Filament Interaction Monitored by Light Scattering in Skinned Fibers

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Abstract The intensity of light scattered by chemically skinned rabbit psoas fibers in relaxed, rigor, and activated states was monitored at 90° to the incident beam. In the relaxed state, scattering varied in proportion to the volume of muscle in the beam. Scattering increased to 2.3 times the resting value when rigor was induced by withdrawal of MgATP or when the myofibrils were activated by the caffeine-induced release of Ca from the sarcoplasmic reticulum. The rigor-induced increase in scattering decreased monotonically when MgATP was reintroduced stepwise (0-100 μM). This decrease in scattering was accompanied by an increase in tension up to an optimum MgATP level of ~10 μM, and then tension decreased at higher concentrations (10-100 μM). The increase in scattering during both rigor and activation was dependent upon fiber length. At lengths when thick-thin filament overlap was near zero, the light signal due to rigor and activation fell to within 10% of the signal for the relaxed fiber at that length. The signal during rigor increased only minimally (~10%) when stretch (~1%) was applied. This increase in signal was small despite a measured 5- to 10-fold increase in tension and an estimated twofold increase in stiffness. Thus, the increased light scattering caused by rigor and activation depends on filament overlap and not tension, stiffness, or substrate binding.

Introduction Both light scattering and birefringence of relaxed muscle have been shown to change upon induction of rigor or activation. During a twitch, light scattering in intact muscle increased (Buchthal et al., 1939; Hill, 1949; Barry and Carnay, 1969), and birefringence decreased (Eberstein and Rosenfalck, 1963) relative to the measurements on relaxed muscle. In glycerinated muscle, there was an increase in scattering (Bozler, 1958) and a decrease in birefringence (Taylor, 1975) when rigor was induced by reducing the concentration of substrate (MgATP) in the medium. Both the change in birefringence upon transition from relaxed to rigor or Ca-activated states (Eberstein and Rosenfalck, 1963; Taylor, 1975) and the change in scattering due to contraction (Barry and Carnay, 1969) depended on the extent of thick-thin filament overlap.

Part of the change in birefringence that occurs upon transition from rigor to relaxation has been ascribed to the extension of myosin cross-bridges towards the thin filaments (Taylor, 1975). The basis for the increase in scattering due to rigor in glycerinated muscle and to activation in intact muscle has not been...
defined beyond the suggested dependence on "aggregation" of filaments (Bozler, 1958) and a possible correlation with molecular events of contraction (Barry and Carnay, 1969).

There is also data on light scattering (turbidity) and birefringence from isolated myofibrils and contractile proteins. In these preparations, the data is believed to reflect degrees of formation of actomyosin from actin and myosin (see Discussion in Kominz, 1971). Our findings lead us to conclude that the increase in scattering by the intact arrays of filaments in skinned fibers also results from interaction between actin and myosin filaments.

This paper deals with the findings on rabbit psoas muscle fibers. Data on crayfish and human skeletal fibers will be reported at another time. Some of the results have been reported at the Biophysical Society Meetings (Katz and Reuben, 1977).

**MATERIALS AND METHODS**

**Preparation**

Small bundles (3 mm diameter, 1 cm length) of psoas muscle were tied at rest length and then cut free from the rest of the muscle. The bundles were "chemically skinned" by immersion in a solution lacking ionized calcium (Wood et al., 1975). The solution contains 5 mM ethylene glycol-bis (β aminoethyl ether)-N,N'-tetraacetic acid (EGTA), 170 mM potassium propionate (KPr), 2.5 mM magnesium acetate, 2.5 mM K₂Na₂ATP and 5 mM imidazole (pH 7.0).

After several hours in the "skinning" solution at 5–10°C, the bundles were stored at −10 to −20°C in "skinning" solution containing 50% glycerol. This storage procedure maintains the viability of both the contractile proteins and the sarcoplasmic reticulum for many months (Reuben et al., 1977). Strands of 10-20 fibers were removed from the stored bundles and single fibers or bundles of 2-8 fibers were dissected for the experiments.

**Solutions**

All solutions had the same ionic strength (200 ± 5 mM) and were buffered for pH (7.0) with imidazole (5 mM). When EGTA, CaEGTA, ethylenediamine-tetraacetic acid (EDTA), or MgEDTA was added, the concentration of KPr was reduced. The solution referred to as relaxing solution (R) had the same composition as the skinning solution given above. The solution used to induce rigor contained 10 mM EDTA and no EGTA, Mg, or ATP. EGTA-buffered Ca solutions were made as previously described (Brandt et al., 1972).

In experiments in which the MgATP concentration was varied from 0 to 100 µM, different amounts of MgEDTA were added to solutions containing fixed levels of ATP (5 mM) and free EDTA (10 mM). The concentrations of MgATP formed were calculated by solving the multiple equilibria with a computer (see Reuben et al., 1971). In these solutions, free Mg did not exceed 10 µM, and free ATP varied between 4.9 and 5.0 mM. The apparent association constants (M⁻¹) that were used are as follows:

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These values are within the range found in the literature for 0.1-0.2 M ionic strength and 20-25°C. The rationale for selecting these particular values has been given in a previous publication (Orentlicher et al., 1977).

**Apparatus**

Two instruments were constructed for different phases of the investigation. The first, with provision for varying the wavelength, has the basic arrangement shown in Fig. 1. An Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring, Md.) with a 100-watt xenon light source and regulated power supply is modified in the following manner: (a) The cuvette chamber is replaced with a front-surface mirror and quartz lens so that the monochromatic light is focused upward through the quartz bottom of a plastic chamber onto a muscle fiber mounted in this chamber. (b) The light scattered by the fiber is detected by a quartz fiber-optics light guide positioned perpendicular to both the axis of the fiber and the incident light. The fiber-optics light guide is stationary and subtends a small angle which includes the 90° component of scattered light (Fig. 2). Light from the distal end of the light guide is passed through the collector monochromator adjusted to the same wavelength as the source monochromator and is measured by a Hamamatsu photomultiplier (type R212, Hamamatsu, Middlesex, N.J.). (c) The photomultiplier power supply and electronic circuitry of the Aminco-Bowman unit are replaced by a unit of our own design to provide greater flexibility in adjusting gain, frequency response, dark current compensation, and voltage offsets. The output voltage derived from the scattering signal is large enough even from single fibers so that photomultiplier noise and circuit drift are negligible problems. Scattering from solutions and chamber is ~20% of the scattering from single resting fibers and is routinely subtracted from experimental readings.

Isometric tensions are monitored with a Bionix type F 100 (Bionix, Richmond, Calif.) strain gauge transducer mounted on a micromanipulator. Recordings are made either with an X-Y recorder to monitor scattered light vs. wavelength, or with a multichannel strip chart recorder to display scattered light simultaneously with tension. Not shown in Fig. 1 is a light-tight lid that closes during the experimental procedure. When the lid is opened for solution exchange or for fiber manipulation, a flag mechanically coupled to...
the lid falls in front of the photomultiplier. This prevents long time-constant sensitivity changes in the photomultiplier caused by the sudden ambient illumination.

The second instrument was constructed for more precise alignment of the fiber with the optical components. This was necessary for the quantitative measurements of light scattering and the comparison of these absolute values between fibers of different sizes. Monochromators are not used. Instead, the incident beam is white light from a quartz-iodide bulb with no wavelength filters. This light is focused by a condensing lens onto a glass fiber-optics light guide, which in turn illuminates the muscle fiber. This method provides a higher intensity and a more even illumination of the fiber than does the first apparatus. A second glass light guide mounted at a right angle to the first conducts light scattered by the fiber directly to a photomultiplier.

![Diagram of fiber-optics setup](image)

**Figure 2.** Dependence of signal on vertical position of fiber. At the right, a section through the chamber showing position of the fiber (oriented perpendicular to the plane of the drawing) in relation to both source and collecting optics. Scale of drawing is shown on ordinate at left. At the left, intensity of light scattering was recorded with the relaxed fiber positioned at different heights above the base of the chamber (arrows). Note that the optimum vertical position is slightly below the mid-point of the collector light-guide. Further description in text.

Fig. 2 shows the position of the muscle fiber in the chamber relative to the source and collector fiber-optics bundles. The variation of scattering intensity with vertical position is also shown. The maximum sensitivity is slightly below the center line of the collector fiber-optics, inasmuch as the incident beam diverges and its intensity decreases with distance from the source. During experiments the muscle fiber rests on two stainless steel spacers (not shown) so that the fiber is positioned at the height of maximum sensitivity. The scattering signal is not sensitive to lateral displacements due to the large diameter of the source. This apparatus (without monochromators) gives a scattered light signal 50–100 times greater than does the apparatus with monochromators.

For the experiments correlating light scattering with muscle mass (Fig. 3), the second experimental setup was used. Uniform bundles of two to six fibers were stretched to 120% of slack length ($L_s$) and positioned on the spacers. Scattering of light in the relaxed state was recorded, and scattering due to the chamber itself was subtracted. The length of the bundle between the clamps was measured with a precision of ~5% of the total length, and the ends were cut where they entered the clamps. With a fine needle, the bundle was transferred to 0.05 ml of NaOH (0.05 N) and the total protein content was
determined essentially as described by Elison et al. (1965). Aliquots of bovine serum albumin were carried through the same procedure to construct a standard curve. Inasmuch as bundles of different lengths were used for the protein determination whereas the scattering intensity was recorded from a region of fixed length (~2 mm; see Fig. 2), the total protein content was divided by the length of the bundle.

RESULTS

Light Scattered by Relaxed Fibers at 1.2 L_s

Earlier studies (Buchthal et al., 1949; Hill, 1953) of the optical properties of intact muscle have shown that absorption of incident light is small in comparison to scattering. The data of Fig. 3 allow us to conclude that the fraction of light absorbed and scattered by bundles of skinned fibers is small in comparison to that transmitted. In this experiment the lengths (4-6 mm) of bundles of two to six fibers were measured (at ×80 magnification) and after recording light-scattering intensity, the bundles were removed from the chamber, and the mass of muscle was determined in terms of micrograms of protein per millimeter (see Methods). The plot of Fig. 3 shows the proportionality between scattering (arbitrary units) and micrograms of protein per millimeter length of muscle. This proportionality implies that the absorption and scattering of light by muscles of this size must be small so that each layer through the muscle experiences virtually the same incident light intensity. An upper limit of muscle size for which scattering and absorption become large and the proportionality no longer holds must exist, but it was not determined in this study.

Light Scattered by Relaxed Fibers at Different Lengths (L_s-1.5 L_s)

The mass of muscle in the beam may also be changed by stretching the muscle to different lengths. Fig. 4 is a plot of relative volume vs. relative scatter for
muscle lengths ranging from $L_s$ to 1.5 $L_s$. The change in volume of muscle in the light beam for a measured increase in length was calculated from the inverse proportionality between length and volume. The light scattering measurements are reasonably well described by the theoretical curve (45° line) for the proportionality between calculated relative volume and relative scatter.

In these experiments no attempt was made to record at higher gain the small transient changes in light scattering that occur when intact resting muscle is stretched by 1-2% (Hill, 1953; Flitney, 1975). Our results are consistent, however, with Flitney's observation that a small decrease in scattering intensity persisted after the transient change had subsided. He, too, ascribed the maintained change in scatter to the decrease in fiber volume. The relationship between volume of relaxed muscle and scattering provides a basis for comparison and normalization of the following data.

The possibility that structures other than the contractile apparatus contribute to scattering by relaxed fibers has not been investigated extensively. However, in two experiments in which skinned fibers were treated with Brij-58 (1%, IC1 United States, Inc., Wilmington, Del.) to disrupt the sarcoplasmic reticulum (Orentlicher et al., 1974), scattering by the relaxed fibers was reduced (10-20%) whereas the increase in scattering due to induction of rigor was unaffected.

**Increase in Light Scattering during Rigor**

Removal of substrate (MgATP) from the medium bathing skinned fibers induces both a tension (Reuben et al., 1971) and an increase in fiber stiffness (Kawai and Brandt, 1976). Simultaneously, there is an increase in the fraction of
light scattered (Fig. 5). Both the amplitude of tension and the light signal can be reproduced numerous times by cyclic removal and addition of substrate. The removal of MgATP was accomplished by washing three times (artifacts visible on tension trace) with a rigor-inducing solution that contained 10 mM EDTA (see Methods). The increase in scattering due to induction of rigor ranged from 113 to 164% of scattering by relaxed muscle at slack length with a mean of 152.9 ± 13.5% (mean ± SD) for 12 single fibers. In three experiments, fibers in rigor were exposed to 1.0 mM N-ethylmaleimide (NEM) for several minutes before reintroduction of MgATP to the bath (data not shown). Treatment of actomyosin systems with NEM can prevent dissociation by MgATP (Pemrick and Weber, 1976), and in our experiments the increase in scattering due to rigor was maintained after adding substrate to the medium.

The responses of Fig. 5 were repeated, but the wavelength (λ) of incident light was varied from 350 to 650 nm (Fig. 6). Although the light signal is larger in rigor than in the relaxed state for all λs, scattering in both states shows a similar dependence on λ. The absence of peaks in the spectrum for both states of the muscle is in keeping with the findings of Hill (1949) on relaxed intact muscle. The data of Fig. 6 also indicate that the components of muscle responsible for scattering light during rest or rigor do not behave as a single population of small (<< λ) randomly oriented particles. If this were the case, then the scattering intensity should vary inversely as λ⁴ (Jenkins and White, 1957).

The following experiments were done to determine whether the increase in signal from relaxed to rigor state correlates with the tension, stiffness, binding of MgATP, extension of cross-bridges, or attachment of cross-bridges to actin.
Signal Change during Stretch of Muscle in Rigor

Extension (by ~1%) of a muscle in rigor causes stiffness to increase about twofold (Kawai and Brandt, 1976); after an initial transient, the tension is maintained at a level 5-10 times above the nonstretched value. In Fig. 7 both the tension (upper trace) and the light intensity (lower trace) were recorded in the relaxed fibers, during rigor, and during rigor plus stretch. The light signal more than doubled upon induction of rigor and the rigor-tension attained 20 mg. Stretch of ~1% increased the tension to ~160 mg, measured at the time just before release, and the light signal increased <10% above the rigor level.

The cycle was repeated with a slightly larger stretch, and the final step was reintroduction of MgATP. In spite of the eightfold increase in tension and presumably a large increase in stiffness during the stretch, the change in signal above that in rigor was small. In six additional experiments of this type the increase in signal during stretch ranged from 3 to 10% of the rigor-signal.

Variation of Light Signal with Changes in MgATP Concentration (0–100 μM)

When the MgATP concentration is increased from 0 to 100 μM in the absence of Ca, skinned fibers stretched to 1.2 Ls, develop tension that attains a maximum
(~50% $P_o$) between 3 and 10 $\mu$M and then decreases to resting level at concentrations above the optimum value (Reuben et al., 1971). The upper trace of Fig. 8 shows the stepwise increase of tension up to 10 $\mu$M MgATP. The lower trace is scattering intensity, which first increased from a low level in the relaxed state to a maximum value when rigor was induced by removing substrate. The scattering signal then declined stepwise from the maximum as the substrate concentration increased from ~0.1 $\mu$M to >100 $\mu$M. Fig. 9 is a plot of the tension and scattering signal obtained from four small bundles exposed to increasing concentrations of MgATP.

Although it was not investigated in detail in this study, it is of interest that light scattering depends on the order in which the MgATP concentration is changed. For example, the reduction in light scattering by ~50% caused by increasing MgATP concentration from 0 to 10 $\mu$M (Fig. 9) was maintained upon returning the fibers to the MgATP-free solution. To obtain the original rigor signal the fibers had to be exposed to a high substrate concentration ($\geq$100 $\mu$M) before reexposure to the MgATP-free medium. The relatively large tensions developed by fibers exposed to 10 $\mu$M MgATP were only slightly reduced (10–20%) when fibers were directly returned to the MgATP-free medium. Thus, tension may increase while scattering decreases, decrease while scattering decreases, decrease while scattering.
decreases, or it may decrease while scattering remains constant, depending upon the order of changing MgATP concentration.

**Dependence of the Rigor-Signal on Filament Overlap**

The data of Fig. 10 were obtained in the following manner. The step increase and decrease in light scattering for transitions from relaxed to rigor and back to relaxed state (as shown in Fig. 5) were first recorded in fibers held at or slightly above slack length \( L_s \). The muscles were then stretched to a new length \( L \), and the procedure of removing and reintroducing substrate was repeated. The cycle was repeated at several different lengths for each fiber.

Because scattering by relaxed fibers also changes with fiber length (Fig. 4), it was necessary to normalize the rigor-induced increase in scattering \( \Delta S_{Ri} \) at each length \( L \) to the relaxed fiber scattering \( S_L \) at that length. The ratio, \[ \frac{\Delta S_{Ri}}{S_L} \], denoted by \( \eta_{Ri} \), is divided by \( \eta_{Ri}^0 \), the ratio obtained at \( L_s \), and plotted against \( L/L_s \). The results from six single fibers tested at lengths ranging from \( L_s \) to 1.7 \( L_s \) are shown in Fig. 10. The linear portion of the plot, when extrapolated, intersects the abscissa at a length of 1.7 \( L_s \). This value is close to the length at which actin filaments are disengaged from myosin filaments. The length of thick filaments is 1.5–1.7 \( \mu \)m, and the thin filaments are 1.1–1.3 \( \mu \)m.\(^1\) At \( L_s \), the sarcomere length is \( \sim 2.6 \mu \)m. If the sarcomere lengths remain uniform along the fiber at all fiber lengths, the point of no overlap should not exceed 1.65 \( L_s \). Although sarcomere uniformity over the entire length of the fiber is not attained (Huxley and Peachey, 1961; Reuben et al., 1977), it is clear that the increase in scattering due to rigor declines with increasing fiber lengths as would

\(^1\) Eastwood, A. B. Unpublished data.
Figure 9. Variations in tension and light scattering induced by increasing substrate concentration in the absence of Ca. Tension (○) and scatter (●) from four experiments like those of Fig. 8 are plotted against the negative logarithm of the MgATP concentration (pS). Each point represents a mean value obtained after normalizing tension and scattering for each bundle to the respective maximum values for that bundle. Bars indicating ±1 SD for the light scattering data at pS 5 are typical of the variability throughout the curve.

Figure 10. Light scattering in rigor at increasing fiber lengths. The increase in scattering during rigor decreases with increasing fiber length. Both scattering and length are normalized as described in the text. Data were obtained on six single fibers; points show means and standard errors of six determinations for lengths of 1.0-1.6 Ls, and five determinations at 1.7 Ls.

be expected if it were proportional to the degree of filament overlap. Inasmuch as the change in concentration of MgATP between the relaxed and rigor conditions was the same at all fiber lengths, scattering does not appear to be correlated with binding of MgATP to myosin. The most plausible interpretation
of these data is that the increase in scattering requires interaction between the thick and thin filaments. However, it is not apparent whether the increase in scattering reflects only an increase in the number of cross-bridges attached to actin, or whether it also depends upon other factors such as a change in the degree of hydration of the proteins (Kominz, 1971).

**Light Scattering Associated with Transient Activation**

Transient tensions whose amplitudes closely approach $P_0$ can be induced by application of caffeine to mammalian skinned fibers that were previously exposed to Ca to load the SR (Wood et al., 1975). In Fig. 11, the increase in light signal concomitant with a caffeine-tension can be directly compared to $\Delta S_{RI}$.

![Diagram](image)

**Figure 11.** Comparison between scattering during rigor and during a transient activation. In the upper trace, two tensions are shown. The first tension was elicited by caffeine after exposing the fiber for 3 min to a solution containing MgATP, EGTA, and Ca (pCa 6.4). Calcium and EGTA were removed (−Ca) before application of 20 mM caffeine (Caff). At (−S), a rigor-tension was induced as described for Fig. 5. In the lower trace, scatter during rest, during the caffeine-induced tension, and during rigor are recorded. The initial step above 0 base line shows light scattering by the relaxed fiber, and the amplitude of this signal did not change noticeably when the buffered Ca solution was introduced (+Ca). Before removal of Ca (−Ca), the 0 base line was again recorded by turning off the photomultiplier. A transient increase in scattering followed the application of caffeine, and a slightly larger, maintained increase in scattering accompanied the induction of rigor. Further description in text.

cau sed by induction of rigor in the same fiber. In this experiment the relaxed fiber was initially exposed for 3 min to a Ca-buffered solution to load the sarcoplasmic reticulum with Ca. Before application of 20 mM caffeine the Ca-buffered solution was replaced by EGTA-free solution. After relaxation of the tension induced by caffeine, MgATP was removed for −1 min and then it was reintroduced.

The amplitude of the light signal change during the 1-min rigor period is comparable to the peak signal change recorded during the transient caffeine-tension. In seven experiments of this type, the amplitude of the peak signal change ranged from 75 to 110% of the rigor signal with a mean of 97.0 ± 11.7 SD. After the peak, the time-course for decline of the caffeine-tension was
similar to that for the scattering to return to its resting value. Although a
systematic study of length vs. peak light signal during the caffeine-response was
not made, in three fibers that were stretched to $1.7L_s$ the increase in light signal
was reduced to a few per cent of the increase which occurred at $L_s$. This
dependence on length is consistent with that reported for intact frog muscle in
which the transient increase in scattering during isometric contractions also
diminished with stretch and disappeared at $1.65L_s$ (Barry and Carnay, 1969).

DISCUSSION

Earlier studies have shown that light scattering power of muscle increases
during rigor in glycerinated muscle (Bozler, 1958) and during contraction in
intact preparations (Buchthal et al., 1939; Hill, 1949; Barry and Carnay, 1969).
The present findings on skinned fibers confirm these observations, and lead us
to conclude that these changes in light scattering power are primarily due to
interaction between thick and thin filaments.

This conclusion is supported by the correlation between the degree of thick-
thin filament overlap and the increase in scattering induced by withdrawal of
substrate (Fig. 10). A similar relationship between increase in scattering during
twitch tension in frog muscle and extent of filament overlap had previously
been demonstrated (Barry and Carnay, 1969). The conclusion that filament
interaction causes increased scattering is reinforced by the experiments with
NEM: when dissociation of rigor bonds in the presence of substrate is blocked
by NEM, the original increase in scattering that accompanies induction of rigor
is maintained.

In that a number of changes in the structure and properties of the filaments
accompanies their interaction, experiments were designed to assess possible
contributions of some of these changes to the scattering signal. The experiments
of Figs. 7, 8, 9, and 11 show that the increase in scattering caused by
development of rigor, Ca activation, or by stretch is not directly related to
tension per se. The data of Fig. 7 eliminate changes in stiffness of the filaments
as a major contributor to the signal. The binding of MgATP to myosin also
appears to be of little importance, because neither addition nor removal of
MgATP caused a change in scattering in fibers stretched to long lengths (Fig.
10). Under the latter condition MgATP should still interact with binding sites on

The experiments at long sarcomere lengths exclude other potential sources of
the increase in scattering. X-ray diffraction data are interpreted to indicate that
Ca binding to troponin causes a shift in the position of the tropomyosin
molecules along the thin filament. This shift occurs even in the absence of
filament overlap (Haselgrove, 1973; Huxley, 1973). Other data are interpreted
to indicate that both rigor and Ca activation induce cross-bridges to extend
(Huxley, 1973; Haselgrove, 1973, 1975) or tilt outward (Lynn, 1976; cf. Nihei et
al., 1974; Sutoh and Harrington, 1977) from the thick filaments toward the thin
filaments, and that these changes also occur at non-overlap lengths. Because, by
contrast, most of the increase in scattering which accompanies Ca activation or
rigor is prevented by stretching fibers to $1.7L_s$ (Fig. 10; Barry and Carnay,
changes in proteins of the thin filaments and cross-bridge extension, or tilting, do not appear to be essential for the increase in scattering. We propose that cross-bridge attachment to actin filaments causes the increase in light scattering. Future work will be directed towards identifying the scatterers and testing this proposal.

This work was supported by grants to the H. Houston Merritt Clinical Research Center for Muscular Dystrophy and Related Diseases from the National Institutes of Health (NS 11766) and the Muscular Dystrophy Association and by a gift from the Ipco Hospital Supply Corporation.

Received for publication 6 September 1977.

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