THE ACTIVE CROSS-BRIDGE MOTIONS OF ISOLATED THICK FILAMENTS FROM MYOSIN-REGULATED MUSCLES DETECTED BY QUASI-ELASTIC LIGHT SCATTERING

S.-F. FAN, M. M. DEWEY, D. COLFLESH, B. GAYLINN, R. A. GREGUSKI, AND B. CHU
Department of Anatomical Sciences, The Medical School, Health Sciences Center; and Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794

ABSTRACT Intensity fluctuation spectroscopy has been used successfully as a probe that can detect an increase in high-frequency internal motions of isolated thick filaments of *Limulus* muscle upon the addition of calcium ions. We have attributed such motions to cross-bridge motion instead of to an increase in the flexibility of the filament backbone. Here we show that after cleavage of the S-1 and then the S-2 moieties with papain, cross-linking the myosin heads to the filament backbone, or heat denaturation (42°C, 10 min), the increase in the high frequency internal motions in the thick filaments no longer occurs. Congo Red, which has been shown to induce shortening of isolated myofibrils, also increases the high-frequency motions of the isolated filaments. Furthermore, the increase is suppressed by treating the filaments with a myosin ATPase inhibitor such as vanadate ions (10 mM) or by replacing ATP with either an equimolar CrADP or the nonhydrolyzable ATP analogue β,γ-imido-adenine-5'-triphosphate (AMP-PNP). Calcium ions have a similar effect on isolated thick filaments from scallop muscle, where the myosin is known to be regulatory. Calcium ions have no such effect on thick filaments isolated from frog muscle, which is believed not to be regulated by calcium binding to myosin. These results confirm our earlier supposition that the additional high frequency internal motions of the thick filaments isolated from striated muscle of *Limulus* are related to the energy dependent, active cross-bridge motions.

INTRODUCTION

The contraction of striated muscle is known to be caused by an active, relative sliding of the thick and thin filaments (Huxley, H. E., 1969; Huxley, A. F., 1974). The most widely held view of the molecular mechanism of the sliding process in striated muscle is that cross-bridges, which are parts of the myosin molecules that project outward from the thick filaments, move cyclically upon activation while interacting with actin filaments. The cross-bridge motion pulls the thin filaments in between the thick filaments. Various methods have been used in an attempt to define or detect the orientation or the motion of the cross-bridges. They include measurement of dichroism with a dichroic label (Fan, 1963; Fan and Hong, 1964; Fan and Wen, 1979), measurement of the polarization of intrinsic fluorescence (Guth, 1980), measurement of the polarization of fluorescence with a fluorescent probe (Niehei et al., 1974; Borejdo, 1979), measurement of electron paramagnetic resonance with spin labels (Thomas et al., 1975; Thomas, 1978; and Thomas et al., 1979; Thomas et al., 1980; Thomas and Cooke, 1980; Arata and Shimizu, 1981; Cooke, 1981; Cooke et al., 1982), measurement of nuclear magnetic resonance (Highsmith et al., 1979; Highsmith and Jardetzhy, 1980, 1981), and small angle x-ray diffraction (Huxley and Brown, 1967; Elliot et al., 1967; Haselgrove, 1975; Huxley et al., 1980, 1981, 1982). Results from x-ray diffraction show that the ordering of the cross-bridge decreases as the muscle is activated. Measurement of the polarization of the fluorescence from tryptophan revealed that it was not sensitive to the orientation of the cross-bridges. Various physical techniques involving the use of labels have provided interesting results. For example, results of the dichroic studies of muscle stained with Congo Red indicate that the mean orientation of the cross-bridges in resting muscle changes with the sarcomere length (Fan and Wen, 1979). Results from high resolution electron paramagnetic resonance (EPR) studies established that during tension generation, the myosin head spends ±20% of its time attached to actin. For most of that time, the orientation of the probed region is identical to that in the rigor state (Cooke et al., 1982). However, the use of labels is always subject to criticism because of possible perturbing effects of the labeling molecules themselves. Congo Red seems to compete with ATP for the same reactive site (Fan and Wen, 1979). The attachment of the iodoacetamide molecule, which was used both as a fluorescence probe and

Dr. Fan's permanent address is Shanghai Institute of Physiology, Academia Sinica, Shanghai, China.

as a spin label, changed the ionic dependence of the myosin ATPase activity. Furthermore, an iodoacetamide spin label does not remain rigidly immobilized on the myosin head during ATP hydrolysis, as indicated by large changes in the EPR spectrum (Seidel et al., 1970). Labeling with another commonly used spin label, a maleimide derivative, also affects the myosin ATPase activity, although it does remain immobilized on the myosin head during ATP hydrolysis (Thomas et al., 1980).

Quasi-elastic light scattering has been used successfully to investigate the dynamics of submicroscopic structures in solution (Chen et al., 1981; Maeda and Fujime, 1981) including suspensions of thin filaments from skeletal muscle (Newman and Carlson, 1980; Maeda and Fujime, 1981), suspensions of heavy meromyosin (HMM) or S-1 and actin (Fujime et al., 1972; Maeda et al., 1974; Fraser et al., 1975) and also in situ myofibrils (Haskell and Carlson, 1981). The advantage of this method is that it does not require any label to detect the motion of the relevant structures. Recently we have used this method to study the behavior of isolated thick filaments from striated muscle of *Limulus* (Kubota et al., 1983). The average linewidth, $\bar{\Gamma}$, over large ranges of KL (up to $\simeq 100$) has been calculated with K and L being, respectively, the magnitude of the momentum transfer vector and the length of the filament. Upon the addition of calcium ions to the solution suspending the filaments, which contained K^+, Mg^{2+}, ATP, and Tris buffer, the $\bar{\Gamma}$ values showed a remarkable increase at high KL. There are two possible explanations: (*a*) The first possible explanation is that the overall flexibility of the filament backbone is increased by calcium ions. Maeda and Fujime (1981) have shown that with semiflexible filaments an increase in flexibility results in an increase in the $\bar{\Gamma}$ values at higher KL. (*b*) The second possible explanation is that the cross-bridges, when activated, move cyclically. Lehman and Szent-Gyorgi (1975) have shown that the contractile activity of *Limulus* striated muscle is regulated through both thick and thin filaments by calcium ions. Thus calcium ions should activate the cross-bridge motions in isolated thick filaments. Since the thick filaments of *Limulus* striated muscle shorten upon the addition of calcium ions in the presence of ATP, we would have expected that the filaments would become stiffer instead of being more flexible. We attributed the increase of $\bar{\Gamma}$ values at higher KL value as being due to the motions of cross-bridges. Here we present evidence that the increase of $\bar{\Gamma}$ values is due to active cross-bridge motions. We also demonstrate that thick filaments isolated from the striated muscle of scallop in which the muscle activity is regulated through myosin (Kendrick-Jones et al., 1970, 1976) gives similar results. Conversely, thick filaments isolated from skeletal muscle of frog do not. The thick filment of this muscle has not been convincingly shown to be involved in regulation. Preliminary accounts of some of our results have been presented elsewhere (Fan et al., 1983*a*, *b*, and *c*).

THEORETICAL BACKGROUND

For a dilute solution of rod-shaped flexible particles (filaments), the translational and rotational diffusions of a particle, as well as other intrafilamentous motions, i.e., motions of segments related to each other, will contribute to the intensity and linewidth of the spectrum of light being scattered. The field intensity correlation function $g^{(1)}(\tau)$ can be expressed as (Pecora, 1968; 1981)

$$g^{(1)}(\tau) = \alpha^2 \left\langle \sum_i \sum_j e^{i\mathbf{K}\cdot[\mathbf{r}_i(t) - \mathbf{r}_j(0)]} \right\rangle \tag{1}$$

where α is the polarizability, $\mathbf{r}_i(t)$ is the position of ith scatterer at time t, $K = 4\pi/\lambda \sin \Theta/2$, λ is the wave length of light in the scatter medium and Θ the scattering angle. The angular bracket denotes an ensemble average.

In dilute solution, scatterers on different filaments are uncorrelated, scatterers i and j can be considered to be different segments on the same filament. The time correlation function in Eq. 1 becomes

$$g^{(1)}(\tau) = \alpha_M^2 \left\langle e^{i\mathbf{K}\cdot[\mathbf{R}(t) - \mathbf{R}(0)]} \sum_i \sum_j 1/N^2 \, e^{i\mathbf{K}\cdot[\mathbf{b}_j(t) - \mathbf{b}_i(0)]} \right\rangle, \tag{2}$$

where α_M is the particle polarizability. The first exponential term on the right hand side of Eq. 2 depends on the translational motion of the particle as a whole and the second term depends upon the relative motion of the segments. If $KL < 1$, the second exponential term contributes a constant factor $\simeq 1$, and (omitting the polarizability factor)

$$g^{(1)}(\tau) = e^{-K^2 D_T \tau}. \tag{3}$$

Thus, if $KL < 1$, $g^{(1)}(\tau)$ is only related to the translational diffusional motion of the particle. As $3 \leq KL \leq 8$, the contribution of rotational diffusion motion will become appreciable. As $KL > 8$, the higher order terms, which reflect the attributions of the intraparticle motions such as bending or twisting, should be taken into consideration.

The limiting form of the first cumulant, $\bar{\Gamma}$ of the field correlation function $g^{(1)}(\tau)$ for a very long and semiflexible filament has been shown by Maeda and Fujime (1984) to be

$$(\bar{\Gamma}/K^2)_{(KL \gg 1)} \rightarrow [D_T - (D_3 - D_1)/3] + L^2 D_R/12 + (k_B T/\zeta L) \Sigma_m'' 1, \tag{4}$$

where $D_T = (2D_1 + D_3)/3$, D_1 and D_3 are, respectively, the sidewise and the lengthwise translational diffusion constants of the filament. D_R is the diffusion constant of the filament as it rotates perpendicular to its axis. L is the length of the filament; ζ is the friction constant; $\Sigma'' 1$ means the number of bending modes of motion involved in the scattering process; k_B and T have the usual meaning, i.e., the Boltzmann constant and the absolute temperature.

Take the long-rod limit of diffusion constants, i.e., $D_3 = 2D_1$,

$D_T - (D_3 - D_1)/3 = D_1$, $(L^2 D_R/12) D_R = D_1$ and $k_B T/\zeta L = D_1$,

Eq. 4 becomes

$$\bar{\Gamma}/K^2_{(KL \gg 1)} \rightarrow D_1 + D_1 + D_1 + \sum_m'' 1. \tag{5}$$

The value of D_1 can be calculated from the equation given by Newman et al., (1977):

$$D_T = (k_B T/3\pi n L) \cdot (\ln(2L/d) - \tfrac{1}{2}\{1.46 - 7.4[1/\ln(2L/d) - 0.34]^2 - 4.2[1/\ln(2L/d) - 0.39]^2\}), \tag{6}$$

using the relation for long-rod limiting case $D_1 = 3D_T/4$ and defining η and d in Eq. 6 as, respectively, the viscosity of the medium and the

effective diameter of the filament. For *Limulus* thick filaments suspended in relaxing solution and after treated with calcium ions, the values of D_1, as calculated from Eq. 6 are, respectively, 4.8×10^{-9}cm^2/s and 5.8×10^{-9} cm^2/s at our experimental temperature (25°C). For a very long filament without other intrafilamentous modes of motion but with realistic flexibility, the value of Σ''l could not be higher than 2–3 D_1 or $\bar{\Gamma}/K^2$ could not exceed 4–5 D_1. The value of $\bar{\Gamma}/K^2$ at $K^2 = 10 \times 10$ cm^{-2} for the filaments suspended in solution free of or with calcium ions are, respectively, $(2.7 \pm 0.4)D_1$ and $(7.3 \pm 1.3)D_1$ ($n = 22$). In a previous paper (Kubota et al., 1983), we attributed the extra line width to cross-bridge motions activated by calcium ions. In this article we show that such extra line width does disappear if we cleave the cross-bridge or render it functionally inhibited. It also disappears if we deplete the energy supply of the filament preparations.

MATERIALS AND METHODS

Isolation of Thick Filaments

Muscle bundles of the levators of the telson of *Limulus* (*Tachypleus polyphemus*) sartorius of the frog, *Rana pipiens*, and the striated adductor muscle of the scallop, *Aequipectin irradians*, were isolated and tied at a fixed length. The bundles were then soaked in a solution containing 100 mM KCl, 2 mM $MgCl_2$, 5 mM Tris, 5 mM EGTA, 5 mM ATP, and 1 mM DTT at pH 7.4 and 4°C for 24 h. After homogenization, the thick filaments were separated by gradient centrifugation at 1.2×10^5 g for 45 min. The gradient consisted of deionized glycerol mixed with the above solution and was either step, one volume each of 10 and 60%, or continuous to 68% glycerol, vol/vol. Thick filaments were collected at the 60% interface or from the 47–55% layers of the continuous gradient. Before light-scattering measurements, the filament suspensions were dialyzed at 4°C for 2–4 d against a solution containing the required composition and containing 0.1% glycerol to control viscosity. The dialyzing solution was changed several times. The total protein concentration of our filament suspensions in relaxing solution (100 mM KCl, 2 mM $MgCl_2$, 0.1 mM EGTA, 5 mM Tris, 2 mM ATP at pH 7.0) was usually ~100 μg/ml. The number of thick filaments in the suspension at this concentration is $\sim 1 \times 10^{11}$/ml, which is higher than the overlap concentration, 1.6×10^{10}/ml. Previously (Kubota et al., 1983) we found that the $\bar{\Gamma}$ values obtained at this concentration are practically the same as those obtained at a concentration of 1×10^9 filaments/ml, which is ~1/10 of the overlap concentration. The ratio of actin to myosin content, A/M, in the suspension was determined by means of densitometric tracings of sodium dodecyl sulfate/polyacrylamide gel electrophoresis patterns. A/M was reduced to <15% of the original A/M in the homogenate. The total protein concentration of our filament suspensions in activating solution (5 mM $CaCl_2$ added to relaxing solution giving a pCa of 2.4 as measured with a calcium electrode) was usually ~25 μg/ml and the A/M ratio was reduced to <3% of the original value. Fig. 1 shows the SDS gel electrophoretic results of the original homogenate and the purified sample we used.

All light-scattering measurements were performed immediately after centrifuging the suspensions at 5×10^3 g for ½ to 1 h at 4°C to minimize the dust particles and aggregates, if any.

Light-Scattering Measurement

The light-scattering spectrometer used has been described previously (Kubota et al., 1983). The measured single-clipped photoelectron autocorrelation function $G_k^{(2)}(\tau)$ due to self-beating for a detector of finite effective photocathode has the form (Hanson, 1971)

$$G_k^{(2)}(\tau) = \langle n_k(t)\,(t+\tau)\rangle \simeq A[1 + b|g^{(1)}(\tau)|^2], \quad (7)$$

where $\langle n_k(t)\rangle$ is the mean clipped photo-electron counts per sample time at time t and clipping level k. $n(t)$ is that of the unclipped value. A is a background equal to $N_s\langle n_k(t)\rangle\langle n(t)\rangle$ with N_s being the total number of samples, which can be computed or measured at longer delay times (usually 5 × the maximum delay time). In our experiments, the difference between the measured and the calculated background is usually of the order of 10^{-3}. b is a fitting parameter that depends on various experimental conditions such as coherence and receiving area. τ is the delay time.

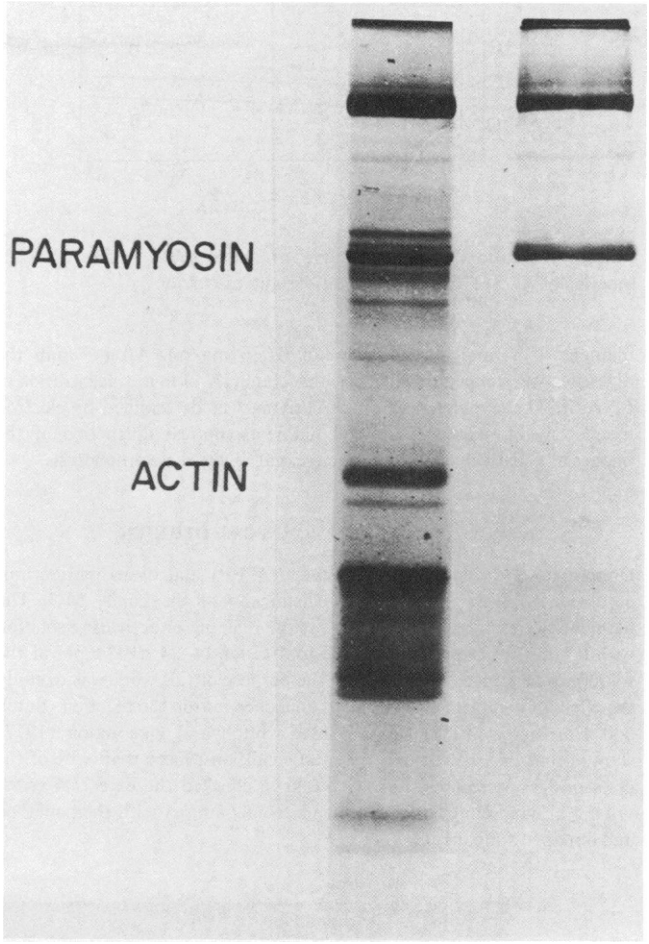

FIGURE 1 An electrophoretic SDS gel of the muscle homogenate (*left* lane) and the purified thick filaments (*right* lane).

For monodisperse, long semiflexible filaments of length L and $KL > 8$, high frequency internal motions increase the $\bar{\Gamma}$ where

$$\bar{\Gamma} = \int G(\Gamma)\Gamma d\Gamma, \quad (8)$$

with $G(\Gamma)$ being the normalized linewidth distribution function. We used the cumulants method (Koppel, 1972) to determine $\bar{\Gamma}$ and μ_2; the latter is defined as

$$\mu_2 = \int G(\Gamma)\,(\Gamma - \bar{\Gamma})^2 d\Gamma, \quad (9)$$

with $\mu_2/\bar{\Gamma}$ being the variance of the linewidth distribution function. In studying the thick filament suspensions, we found that $\mu^2/\bar{\Gamma} \leq 0.3$ over all accessible ranges of K. Thus our linewidth measurements were carried out at a delay time range such that $\bar{\Gamma}\tau_{max} \simeq 3$, where τ_{max} denotes the maximum delay time range. We used the second-order cumulants fit for most data analysis. Occasionally, a third-order cumulants fit was performed to check the goodness of the second-order analysis.

Cleaving of the HMM Moieties of the Thick Filament

Cross-bridges were cleaved by papain (Komitz et al., 1965; Yamanaotik and Schiao, 1980). Papain was added directly to the suspensions of

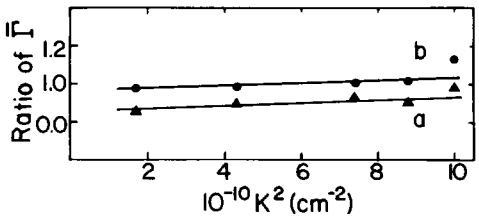

FIGURE 2 Ratio of $\bar{\Gamma}_{p,a}/\bar{\Gamma}_{r,p}$ (curve *a*) and $\bar{\Gamma}_{a,p}/\bar{\Gamma}_{r,p}$ (curve *b*) as a function of K^2. See Table I for notations and discussion.

filament to a final concentration of 0.046 mg/ml. After 7 min the digestion was stopped by adding iodoacetate (IAA) to a concentration of 1.5 mM. The completeness of the cleaving was determined by electron microscopy of negatively stained filaments, optical diffraction of the images of negatively stained filaments, and SDS gel electrophoresis.

Cross-linking of the Cross-bridges

Dimethyl 3,3'-dithiobispropionimidate (DTBP) and dimethylsuberimidate (DMS) were from Sigma Chemical Co, St. Louis, MO. The filaments were dialyzed either against the relaxing or activating solution with 0.5 mg/ml cross linker added to 4°C for 14–24 h. The pH of the solution was adjusted to 7.0. Since the purpose of this work was to study the effect of complete cross-linking, quencher to stop the reaction (Sutoh and Harrington, 1977) was not used. Sutoh and Harrington (1977) showed that under their experimental conditions, more than 95% of the cross-bridges were cross-linked. We have checked the extent of cross-linking in our isolated thick filaments from *Limulus* with their method and obtained similar results.

Preparation of CrADP

CrADP was prepared essentially according to the method of Dunaway-Mariano and Cleland (1980*a*). An ATP solution was first heated in a boiling water bath for 7 min and an equimolar quantity (to ATP) of $CrCl_3$ was added. The pH of the solution was lowered to 3.0; than the solution was heated in an 80°C bath for 10 min and the pH was finally adjusted to 6.0. At higher pH (>6.0) a gel-like suspension often formed.

Replacement of ATP with β, γ-imido-adenine-5'-triphosphate (AMP-PNP)

AMP-PNP contains trace amounts of ATP. To remove the ATP contamination, as well as that remaining in the filament preparation 2 mM glucose was added to the dialyzing solution and 50 μg/ml of hexokinase was added to the dialysate (see Meisner and Beinbrech, 1979) and incubated at room temperature for 2 h. The suspension was then dialyzed at 4°C before measurements were made.

RESULTS AND DISCUSSION

Thick Filaments of the Striated Muscle of Limulus

A. Cleaving or Rendering the Cross-bridge Nonoperative

Cleaving of the S-1 Moiety of the Filaments in Suspension. As we have shown, the average ratio of the $\bar{\Gamma}$ values of thick filaments suspended in an activating solu-

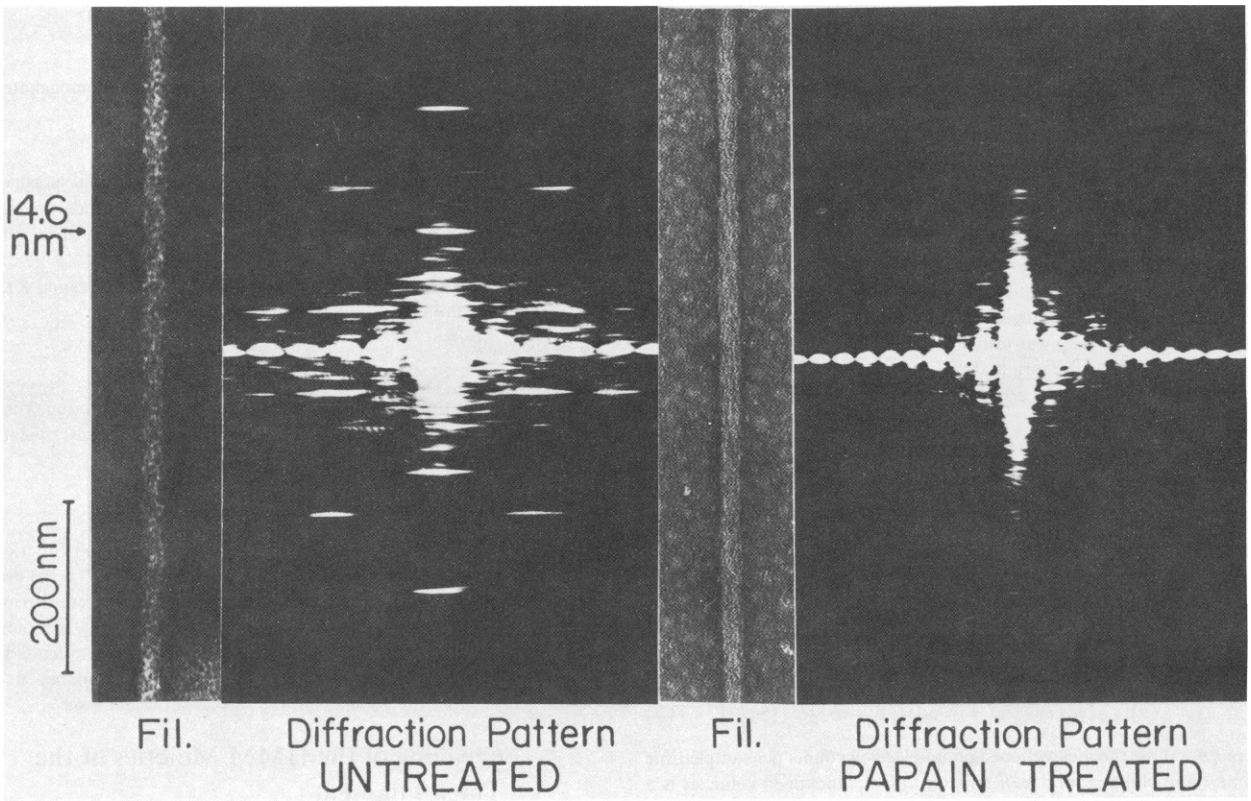

FIGURE 3 Electron micrographs of nontreated and papain treated thick filaments and their optical transforms. These illustrate the completeness of the digestion. Note the absence of the 14.6-nm meridional reflection in the transform of the treated filament.

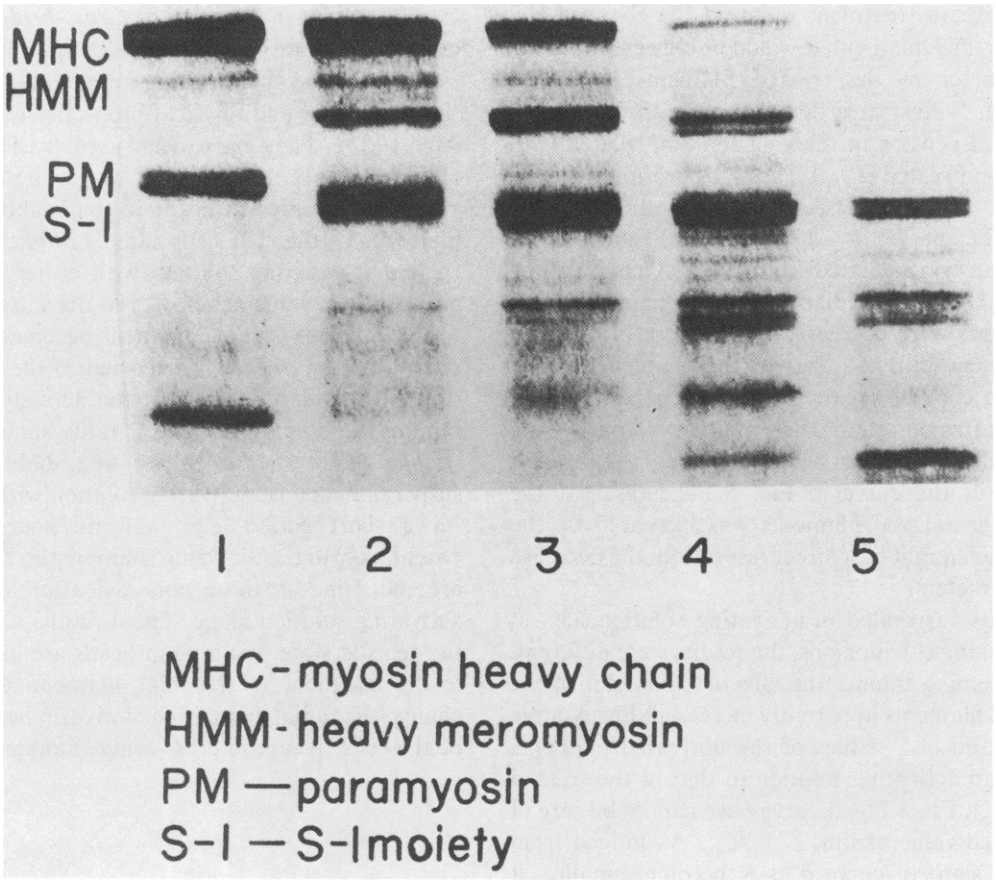

FIGURE 4 An electrophoretic SDS gel of papain-treated preparation of isolated thick filaments. Digestion was stopped by addition of IAA. Lanes 1–5 are 0, 1, 3, 6, and 12 min of digestion.

tion to that suspended in a relaxing solution was ~1.4 at $K^2 = 0.7 \times 10^{10}\text{cm}^{-2}$. The ratio increased more or less linearly to ~2.3 at $K^2 = 10^{10}\text{cm}^{-2}$ (Kubota et al., 1983). If these increases are due to activation of cross-bridge motions, then removing cross-bridges from the filaments should eliminate the corresponding high-frequency components of the characteristic time. This was what actually happened after the cross-bridges were cleaved by treating with papain.

Papain treatment was performed either before or after the sample was activated. We specify the $\bar{\Gamma}$ values obtained with the two different treatments, respectively, by $\bar{\Gamma}_{p,a}$ and $\bar{\Gamma}_{a,p}$. In this notation, p denotes papain treatment and a denotes activated filaments; thus p,a indicates that the filaments were treated with papain before activation and a,p vice versa. Since the filaments were isolated in relaxing solution, only $\bar{\Gamma}_{r,p}$ could be measured. $\bar{\Gamma}_r$ and $\bar{\Gamma}_a$ is used to denote the $\bar{\Gamma}$ values obtained from relaxed and activated samples respectively.

After treatment with papain, the ratio of the $\bar{\Gamma}$ values from suspensions of filaments in activating and relaxing solutions remained $\simeq 1$ through the whole range of K^2 we used, i.e., from $0.8 \times 10^{10}\text{cm}^{-2}$ to $10 \times 10^{10}\text{cm}^{-2}$. The high frequency component of the characteristic time was not present following papain treatment. Curve *a* in Fig. 2 shows $\bar{\Gamma}_{p,a}/\bar{\Gamma}_{r,p}$, while curve *b* in Fig. 2 shows the results $\bar{\Gamma}_{a,p}/\bar{\Gamma}_{r,p}$. Papain digestion was terminated by the addition of 1.5 mM iodoacetate (IAA). IAA alone at 1.5 mM had no effect on the increase of $\bar{\Gamma}$ values following calcium activation. Fig. 3 illustrates negatively stained isolated filaments and their optical diffraction patterns before and after papain treatment and Fig. 4 presents the results obtained on an SDS gel of papain treated and untreated, isolated filaments. These results confirmed that the thick filaments lost over 95% of their S-1 and S-2 moieties following the papain treatment.

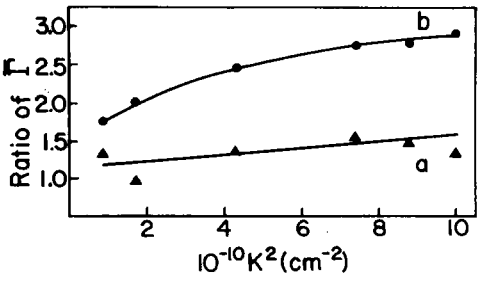

FIGURE 5 Ratio of $\bar{\Gamma}_{r,p}/\bar{\Gamma}_r$ (curve *a*) and $\bar{\Gamma}_a/\bar{\Gamma}_{a,p}$ (curve *b*) as a function of K^2. See Table I for notations and discussion.

Since the papain treatment removed the S-1 and S-2 moieties of the thick filament, it would be expected that the effective diameter of the treated filaments would be decreased. Fig. 5 illustrates this finding in the ratio of $\bar{\Gamma}$ values of the suspension in relaxing solution treated with papain to that untreated ($\bar{\Gamma}_{r,p}/\bar{\Gamma}_r$) vs. K^2. The limiting value of $\bar{\Gamma}_{r,p}/\bar{\Gamma}_r$ as $K \rightarrow 0$ is ~1.2 As $K \rightarrow 0$, the translational diffusion coefficient $D_T \equiv \bar{\Gamma}/K^2$. For a rigid rod in solution, D_T can be calculated from Eq. 6. Thus it can be estimated that the effective diameters of the papain treated relaxed filaments were decreased by ~40%, provided that papain treatment did not change the filament length. Electron microscopy of negatively stained, papain treated filaments confirmed that the filament length was unchanged and the filaments became thinner (Fig. 2). The positive slope of the curve in Fig. 5 indicates that the flexibility of the relaxed filaments was increased by the treatment, which might be a direct result from the decrease in filament diameters.

For filaments suspended in activating solution, i.e., in solution containing calcium ions, the results were different. Instead of becoming thinner the effective diameter of the papain treated filaments apparently increased. Fig. 5 curve *b* shows the ratio of $\bar{\Gamma}$ values of the untreated filaments suspended in an activating solution to that of the treated sample ($\bar{\Gamma}_a/\bar{\Gamma}_{a,p}$). From Fig. 5 curve *b* we cannot be sure of the extrapolated value of $\lim_{K\rightarrow 0} \bar{\Gamma}_a/\bar{\Gamma}_{a,p}$. As judged from the change of slope of curve *b* as K becomes smaller, it seems highly probable that $\lim_{K\rightarrow 0} \bar{\Gamma}_a/\bar{\Gamma}_{a,p} > 1$. This accessment implies that the effective diameter of the treated filaments increased provided that the length of the filaments had not changed following treatment with papain. Again, electron micrographs showed that the length of the activated filaments was not changed by such treatment. This contention is strengthened by the fact that $\bar{\Gamma}_{a,p}/\bar{\Gamma}_{r,p}$ is close to one as shown in curve *b* of Fig. 2. This implies that the effective diameter, d, of papain treated and activated filaments ($d_{a,p}$) is increased by >40% and is equal to ~44 nm, if we take the effective diameter of the treated relaxed filaments to be equal to 14 nm. If we adopt $d_{a,p}$ = 44 nm, then d_a, the diameter of the activated untreated filaments, is equal to 30 nm. $L_a = L_{a,p} = 3\ \mu m$, then the calculated value of $\bar{\Gamma}_a/\bar{\Gamma}_{a,p} \simeq 1.1$, is in reasonable agreement with the observed tendency of the results shown in Fig. 5 curve *b* as $K \rightarrow 0$. The increase in the effective diameter of the shortened filaments after papain treatment suggests that papain has not only cleaved the cross-bridge, but also caused a loosening or swelling of the shortened filaments in the presence of calcium ions.

The $\bar{\Gamma}$ values of papain-treated filaments show larger experimental variations than that of untreated filaments in either a relaxing solution or an activating solution. Therefore, the percentage change in the effective diameter of the filament after papain treatment is largely qualitative in nature.

Cross-linking of the Cross-bridge. Imido ester cross-linkers are believed to react specifically with lysine side chains and the resulting amidinate protein maintains their chemical and physical properties (Hunter and Ludwig, 1962). They have been used by Harrington et al. (Reisler et al., 1973; Sutoh and Harrington, 1977) to cross-link the myosin heads to the backbone of the thick filament. In the case of isolated *Limulus* thick filaments treated in relaxing solution with either DTBP or DMS added, the $\bar{\Gamma}$ value at KL = 100 decreases to ~$2D_1$. This result indicates that the filament becomes quite stiff after cross-linking as would be expected. If the filament suspension is then dialyzed against the activating solution containing the cross-linker, the $\bar{\Gamma}$ value shows practically no change (Fig. 6). But if we first dialyze the filament suspension against activating solution without cross-linker for a short period, e.g., half an hour, than add the cross-linker to the dialyzing solution, the $\bar{\Gamma}$ values obtained are the same as those obtained after dialyzing against activating solution alone. These results also imply that in the relaxed state, the myosin heads are in close proximity to the backbone of the thick filament. Calcium ions do change the radial disposition of myosin heads, which could be the consequence of cross-bridge motion.

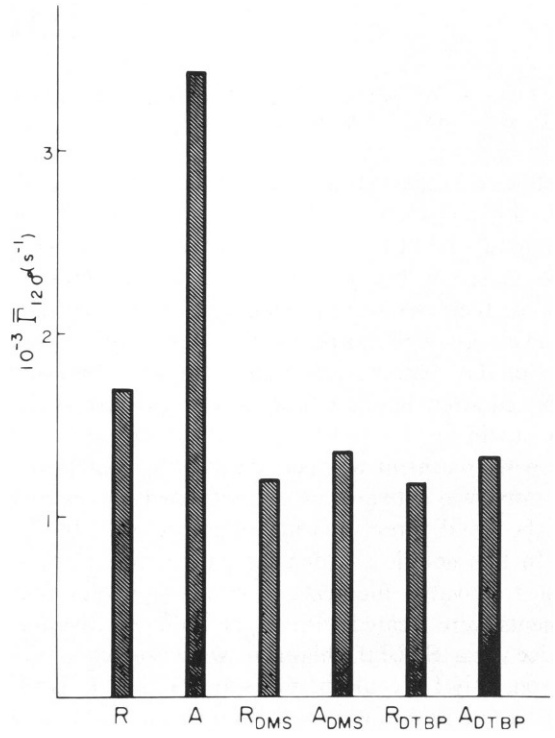

FIGURE 6 $\bar{\Gamma}$ values at θ = 120° of filaments suspended in relaxing solution (R), activating solution (A), relaxing solution with 0.5 mg/ml DMS (R_{DMS}), activating solution with 0.5 mg/ml DMS (A_{DMS}), relaxing solution with 0.5 mg/ml DTBP (R_{DTBP}), and activating solution with 0.5 mg/ml DTBP (A_{DTBP}). The effect of calcium ions on $\bar{\Gamma}$ value is suppressed by both.

Heat Denaturation of the Cross-bridges. X-ray diffraction studies of the sartorius of the frog have shown that after the muscle is heated to ≃42°C for 10 min, the diffraction layer line believed to originate from myosin and the off-meridional 38.0-nm layer lines disappeared while the layer lines believed to originate from actin do not change (Huxley, 1972). The dichroism of the sartorius of frog or toad and the psoas of rabbit stained with Congo Red also disappeared after heat treatment (Fan and Wen, 1979). Both results have been interpreted to mean that the cross-bridges are denatured by heating. Such interpretation is not proved. While this has not been proven directly, following heat treatment both glycerinated amphibian

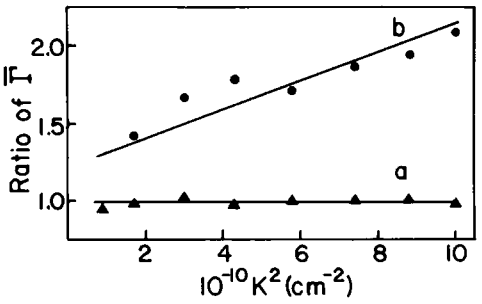

FIGURE 7 Ratio of $\bar{\Gamma}_r/\bar{\Gamma}_{r,H}$ (curve *a*) and $\bar{\Gamma}_a/\bar{\Gamma}_{a,H}$ (curve *b*) as a function of K^2. See Table I for notations and discussion.

TABLE I
EFFECT OF CLEAVAGE, CROSS-LINKING, OR HEAT DENATURATION OF CROSS-BRIDGES

Filament suspension A		Filament suspension B				
Treatments	Notation of $\bar{\Gamma}$ value at $K^2 = 8.8 \times 10^{-10}$ cm^{-2} $(\bar{\Gamma}_A)$*	Treatments	Notation of $\bar{\Gamma}$ value at $K^2 = 8.8 \times 10^{-10}$ cm^{-2} $(\bar{\Gamma}_B)$*	$\bar{\Gamma}_A/\bar{\Gamma}_B$	Implication	Remarks
1) Suspended in an activating solution§	$\bar{\Gamma}_a$	Suspended in a relaxing solution‡	$\bar{\Gamma}_r$	2.1	Calcium ions increase the $\bar{\Gamma}$ value of the filament suspension.	Data taken from Kubota et al. (1983). Results imply that calcium ions (in the presence of ATP) activate cross-bridge motions.
2) First treated with papain while filaments were suspended in a relaxing solution,‡ then dialysed against an activating solution§	$\bar{\Gamma}_{p,a}$	Treated with papain while filaments were suspended in a relaxing solution‡	$\bar{\Gamma}_{r,p}$	≃0.8–1.1	After papain treatment calcium ions can no longer increase the $\bar{\Gamma}$ value of the filament suspension.	Papain cleaves cross-bridges. Therefore, no cross-bridge motion can be expected to be present. $\lim_{K\to 0} \bar{\Gamma}_{p,a}/\bar{\Gamma}_{r,p} \simeq 0.9$ denotes the the effective diameter of the papain treated activated filaments may have been increased since $L_{p,a} \simeq L_{r,p} \simeq 4$ μm where L denotes the average length of the isolated filament from electron microscopic measurements.
3) First dialyzed against an activating solution,§ then treated with papain	$\bar{\Gamma}_{a,p}$	Same as above	$\bar{\Gamma}_{r,p}$	≃0.5–1.1	Papain treatment suppresses the $\bar{\Gamma}$ value of the activated filament suspension.	$\lim_{K\to 0} \bar{\Gamma}_{a,p}/\bar{\Gamma}_{r,p} \simeq 1$ confirms that the effective diameter of of papain treated activated filaments may have been increased since $L_{a,p} \simeq 3.0$ μm.
4) Treated with papain while filaments were suspended in a relaxing solution‡	$\bar{\Gamma}_{r,p}$	Suspended in a relaxing solution‡	$\bar{\Gamma}_r$	≃1.5	Papain treatment increased the flexibility of filaments suspended in a relaxing solution.‡	$\lim_{K\to 0} \bar{\Gamma}_{r,p}/\bar{\Gamma}_r = 1.1 \sim 1.2$. 1.2 denotes that the effective diameter of the papain treated relaxed filaments may have decreased with $L_{r,p} \simeq L_r \simeq 4$ μm, while the effective diameter of papain treated filaments increased as in activating solution, as demonstrated in 2) and 3).

(*continued on next page*)

TABLE I (*continued*)

Filament suspension A		Filament suspension B				
Treatments	Notation of $\bar{\Gamma}$ value at $K^2 = 8.8 \times 10^{-10}\ cm^{-2}$ $(\bar{\Gamma}_A)$*	Treatments	Notation of $\bar{\Gamma}$ value at $K^2 = 8.8 \times 10^{-10}\ cm^{-2}$ $(\bar{\Gamma}_B)$*	$\bar{\Gamma}_A/\bar{\Gamma}_B$	Implication	Remarks
5) Suspended in an activating solution§	$\bar{\Gamma}_a$	First dialyzed against activating solution,§ then treated with papain	$\bar{\Gamma}_{a,p}$	$\simeq 2.7$	Papain treatment suppresses the $\bar{\Gamma}$ value of the activated filaments as well as increases their effective diameter.	With $L_a = L_{a,p} \simeq 3\ \mu m$ and $\lim_{K\to 0} \bar{\Gamma}_a/\bar{\Gamma}_{a,p} \geq 1$. $d_a \leq d_{a,p}$ the large increases in $\bar{\Gamma}_a/\bar{\Gamma}_{a,p}$ again signifies the presence of cross-bridge motions since papain cleaved the cross-bridges.
6) Suspended in a relaxing solution‡	$\bar{\Gamma}_r$	Suspended in a relaxing solution‡ with cross-linker added	$\bar{\Gamma}_{r,c}$	$\simeq 0.7$	Cross-linker makes the filament stiffer.	The cross-linking agent cross-links the myosin head to the filament backbone, rendering the filament stiffer.
7) Suspended in a relaxing solution‡ with cross-linker	$\bar{\Gamma}_{r,c}$	First dialyzed against relaxing solution‡ with cross-linker, then dialyzed against activating solution§ with cross-linker	$\bar{\Gamma}_{c,a}$	$\simeq 0.8$	Calcium ions can no longer activate the cross-bridge motion.	Since the cross-bridges are cross-linked to the backbone of the filament no cross bridge motion can be expected. The difference between the $\bar{\Gamma}_A/\bar{\Gamma}_B$ of 6) and 7) might reflect either a slight difference in flexibility or that a certain proportion of cross-bridges are not cross-linked and can still be activated.
8) Suspended in a relaxing solution‡	$\bar{\Gamma}_r$	Heating of filaments suspended in a relaxing solution‡	$\bar{\Gamma}_{r,H}$	1.0	Heating has no effect on the $\bar{\Gamma}$ value of relaxed filaments.	Heating at 42°C for 10 min is believed to denature the cross-bridges. For filaments in a relaxing solution little or no active cross-bridge motions are present. Therefore, $\bar{\Gamma}_r \simeq \bar{\Gamma}_{r,H}$ also suggests that the filament flexibility has remained essentially the same.
9) Suspended in an activating solution§	$\bar{\Gamma}_a$	Heating of filaments suspended in an activating solution§ at ~42°C for 10 min	$\bar{\Gamma}_{a,H}$	2.1	Heating suppresses the $\bar{\Gamma}$ value of the activated filaments.	Since 8) suggests that there is no change in filament flexibility by heating the filaments in a relaxing solution, $\bar{\Gamma}_a/\bar{\Gamma}_{a,H} = 2.1$ denotes that we detect the active cross-bridge motions.

*$\theta = 120°$ for $K^2 = 8.8 \times 10^{-10}\ cm^{-2}$.
‡Relaxing solution: 100 mM KCl, 2 mM $MgCl_2$, 0.1 mM EGTA, 2 mM ATP, 5 mM Tris, pH ~7.0.
§Activating solution: 100 mM KCl, 2mM $MgCl_2$, 0.1 mM EGTA, 2 mM ATP, 5 mM Tris, 5 mM $CaCl_2$, pH ~7.0.

skeletal muscle and *Limulus* striated muscle lose their ability to shorten in the presence of calcium ions and ATP, thus indicating that the contractile mechanism of the muscle is no longer functional. Heating of isolated thick filaments in relaxing solution to 42°C for 10 min did not change the $\bar{\Gamma}$ values as shown in curve *a* Fig. 7, where $\bar{\Gamma}_r/\bar{\Gamma}_{r,H} \simeq 1$ (the subscript H denotes that the filaments had been heated to 42°C for 10 min). However, heating the filaments suspended in activating solution resulted in $\bar{\Gamma}$ values decreased to the values similar to those obtained from filaments in relaxing solution (Fig. 7 curve) where $\bar{\Gamma}_a/\bar{\Gamma}_{a,H} \simeq \bar{\Gamma}_a/\bar{\Gamma}_r$ (Kubota et al., 1983). The results pre-

sented here and in the previous sections are summarized in Table I.

B. The Effect of Congo Red on Isolated Thick Filaments

If the increase of $\bar{\Gamma}$ values is due to cross-bridge motion, other forms of activation of the bridges should produce similar increases. Isolated myofibrils of both the sartorius muscle of frog and the indirect flight muscle of the honey bee can be shortened by Congo Red (Fan, 1964). Addition of Congo Red to a suspension of filaments in relaxing solution caused an increase in the $\bar{\Gamma}$ value even above that obtained with calcium ions. Fig. 8 shows the effect of 0.01% (wt/vol) Congo Red on the $\bar{\Gamma}$ value of filaments suspended in relaxing solution with 5 mM EGTA. The $\bar{\Gamma}$ value increased by a factor of ~4 at a K^2 value of $8.8 \times 10^{10}\text{cm}^{-2}$ ($KL \simeq 106$) as compared to a factor of ~2.2 after the addition of calcium ions. Since the pCa value of the 5 mM EGTA relaxing solution with Congo Red added is only ~8, the Congo Red effect cannot be due to any possible calcium ion contamination and must be due to Congo Red itself.

The mechanism of shortening of the isolated myofibril by Congo Red has not been elucidated, yet the following facts have been established: (*a*) Congo Red affects the myosin ATPase activity (Acemann, 1953; Teranishi, 1957*a, b, c;* Filatova et al., 1968); (*b*) Congo Red enhances the contractile properties of rabbit myosin preparation (Ivanov et al, 1976); and (*c*) The A-band remains constant during Congo Red shortening of the isolated myofibril until the contraction band appears. Therefore, Congo Red shortening of the isolated myofibril is probably brought about by the sliding filaments mechanism, i.e., through the activation of cross-bridges.

The fact that Congo Red increased the $\bar{\Gamma}$ value more than calcium ions did suggests that Congo Red not only activates the cross-bridge motions but also increases the flexibility of the thick filaments. This supposition is supported by the fact that the $\bar{\Gamma}$ values of heated Congo Red-treated filaments were always greater than those of simply heat-treated filaments.

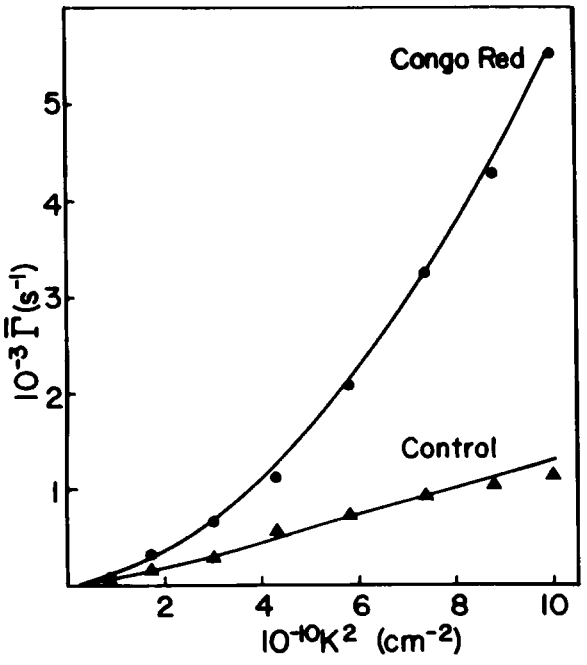

FIGURE 8 Plot of $\bar{\Gamma}$ vs. K^2 of suspensions of thick filaments from *Limulus* striated muscle. The "control" curve denotes filaments suspended in a relaxing solution containing 5 mM EGTA and the "Congo Red" curve corresponds to filaments suspended in a relaxing solution containing 0.01% Congo Red and 5 mM EGTA.

C. The Effect of Depletion of Energy Supply on Isolated Thick Filaments

Effect of Inhibition of the Myosin ATPase Activity. Vanadate has been reported to form a complex with myosin and ADP (Goodno, 1979; Goodno and Taylor, 1982) that was supposed to be a stable analogue of the myosin-ADP-phosphate complex. The complex is believed to be a key intermediate in the myosin ATPase cycle. Fig. 9 shows the ratio of $\bar{\Gamma}$ values obtained from filaments suspended in an activating solution to that from filaments in a relaxing solution with both containing 10 mM vanadate. This experiment was done with a neon laser source with wave length of 632.8 nm. Thus the maximum value of K^2 is only $\sim 4.5 \times 10^{10}\ \text{cm}^{-2}$. The ratio of $\bar{\Gamma}$ values between $K^2 = 0.8 \times 10^{10}$ and $4.5 \times 10^{10}\text{cm}^{-2}$ remains practically unchanged and is close to one, indicating that in the presence of vanadate, calcium ions can no longer appreciably activate cross-bridge motions.

Effect of Replacement of ATP with CrADP. CrADP has been shown to be a competitive inhibitor to MgADP for myokinase (Dunaway-Mariano and Cleland, 1980*b*). Replacement of ATP by CrADP eliminates the regeneration of ATP by myokinase. When CrADP, instead of ATP, was added to suspensions of filaments both in relaxing and activating solutions, the same $\bar{\Gamma}$ values were obtained up to $K^2 \simeq 7 \times 10^{10}\ \text{cm}^{-2}$, as shown in Fig. 10.

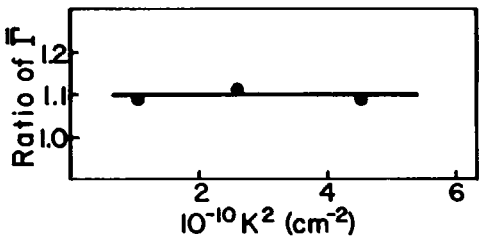

FIGURE 9 Ratio of the $\bar{\Gamma}$ value of filaments suspended in an activating solution ($\bar{\Gamma}_{a,v}$) to that in a relaxing solution ($\bar{\Gamma}_{r,v}$). Both solutions contained 1 mM vanadate. The effect of calcium ions on the active cross-bridge motions was suppressed by vanadate.

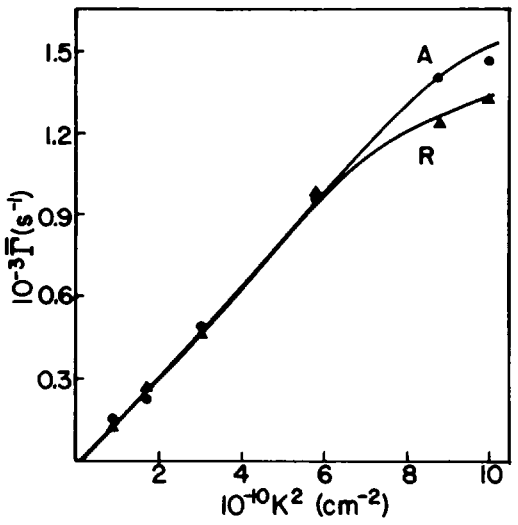

FIGURE 10 Plot of $\bar{\Gamma}$ vs. K^2 of thick filaments from *Limulus* suspended in a relaxing solution (curve *R*) and in an activating solution (curve *A*) with ATP in both solutions replaced by CrADP. The effect of calcium ions activating cross-bridge motions was suppressed by replacing ATP with CrADP. The effect of calcium ion on $\bar{\Gamma}$ value is suppressed by both DMS and DTBP.

Effect of Replacement of ATP with AMP-PNP. AMP-PNP is a nonhydrolyzable ATP analogue. Filament suspensions were dialyzed overnight first against an activating solution which contained no ATP. Presumably, due to the residual ATP contained in the filament preparation, the $\bar{\Gamma}$ value (Fig. 11, bar *A*, which shows the $\bar{\Gamma}$ value at $K^2 = 8.8 \times 10^{-10}cm^{-2}$) is still higher than that obtained with suspensions of filaments dialyzed against relaxing solution (Fig. 11, line *R*, bar *A*, which also shows the $\bar{\Gamma}$ value at $K^2 = 8.8 \times 10^{-10}cm^{-2}$). Then 2 mM AMP-PNP together with 2 mM glucose were added to the dialyzing solution and an additional 50 μg/ml of hexokinase was added directly into the dialyzing tube containing the filament preparation. After such treatment, the $\bar{\Gamma}$ value drops down to that of bar *B* in Fig. 11, which is close to the value of *R* in Fig. 10, the $\bar{\Gamma}$ value of filaments suspended in relaxing solution. This demonstrates that, without ATP, calcium ions can no longer activate the cross-bridge motions. If the sample was then dialyzed against normal activating solution, i.e., solution with 2 mM ATP, the $\bar{\Gamma}$ value increased tremendously to bar *C* in Fig. 10, which is comparable to the usual $\bar{\Gamma}_a$.

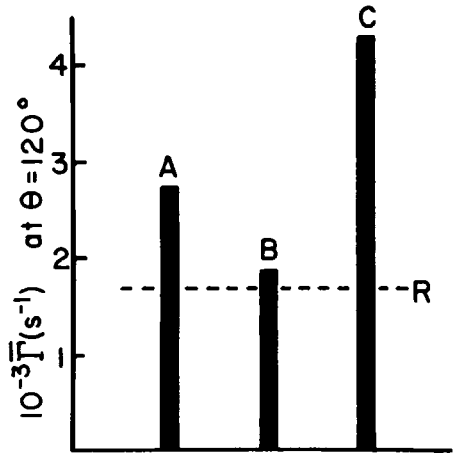

FIGURE 11 The effect of replacing ATP with AMP-PNP. (*A*) Filaments dialyzed against an activating solution with no ATP added. (*B*) Filaments dialyzed further in an activating solution containing 2 mM AMP-PNP, with 2 mM glucose and 50 μg/ml hexokinase added. (*C*) The result obtained after dialyzing in a standard ATP-containing solution. *R* shows the $\bar{\Gamma}$ value of a corresponding sample in a relaxing solution. All the $\bar{\Gamma}$ values shown were obtained at $K^2 = 8.8 \times 10^{10}\ cm^{-2}$ or $\theta = 120°$.

Thick Filaments from the Striated Adductor of Scallop

The activity of striated adductor muscle of the scallop is also believed to be regulated through myosin (Kendrick-Jones, et al., 1970; 1976). Fig. 12, curve *R*, illustrates the $\bar{\Gamma}$ values of isolated thick filaments from scallop muscle in relaxing solution. The size of thick filaments from striated muscle of the scallop is much less than that of thick filaments of *Limulus* with a length of 1.4 μm and a diameter of 21 nm. Therefore, it is not surprising that the $\bar{\Gamma}$ values are usually two to three times higher than those of the thick filaments from *Limulus* at $K^2 \cong 10 \times 10^2\ cm^{-2}$. After the addition of calcium ions, the $\bar{\Gamma}$ value increases from 2 to 4 kHz at $K^2 = 10 \times 10^{10}cm^{-2}$s shown by the difference between curves *A* and *R* in Fig. 12. This result established that calcium ions did induce a new mode of motion in isolated thick filaments from the scallop with

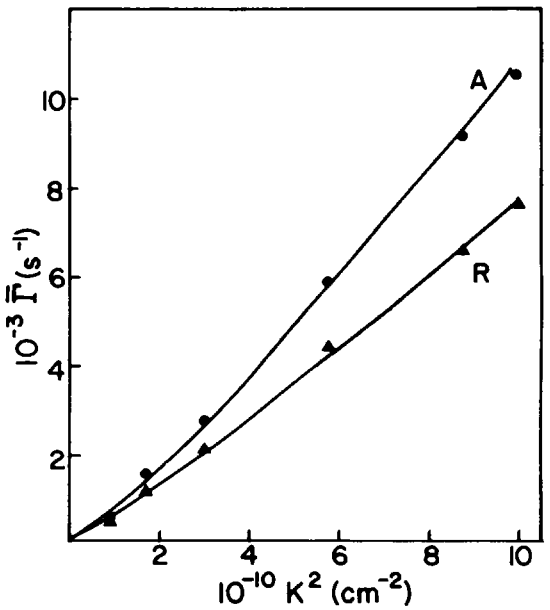

FIGURE 12 Plot of $\bar{\Gamma}$ vs. K^2 of isolated thick filaments from the striated adductor muscle of the scallop suspended in relaxing solution (curve *R*) and in activating solution (curve *A*). Calcium ions have similar effects on the $\bar{\Gamma}$ values of thick filaments from scallop as on $\bar{\Gamma}$ values of isolated thick filaments from *Limulus*.

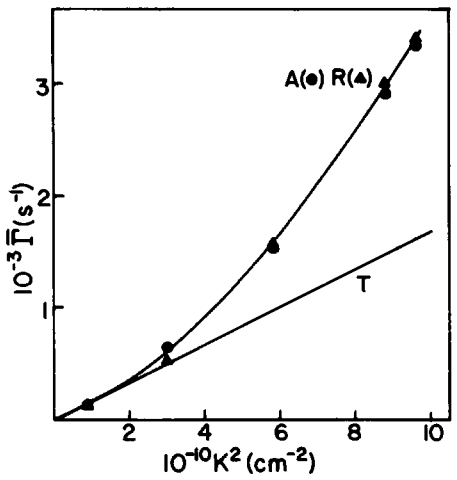

FIGURE 13 Plot of $\bar{\Gamma}$ vs. K^2 of isolated thick filaments from the sartorius of the frog suspended in a relaxing solution (R) and in an activating solution (A). Both suspensions gave the same $\bar{\Gamma}$ values showing calcium ions have no effect on the $\bar{\Gamma}$ value of the thick filaments isolated from the frog. Line T represents the computed $\bar{\Gamma}$ due to pure translational motions according to Eq. 4 for a rigid rod with length L = 1.5 μm and diameter d = 10 nm.

frequencies similar to those induced in thick filaments isolated from *Limulus* muscle.

Thick Filaments from the Sartorius of the Frog

The activity of skeletal muscle in the frog is known to be regulated by calcium ions through the thin filaments. Calcium ions were shown to have no effect on the quasi-elastic light scattering results from synthetic filaments of rabbit myosin (Suzuki and Wada, 1981). Our findings from isolated thick filaments from sartorius are in agreement. The $\bar{\Gamma}$ values of isolated thick filaments from sartorius suspended in relaxing solution and from filaments suspended in activating solution show no difference (Fig. 13). Heating to 42°C for 10 min had no effect on either. In Fig. 13, the straight line T represents the $\bar{\Gamma}$ due to pure translational motion according to Eq. 6 for a rigid rod with length L = 1.5 μm and diameter d = 10 nm.

CONCLUSION

The experimental results presented here unambiguously confirm our earlier supposition that the increase in the average linewidth, $\bar{\Gamma}$, of the autocorrelation function from isolated thick filaments of *Limulus* due to the presence of calcium ions (Kubota et al., 1983) is related to activation of cross-bridges. (*a*) The increase disappeared after cleavage of the S-1 and S-2 moieties from the thick filaments with papain. (*b*) The increase disappeared after cross-linking the myosin heads to the backbone of the thick filament. (*c*) The increase disappeared following heat denaturation of the S-1. (*d*) Congo Red, known to activate other muscles, caused an increase in $\bar{\Gamma}$. (*e*) Myosin ATPase inhibitors, vanadate, or replacing ATP with CrADP or AMP-PNP, suppressed the increase. (*f*) Calcium ions have similar effects on thick filaments isolated from scallop, also known to be myosin regulated. (*g*) Thick filaments isolated from thin filament-regulated muscle do not show these calcium-activated effects. So far, the quasi-elastic light scattering method is unique when applied to myosin regulated thick filaments in that it can detect cross-bridge motions without the intervention of any labeling molecules. It should be a very promising method to analyse the kinetics and mechanisms of cross-bridge motion.

The increase of $\bar{\Gamma}$ values can be used to estimate the frequency of cross-bridge motions in the isolated thick filament suspension. For *Limulus* and scallop, a 2–4 kHz frequency range was observed with the cross-bridges moving under practically load-free conditions. Obviously this frequency cannot be ascribed to cross-bridges in a muscle fiber under tension. However, changes in tension (Ford et al., 1977) and in sarcomere length (Barden and Mason, 1978) in muscle fibers have been observed that occur in this time scale. Thomas et al. (1980) have reported that for rabbit skeletal muscle in the relaxed state, the myosin head undergoes a rapid rotational motion with an effective rotational correlation time of 10 μs, which is insensitive to the presence or absence of calcium ions. Thus, the active cross-bridge motions reported in this article may be different from those reported by Thomas et al. (1980).

REFERENCES

Acemann, E. P. 1953. Enzymatic degradation of adenosine triphosphate and the shortening of actomyosin. *Biochemistry (Moscow)*. 18:71–78.

Arata, T., and H. Shimizu. 1981. Spin label study of actin-myosin-nucleotide interaction in contracting glycerinated muscle fibers. *J. Mol. Biol.* 151:411–437.

Barden, J. A., and P. Mason. 1978. Muscle crossbridge stroke and activity revealed by optical diffraction. *Science (Wash. DC)*. 199:1212–1213.

Borejdo, J., S. Putman, and M. F. Morale. 1979. Fluctuation in polarized fluorescence. Evidence that muscle cross bridges rotate repetitively during contraction. *Proc. Natl. Acad. Sci. USA*. 76:6346–6350.

Chen, S. J., B. Chu, and R. Nossal, editors. 1981. Scattering Technique Applied to Supramolecular and Non-equilibrium Systems. Plenum Publishing Corp., New York.

Cooke, R. 1981. Stress does not alter the conformation of a domain of the myosin cross-bridge in rigor muscle fiber. *Nature (Lond.)*. 294:570–571.

Cooke, R., M. S. Crowden, and D. D. Thomas. 1982. Orientation of spin labels attached to cross-bridges in contracting muscle fibre. *Nature (Lond.)*. 300:776–778.

Dunaway-Mariano, D., and W. W. Cleland. 1980*a*. Preparation and properties of chromium (III) adenosine 5'-triphosphate chromium (III) adenosine 5'-diphosphate and related chromium (III) complex. *Biochemistry*. 19:1496–1505.

Dunaway-Mariano, D., and W. W. Cleland. 1980*b*. Investigations of substrate specificity and reaction mechanism of several kinases using chromium (III) adenosine 5'-triphosphate and chromium (III) adenosine 5'-diphosphate. *Biochemistry*. 9:1506–1515.

Elliot, G. F., J. Lowey, and B. M. Millman. 1967. Low-angle x-ray diffraction studies of living striated muscle during contraction. *J. Mol. Biol.* 25:31–45.

Fan, S.-F. 1963. The dichroism of Congo Red stained toad and honey bee striated muscle. *Acta Physiol. Sinica*. 25:240–250.

Fan, S.-F. 1964. Shortening in Congo Red solution of myofibrils isolated from glycerinated muscle fiber. *Sci. Sinica.* 13:692–693.

Fan, S.-F., M. M. Dewey, D. Colflesh, B. Chu, and K. Kubota. 1983*a*. Active cross-bridge motion of isolated *Limulus* thick myofilaments as revealed by quasi-elastic light-scattering studies. *Biophys. J.* 41 (2, Pt. 2): 261*a*. (Abstr.)

Fan, S.-F., M. M. Dewey, D. Colflesh, and B. Chu. 1983*b*. Further evidence of cross-bridge motions in *Limulus* thick myofilament suspensions. *In* The Application of Laser Light Scattering to the Study of Biological Motions. J. C. Earnshaw and M. W. Steer, editors. Plenum Publishing Corp., New York. 477–483.

Fan, S.-F., M. M. Dewey, D. Colflesh, P. Brink, and B. Chu. 1983*c*. Dynamic laser light scattering of papain-treated thick filaments from *Limulus* striated muscle in suspension. *In* Contractile Mechanisms in Muscle: Structural Dynamics. G. Pollack and H. Sugi, editors. Plenum Publishing Corp., New York.

Fan, S.-F., and M.-X. Hong. 1964. Effect of salt concentrations, adenosine triphosphate, pH, alcohol and urea on the binding of Congo Red with glycerinated striated muscle fiber. *Acta Physiol. Sin.* 27:178–188.

Fan, S.-F., and Y.-S. Wen. 1979. Concerning the binding sites of myofibril with Congo Red and the dichroism change with myofibril length of Congo Red stained glycerinated sartorious muscle fiber. *Acta Physiol. Sin.* 31:227–238.

Filatova, L. G., and J. G. Shrankfield. 1968. Interaction between the dye, Congo Red and the contractile proteins of muscle. *Biophysics (Moscow).* 13:899–902.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1977. Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *J. Physiol. (Lond.).* 269:441–575.

Fraser, A., E. Eisenberg, W. W. Kielley, and F. D. Carlson. 1975. The interaction of heavy meromyosin and subfragment 1 with actin. Physical measurements in the presence and absence of adenosine triphosphate. *Biochemistry.* 14:2207–2214.

Fujime, S., S. Ishiwata and T. Maeda. 1972. F-actin-heavy meromyosin complex studied by optical homodyne and hetrodyne methods. *Biochim. Biophys. Acta.* 283:351–362.

Goodno, C. C. 1979. Inhibition of myosin ATPase by vanadate ion. *Proc. Natl. Acad. Sci. USA.* 76:2620–2624.

Goodno, C. C., and E. W. Taylor. 1982. Inhibition of actomyosin ATPase by vanadate. *Proc. Natl. Acad. Sci. USA.* 79:21–25.

Guth, K. 1980. Polarization of tryptophane fluorescence measurements in muscle. A re-evaluate. *Biophys. Struct. Mechanism.* 6:81–93.

Hanson, R. J. 1971. A numerical method for solving Fredholm integral equation of the first kind using singular values. *SIAM (Soc. Ind. Appl Math. J. Numer. Anal.* 8:616–622.

Haskell, R. C., and F. D. Carlson. 1981. Quasi-elastic light-scattering studies of single skeletal muscle fibres. *Biophys. J.* 33:39–62.

Haselgrove, J. C. 1975. X-ray evidence for conformation changes in the myosin filaments of vertebrate striated muscle. *J. Mol. Biol.* 92:113–143.

Highsmith, S., K. Akasaka, M. Konard, M. Goody, K. Holmes, N. Wade-Jardetzky, and O. Jardetzky. 1979. Internal motions in myosin. *Biochemistry.* 18:4238–4244.

Highsmith, S., and O. Jardetzky. 1980. G-actin binding quenches internal motions in myosin subfragment-1. *FEBS (Fed. Eur. Biochem. Soc.). Lett.* 121:55–60.

Highsmith, S., and O. Jardetzky. 1981. Internal motions in myosin. 2. *Biochemistry.* 20:780–783;

Hunter, M. J., and M. L. Ludwig. 1962. The reaction of imidoesters with proteins and related small molecules. *J. Am. Chem. Soc.* 84:3491–3504.

Huxley, A. F. 1974. Muscle contraction. *J. Physiol. (Lond.).* 243:1–43.

Huxley, H. E. 1969. The mechanism of muscular contraction. *Science (Wash. DC).* 164:1356–1366.

Huxley, H. E. 1972. Structural changes in the actin- and myosin-containing filaments during contraction. *Cold Spring Harbor Symp. Quant. Biol.* 37:1336–1376.

Huxley, H. E., and H. Brown. 1967. The low-angle x-ray diagram of vertebrate striated muscle and its behavior during contraction and rigor. *J. Mol. Biol.* 30:383–434.

Huxley, H. E., A. R. Faruqi, J. Bordas, H. M. J. Koch, and J. R. Milch. 1980. The use of synchrotron radiation in time-resolved x-ray diffraction studies of myosin layer-line reflections during muscle contraction. *Nature (Lond.).* 284:140–143.

Huxley, H. E., A. R. Faruqi, M. Kress, J. Bordas, and M. H. J. Koch. 1982. Time resolved x-ray diffraction studies of the myosin layer-line reflection during muscle contraction. *J. Mol. Biol.* 158:637–648.

Huxley, H. E., R. M. Simmons, A. R. Faruqi, M. Kress, J. Bordas, and M. H. J. Koch. 1981. Millisecond time-resolved change in x-ray reflection from contracting muscle during rapid mechanical transients, recorded using synchrotron radiation. *Proc. Natl. Acad. Sci. USA.* 78:2297–2301.

Ivanov, V. I., O. N. Barovikova, E. P. Ivantecva, N. A. Lebedeva, and V. I. Shavobaiko. 1976. Absence of the ability of highly purified myosin preparations to form contractile complexes with DNA. *In* Biophysics and Biochemistry of Muscular Contraction. G. M. Frank, editor. Nauka, Moscow. 33–36.

Kenrick-Jones, J., W. Lehman, and A. G. Szent-Gyorgyi. 1970. Regulation in mollusca muscle. *J. Mol. Biol.* 54:313–326.

Kendrick-Jones, J., E. M. Szentkiralyi, and A. G. Szent–Gyorgyi. 1976. Regulatory light chain in myosin. *J. Mol. Biol.* 104:747–775.

Komitz, D. P., F. R. Mitchell, T. Niehei, and C. M. King. 1965. The papain digestion of skeletal myosin A. *Biochemistry.* 4:2273–2381.

Koppel., D. R. 1972. Analysis of macromolecular polydispersity in intensity correlation spectroscopy. The method of cumulants. *J. Chem. Phys.* 57:4814–4820.

Kubota, K., B. Chu, S.-F. Fan, M. M. Dewey, P. Brink, and D. Colflesh. 1983. Quasi-elastic light scattering of suspensions of *Limulus* thick myofilaments in relaxed (long) activated and rerelaxed (short) states. *J. Mol. Biol.* 166:329–340.

Lehman, W., and A. G. Szent-Gyorgyi. 1975. Regulation of muscular contraction. Distribution of actin control and myosin control in the animal kingdom. *J. Gen. Physiol.* 66:1–30.

Maeda, T., and S. Fujime. 1981. Effect of filament flexibility on the dynamic light-scattering spectrum with special reference to fd virus and muscle thin filaments. *Macromolecules.* 14:809–818.

Maeda, T., and S. Fujime. 1984. Spectrum of light quasi-elastically scattered from solution of semiflexible filaments in the dilute and semidilute regimes. *Macromolecules.* 17:2381–2391.

Maeda, T., S. Ishiware, and S. Fujime. 1974. Light-beating study of the effect of β-actinin on the interaction between F-actin and heavy meromyosin. *Biochim. Biophys. Acta.* 336:445–452.

Meisner, D., and G. Beinbrech. 1979. Alteration of crossbridge angle induced by β,γ-imido-ATP. Electron microscope and optical diffraction studies on myofibrillar fragments of abdominal muscle of the crayfish, *Orconectes limesus. Eur. J. Cell. Biol.* 9:189–193.

Newman, J., and F. D. Carlson. 1980. Dynamic light-scattering evidence for the flexibility of native muscle thin filaments. *Biophys. J.* 29:37–48.

Newman, J., H. L. Swinney, and L. A. Day. 1977. Hydrodynamic properties and structure of virus. *J. Mol. Biol.* 116:593–606.

Niehei, T., R. A. Mendelson, and J. Botts. 1974. Use of fluorescence polarization to observe change in attitude of S-1 moieties in muscular fiber. *Biophys. J.* 14:236–242.

Pecora, R. 1968. Spectral distribution of light scattered from flexible-coil macromolecules. *J. Chem. Phys.* 49:1032–1035.

Pecora, F. 1981. Polarized dynamic light scattering as a probe of macromolecular interamolecular motions. *In* Scattering Technique Applied to Supramolecular and Non-equilibrium Systems. S. H. Chen, B. Chu, and R. Nossal, editors. Plenum Publishing Corp., New York. 161–172.

Reisler, D., N. Burke, R. Josephs, and W. J. Harrington. 1973. Crosslinking of myosin and myosin filaments. *J. Mechanochem. Cell Motil.* 2:163–179.

Sëidel, J. C., M. Chopek, and J. Gergely. 1970. Effect of nucleotides and pyrophosphate on spin labels bound to S. thiol groups of myosin. *Biochemistry.* 9:3265–3272.

Sutoh, K., and W. F. Harrington. 1977. Cross-linking of myosin thick filaments under activity and rigor conditions. A study of the radial disposition of cross-bridges. *Biochemistry.* 16:2441–2449.

Suzuki, N., and A. Wada. 1981. Quasielastic light scattering stucy of solutions of synthetic myosin filaments. *Biochim. Biophys. Acta.* 670:408–420.

Teranishi, M. 1957. Interaction between Congo Red-myosin and adenosinetriphosphate. I. The effects of pH, potassium chloride and ATP concentration on the interaction between Congo Red myosin and ATP. *Sapporo Igaku Zasshi.* 11:239–244.

Teranishi, M. 1957. Interaction between Congo Red-myosin and adenosinetriphosphate. II. The interaction between Congo Red-myosin and pyrophosphate. *Sapporo Igaku Zasshi.* 12:173–177.

Teranishi, M. 1957. Interaction between Congo Red-myosin and adenosinetriphosphate. III. Effect of Congo Red on myosin and adenosinetriphosphatase. *Sapporo Igaku Zasshi.* 12:318–322.

Thomas, D. D. 1978. Large-scale rotational motions of proteins detected by electron paramagnetic resonance and fluorescence. *Biophys. J.* 24:439–462.

Thomas, D. D., and R. Cooke. 1980. Orientation of spin-labeled myosin heads in glycerinated muscle fibers. *Biophys. J.* 32:891–906.

Thomas, D. D., S. Ishiwata, J. C. Seidal, and J. Gergely. 1980. Submillisecond rotational dynamics of spin-labeled myosin heads in myofibrils. *Biophys. J.* 32:873–890.

Thomas, D. D., J. C. Seidel, and J. Gergely. 1979. Rotational dynamics of spin labeled F-actin in the submillisecond time range. *J. Mol. Biol.* 132:257–273.

Thomas, D. D., J. C. Seidel, J. S. Hyde, and J. Gergely. 1975. Motions of subfragment-1 in myosin and its supramolecular complexes: Saturation transfer electron paramagnetic resonance. *Proc. Natl. Acad. Sci. USA.* 72:1729–1733.

Yamanaotik, K., and T. Schiao. 1980. Substructure of myosin subfragment-1 as revealed by digestion with proteolytic enzyme. *J. Biochem. (Tokyo).* 87:219–226.