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F-ACTIN–HEAVY MEROMYOSIN COMPLEX STUDIED BY OPTICAL HOMODYNE AND HETERODYNE METHODS

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SUMMARY

Quasi-elastic scattering of laser light is now becoming a powerful tool for the study of dynamic properties of both biological and non-biological macromolecules. In solutions of biological macromolecules, aggregation–disaggregation of molecules under study usually depends on solvent conditions. Therefore, special attention must be paid in light-beating spectroscopy.

By use of both homodyne and heterodyne methods, the spectral densities of solutions of F-actin and of a complex of F-actin and heavy meromyosin were measured. The half-width of the heterodyne spectrum was much wider than that of the homodyne spectrum. This was probably due to polydispersity of F-actin. The results showed that laser light scattering gave information about the dynamics of free filaments, although low frequency rheometry has suggested the rubber-like elasticity of the solution of F-actin and heavy meromyosin.

The interaction between F-actin and heavy meromyosin was studied in the presence of pyrophosphate (PP_i). At PP_i concentrations between 10 and 100 μM, the spontaneous bending motion of F-actin occurred as if heavy meromyosin were not present, although turbidity of the solution indicated that heavy meromyosin was, in fact, bound to F-actin. This meant that there were two types of binding state of the F-actin–heavy meromyosin complex. A plausible model is that, in the absence of PP_i, the two heads of one heavy meromyosin molecule simultaneously interact with two neighbouring monomers in F-actin, whereas a single head binding occurs in the presence of PP_i. Above 100 μM PP_i, heavy meromyosin seemed to dissociate from F-actin. However, it was not clear whether or not this was due to a direct effect of PP_i on heavy meromyosin alone, because the dynamic properties of F-actin also changed.

Assuming that actin filaments are cross-linked by myosin, a simple model is proposed to explain qualitatively the rubber-like elasticity of the solution of an F-actin–heavy meromyosin complex.

INTRODUCTION

Since the pioneering work of Pecora[1] in 1964, quasi-elastic scattering of laser light has become a useful tool for the study of dynamic properties of macromolecules. We have previously studied spectral densities of some biological macromolecules[2-12].

To measure very narrow spectral densities, there are two methods most commonly used at present; one is the optical heterodyne method[13] and the other the optical homodyne method[14]. In our studies, the latter method has been employed[2,9]. However, the question arises of whether the experimental spectrum is a pure homodyne one or not. When, for example, there is a large aggregate(s) of the macromolecules under study, the scattered light from the aggregate will act as a reference signal in the heterodyne method (see the next section). In solutions of biological macromolecules, aggregation–disaggregation usually depends on solvent conditions. In this paper, we will examine whether the homodyne spectra are pure optical homodyne or not, with special reference to muscle F-actin and a complex of F-actin and heavy meromyosin in the absence of ATP.

F-actin is a two-stranded helical polymer, with a contour length longer than 1 μm. In such a case, the spontaneous bending motion of the polymer can be observed to contribute to the broadening of the scattered light[3,9]. When F-actin and heavy meromyosin are mixed in a solvent without ATP, heavy meromyosin binds to F-actin up to a molar ratio of heavy meromyosin to actin of unity[15]. We have studied this complex by the homodyne method[4,9]. The result of the spectral analysis is schematically quoted in Fig. 1, where τ is the relaxation time of the spontaneous bending motion and D the translational diffusion coefficient of the complex filament[3]. We concluded from these data that F-actin becomes flexible when heavy meromyosin binds to F-actin in a molar ratio within a certain range[9]. According to Abe and Maruyama[16], the dynamic rigidity (or rigidity modulus for a shear stress) of the complex markedly increases as the molar ratio increases (see Fig. 1). This must be related to the rubber-like elasticity (*i.e.* the formation of cross-links). Furthermore, they observed that the dynamic rigidity of the complex solution became about 3 times larger than the original value after added ATP was split into ADP and inorganic phosphate. The dynamic rigidity is proportional to the 3.5th power of the concentration of the complex, in the range they studied (0.5 to 3 mg/ml F-actin at the molar ratio of about 1:2). The aggregate seems to be very fragile, because the rigidity modulus strongly depends on the velocity gradient. On the other hand, spectral analysis showed no appreciable change in the dynamic properties of the complex after added ATP was split. A solution of the present complex was very viscous when allowed to stand. Even in such a case, the $1/\tau$ values of low frequency spectra were the same as those of a fresh solution. Furthermore, the concentration dependence of both D and $1/\tau$ was not observable in the range from 1 to 3 mg/ml F-actin at the

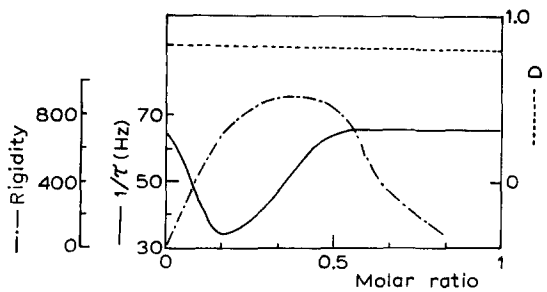


Fig. 1. Schematic illustration of $1/\tau$ (Hz), D (arbitrary units)[4,9] and dynamic rigidity (dynes/cm^2)[16] against the molar ratios of heavy meromyosin to actin.

molar ratio of 1:6, where the value of $1/\tau$ was minimal[9]. From the differences in the behaviour of the internal relaxation time ($1/\tau$) and of the dynamic rigidity, we believe that the light scattering experiment deals with free filaments, whereas the rigidity experiment deals with aggregates of filaments. In order to clarify this situation, the present experiments were carried out. The interaction between F-actin and heavy meromyosin was also studied in the presence of pyrophosphate.

THEORETICAL

Since macromolecules in solution undergo Brownian motion, the frequency of light scattered from these molecules will be shifted to some extent due to the Doppler effect. The amount of this shift is related to the dynamics of molecular motion[1]. The most dominant contribution of the molecular motion to the line broadening comes from the translational diffusive motion of the molecule. However, the conformational fluctuations of macromolecules (such as F-actin presented here) also contribute to the line broadening under favorable experimental conditions[3]. A theoretical study based on a simple model of molecular motion gives the following expressions for the frequency spectrum of scattered light[3,12]:

(*i*) *Heterodyne case*

$$S(\boldsymbol{K},\omega) = P_0(\boldsymbol{K})L_h(0) + P_2(\boldsymbol{K})L_h(2) + P_4(\boldsymbol{K})L_h(4) + \ldots, \tag{1}$$

$$L_h(N) \equiv \frac{(DK^2 + N/\tau_1)/\pi}{\omega^2 + \{DK^2 + N/\tau_1\}^2}$$

(*ii*) *Homodyne case*

$$S(\boldsymbol{K},\omega) = P_{00}L(0) + 2P_{02}L(2) + (P_{22} + 2P_{04})L(4) + \ldots, \tag{2}$$

$$P_{MN} \equiv P_M(\boldsymbol{K})P_N(\boldsymbol{K}),$$

$$L(M+N) \equiv \frac{\{2DK^2 + (M+N)/\tau_1\}/\pi}{\omega^2 + \{2DK^2 + (M+N)/\tau_1\}^2}$$

Here $P_N(\boldsymbol{K})$ values (N: even integers including zero) are the scattering form factors, D is the translational diffusion coefficient, $\boldsymbol{K}$ is the scattering vector, whose length is given by $K = (4\pi/\lambda)\sin(\Phi/2)$ (λ, the wavelength of incident light in a medium; Φ, the scattering angle). τ_1 is the slowest relaxation time of the spontaneous bending motion of the polymer:

$$\tau_1 = \frac{16}{81\pi^4} \cdot \frac{L^3}{D\varepsilon} \cdot kT \tag{3}$$

where L is the extended length of the polymer and ε is the flexural rigidity. The longer the relaxation time, the more flexible is the polymer (or the smaller is the ε). To sum up, the heterodyne spectrum consists of Lorentzians with half-widths at half-height

$$\Gamma_h = DK^2 + N/\tau_1 \qquad \text{(in Hz units)} \tag{4}$$

whereas the homodyne spectrum consists of Lorentzians with widths

$$\Gamma = 2DK^2 + (M + N)/\tau_1 \qquad \text{(in Hz units)}. \tag{5}$$

Next we consider the case where large aggregates coexist with free filaments. If the beat note occurs between scattered light beams from large aggregates and from free filaments, partial heterodyning will occur. In such a case, the half-width at half-height of the spectrum will not be equal to either Eqn 4 or Eqn 5.

In Eqns 1 and 2, the experimentally important terms may be P_0, P_2 and P_4 (or P_{00}, $2P_{02}$ and $P_{22} + 2P_{04}$)[12]. At the present stage, a multi-Lorentzian approximation is unfortunately difficult because of the lack of reliable values of $P_N(\boldsymbol{K})$. However, if it is possible to approximate the experimental spectrum using a single Lorentzian having an appropriate width, we can discuss the qualitative nature of the dynamics of the polymer[2,9,12]. In the $\boldsymbol{K}$ range of our measurements, the homodyne spectrum of F-actin is well approximated by $(P_{22} + 2P_{04})L(4)$ (ref. 3) and the heterodyne spectrum by $P_2L_h(2)$ (ref. 12). Throughout this paper, we will adopt a single Lorentzian approximation, where the half-width Γ is given by (*cf.* Eqns 4 and 5)

$$\Gamma = DK^2 + 1/\tau \qquad \text{(heterodyne)}$$

or

$$\Gamma = 2DK^2 + 1/\tau \qquad \text{(homodyne)}$$

The slope and the intercept with the ordinate of the $\Gamma - K^2$ curve give apparent values of D and $1/\tau$, respectively.

MATERIALS AND METHODS

Actin and heavy meromyosin were prepared from rabbit skeletal muscle as described previously[9]. The molecular weights of G-actin and heavy meromyosin were assumed to be $5 \cdot 10^4$ and $3 \cdot 10^5$, respectively. The concentration of F-actin was determined by measuring the degree of flow birefringence[17]. A Zeiss spectrophotometer was used to determine the concentration of heavy meromyosin[18] and to measure the absorbances at 330 nm and 350 nm.

The light-scattering apparatus used in this work was the same as that used previously[2]. A 632.8-nm light beam from an He–Ne laser (approx. 15 mW) was passed through the solution. The scattered light was frequency analysed. The bandwidth of the analyser was 3 Hz. In this study, a teflon wedge was used in order to produce the reference signal in the heterodyne method (see Fig. 2)[19]. As a standard scatterer to test the method, a dilute aqueous solution of polystyrene latex spheres (Dow Chemical Co.) was used, whose diameter were 234.0 ± 2.5 nm. All the measurements were made at room temperature (20 to 22 °C).

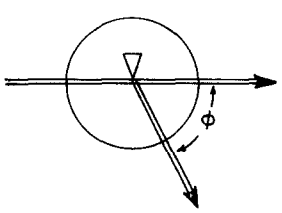


Fig. 2. Top view of the scattering cell. A teflon wedge was placed to produce a local oscillator signal in the heterodyne method. Φ is the scattering angle.

RESULTS AND DISCUSSION

(1) *Polystyrene latex spheres*

In order to test the theory and the experimental method, an aqueous solution of polystyrene latex spheres was studied by both the homodyne and heterodyne methods. In this case, the Γ values in Eqns 4 and 5 are simply written as

$$\Gamma_h = DK^2, \qquad \Gamma = 2DK^2 \qquad \text{(in Hz units)}, \tag{6}$$

$$D = kT/6\pi\eta_0 a \tag{7}$$

where k is the Boltzmann constant, T is the absolute temperature, η_0 is the solvent viscosity and a is the radius of the sphere. Fig. 3 shows the half-widths at half-height of spectra measured at various values of K. Satisfactory straight lines were obtained for both homodyne and heterodyne spectra. The heterodyne spectra usually showed high noise levels because of the poor isolation of the apparatus from mechanical vibration. Experimental data at 20 °C gave the following figures:

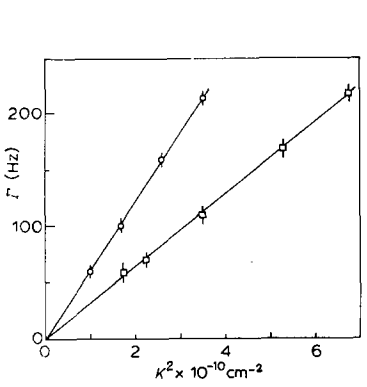
$$D \text{ (homodyne)} = (0.193 \pm 0.002) \cdot 10^{-7} \text{ cm}^2/\text{s}$$

$$D \text{ (heterodyne)} = (0.198 \pm 0.004) \cdot 10^{-7} \text{ cm}^2/\text{s}$$

which must be compared with the theoretical value:

$$D \text{ (Eqn 7)} = (0.194 \pm 0.001) \cdot 10^{-7} \text{ cm}^2/\text{s}$$

Good coincidence between these values proves that the present method can be used in the following experiments.



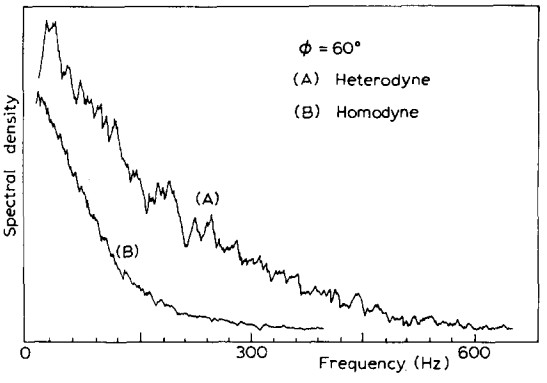


Fig. 3. Half-widths at half-height of heterodyne and homodyne spectra of an aqueous solution of polystyrene latex spheres against the square of the scattering vector. $K = (4\pi/\lambda) \sin(\Phi/2)$, where, λ is the wavelength of the incident light in a medium. Concentration: about 0.03 mg/ml; the diameter of spheres: 234.0 ± 2.5 nm. ○, homodyne; □, heterodyne.

Fig. 4. Examples of spectral densities of an F-actin solution in heterodyne and homodyne methods. Concentration: 3 mg/ml F-actin; solvent: 0.1 M KCl, 2.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.0) and 50 μM ATP.

(2) F-actin

Fig. 4 shows examples of spectral densities measured by homodyne and heterodyne methods. The reproducibility of recorded spectra was so good that successive spectra from the same solution could be superimposed in order to average out accidental noise. All the spectra could be approximated with a single Lorentzian having the correct widths[3,12]. Fig. 5 shows the half-widths at half-height *versus* K^2 of spectra of an F-actin solution by the two methods. The result using the homodyne method coincided with the previous one[3,9]. On the other hand, the half-widths at half-height of heterodyne spectra were very broad. (We have experienced a similar situation in the case of bacterial flagella[12].) This presents a marked contrast to the case of polystyrene latex spheres, where the half width of the heterodyne spectrum is one half that of the homodyne spectrum. This apparent contradiction undoubtedly came from the sample polydispersity. The length distribution of F-actin has been known to be of an exponential type[20,21]. At the present stage, however, we have little knowledge about an averaging procedure of the spectrum with respect to the length distribution of polymers. Although a treatment in a previous paper[12] seems also to explain the present result, such a discussion is not of interest at present, so that we have not investigated this problem. However, the experimental heterodyne spectra were indeed wider than the homodyne ones. We wish only to emphasize here that when partial heterodyning occurs, the spectrum is more or less wider than the pure homodyne one.

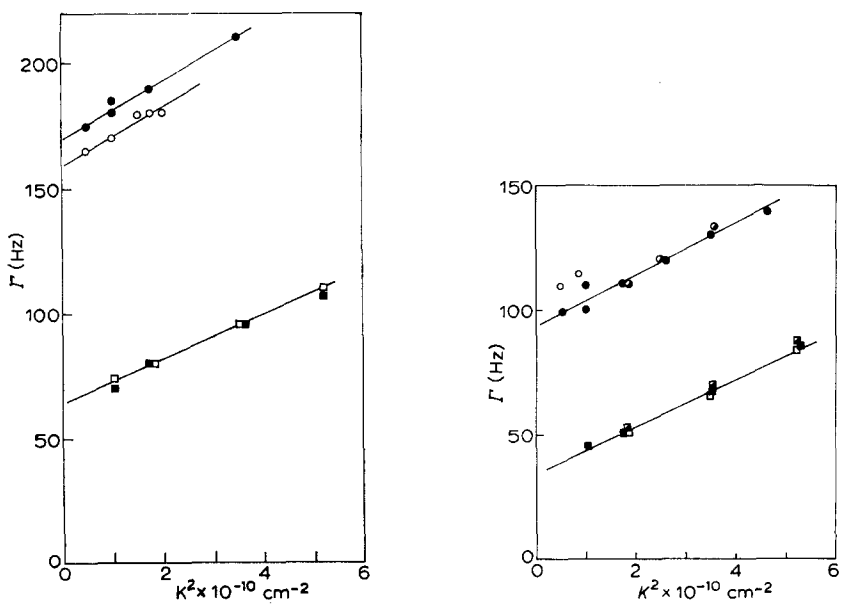


Fig. 5. Half-widths at half-height of heterodyne (○, ●) and homodyne (□, ■) spectra of an F-actin solution against the square of the scattering vector. Concentration: ●, ■, 5 mg/ml F-actin; ○, □, 3 mg/ml F-actin; solvent: the same as in Fig. 4. Experimental error of each point is about ± 10 Hz (○, ●) and ± 5 Hz (□, ■).

Fig. 6. Half-widths at half-height of heterodyne (○, ●, ◑) and homodyne (□, ■, ◨) spectra of an F-actin–heavy meromyosin complex solution against the square of the scattering vector. Molar ratio of heavy meromyosin to F-actin: 1:6; concentration of F-actin: 0.5 mg/ml (○, □) and 1 mg/ml (●, ◑, ■, ◨); solvent: 0.1 M KCl, 2.5 mM $MgCl_2$ and 10 mM Tris–HCl (pH 8.0).

(3) *F-actin–heavy meromyosin complex*

The solution of an F-actin–heavy meromyosin complex at a molar ratio of heavy meromyosin to actin of 1:6 was studied by the two methods (Fig. 6). The results clearly showed that heterodyne spectra were wider than homodyne ones. From Figs 5 and 6, we see that the values of $1/\tau$ of the complex are smaller than those of F-actin alone in both cases. Therefore, the change in $1/\tau$ of homodyne spectra on the addition of heavy meromyosin is opposite to the change in $1/\tau$ expected from the occurrence of partial heterodyning. That is, if partial heterodyning had occurred in the case of the complex solution, the $1/\tau$ of the pure homodyne spectra would be smaller than the $1/\tau$ of the present homodyne spectra. If so, F-actin in the complex might be more flexible than we have expected.

Aggregates of filaments, as shown by the measurement of dynamic rigidity, seem not to affect the present light-beating spectroscopy. Why does laser light scattering not detect the effect of cross-linking between filaments? This is probably due to the following facts: Eigen values (*i.e.* relaxation times) of the transverse vibrations of a rod are the same in both cases, *i.e.* both ends free or both ends clamped, provided that the end-to-end distance is equal[3,22]. Thus, if cross-links occur at both ends of filaments, the value of $1/\tau$ of filaments in the tightly bound network will be nearly equal to that of free filaments. However, if the distance between two cross-links on a filament is shorter than the end-to-end distance of a free filament, the value of $1/\tau$ becomes very large, because the τ is proportional to the 3rd (or 4th) power of the length (see Eqn 3). In such a case, the spectral component due to network becomes weak and wide, only contributing to the leveling up of the base line of the narrow component due to free filaments, just as does the shot noise. To sum up, the present spectral analysis shows that the change in $1/\tau$ observed by the homodyne method reflects changes in the intrinsic flexibility of individual actin filaments as a result of the interaction with heavy meromyosin. This supports our previous proposal that F-actin becomes flexible when heavy meromyosin binds to it[4,9].

(4) *F-actin–heavy meromyosin complex in the presence of pyrophosphate*

Fig. 7 shows the experimental results of the absorbances (A) at 350 nm and 330 nm, the degree of flow birefringence (Δn), D and $1/\tau$ of F-actin alone (filled symbols) and of the complex of F-actin and heavy meromyosin at a molar ratio of 1:6 (open symbols) at various concentrations of pyrophosphate (PP_i). As the concentration of PP_i increased, a marked decrease of D and a slight increase of A of the complex were observed, whereas both D and A of F-actin alone remained constant. These facts suggest that the complex tends to form a kind of aggregate or micro-network. The value of D suggests that this aggregate consists of only a few filaments. On the other hand, the $1/\tau$ of the complex above 10 μM PP_i became almost the same value as that of F-actin alone. That is, the bending motion of the complex occurred as if heavy meromyosin were not present. Experimental results by the heterodyne method showed that, in the presence of 30 μM PP_i, the value of $1/\tau$ of the complex was nearly the same as that of F-actin alone (Fig. 8). This suggested that the change in $1/\tau$ of the complex against the PP_i concentration did not correspond to the occurrence of partial heterodyning, but to the change in the intrinsic flexibility of the complex. The changes in A and Δn of the complex above 100 μM PP_i could be attributable to the dissociation of heavy meromyosin from F-actin, as in the

presence of ATP[15]. Nevertheless, it was difficult to conclude that the dissociation of heavy meromyosin from F-actin was due to the direct effect of PP_i on the former alone, because $1/\tau$ of both the complex and F-actin alone changed in parallel (Fig. 7). The light scattering experiment suggested a large change in F-actin structure above 100 μM PP_i, regardless of the presence or absence of heavy meromyosin. Therefore, the effect of PP_i above 100 μM could not be unequivocally explained at present.

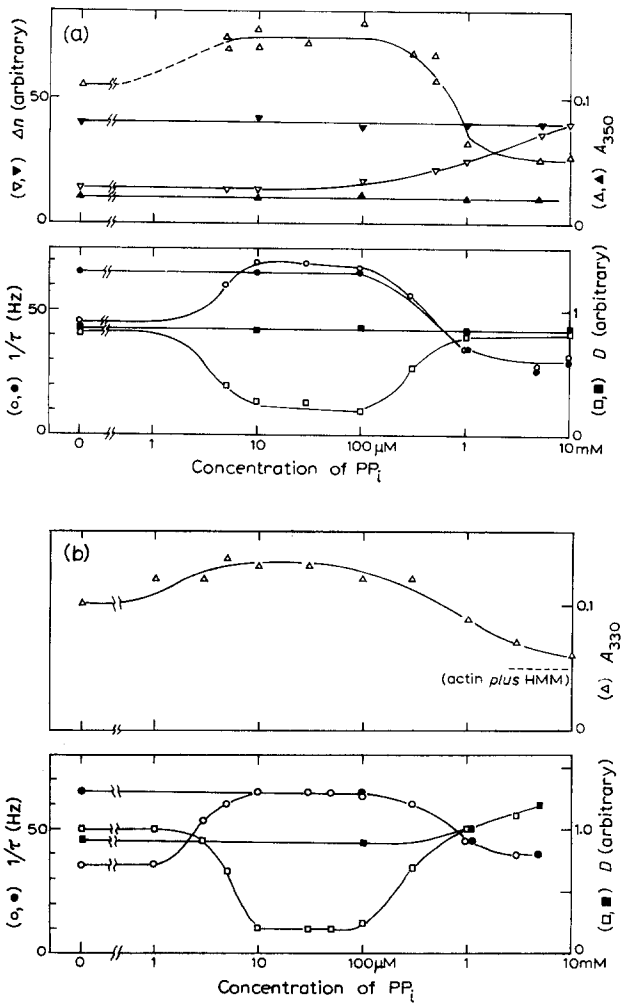


Fig. 7. The effect of pyrophosphate (PP_i) on F-actin and an F-actin–heavy meromyosin complex. Solutions were allowed to stand in a cold bath at 4 °C for 15 h after PP_i was added. Open symbols: an F-actin–heavy meromyosin complex; filled symbols: F-actin alone. (a) △, ▲, optical density (A) at 350 nm; ▽, ▼, degree of flow birefringence at a shear rate of 17 s^{-1} (Δn); ○, ●, $1/\tau$ (Hz); □, ■, D (in an arbitrary unit). F-actin: 0.5 mg/ml; heavy meromyosin: 0.5 mg/ml; solvent: 0.3 M KCl, 2.5 mM $MgCl_2$ and 10 mM Tris–HCl (pH 8.0). (b) Symbols are the same as in (a). A was measured at 330 nm. F-actin: 0.5 mg/ml; heavy meromyosin (HMM): 0.5 mg/ml; solvent: 0.1 M KCl, 2.5 mM $MgCl_2$ and 25 mM Tris–HCl (pH 8.0). Light-scattering data were taken by the homodyne method.

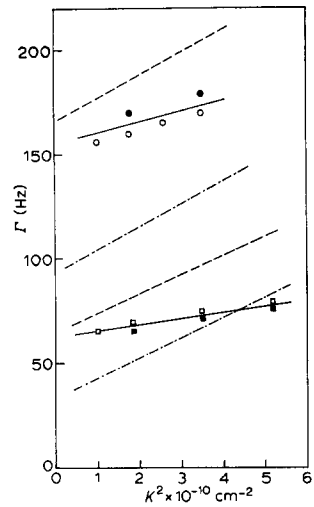


Fig. 8. Half-widths at half-height of heterodyne (○, ●) and homodyne (□, ■) spectra of an F-actin-heavy meromyosin complex in the presence of 30 μM PP_i. Molar ratio: 1:6; concentration of F-actin: 0.8 mg/ml (○, □) and 0.5 mg/ml (●, ■); solvent: 0.1 M KCl, 2.5 mM $MgCl_2$, 30 μM PP_i and 20 mM Tris–HCl (pH 8.0). – – – –, the same as in Fig. 5; —·—, the same as in Fig. 6.

The change in the flexibility of the complex at low concentrations of PP_i probably reflects the manner of binding of heavy meromyosin to F-actin. If the change in $1/\tau$ (and D) is assumed to be due to the binding of one PP_i molecule per one heavy meromyosin, the concentration of PP_i, where the midpoint of the change in $1/\tau$ (and D) appeared, can be regarded as a measure of the binding affinity of PP_i to heavy meromyosin. The apparent binding constant thus estimated was $2 \cdot 10^5$ to $3 \cdot 10^5$ M^{-1}, in good accordance with the results of equilibrium dialysis[23]. 2 moles of PP_i bind to 1 mole of heavy meromyosin in the absence of F-actin, whereas 1 (about 0.6) mole of PP_i binds to 1 mole of heavy meromyosin in the presence of F-actin[23]. Each heavy meromyosin molecule has two heads[24]. Then, the above result (below 100 μM PP_i) will be understandable as follows. In the absence of PP_i, the two heads of one heavy meromyosin molecule simultaneously interact with two neighbouring actin monomers in F-actin, resulting in the increase of the F-actin flexibility[9]. In the presence of PP_i, one of the two heads of one heavy meromyosin molecule is probably occupied by PP_i and only the other can interact with F-actin. In such a case, the F-actin flexibility does not increase, as in the case of the interaction between S1 (a single head of myosin formed by proteolysis) and F-actin[9].

This statement, however, does not necessarily mean the non-identity of the two heads of one heavy meromyosin molecule. For an explanation of the change in $1/\tau$ upon addition of PP_i, it is sufficient to assume only that the interaction between actin monomers and the two heads of one heavy meromyosin molecule becomes weak when PP_i exists. If we assume the lack of "size-fitting" between substructures of heavy meromyosin and of F-actin, the simultaneous binding of the two heads of one heavy meromyosin molecule with two neighbouring actin monomers is supposed to induce a kind of strain in the F-actin (and/or myosin) structure. This strain is assumed to be released by loosening the bonds between strands, resulting in the

increase of the F-actin flexibility. Let E represent the energy gain due to the formation of a bond between an actin monomer and a single head of heavy meromyosin, and S the energy loss (or strain energy) induced by the two-head binding. For simplicity, the bond energy in the two-head binding is assumed to be $2E$. When one heavy meromyosin molecule binds to F-actin with both heads ($|$ single head$|$) the energy gain due to binding of heavy meromyosin will be $2E(|E|)$ and the strain energy be $S(|o|)$. Thus the net energy gain of the system will be $2E - S(|E|)$. The difference is $E - S$. Therefore, the two-head binding will occur only when $E > S$. Since equilibrium in the solution of F-actin and heavy meromyosin is greatly shifted to the complex side, the magnitude of E must be large. On the other hand, the difference, $E - S$, seems to be small and its sign may depend on the environment. As far as the present results are concerned, it may be sufficient to assume that the magnitudes of both E and S are determined by the state of myosin only. The presence of PP_i probably decreases (and/or increases) the magnitude of E (and/or S), resulting in $E - S < 0$. However, it seems to be necessary in some cases to assume that the magnitude of S is mainly determined by the state of F-actin. For example, the removal of free calcium ions from a solution of the complex of F-actin, tropomyosin, troponin and heavy meromyosin increases $1/\tau$ and decreases D (ref. 7). Since the removal of Ca^{2+} makes an F-actin–tropomyosin–troponin complex rigid[10], the magnitude of S probably increases, resulting also in $E - S < 0$. Details about the interaction between F-actin and (heavy mero)myosin under various conditions and the physiological implication of the F-actin flexibility will be published elsewhere*.

The decrease of D of the complex in the presence of PP_i can probably be ascribed to the occurrence of loose binding between another head of one heavy meromyosin molecule bound to one actin filament and a nearby monomer of another filament. This is diagramatically represented in Fig. 9a. Such loose binding may be expected in a concentrated solution. (At present, the most dilute solution giving a good signal-to-noise ratio is about 0.5 mg/ml F-actin.) We have observed the occurrence of such a kind of loose binding between actin filaments in many cases[6,7,9,10*]. At the present stage, however, a definite physical picture of loose binding cannot be visualized. Aside from the loose binding, it is possible to assume the occurrence of tight binding (or cross-links), producing a network structure (Fig. 9b)[25]. The ceaseless creation and annihilation of loose binding presumably occur as the result of the

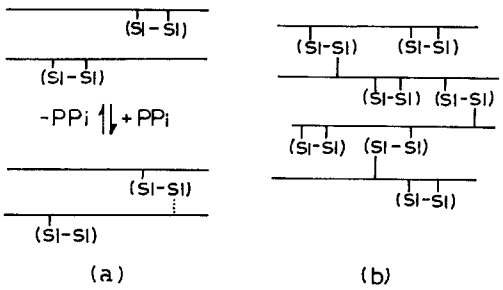


Fig. 9. Diagramatic representation of inter-filamental linking by myosin. ———, F-actin; (SI–SI), heavy meromyosin. (a) Loose link between filaments (· · · · · ·). (b) Tight link. For details, see text.

* S. Ishiwata and S. Fujime, unpublished.

Brownian motion of filaments, but do not occur in the case of tight binding. The occurrence of tight binding is supposed to be an origin of the large rubber-like elasticity of the present solutions.

In the present model, loose binding mainly occurs in the case of a single head binding. The occurrence of tight binding may have no direct correlation to the occurrence of loose binding. The small value of Δn of the solution of the complex of F-actin and heavy meromyosin compared with the Δn of the solution of F-actin alone is thought to come partly from the formation of a network structure of the complex filaments. It should be noted that the change in Δn was not observed at around 10 μM PP_i (Fig. 7a). This seems to support our explanation that the changes in $1/\tau$, D and A at around 10 μM PP_i are due not to tight but to loose binding.

(5) *On the rigidity modulus of the solution of an F-actin heavy meromyosin complex*

The two heads of one heavy meromyosin molecule will bind to two neighbouring monomers in F-actin as mentioned before. Above a molar ratio (X) of heavy meromyosin to actin of 0.5, some of the heavy meromyosin are forced to bind to F-actin with their single head, because the stoichiometric ratio is unity in a molar basis[15]. (In this case, loose binding as mentioned before is not expected to occur because all interaction sites (or areas) on F-actin with heavy meromyosin are occupied.) In a not very dilute solution, however, the two heads of one heavy meromyosin molecule will have a chance to bind with actin monomers belonging to different filaments, resulting in the formation of crosslinks (*i.e.* tight binding). It must be noted that the large rigidity modulus was also observed in the case of F-actin and myosin in 0.6 M KCl, but not in the case of F-actin and S1[26]. The Brownian motion of filaments between cross-links will result in the visco-elastic property of the solution[17]. For a shear stress, rigidity modulus, R, of the network composed of ideal Gaussian chains is given by $R = Y \cdot kT$, where Y is the number density of cross-links[27]. The present filaments are not Gaussian and, furthermore, their elastic property is also a function of X. Since kT in $R = Y \cdot kT$ originated from the flexibility of the chain, we simply assume here that the dynamic rigidity of the present solution is proportional to the product of Y and the flexibility of the chain between cross-links, and the flexibility to τ of our experiments.

A remark is necessary here (for details, see ref. 3). The ε in Eqn 3 is expressed in terms of γ (*i.e.* the inverse of the statistical length) as

$$\varepsilon/L = 3kT/4\gamma L$$

The mean-square end-to-end distance, $\langle R^2 \rangle$, and the elastic constant for stretching, κ, of a chain are also expressed as

$$\langle R^2 \rangle = (\exp(-2\gamma L) - 1 + 2\gamma L)/2\gamma^2 \quad \text{and} \quad \kappa/L = 3kT\gamma/L$$

Since $\langle R^2 \rangle = L/\gamma$ for $\gamma L \gg 1$, it holds that

$$\kappa/L = 3kT/\langle R^2 \rangle \quad \text{for} \quad \gamma L \gg 1$$

The kT in this last equation is that expressed in $R = Y \cdot kT$. The increase of τ_1 means the increases of both γ (see Eqn 3) and κ/L. The decrease of flexural rigidity ε, and the increase of κ correspond to the fact that a polymer becomes flexible in our

terminology. The term "flexibility" has been used as a measure of resistance to the spontaneous bending motion of a filament[3-12]. "Rigidity" of a solution and "flexibility" of a filament in the solution must not be confused.

Then, from our results[4,9] and those of Abe and Maruyama[16], the number of cross-links can be estimated in an arbitrary unit (Fig. 10). On the other hand, the probability that the two heads of one heavy meromyosin simultaneously interact with two monomers belonging to different filaments, may be assumed proportional to $X(1 - X)$, or simply $X(X \leq 0.5)$ and $1 - X(0.5 \leq X \leq 1)$. Comparing the number of cross-links estimated above with these assumed probability functions (Fig. 10), the agreement is fairly well below $X = 0.5$. Disagreement at higher X may simply be attributed to some steric restrictions in the cross-link formation. If the F-actin flexibility is assumed to be constant against X, a complicated form of the probability function of the cross-link formation must be assumed. The present model is very simple for a qualitative interpretation of the dynamic rigidity of the complex solution.

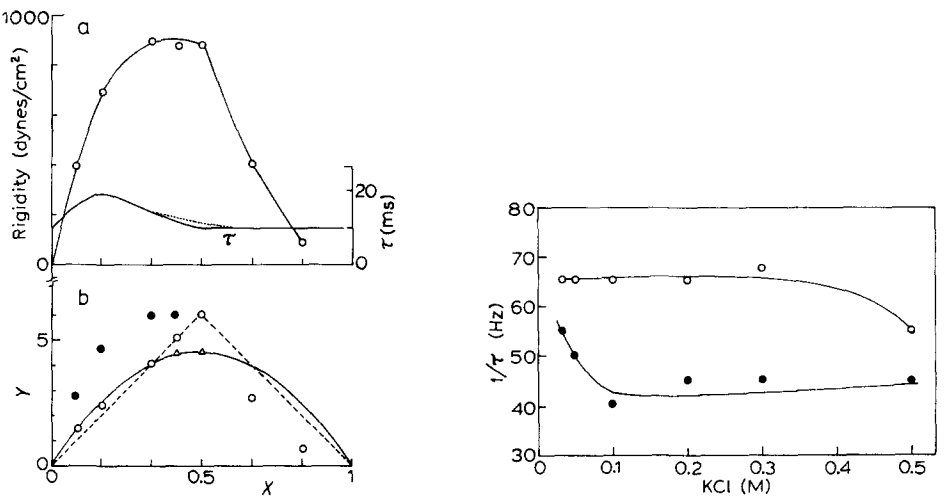


Fig. 10 (a) Dynamic rigidity (F-actin, 2 mg/ml)[16] and the internal relaxation time (τ)[4,9] of an F-actin–heavy meromyosin complex solution against the molar ratio, X, of heavy meromyosin to actin. (b) Number of cross-links, Y, in arbitrary units. We assumed $Y \propto$ (rigidity)/τ. △, Y in the case of dotted line of τ; ○, Y in the case of full line of τ; ●, Y if τ is assumed to be constant against X. ———, $Y = 18X(1 - X)$; – – – –, $Y = 12X$ $(X \leq 0.5)$ and $Y = 12$ $(1 - X)$ $(0.5 \leq X \leq 1)$. For details, see text.

Fig. 11. The $1/\tau$ *versus* KCl concentrations of the complex of heavy meromyosin and F-actin at the molar ratio of 1:6. Concentrations: 0.5 mg/ml F-actin and 0.5 mg/ml heavy meromyosin; 10 mM Tris–HCl (pH 8.0). ○, F-actin alone; ●, a complex of F-actin and heavy meromyosin. Data were taken by the homodyne method. (D was almost independent of KCl concentrations.)

The rigidity modulus decreases below, and stays constant above, 0.07 M KCl[16]. In this connection, the values of $1/\tau$ of the complex at the molar ratio of 1:6 were also measured at various concentrations of KCl (Fig. 11). The $1/\tau$ of F-actin alone stayed constant up to 0.3 M KCl. Contrary to this, the $1/\tau$ of the complex largely increased below 0.1 M KCl. This probably indicates that, at low salt concentrations, the interaction between F-actin and heavy meromyosin becomes weak. According

to the above interpretation, the KCl-concentration dependence of the rigidity modulus of the complex solution will be due to the changes in $1/\tau$ and/or Y.

(6) Concluding remark

It must be noted that the actin-activated Mg^{2+}-ATPase activity of heavy meromyosin is higher at 30 mM KCl than at 60 mM KCl[28], which means that the interaction between F-actin and heavy meromyosin is stronger at lower KCl concentrations. This is contrary to the present results (Fig. 11). A study will be made in the future whether or not this discrepancy is due to the absence of ATP in the present experiment. A recent study suggests that the complex of F-actin and heavy meromyosin is more flexible than F-actin alone even in the clearing phase, provided that the concentration of ATP is appropriate*. Combination of the results obtained by quasi-elastic light scattering with biochemical data will lead to a deeper understanding of the molecular mechanism of muscle contraction.

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REFERENCES

1 R. Pecora, *J. Chem. Phys.*, 40 (1964) 1604.
2 S. Fujime, *J. Phys. Soc. Japan*, 29 (1970) 416.
3 S. Fujime, *J. Phys. Soc. Japan*, 29 (1970) 751.
4 S. Fujime and S. Ishiwata, *J. Phys. Soc. Japan*, 29 (1970) 1651.
5 S. Ishiwata and S. Fujime, *J. Phys. Soc. Japan*, 30 (1971) 302.
6 S. Ishiwata and S. Fujime, *J. Phys. Soc. Japan*, 30 (1971) 303.
7 S. Ishiwata and S. Fujime, *J. Phys. Soc. Japan*, 31 (1971) 1601.
8 S. Fujime, *J. Phys. Soc. Japan*, 31 (1971) 1805.
9 S. Fujime and S. Ishiwata, *J. Mol. Biol.*, 62 (1971) 251.
10 S. Ishiwata and S. Fujime, *J. Mol. Biol.*, 68 (1972) 511.
11 S. Fujime and S. Hatano, *J. Mechanochem. Cell Motility*, 1 (1972) 81.
12 S. Fujime, M. Maruyama and S. Asakura, *J. Mol. Biol.*, 68 (1972) 347.
13 H. Z. Cummins, N. Knable and Y. Yeh, *Phys. Rev. Lett.*, 12 (1964) 150.
14 N. C. Ford, Jr and G. B. Benedek, *Phys. Rev. Lett.*, 15 (1965) 649.
15 K. Tawada, *Biochim. Biophys. Acta*, 172 (1969) 311.
16 S. Abe and K. Maruyama, *Biochim. Biophys. Acta*, 243 (1971) 98.
17 M. Kasai, H. Kawashima and F. Oosawa, *J. Polymer Sci.*, 44 (1960) 51.
18 D. M. Young, S. Himmelfarb and W. F. Harrington, *J. Biol. Chem.*, 240 (1965) 2428.
19 H. Z. Cummins, F. D. Carlson, T. J. Herbert and G. Woods, *Biophys. J.*, 9 (1969) 518.
20 M. Kawamura and K. Maruyama, *J. Biochem. (Tokyo)*, 67 (1970) 437.
21 F. Oosawa, *J. Theor. Biol.*, 27 (1970) 69.
22 R. Courant and D. Hilbert, *Methoden der Mathematischen Physik I*, Berlin Verlag von Julius Springer, Berlin, 1931, p. 253.
23 K. M. Nauss, S. Kitagawa and J. Gergely, *J. Biol. Chem.*, 244 (1969) 755.
24 S. H. Slayter and S. Lowey, *Proc. Natl. Acad. Sci. U.S.*, 58 (1967) 1611.
25 P. J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, N.Y., 1953, p. 347.
26 S. Abe and K. Maruyama, *J. Biochem. (Tokyo)*, 71 (1972) 169.
27 P. J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, N.Y., 1953, p. 432.
28 K. Tawada and F. Oosawa, *J. Mol. Biol.*, 44 (1969) 309.

* S. Ishiwata, unpublished.