviation function is also shown in Figure 1.

The inverse of the correlation time θ_2 and the average fluorescence lifetime $\bar{\tau}$ were plotted as functions of the molar ratios of HMM and S_1 to F-actin monomer in Figures 2 and 3, respectively. $\bar{\tau}$ varied slightly with these molar ratios. When

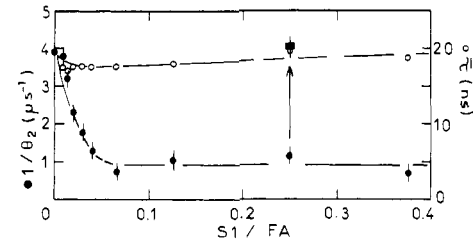

FIGURE 3: Inverse of correlation time θ_2 (●) and average lifetime (○) of F-actin as a function of the molar ratio of myosin subfragment 1 and F-actin in buffer F plus 0.1 mM $CaCl_2$ at 20 °C. (■) denotes added 10 mM ATP.

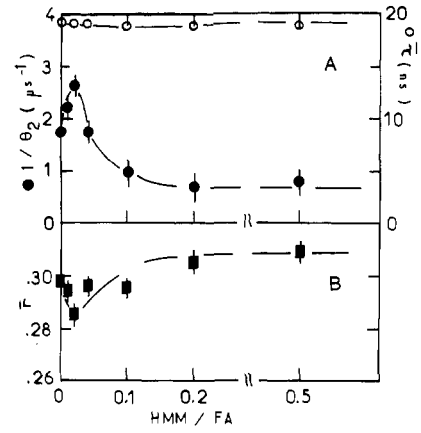

FIGURE 4: (A) Inverse of correlation time θ_2 (●) and average lifetime (○) and (B) average anisotropy of F-actin as a function of the molar ratio of heavy meromyosin and F-actin in buffer F plus 1 mM $MgCl_2$ at 20 °C.

[HMM]/[A] or $[S_1]$/[A] increased, $1/\theta_2$ decreased sharply and then reached a plateau value, where [HMM], [A], and $[S_1]$ designate the molar concentrations of HMM, actin, and S_1 in the solution, respectively. The midpoint of the $1/\theta_2$ change was obtained at [HMM]/[A] = 0.01 and $[S_1]$/[A] = 0.02. Since HMM has two myosin heads and S_1 only one, this midpoint occurred at the same [myosin head]/[actin] molar ratio (0.02).

In order to show that the high value of the correlation time of labeled F-actin in the F-actin–S_1 complex was not due to the denaturation of labeled F-actin, we performed the following experiments: we measured the fluorescence anisotropy decay of the F-actin–S_1 complex and found the usual high θ_2 value. Then we added 10 mM ATP to this solution and immediately measured it again. The correlation time was found equal to that of F-actin alone (Figure 3).

Interaction of F-Actin with HMM and S_1 in the Presence of Mg^{2+} Ions. The fluorescence decay and the anisotropy decay of labeled F-actin (0.2 mg/mL) in buffer F containing 1 mM $MgCl_2$ at 20 °C was measured. In the presence of Mg^{2+} ions, θ_2 was larger than that in the presence of Ca^{2+} as previously found (Ikkai et al., 1979). The anisotropy decay parameters were $\alpha_1 = 0.04$, $\theta_1 = 5.8$ ns, $\alpha_2 = 0.25$, $\theta_2 = 682$ ns, and $\chi^2 = 1.2$. We added HMM in various amounts to this F-actin solution. The average fluorescence lifetime and the inverse of θ_2 were plotted as functions of the molar ratio of HMM to actin in Figure 4A. As this molar ratio increased, $1/\theta_2$ first increased to a maximum (molar ratio 1/50) and then decreased and reached a constant value for molar ratios larger than 0.2. The decrease of $1/\theta_2$ at [HMM]/[A] larger than 0.1 was previously found by Tawada et al. (1978). The steady-state fluorescence anisotropy showed a minimum value at a molar ratio of HMM to F-actin also equal to 1/50 (Figure 4B). Figure 5 shows the fluorescence decay and anisotropy

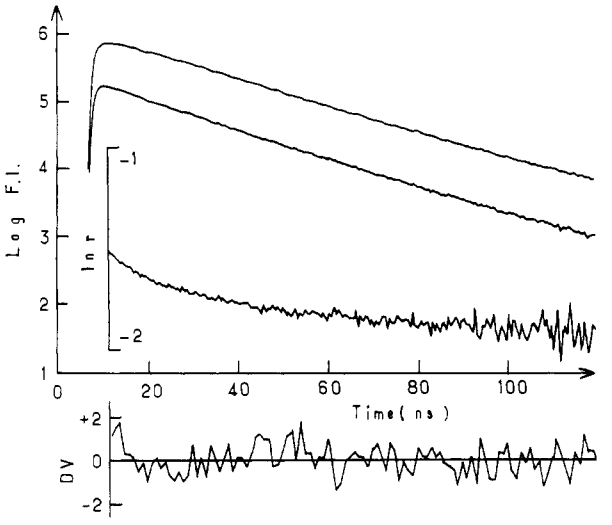

FIGURE 5: Transient fluorescence of labeled F-actin and myosin subfragment 1 complex (50/1 molar ratio) in buffer F plus 1 mM $MgCl_2$ at 20 °C. The curves have the same meaning as in Figure 1. Parameters of $R(t)$: $\alpha_1 = 0.062$, $\theta_1 = 7.1$ ns, $\alpha_2 = 0.182$, and $\theta_2 = 303$ ns.

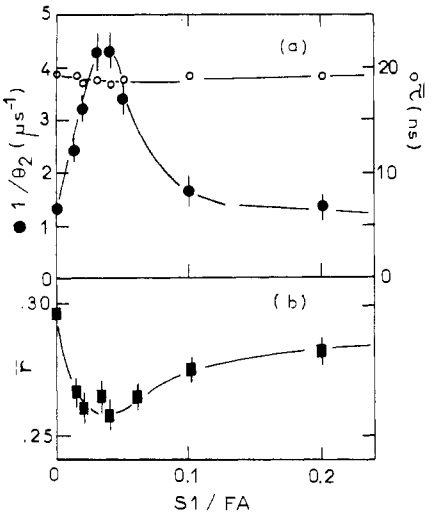

FIGURE 6: (a) Inverse of correlation time θ_2 (●) and average lifetime (○) and (b) average anisotropy of F-actin as a function of the molar ratio of myosin subfragment 1 and F-actin in buffer F plus 1 mM $MgCl_2$ at 20 °C.

decay of labeled F-actin–S_1 complex (molar ratio 50/1) in buffer F containing 1 mM $MgCl_2$ at 20 °C. The decay parameters were $\alpha_1 = 0.062$, $\theta_1 = 7.1$ ns, $\alpha_2 = 0.182$, $\theta_2 = 303$ ns, and $\chi^2 = 0.84$, respectively. The inverse of the correlation time θ_2, the average fluorescence lifetime $\bar{\tau}$, and the average anisotropy $\bar{r}$ were plotted against the molar ratio of S_1 to F-actin in Figure 6. $1/\theta_2$ and $\bar{r}$ also showed a biphasic change with the ratio $[S_1]/[A]$. The maximum of $1/\theta_2$ and the minimum of $\bar{r}$ were obtained at $[S_1]/[A] = 1/25$.

Reversibility of Conformation Change of F-Actin Induced by S_1. We measured the anisotropy decay of a solution of the labeled F-actin–S_1 complex (molar ratio 25/1) in buffer F containing 1 mM $MgCl_2$. This solution was then dialyzed 2 times against 20 times its volume of buffer F plus 0.2 mM $CaCl_2$ in order to exchange Mg^{2+} for Ca^{2+}. The correlation time θ_2 was equal to 226 ± 12 ns before dialysis and to 985 ± 100 ns after dialysis. On the other hand, we measured the anisotropy decay of a solution of the F-actin–S_1 complex (molar ratio 25/1) in buffer F plus 0.2 mM $CaCl_2$. This solution was then dialyzed 2 times against 20 times its volume

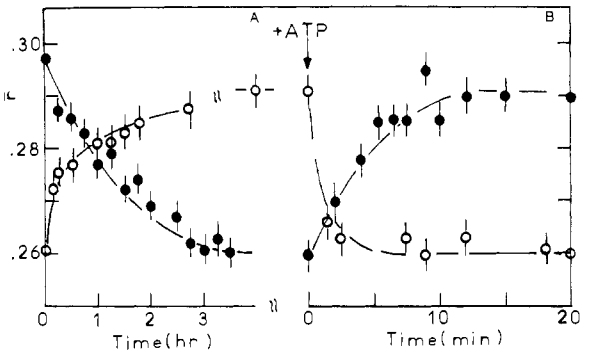

FIGURE 7: (A) Kinetics of conformation change of F-actin after adding heavy meromyosin in buffer F plus 0.1 mM $CaCl_2$ (○) or myosin subfragment 1 in buffer F plus 1 mM $MgCl_2$ (●) and (B) then after adding 4 mM ATP. Concentrations: F-actin, 0.2 mg/mL; heavy meromyosin, 0.2 mg/mL; myosin subfragment 1, 0.02 mg/mL.

of buffer F plus 1 mM $MgCl_2$ in order to exchange Ca^{2+} for Mg^{2+}. Before dialysis, the correlation time θ_2 was equal to 1160 ± 270 ns and equal to 1211 ± 150 ns after dialysis. Starting therefore from the Mg–F-actin–S_1 complex, one could obtain by dialysis against Ca^{2+} ion the anisotropy decay that characterizes the Ca–F-actin–S_1 complex. The reverse process was impossible.

When we added 0.2 mg/mL HMM to 0.2 mg/mL F-actin in buffer F containing 0.1 mM $CaCl_2$, the average anisotropy increased with time and reached the equilibrium value after 3 h (Figure 7). On the contrary, when we added 0.02 mg/mL S_1 to 0.2 mg/mL F-actin in buffer F containing 1 mM $MgCl_2$, the average anisotropy decreased with time and reached the equilibrium value after 3 h (Figure 7). These processes were reversible. When a small amount of concentrated ATP (100 mM) was added to these solutions in order to make a final ATP concentration of 4 mM, the average anisotropies recovered their initial values.

Discussion

It has been shown that F-actin interacted strongly with the soluble subfragments of myosin in solutions that do not contain ATP. The binding constant of the complex has been measured by several authors (Marston & Weber, 1975; Margossian & Lowey, 1978; Highsmith, 1978; Green & Eisenberg, 1980). The value reported for S_1 is about 2×10^7 M^{-1}. The saturated complex was obtained for a molar ratio of myosin head to actin equal to 1 (Takeuchi & Tonomura, 1971). It has been recently found that one myosin head is bound to two F-actin protomers (Mornet et al., 1981).

As a result of the myosin head binding, the F-actin molecule undergoes conformation changes as shown by numerous physical techniques (Tawada, 1969; Fujime & Ishiwata, 1971; Los Calzo et al., 1975; Miki et al., 1976; Tawada et al., 1978; Thomas et al., 1979). But the physical parameters associated with the F-actin conformation did not vary proportionally to the number of interacting actin monomers. A maximum variation of these parameters was obtained at a molar ratio of myosin head to actin well below the saturation value. These results suggested that one myosin head interacting with two actin protomers (Mornet et al., 1981) induced a cooperative conformation change propagating to a number of neighboring protomers of the same F-actin filament.

The inverse of the correlation time $1/\theta_2$ determined in the present work was a measure of the mobility of the fluorescent label attached to actin (Tawada et al., 1978; Ikkai et al., 1979). $1/\theta_2$ changed when HMM or S_1 was added to the F-actin solutions. These changes reached a maximal value at a molar ratio of myosin head to actin well below the stoichiometric

complex. Our measurements therefore also suggested cooperative conformation changes of the actin protomers induced by the interaction of these protomers with the myosin head. But our work showed for the first time that these conformation changes of F-actin depended on the nature of the divalent cation present in the solution.

According to its magnitude, θ_2 reflected some contribution of the internal motions of the actin protomer. Then we may explain the decrease of θ_2 by a loosening of the physicochemical links between the protomers in the F filament when a small amount of HMM or S_1 binds to F-actin in the presence of Mg^{2+}. The opposite effect is found when the complex is formed in the presence of Ca^{2+}.

Measurements of the dynamic flexibility of F-actin filaments and of its changes by interaction with myosin heads have been performed by other techniques. Fujime & Ishiwata (1971) measured the quasi-elastic light scattering of F-actin solutions and determined a relaxation time of the F-actin filament bending motion of the order of 10 ms. The flexibility of F-actin was increased by HMM but was not changed by S_1. The solutions used by these authors contained no Mg^{2+}. By saturation transfer electron paramagnetic resonance, Thomas et al. (1979) found that a spin-label bound to actin had a correlation time of 10^{-4} s. This correlation time increased 4 times when HMM or S_1 interacted with F-actin in the presence of Mg^{2+}. These authors concluded that the flexibility of F-actin decreased in the actomyosin complex.

These results may seem to be inconsistent with our findings. But the correlation times that are measured by quasi-elastic light scattering, saturation transfer electron paramagnetic resonance, and fluorescence polarization show quite different orders of magnitude, and there is no theory that allows one to relate these parameters between them. On the other hand, we used here small concentrations of actin, which decreased the influence of the interfilament interactions, while in the other techniques, the concentrations were higher than ours. This might also be a reason for the different results obtained by these various techniques.

Each myosin head has a binding site of high affinity for the divalent cations located on the DTNB light chain. But this site does not exist on our S_1 sample since the chymotryptic digestion hydrolyzes the DTNB light chain (Weeds & Pope, 1977). On the other hand, divalent cations bind to a high-affinity site of actin. The nature of the ion bound to this site may be involved in the conformation of an actin monomer interacting with a myosin head. It remains to be established whether there is a relation between the divalent cation effect found in this work and the influence of divalent cations on the mechanochemical transduction in muscles.

Acknowledgments

We thank Professor Oosawa for the interest he showed in our work.

References

Eisenberg, E., & Moos, C. (1968) *Biochemistry 7*, 1486–1489.
Elzinger, M., Collins, J. H., Kuehl, W. M., & Adelstein, R. S. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2687–2691.
Fujime, S., & Ishiwata, S. (1971) *J. Mol. Biol. 62*, 254–265.
Green, L. E., & Eisenberg, E. (1980) *J. Biol. Chem. 255*, 549–554.
Grinvald, A., & Steinberg, I. Z. (1974) *Anal. Biochem. 59*, 583–598.
Highsmith, S. (1978) *Biochemistry 17*, 22–26.
Hudson, E. N., & Weber, G. (1973) *Biochemistry 12*, 4154–4161.
Ikkai, T., Wahl, Ph., & Auchet, J. C. (1979) *Eur. J. Biochem. 93*, 397–408.
Los Calzo, J., Reid, G. H., & Weber, A. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3412–3415.
Lowey, S., & Cohen, C. (1962) *J. Mol. Biol. 4*, 293–308.
Lowey, S., Slater, S., Weeds, A. G., & Baker, H. (1969) *J. Mol. Biol. 42*, 1–29.
Margossian, S. S., & Lowey, S. (1973) *J. Mol. Biol. 74*, 313–330.
Margossian, S. S., & Lowey, S. (1978) *Biochemistry 17*, 5431–5439.
Marston, S., & Weber, A. (1975) *Biochemistry 14*, 3868–3873.
Miki, M., Kouyama, T., & Mihashi, K. (1976) *FEBS Lett. 66*, 98–101.
Mommaerts, W. F. H. M. (1952) *J. Biol. Chem. 198*, 445–458.
Mornet, D., Pantel, P., Andemard, E., & Kassab, R. (1979) *Eur. J. Biochem. 100*, 421–431.
Mornet, D., Bertrand, R., Pantel, P., Audemand, E., & Kassab, R. (1981) *Nature (London) 292*, 301–306.
Onodera, M., & Yagi, K. (1971) *J. Biochem. (Tokyo) 69*, 145–153.
Oosawa, F., Fujime, S., Ishiwata, S., & Mihashi, K. (1973) *Cold Spring Harbor Symp. Quant. Biol. 33*, 277–286.
Straub, F. B. (1943) *Stud. Inst. Med. Chem., Univ. Szeged 2*, 3–15.
Takashi, R. (1979) *Biochemistry 18*, 5164–5169.
Takeuchi, K., & Tonomura, Y. (1971) *J. Biochem. (Tokyo) 70*, 1011–1026.
Tausky, H. H., & Shorr, E. (1953) *J. Biol. Chem. 202*, 675–685.
Tawada, K. (1969) *Biochim. Biophys. Acta 172*, 311–318.
Tawada, K., Wahl, Ph., & Auchet, J. C. (1978) *Eur. J. Biochem. 88*, 411–419.
Thomas, D. D., Seidel, J. C., & Gergely, J. (1979) *J. Mol. Biol. 132*, 257–273.
Wahl, Ph. (1979) *Biophys. Chem. 10*, 91–104.
Wahl, Ph., Mihashi, K., & Auchet, J. C. (1975) *FEBS Lett. 60*, 164–167.
Weeds, A. G., & Pope, B. (1977) *J. Mol. Biol. 11*, 129–157.