BBA 46312

EFFECTS OF TROPOMYOSIN, TROPONIN AND Ca^{2+} ON THE INTERACTION BETWEEN F-ACTIN AND HEAVY MEROMYOSIN

YASUO NAKAOKA

Department of Physics, Faculty of Science, Nagoya University, Nagoya (Japan)

(Received December 17th, 1971)

SUMMARY

1. In the absence of ATP, H-meromyosin (heavy meromyosin) bound with the F-actin–tropomyosin–troponin complex up to the molar ratio of H-meromyosin to actin of 1:1, independently of the concentration of Ca^{2+}.

2. In the presence of free Ca^{2+} above about 1 μM, with an increasing amount of H-meromyosin bound to a fixed amount of the F-actin–tropomyosin–troponin complex, the degree of flow birefringence decreased and the extinction angle increased. The minimum value of the birefringence and the maximum value of the extinction angle were found at the molar ratio of H-meromyosin to actin of 1:2. A further increase of bound H-meromyosin to actin restored both the degree of birefringence and the extinction angle to nearly the same level as the F-actin–tropomyosin–troponin complex only. In the absence of free Ca^{2+}, the birefringence did not change with the binding of H-meromyosin.

3. This sensitivity of birefringence to the concentration of Ca^{2+} appeared only in the presence of tropomyosin and troponin. At a fixed ratio of H-meromyosin, actin and tropomyosin, the birefringence in the absence of Ca^{2+} increased with increasing amount of added troponin up to the weight ratio of troponin to actin of 1:6; whereas the birefringence in the presence of Ca^{2+} did not change.

4. At a fixed ratio of H-meromyosin to actin, the birefringence changed with increasing amount of tropomyosin added up to the weight ratio of tropomyosin to actin of 1:6; above this ratio, the birefringence was constant.

5. Subfragment S-1, prepared by the chymotryptic digestion of myosin, bound to F-actin, but the birefringence did not change even in the presence of tropomyosin and troponin.

INTRODUCTION

The interaction between F-actin and myosin coupled with the splitting of ATP is the elementary process of muscular contraction. According to the excellent investigation of Ebashi and Endo[1], this interaction is controled by Ca^{2+} through tropomyosin and troponin located on F-actin. In the absence of Ca^{2+} the interaction between F-actin and myosin to induce contraction is inhibited by the troponin–tropomyosin

Abbreviation: EGTA, glycol ether diamine tetraacetic acid.

system and the addition of Ca^{2+} releases the inhibition. The molecular mechanism of this control, however, is not yet clear.

For the analysis of this interaction it is more convenient to use H-meromyosin (heavy meromyosin) instead of myosin because H-meromyosin is soluble at the physiological salt concentration. In the absence of ATP, H-meromyosin binds strongly with F-actin. At saturation one H-meromyosin molecule binds with one actin monomer in F-actin[2-4] and the complex shows the "arrow-head" structure in electron micrographs[2]. Properties of the complex were investigated by Tawada[4] at various ratios of two proteins. With increasing binding of H-meromyosin to F-actin the flow birefringence decreases and has a minimum value at the molar ratio of H-meromyosin to actin of about 1:6. With further increases of H-meromyosin the flow birefringence recovers to the level of F-actin. The change of ultraviolet absorption due to the binding also showed similar behaviour. Recently, by using quasielastic light scattering, Fujime and Ishiwata[5] showed that the flexibility of F-actin increases with the binding of H-meromyosin and has a maximum value at the molar ratio of 1:6. These results suggest that the binding of H-meromyosin induces some change of the polymer structure of F-actin in a certain range of the molar ratio.

The present work has been undertaken to see the effects of tropomyosin and troponin and Ca^{2+} on such behavior of the F-actin–H-meromyosin complex. In the presence of tropomyosin and troponin, the change of the polymer structure of F-actin is not caused by H-meromyosin in the absence of Ca^{2+}, but it occurs in the presence of Ca^{2+} similar to that of F-actin without tropomyosin and troponin When S-1, the product of chymotryptic digestion of myosin, is used instead of H-meromyosin, such sensitivity to Ca^{2+} is not found.

MATERIALS

All proteins were prepared from rabbit skeletal muscle. Heavy meromyosin prepared by the method of Lowey and Cohen[6] was purified by treatment with ammonium sulfate. The fraction obtained between 42 and 55 % saturation of $(NH_4)_2SO_4$ was dialysed to remove $(NH_4)_2SO_4$. The solution was lyophilized after the addition of 0.1 M sucrose and stored. Before the experiment, the lyophilized H-meromyosin was dissolved into a 5 mM Tris-maleate buffer (pH 7.0) and dialysed to remove sucrose. The lyophilization of H-meromyosin in sucrose did not change the ATPase activity or the binding ability to F-actin. The molecular weight of H-meromyosin is assumed to be 320000 (ref. 6).

S-1 was prepared by the method of Onodera and Yagi[7]. The crude S-1 eluted from a DEAE-cellulose ion-exchange column by 0.135 M KCl was precipitated at 65 % saturation of $(NH_4)_2SO_4$ and dialysed to remove $(NH_4)_2SO_4$. This S-1 can accelerate the polymerization of G-actin and its ATPase is activated and then inhibited by the addition of *p*-chloromercuribenzoate.

Actin was prepared from acetone-dried powder by the method of Straub[8] after new proteins discovered by Ebashi and co-workers[9-11] were carefully removed before acetone treatment of this myosin extracted minced muscle by incubating it in distilled water for 12 h at room temperature. For further purification the cycle of polymerization at 30 mM KCl (pH 8.0) and depolymerization in a salt-free environment (pH 8.0) was repeated twice. The molecular weight of G-actin was assumed to be 45000 (refs 12, 13).

Tropomyosin and troponin were prepared by the method of Ebashi and coworkers[14,15]. For further purification of troponin the acid precipitation at pH 4.5 was repeated in 1 M KCl.

The F-actin–tropomyosin–troponin complex was made by the addition of tropomyosin and then troponin to F-actin at physiological salt concentration (0.1 M KCl, pH 7.0). Large aggregates produced upon the addition of troponin were eliminated by incubating the solution at 45 °C for 10 min (S. Ishiwata, personal communication). This treatment did not affect the physiological function of the complex. The original solution of the complex contained Ca^{2+}, probably of the order of 10^{-5} M. When a Ca^{2+}–glycol ether diamine tetraacetic acid (EGTA) buffer was used, the binding constant between Ca^{2+} and EGTA was assumed to be $5 \cdot 10^6\ M^{-1}$ at pH 7.0 to calculate the concentration of free Ca^{2+} (ref. 16).

The DEAE-cellulose ion-exchanger was obtained from Brown Company. Other chemicals were of the reagent grade from Katayama Chemical (Osaka, Japan).

METHODS

Flow birefringence and the extinction angle were determined by a Rao type home-made apparatus at the shear rate between 17 and 1700 s^{-1}. The absorbance or the turbidity was measured by a Carl Zeiss (PM QII) Spectrophotometer. Viscosity was measured by an Ostwald viscometer in a thermostat, in which the flow time of water was 46 s at 23 °C.

The concentration of proteins was determined by the biuret method or by the absorbance at 280 nm, using the value calibrated previously[7,14].

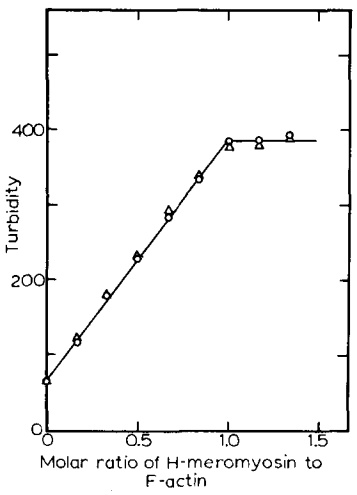
RESULTS

Binding of H-meromyosin to the F-actin–tropomyosin–troponin complex

When H-meromyosin is added to a solution of F-actin at physiological salt concentration, the turbidity of the solution increases linearly with the amount of H-meromyosin added, up to the molar ratio of 1:1 (ref. 4). Further addition of H-meromyosin does not produce a large increase of turbidity. At saturation one H-meromyosin molecule binds with one actin monomer in F-actin. A similar increase of the turbidity is observed when H-meromyosin is added to the F-actin–tropomyosin–troponin complex. In Fig. 1, various amounts of H-meromyosin were added to a fixed amount of the F-actin–tropomyosin–troponin complex (weight ratio 6:1:1) and the turbidity of the solution was measured after incubation for 1 h at 20 °C. H-meromyosin can bind with the F-actin–tropomyosin–troponin complex and maximum binding is attained at the molar ratio of H-meromyosin to actin of 1:1. Binding does not depend on the concentration of Ca^{2+}, as shown by the fact that the addition of EGTA had no effect on the turbidity.

Flow birefringence of the F-actin–tropomyosin–troponin complex with bound H-meromyosin

Flow birefringence of the solution of the F-actin–tropomyosin–troponin complex with various amounts of bound H-meromyosin was measured. As shown in Fig. 2, the degree of birefringence decreases and the extinction angle increases with in-



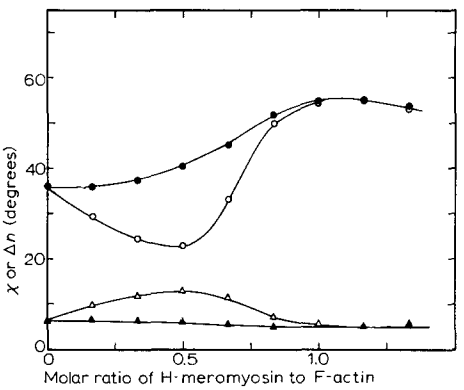


Fig. 1. Turbidity of the solution of the F-actin–tropomyosin–troponin and H-meromyosin complex. Protein concentrations: F-actin 0.4 mg/ml, tropomyosin 0.07 mg/ml, troponin 0.07 mg/ml. Solvent conditions: 0.1 M KCl, 10 mM Tris–maleate buffer (pH 7.0). △, in the presence of 0.5 mM EGTA. The turbidity of the solution was measured at 350 nm in a cell of 1-cm path length.

Fig. 2. Degree of the flow birefringence (○, ●) and the extinction angle (△, ▲) of the solution of the F-actin–tropomyosin–troponin and H-meromyosin complex. The sample solution was the same as in Fig. 1. Filled symbols: in the presence of 0.5 mM EGTA. Shear rate of flow: $G = 17\ s^{-1}$. At room temperature.

creasing amounts of bound H-meromyosin up to the molar ratio of H-meromyosin to actin of 1:2. With further increase of H-meromyosin, the degree of birefringence increases, and the extinction angle decreases to the same level as the F-actin–tropomyosin–troponin complex without bound H-meromyosin.

The decrease of the degree of birefringence and the increase of the extinction angle caused by H-meromyosin are both eliminated by the addition of EGTA to remove free Ca^{2+} from the solution. In the absence of free Ca^{2+}, the degree of birefringence increases slightly with increasing amount of bound H-meromyosin and the extinction angle is kept constant. The addition of Ca^{2+} restores the original flow birefringence behavior.

Thus, at low molar ratios of H-meromyosin to actin, Ca^{2+} has the effect of decreasing the degree of birefringence and increasing the extinction angle of the solution of the F-actin–tropomyosin–troponin complex. This effect of Ca^{2+} becomes maximum at the molar ratio of about 1:2 and almost vanishes above the molar ratio of 1:1, as shown in Fig. 2.

The experiment shown in Fig. 3 was made to show that early decrease and later increase of the degree of birefringence with binding of H-meromyosin are independent of the shear rate where the birefringence was measured. The minimum of the degree of birefringence is always found at the molar ratio of about 1:2.

Viscosity was also measured in solutions of the F-actin–tropomyosin–troponin complex at various amounts of added H-meromyosin. With increasing the amount of bound H-meromyosin the viscosity increases. The viscosity increase is a little larger

in the absence of Ca^{2+} (in the presence of EGTA) than in the presence of Ca^{2+} but the difference is rather small (Fig. 4). Above the molar ratio of H-meromyosin to actin of about 1:2, the viscosity increase becomes smaller.

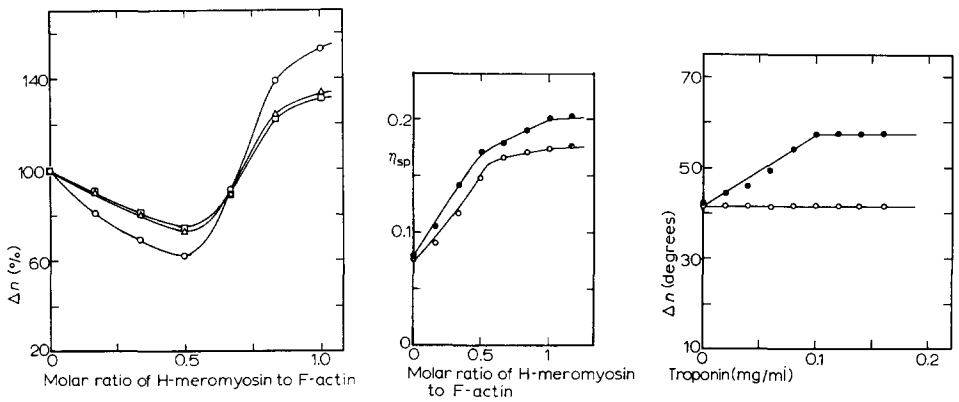


Fig. 3. Effect of the shear rate of flow on the degree of birefringence of the F-actin–tropomyosin–troponin and H-meromyosin complex. The 100% in the ordinate corresponds to the flow birefringence of F-actin–tropomyosin–troponin complex only. Protein concentrations: F-actin 0.5 mg/ml, tropomyosin 0.08 mg/ml, troponin 0.08 mg/ml. Solvent conditions: 0.1 M KCl, 10 mM Tris–maleate buffer (pH 7.0). Shear rate of flow: $G = 17\ s^{-1}$ (○), $G = 260\ s^{-1}$ (△), $G = 1700\ s^{-1}$ (□).

Fig. 4. Viscosity of the solution of the F-actin–tropomyosin–troponin and H-meromyosin complex. The sample solutions were obtained from the solution of Fig. 1 by 3 times dilution with the same solvent. ●: in the presence of 0.5 mM EGTA.

Fig. 5. Effect of troponin and Ca^{2+} on the flow birefringence of the F-actin–troponin complex. After various amounts of troponin were added to the F-actin–tropomyosin complex, the temperature was raised to 45 °C for about 10 min, to eliminate the turbidity due to large aggregates. Then, H-meromyosin was added at room temperature. Protein concentration: F-actin 0.5 mg/ml, tropomyosin 0.08 mg/ml, H-meromyosin 1.5 mg/ml, the molar ratio of H-meromyosin to F-actin was 1:2. Solvent conditions were the same as in Fig. 1. ●: in the presence of 0.5 mM EGTA. Shear rate of flow: $G = 17\ s^{-1}$.

Effect of troponin and Ca^{2+}

As shown in Fig. 2, the degree of birefringence of the F-actin–tropomyosin–troponin complex with bound H-meromyosin is affected by Ca^{2+}. In the absence of troponin, however, the birefringence is not sensitive to Ca^{2+}. This was proved by the experiment in Fig. 5 where the degree of birefringence of the F-actin–tropomyosin complex (weight ratio of tropomyosin to actin 1:6) with bound H-meromyosin (the molar ratio of H-meromyosin to actin 1:2) was measured at various amounts of troponin added. In the presence of free Ca^{2+} the birefringence is not changed by troponin; whereas in its absence (in the presence of EGTA) the birefringence is increased by troponin until the weight ratio of troponin to actin reaches about 1:6. Further increase of troponin has no effect on the birefringence. If Ca^{2+} is added to these solutions, the birefringence decreases to the same level as the complex without troponin.

The flow birefringence of the F-actin–tropomyosin–troponin complex with bound H-meromyosin was investigated at various concentrations of free Ca^{2+} by using

the Ca^{2+}–EGTA buffer. The decrease of the degree of birefringence and the increase of the extinction angle with increasing concentration of free Ca^{2+} were found at the Ca^{2+} concentration of around 1 μM, as shown in Fig. 6.

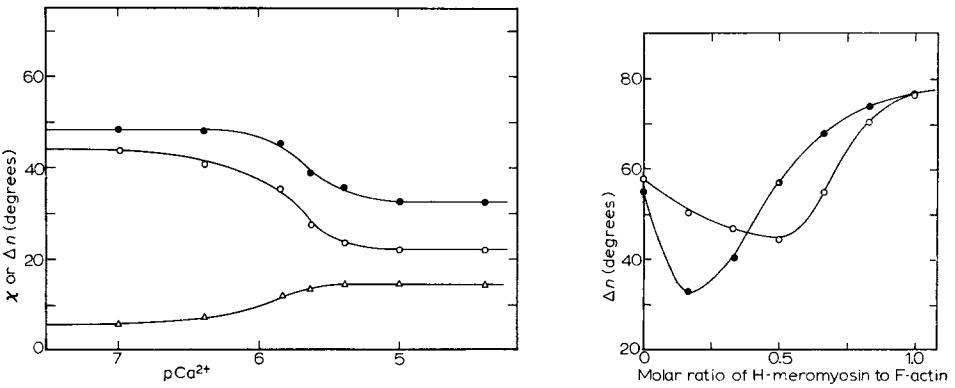


Fig. 6. Effect of the concentration of Ca^{2+} on the flow birefringence (○, ●) and the extinction angle (△) of the F-actin–tropomyosin–troponin and H-meromyosin complex. Protein concentration: F-actin 0.5 mg/ml, tropomyosin 0.08 mg/ml, H-meromyosin 1.5 mg/ml; the molar ratio of H-meromyosin to F-actin was 1:2. Solvent conditions: 0.1 M KCl, 10 mM Tris–maleate buffer (pH 7.0), and Ca^{2+}–EGTA buffer; various concentrations of Ca^{2+} were added to 0.5 mM EGTA. Shear rate of flow: $G = 17\ s^{-1}$ (○, △), $G = 270\ s^{-1}$ (●).

Fig. 7. The flow birefringence of the F-actin–H-meromyosin complex in the presence and the absence of tropomyosin. F-actin concentration: 0.5 mg/ml, tropomyosin: 0.08 mg/ml (○), no tropomyosin (●). Solvent conditions were the same as in Fig. 3.

Effect of tropomyosin

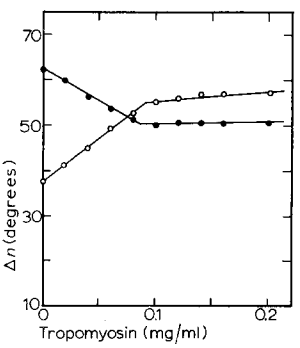
It was shown previously[4] that in the case of pure F-actin, the degree of birefringence decreases and then increases with the binding of H-meromyosin. In that case, however, the minimum of birefringence appeared at the molar ratio of H-meromyosin to actin of about 1:6. That is, the addition of tropomyosin and troponin to F-actin produces the shift of the molar ratio giving the greatest decrease of birefringence from 1:6 to 1:2.

This shift of the relation between the birefringence and the molar ratio was found to be caused by simple addition of tropomyosin to F-actin without troponin, as shown in Fig. 7. This situation was confirmed by the following experiment where various amounts of tropomyosin were added to the complex of F-actin and H-meromyosin at fixed molar ratios. Fig. 8 shows that at the molar ratio of H-meromyosin to actin of 1:6 the degree of flow birefringence increases with the addition of tropomyosin, whereas at the molar ratio of 1:2 it decreases.

The change of birefringence due to tropomyosin continues up to the weight ratio of tropomyosin to actin of 1:6. Further addition of tropomyosin does not change the birefringence. It must be also remarked that the value of birefringence does not depend on the order of mixing of proteins in sample solutions. Tropomyosin changes the birefringence of the complex of F-actin and H-meromyosin, and also H-meromyosin changes the birefringence of the complex of F-actin and tropomyosin; however, the final values are the same at the same ratios of the three proteins.

Binding of S-1 to F-actin

Similar experiments were carried out by using, instead of H-meromyosin, S-1 which was prepared by the chymotryptic digestion of myosin. S-1 is tightly bound to F-actin as shown by the turbidity measurement in Fig. 9. However, the extinction angle is kept constant and the degree of birefringence only slightly increases with increasing amount of bound S-1.



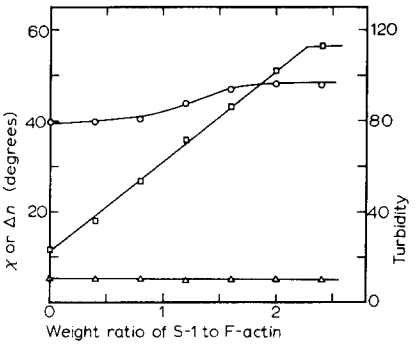


Fig. 8. Effect of tropomyosin on the flow birefringence of the F-actin–H-meromyosin complex. Increased amount of tropomyosin was added to the solution of the F-actin–H-meromyosin complex. F-actin concentration: 0.5 mg/ml, the molar ratio of H-meromyosin to F-actin, 1:6 (○) or 1:2 (●). Solvent conditions were the same as in Fig. 3. Shear rate of flow: $G = 17\ s^{-1}$.

Fig. 9. Flow birefringence (○), extinction angle (△) and turbidity (□) of the solution of the F-actin and S-1 complex. F-actin concentration: 0.4 mg/ml. Solvent conditions were the same as in Fig. 3. Shear rate of flow: $G = 17\ s^{-1}$.

DISCUSSION

The main finding of this work is that the degree of flow birefringence of the F-actin–tropomyosin–troponin complex is decreased and its extinction angle is increased by the binding of H-meromyosin at a certain range of their molar ratio, and that such changes of flow birefringence are inhibited by removal of Ca^{2+} from the solution. Several mechanisms can be supposed for this phenomenon. Formation of large aggregates of F-actin or the F-actin–tropomyosin–troponin complex by binding of H-meromyosin might decrease the birefringence. Measurements of dynamic rigidity showed the presence of a gel-like structure in a mixture of F-actin and H-meromyosin[17]. Such a structure, however, was found to be broken by the shearing force[17] in the measurement of flow birefringence. Nevertheless, in the present experiment the decrease of birefringence remained even at high shear rates. Moreover, in spite of a large difference in the birefringence, there was only a very small difference in the viscosity between the F-actin–tropomyosin–troponin complex with bound H-meromyosin in the presence of Ca^{2+} and that in its absence. Therefore, it is unlikely that formation of large aggregates is the main cause of the observed decrease of flow birefringence.

Fragmentation of F-actin or the complex by binding of H-meromyosin, if it happened, could produce the decrease of birefringence and the increase of extinction angle. However, both hypotheses are improbable. The response of the birefringence

of the F-actin–tropomyosin–troponin complex with H-meromyosin to Ca^{2+} was very quick and viscosity was changed only a little, as mentioned above.

It is more likely that the decrease of flow birefringence is due to some change in the state of individual filaments of F-actin or the F-actin–tropomyosin–troponin complex. One possibility is that the binding of H-meromyosin directly caused the decrease of the birefringence. If the long axis of bound H-meromyosin were perpendicular to the axis of the F-actin filament, the degree of birefringence would be decreased by the binding and the extinction angle would be increased because of the increase of the frictional constant. If so the recovery of birefringence at the saturation point of H-meromyosin binding could be interpreted as being due to the change of the manner of H-meromyosin binding depending on the molar ratio of H-meromyosin to actin[4]. However, recent experiments by quasielastic light scattering showed that the translational diffusion constant of pure F-actin was not changed by the binding of H-meromyosin[5]. In the case of the F-actin–tropomyosin–troponin complex, the diffusion constant was a little increased by H-meromyosin in the presence of Ca^{2+} (ref. 18). Therefore, the increase of the extinction angle seems not to be wholly attributable to the direct contribution of bound H-meromyosin.

The other possibility is the increase of flexibility of F-actin, that is, the decrease of the mean end-to-end distance. Such an increase of the flexibility of F-actin due to the binding of H-meromyosin was previously concluded from the increase of the relaxation time of the micro Brownian motion of the F-actin filament in the quasielastic light scattering experiment[5,19]. According to this experiment, the mean end-to-end distance of pure F-actin of the contour length 2.5 μm was estimated to be about 2.2 μm and that of F-actin having bound H-meromyosin at the molar ratio of 1:6 was about 1.6 μm (ref. 19). These values explain very well the observed increase of the extinction angle from 6° to 13° at a shear rate of 17 s^{-1}.

The flexibility increase creates the decrease of birefringence although the direct contribution of bound H-meromyosin must also be included in the birefringence. The flexibility increase may be due to loosening or partial breaking of actin–actin bonds in F-actin. This interpretation is supported by the finding that the ultraviolet absorption difference between the complex of F-actin and H-meromyosin and the simple sum of two proteins appeared in a direction corresponding to the depolymerization of actin[4].

In the case of pure F-actin, the degree of birefringence was at a minimum at the molar ratio of H-meromyosin to actin of 1:6. One H-meromyosin molecule seems to influence many actin monomers in F-actin. When tropomyosin and troponin were added to F-actin, however, minimum birefringence was found at the molar ratio 1:2. Since H-meromyosin has two heads to interact with actin, the ratio 1:2 means saturation of all actin monomers by H-meromyosin heads. With increasing amounts of bound H-meromyosin, each H-meromyosin molecule is forced to bind with only one actin monomer by one of the two heads. Then, in the final state of the molar ratio 1:1, the extinction angle has returned to the original level and the degree of birefringence becomes a little larger than that of F-actin having no H-meromyosin. Namely, the effect of bound H-meromyosin on the structure of the F-actin–tropomyosin–troponin complex depends on the molar ratio or the manner of binding. Simultaneous binding of both heads of H-meromyosin with F-actin seems to be necessary for a large decrease of birefringence or the increase of flexibility.

The change of birefringence of the F-actin–tropomyosin–troponin complex by binding of H-meromyosin is inhibited by removal of Ca^{2+} from the solution. Even in the absence of Ca^{2+}, H-meromyosin can bind with F-actin, as shown by the turbidity increase. Therefore, as suggested by the fact that activation of H-meromyosin Mg^{2+}-ATPase by the F-actin–tropomyosin–troponin complex requires Ca^{2+} (refs 20, 21), the manner of binding of H-meromyosin to the complex must depend on the presence or the absence of Ca^{2+}. This sensitivity of the binding manner to Ca^{2+} is realized only in the presence of tropomyosin and troponin. The amount of tropomyosin and troponin to give enough sensitivity to Ca^{2+} was found to be of the same order as that in the thin filament *in vivo*[1,22]. It was reported previously that tropomyosin can bind with F-actin up to the weight ratio of tropomyosin to actin of 1:3 (refs 23, 24). Recent experiments, however, showed that tropomyosin of the weight ratio to actin of 1:6 only can bind directly and tightly to F-actin[18,25]. The result of the present work is consistent with these data.

The concentration of Ca^{2+} necessary to produce the birefringence change by H-meromyosin is also of the same order as that necessary to initiate the muscular contraction or superprecipitation[1].

The S-1 fragment, the product of chymotryptic digestion of myosin, has only one head to interact with actin[7]. Binding of this S-1 was found not to decrease the flow birefringence of F-actin or the F-actin–tropomyosin–troponin complex. The quasielastic light scattering experiment also showed that S-1 cannot change the flexibility of F-actin or the F-actin–tropomyosin–troponin complex[18]. Therefore, it is reasonable to suppose that only when both heads of heavy meromyosin are interacting with actin, the change of structure of F-actin or the F-actin–tropomyosin–troponin complex can be induced.

ACKNOWLEDGEMENTS

The author would like to thank Professor F. Oosawa and collaborators for many helpful suggestions and stimulating discussions.

REFERENCES

1 S. Ebashi and M. Endo, *Prog. Biophys. Mol. Biol.*, 18 (1968) 123.
2 H. Huxley, *J. Mol. Biol.*, 7 (1963) 281.
3 M. Young, *Proc. Natl. Acad. Sci. U.S.*, 58 (1967) 2393.
4 K. Tawada, *Biochim. Biophys. Acta*, 172 (1969) 311.
5 S. Fujime and S. Ishiwata, *J. Phys. Soc. Jap.*, 29 (1970) 1651.
6 S. Lowey and C. Cohen, *J. Mol. Biol.*, 4 (1962) 293.
7 M. Onodera and K. Yagi, *J. Biochem. Tokyo*, 69 (1971) 145.
8 F. B. Straub, *Studies Inst. Med. Chem. Univ. Szeged.*, 1 (1941) 5.
9 S. Ebashi, F. Ebashi and K. Maruyama, *Nature*, 203 (1964) 645.
10 S. Ebashi and F. Ebashi, *J. Biochem. Tokyo*, 58 (1965) 7.
11 S. Ebashi and K. Maruyama, *J. Biochem. Tokyo*, 58 (1965) 20.
12 M. Rees and M. Young, *J. Biol. Chem.*, 242 (1967) 449.
13 K. Tsuboi, *Biochim. Biophys. Acta*, 160 (1968) 420.
14 S. Ebashi, A. Kodama and F. Ebashi, *J. Biochem. Tokyo*, 64 (1968) 465.
15 S. Ebashi and A. Kodama, *J. Biochem. Tokyo*, 58 (1965) 107.
16 G. Schwarzenbach, H. Senn and G. Anderegg, *Helv. Chim. Acta*, 40 (1957) 1886.
17 S. Abe and K. Maruyama, *Biochim. Biophys. Acta*, 243 (1971) 98.
18 S. Ishiwata and S. Fujime, *J. Phys. Soc. Jap.*, 30 (1971) 302.
19 S. Fujime and S. Ishiwata, *J. Mol. Biol.*, 62 (1971) 251.

20 E. Eisenberg and W. W. Kielley, *Biochem. Biophys. Res. Commun.*, 40 (1970) 50.
21 J. Spudich and S. Watt, *J. Biol. Chem.*, 246 (1971) 4866.
22 S. Ebashi, M. Endo and I. Ohtsuki, *Quart. Rev. Biophys.*, 2 (1969) 351.
23 K. Maruyama, *Arch. Biochem. Biophys.*, 105 (1964) 142.
24 H. Tanaka and F. Oosawa, *Biochim. Biophys. Acta*, 253 (1971) 274.
25 W. Drabikowski, D. Kominz and K. Maruyama, *J. Biochem. Tokyo*, 63 (1968) 802.

Biochim. Biophys. Acta, 267 (1972) 558–567