Microsecond rotational motion of spin-labeled myosin heads during isometric muscle contraction

Saturation transfer electron paramagnetic resonance

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ABSTRACT We have used saturation transfer electron paramagnetic resonance (ST-EPR) to detect the microsecond rotational motions of spin-labeled myosin heads in bundles of skinned muscle fibers, under conditions of rigor, relaxation, and isometric contraction. Experiments were performed on fiber bundles perfused continuously with an ATP-regenerating system. Conditions were identical to those we have used in previous studies of myosin head orientation, except that the fibers were perpendicular to the magnetic field, making the spectra primarily sensitive to rotational motion rather than to the orientational distribution. In rigor, the high intensity of the ST-EPR signal indicates the absence of microsecond rotational motion, showing that heads are all rigidly bound to

actin. However, in both relaxation and contraction, considerable microsecond rotational motion is observed, implying that the previously reported orientational disorder under these conditions is dynamic, not static, on the microsecond time scale. The behavior in relaxation is essentially the same as that observed when myosin heads are detached from actin in the absence of ATP (Barnett and Thomas, 1984), corresponding to an effective rotational correlation time of ~ 10 μs. Slightly less mobility is observed during contraction. One possible interpretation is that in contraction all heads have the same mobility, corresponding to a correlation time of ~ 25 μs. Alternatively, more than one motional population may be present. For example, assuming that the spectrum in contraction is a linear com-

bination of those in relaxation (mobile) and rigor (immobile), we obtained a good fit with a mole fraction of 78-88% of the heads in the mobile state. These results are consistent with previous ST-EPR studies on contracting myofibrils (Thomas et al., 1980). Thus most myosin heads undergo microsecond rotational motions most of the time during isometric contraction, at least in the probed region of the myosin head. These motions could arise primarily from the free rotations of heads detached from actin. However, if most of these heads are attached to actin during contraction, as suggested by stiffness measurements, this result provides support for the hypothesis that sub-millisecond rotational motions of actin-attached myosin heads play an important role in force generation.

INTRODUCTION

Molecular reorientation of the myosin cross-bridge in active muscle is an essential feature of many current models for the molecular mechanism of muscle contraction (H. E. Huxley, 1969; A. F. Huxley and Simmons, 1971; reviewed by Cooke, 1986). A cross-bridge consists of two actin-binding globular domains (myosin heads, designated S1) connected through a double-stranded alpha-helical domain (designated S2) to the thick filament core. Some evidence for changes in the orientation of cross-bridges has been drawn from electron microscopy and x-ray diffraction studies on muscle fibers in rigor (no ATP) and relaxation (ATP but no Ca^{++}). Electron micrographs of insect flight muscle have been interpreted as demonstrating a change in cross-bridge orientation from 45° for rigor fibers to 90° for relaxed fibers (Reedy, et al., 1965; Reedy, 1967). X-ray diffraction patterns of

frog sartorius muscle have also been interpreted as demonstrating a similar change in cross-bridge orientation (Haselgrove, 1975). On the other hand, Poulsen and Lowy (1983) have analyzed the diffuse scatter in x-ray diffraction patterns, concluding that considerable orientational disorder of heads occurs in relaxed frog muscle fibers. The x-ray data suffer from ambiguity in assigning diffraction features to specific molecular orientations, and electron microscopy is not appropriate for measuring the rates of sub-millisecond molecular motions. In addition, most of these measurements lack the resolution to determine what part of the cross-bridge moves, or whether the motion is rotational or translational.

Spectroscopic probes have provided complementary information that is more site-specific and more directly related to rotational motion (reviewed by Thomas, 1987). Conventional electron paramagnetic resonance spectroscopy (EPR) of skinned rabbit psoas fibers, whose myosin heads have been specifically spin-labeled at a reactive sulfhydryl on the myosin head (SH1), has been shown to

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be sensitive to the orientation of the long axis of the myosin head with respect to the fiber axis (Thomas et al., 1980; Thomas and Cooke, 1980). In rigor, all the myosin heads are attached to actin in a narrow orientational distribution (Thomas and Cooke, 1980; Barnett and Thomas, 1984; Barnett et al., 1986). Relaxation of the fiber results in a nearly complete disorientation of the myosin heads (Thomas and Cooke, 1980), presumably due to cross-bridge detachment. In isometric contraction, there is almost as much disorientation as in relaxation (Cooke et al., 1982, 1984), and no evidence was found for a distinct orientation of attached myosin heads differing by more than 2° from the rigor angle. Those results put in doubt the validity of models that describe force generation as a simple axial rotation of the entire myosin head from one distinct angle to another. One interpretation of the conventional EPR data is that the disoriented heads in isometric contraction are statically disordered, presumably because they bind to actin rigidly at a wide range of angles. Alternatively, their disorder may be dynamic, due either to the free rotational motion of detached myosin heads or to myosin heads that are rotating while attached to actin. In order to distinguish between static and dynamic disorder, it is necessary to measure directly the rotational motion of myosin heads during isometric contraction.

Saturation transfer EPR (ST-EPR) is a technique optimally sensitive to rotational motion of nitroxide spin labels on the sub-millisecond time scale (Thomas et al., 1976; Squier and Thomas, 1986; reviewed by Thomas, 1986), and ST-EPR has been extensively applied to the study of spin-labeled myosin heads from rabbit skeletal muscle (reviewed by Thomas, 1987). For myofibrils in rigor, the ST-EPR spectrum indicates no sub-millisecond rotational motion (Thomas et al., 1980). The ST-EPR spectrum of relaxed myofibrils indicates a rotational correlation time on the order of 10 μs (Thomas et al., 1980), and this has been confirmed in relaxed fibers (Fajer et al., 1988). When spin-labeled fibers are stretched, so that myosin heads reside in the nonoverlap region of the sarcomere, the ST-EPR spectrum also indicates mobility on the microsecond time-scale (Barnett and Thomas, 1984). Therefore, it appears that myosin heads are dynamically disordered if they are detached from actin, either by addition of ATP or by stretching to a nonoverlap position. ST-EPR spectra of contracting myofibrils show almost as much microsecond motion as in relaxation (Thomas et al., 1980). However, the structural order of contracting myofibrils is poor, and their mechanical properties are not easily determined, so it is essential to extend these measurements to contracting fibers. Therefore, here we have performed ST-EPR experiments on spin-labeled muscle fibers during isometric contraction, in order to determine whether the orientational disorder observed previously by conventional EPR (Cooke et al., 1982) is static or dynamic.

METHODS

Solutions

Solutions used here were essentially the same as those used in studies of contracting fibers using conventional EPR (Cooke et al., 1982). Rigor solution contained 0.12 M KCl, 5 mM $MgCl_2$, 2 mM EGTA and 20 mM MOPS, pH 7.0. The relaxing solution for most experiments was prepared by the addition of 5 mM ATP, 20 mM creatine phosphate, and 0.4 mg/ml creatine kinase. The solution for isometric contraction was prepared by addition of 2 mM $CaCl_2$ to the relaxing solution. Increasing the ATP concentration from 5 to 10 mM did not have a significant effect on the spectra of contracting fibers. All preparations were done at 0–4°C, and all EPR and functional measurements were done at 20°C, adjusting the pH at the appropriate temperature.

Preparations

Psoas muscle from New Zealand white rabbits was dissected into small strips (2 to 5 mm in diameter) and glycerinated (Cooke and Bialek, 1979). Myofibrils were prepared from white muscles of the rabbit back and legs (Thomas et al., 1980). Fibers and myofibrils were spin-labeled as previously described (Thomas and Cooke, 1980; Thomas et al., 1980).

Muscle fiber mechanics

Isometric tension and stiffness of single muscle fibers were measured as described previously (Fajer et al., 1988; Pate and Cooke, 1988), under the same conditions used for EPR experiments, although these measurements were not made during EPR experiments. The fraction of spin-labeled heads was 0.5 to 0.7 (Fajer et al., 1988). There was no significant change in active fiber tension (typically 2.2 ± 0.3 kg/cm^2) upon spin labeling, although ferricyanide treatment of labeled fibers (used to eliminate the signal from nonspecifically attached probes) did result in a decrease of 10–20%. EPR experiments were done both with (shown in all figures below) and without (not shown) ferricyanide treatment. When corrected for nonspecific labeling, the results reported in the present study were independent of whether the muscle fibers were treated with ferricyanide. Spin-labeled fibers maintained a steady level of isometric tension during the time period required for spectral acquisition, both labeled and unlabeled fibers had 70% ± 10% of the rigor stiffness during contraction, and both tension and stiffness in relaxation were <10% of the values in isometric contraction (P. Fajer, personal communication).

EPR spectroscopy

Conventional (V_1) and saturation transfer (V_2') EPR experiments were performed on muscle fibers essentially as previously described (Barnett and Thomas, 1984). Digitized EPR spectra were acquired using a spectrometer (model E-109; Varian Associates, Palo Alto, CA) interfaced to a North Star computer (Lipscomb and Salo, 1983), and analyzed on a Zenith-158 computer, using a program developed by Piotr Fajer and Robert L. H. Bennett. The spectra were recorded with the muscle fiber axis perpendicular to the static magnetic field, in order to minimize the spectral effects of myosin head orientation and to maximize the sensitivity to rotational motion (Barnett and Thomas, 1984). Acquisition of conventional EPR spectra required scans of ~2–4 min duration.

Full (120 Gauss) ST-EPR scans were 8–10 min in duration. For most of the ST-EPR spectra obtained here, 4 to 16 scans (0.5 to 1 min in duration) of a 40 Gauss low-field region were acquired and averaged, providing that the first and last scans were not significantly different.

For a typical EPR sample, a bundle of spin-labeled fibers (0.3–0.5 mm in diameter, 7–10 cm in length) was secured at mid-length with a loop of 6-0 surgical silk (Deknatel, Howmedica, Inc., Queens Village, NY) and pulled into a 50 μL capacity glass capillary tube (0.7 mm i.d.). Before the bundle completely entered the capillary, its trailing ends were tied together with a length of surgical silk. The ends of the capillary were inserted into tight-fitting Tygon tubing, securing the thread so that constant fiber length was maintained during EPR experiments. The tubing also provided a means of continuously perfusing the fibers during EPR spectral acquisition. A fiber-loaded capillary, with perfusion tubing attached to each end, was fitted into a quartz tube (length = 19 cm, o.d. = 5 mm, i.d. = 3 mm) and positioned in a TE_{102} EPR cavity (model E231; Varian Instruments) such that the center of the fiber was coincident with the center of the cavity. The fiber bundle was positioned vertically, perpendicular to the magnetic field (Barnett and Thomas, 1984). Temperature was monitored with a thermocouple (BAT-12; Bailey Instruments, Clinton, NJ) either inserted into the perfusion tubing or inside the quartz tube. Temperature was regulated by a stream of nitrogen gas that was cooled in a dry ice/isopropanol bath then warmed to the desired temperature before passing over the sample. The heater/sensor and feedback circuit maintaining the nitrogen temperature was provided by a V4540 variable temperature controller (Varian Instruments). The ends of the tubing were connected to a transfusion system including a peristaltic pump (LKB MicroPerpex, LKB-Produkter AB, Bromma, Sweden) and a 2 to 5 mL buffer reservoir. Typical rates of flow were 0.5–2 mL/min.

RESULTS

Rigor

EPR spectra of nitroxide spin labels depend almost exclusively on the orientation and rotational motion of the label relative to the magnetic field. Conventional (V_1) EPR spectra are sensitive to orientation and to sub-microsecond motion, and ST-EPR (V'_2) spectra are sensitive to orientation and sub-millisecond motion. Because the spin label used here (MSL) remains rigidly bound to the myosin head under all physiologically relevant conditions (Thomas et al., 1980), its spectra provide direct information about the orientation and rotational motion of heads. We first consider EPR spectra of fibers and myofibrils in rigor (no ATP). Previous EPR studies on these preparations showed that spin-labeled heads are highly oriented relative to the fiber axis, as demonstrated by EPR spectra of fibers oriented parallel to the magnetic field (Thomas and Cooke, 1980). Nevertheless, the conventional EPR (V_1) spectrum of fibers mounted perpendicular to the magnetic field is similar to that of randomly oriented myofibrils (Fig. 1, left), which give the signature spectrum of randomly oriented but immobilized (on the nanosecond time scale) spin labels (Thomas et al., 1980). Thus, although it is not technically feasible to acquire spectra of randomly oriented muscle fibers during isometric con-
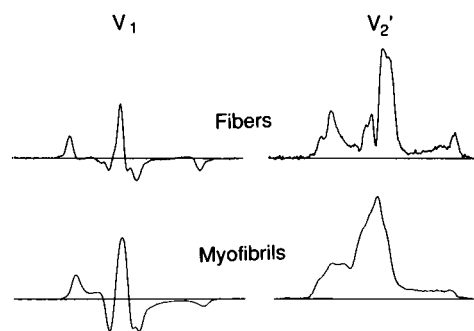

FIGURE 1. EPR spectra in rigor. Conventional EPR $(V_1,$ left) and ST-EPR $(V'_2,$ right) spectra were recorded from a spin-labeled fiber bundle perpendicular to the magnetic field (top) or from randomly oriented myofibrils prepared from spin-labeled fibers (bottom). For V_1, the modulation amplitude (H_m) was 2 G peak-to-peak, and the microwave field strength (H_1) was 0.14 G. For V'_2, H_m was 5 G and H_1 was 0.25 G. The temperature was 20°C, and the baseline in each spectrum is 120 G wide.

traction, the perpendicular orientation of fibers used here minimizes the contribution of orientational effects on EPR spectra (Barnett and Thomas, 1984).

The ST-EPR spectra (V'_2) of the same samples (Fig. 1, right) are also quite similar. The spectrum of myofibrils is typical of randomly oriented spin labels that are immobile on the microsecond time scale, showing that virtually all (>90%) of myosin heads in a rabbit skeletal muscle fiber bind rigidly to actin (Thomas et al., 1980; Barnett and Thomas, 1984). When rigor fibers are disoriented by mincing, both their V_1 and V'_2 spectra are identical to those of myofibrils (Barnett and Thomas, 1984), indicating that the same rigid immobilization occurs in fibers and myofibrils. While the ST-EPR spectrum of unminced fibers in rigor (Fig. 1, top right) is not identical to that of myofibrils (Fig. 1, top left), it contains essentially the same motional information. In particular, any decrease (relative to rigor) in the overall intensity of the V'_2 spectrum implies sub-millisecond rotational motion (Squier and Thomas, 1986).

Relaxation and contraction

Fig. 2 shows the conventional EPR spectra of a single fiber bundle, perpendicular to the magnetic field, under conditions of rigor, relaxation, and contraction. Although slight effects are observed, the spectra are all very similar to that of randomly oriented myofibrils (Fig. 1, left). Thus any substantial effects on ST-EPR spectra under the same conditions must be due to rotational motion, not to changes in the orientational distribution.

In Fig. 3, the ST-EPR spectra of spin-labeled fibers are shown in rigor, relaxation, and isometric contraction. In
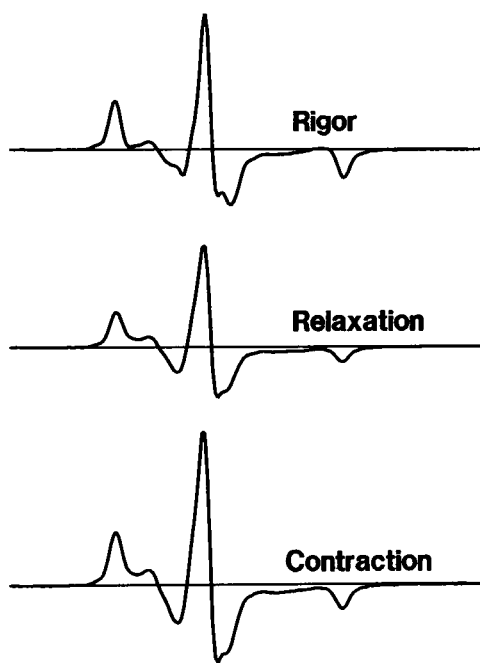
FIGURE 2. Effects of ATP and Ca on Conventional EPR Spectra. Conventional EPR spectra (V_1) were recorded from a spin-labeled fiber bundle perpendicular to the magnetic field in rigor, relaxation, and isometric contraction. Other conditions were as in Fig. 1.

order to minimize data acquisition time, only the first 40 Gauss of the spectrum was acquired. This spectral region has high sensitivity to changes in rotational motion on the sub-millisecond time scale (Thomas et al., 1976). In rigor the intensity is high at both P1 and P2, which are defined
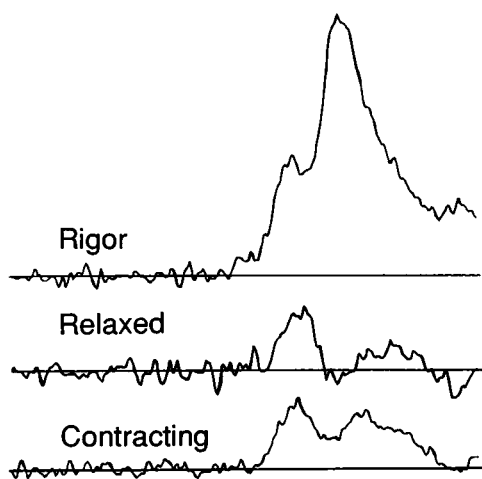

FIGURE 3. Effects of ATP and Ca on ST-EPR Spectra. V_2' spectra were recorded from a spin-labeled fiber bundle perpendicular to the magnetic field, in rigor, relaxation, and isometric contraction. Only the first 40 G are displayed. Other conditions were as in Figs. 1 and 2.

as the spectral positions in this region that have maximum intensity in rigor (Fig. 3). The high intensity at P2 indicates no sub-millisecond rotational motion (Barnett et al., 1984). When the muscle fibers are perfused with a relaxing solution, the spectrum changes dramatically (Fig. 3), with a large decrease in spectral intensity, especially at P2. This spectrum is similar to that observed for synthetic myosin filaments (Thomas et al., 1980) and is indicative of large-amplitude rotational motion on the microsecond time scale (Thomas et al., 1980; Barnett and Thomas, 1984; Fajer et al., 1988). Isometric contraction results in a spectrum (Fig. 3) with an intensity intermediate between that of rigor and relaxation. For example, the intensities at P_2 (relative to rigor) are -0.01 ± 0.04 for relaxation and 0.17 ± 0.04 for contraction. The spectral changes in Fig. 3 were completely reversible. The simplest interpretation of the data is that all myosin heads are undergoing the same rotational motion, with a single effective rotational correlation time τ_r (Squier and Thomas, 1986). Because τ_r is 10 μs in relaxation (Thomas et al., 1980), and because the intensity (P_2) in contraction is 17% of the value in the rigid limit (rigor), the effective correlation time is ~25 μs in contraction (Squier and Thomas, 1986).

However, considering the complex mixture of chemomechanical states likely to be present during isometric contraction, is it likely that more than one motional state is present. This is supported by previous conventional EPR studies (with the long axis of the fiber parallel to the magnetic field), indicating that the orientational distribution of myosin heads in contraction is well modeled by two populations that do not exchange on the nanosecond time scale (Cooke et al., 1982). In fact, the contraction spectrum was shown to be an excellent fit to a linear combination of the rigor and relaxation spectra (Cooke et al., 1982, 1984). To determine whether the ST-EPR spectrum in contraction could be similarly composed of a linear combination of the rigor and relaxation spectra of the same sample, we compared the contraction spectrum (Fig. 3, bottom) with composite spectra constructed from the digitized rigor and relaxation spectra. For purposes of this analysis, we constructed composite ST-EPR spectra according to the formula:

Composite spectrum $= x \cdot$ (rigor spectrum)

$$+ (1 - x) \cdot \text{(relaxation spectrum)}.$$

The composite spectrum was compared with the spectrum of the fiber during contraction, and a difference spectrum (residual) was produced by digital subtraction. An iterative search (varying x) yielded a composite whose residual intensity was a minimum (Fig. 4). For the example in Fig. 4, x was 0.18. Averaging over all of the preparations used in the present study, the mean value of x was $0.17 \pm$
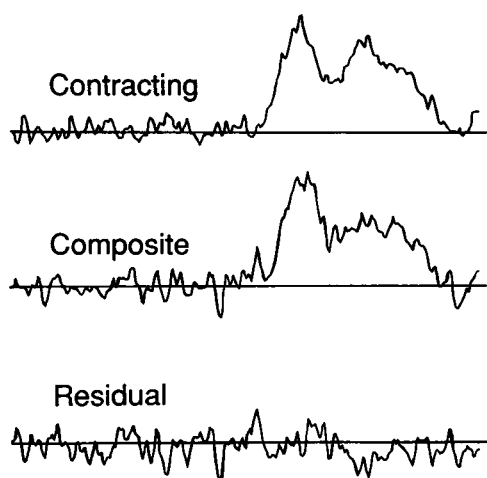
Contracting

Composite

Residual

FIGURE 4. Composite spectral analysis. The ST-EPR spectrum of a spin-labeled fiber bundle in isometric contraction (Fig. 3, *bottom*) is compared with a composite spectrum synthesized by computer addition of the rigor and relaxation spectra. The composite shown represents 18% of the rigor spectrum (Fig. 3, *top*) plus 82% of the relaxation spectrum (Fig. 3, *center*). This is the composite spectrum that minimized the peak-to-peak amplitude of the residual (*bottom*), obtained by subtracting the composite spectrum from the contraction spectrum.

0.05 (SEM, $n = 7$). Thus x falls within the range of 12 to 22%.

DISCUSSION

Rotational motion of myosin heads in contraction

Here, ST-EPR has been used to measure myosin head rotational motion in isometric contraction, extending the knowledge of active cross-bridge dynamics into the microsecond time scale. In rigor the myosin heads are rigidly bound to actin on the thin filaments and have little or no sub-millisecond rotational mobility, while relaxation gives rise to dynamically disordered heads with microsecond mobility (Fig. 3). During isometric contraction, the ST-EPR spectrum resembles that of relaxation more than that of rigor (Fig. 3), indicating that most of the myosin heads spend most of their time undergoing microsecond rotational motions during isometric contraction. If a single motional population is assumed, the spectrum in contraction (Fig. 3) corresponds to an effective rotational correlation time of ~25 μs (Squier et al., 1986). Alternatively, a composite spectrum closely fitting the contraction spectrum was constructed from rigor and relaxation spectra, with 12–22% of the rigor spectrum needed for the best fit (Fig. 4).

Relationship to previous spectroscopic studies

Thomas et al. (1980) used ST-EPR to show that the microsecond rotational mobility of myosin heads in contracting myofibrils is nearly as great as in relaxation. However, because the lattice structure within isolated myofibrils becomes disordered upon extensive contraction, the observed motion might not occur in a more structurally intact fiber during isometric contraction. The present study shows that the myofibril result is valid for isometric fibers; heads are nearly as rotationally mobile in contraction as in relaxation.

Previous conventional EPR studies, with fibers parallel to the field, resolved two orientational populations of heads during contraction, with the major component highly disordered (Cooke et al., 1982), having at least a 90° range of rotation (Barnett and Thomas, 1984). However, because conventional EPR can not detect microsecond motions, it remained unclear whether these heads were statically or dynamically disordered on the microsecond time scale. The present study shows that the disorder is dynamic, with an effective rotational correlation time comparable with that observed in relaxation (10 μs; Thomas et al., 1980).

The model of a rotationally mobile actin-attached head that is predominant during the actomyosin ATPase cycle, is supported by ST-EPR experiments on cross-linked acto-S1 (Svensson and Thomas, 1986). Spin-labeled myosin heads (S1) were cross-linked covalently to actin, producing ATPase activity comparable with the V_{MAX} obtained at infinite actin concentration. The heads were rigidly immobilized in the absence of ATP but became mobile upon the addition of ATP. Although this result might be questioned because of the nonphysiological covalent linkage, it has recently been verified for a noncovalent actin-S1 complex at low ionic strength, using photolysis of caged ATP to initiate the ATPase reaction (Berger et al., 1989). The rotational motion appears to require a hydrolyzable nucleotide, since AMPPNP and pyrophosphate do not produce microsecond rotation of actin-attached S1.

While ATP can mobilize attached heads, its nonhydrolyzable analogues can not mobilize heads without detaching them from actin, as shown by previous studies on MSL-heads. This has been shown for ADP in acto-S1 (Manuck et al., 1986) and for AMPPNP and pyrophosphate in myofibrils (Ishiwata et al., 1986), and in cross-linked acto-S1 (Thomas et al., 1988). Fajer et al. (1988) showed that AMPPNP can mobilize up to 50% of the myosin heads in fibers without decreasing stiffness, but the mobile heads are detached from actin, so the cross-bridges remain attached by a single (strongly bound)

immobile head. Pate and Cooke (1988) obtained similar results with pyrophosphate.

Optical studies using polarized light have been used to probe myosin head orientation and rotational motion (reviewed by Thomas, 1987). Fluorescence depolarization studies of contracting fibers (Guth, 1980; Burghardt and Ajtai, 1985) are restricted to the nanosecond time range and thus do not have the sensitivity needed to detect the microsecond motions reported in the present study. Besides the present study, there is one other microsecond-sensitive measurement of myosin head rotational motion during contraction, in which time-resolved phosphorescence anisotropy measurements produced conclusions consistent with those of the present study: large-amplitude rotational motion in relaxation, with only slightly less motion in contraction (Stein et al., 1988)

CONCLUSIONS

We conclude that the spin-labeled domain of each myosin head (and probably the whole head) in an isometrically contracting muscle spends most (at least 78–88%) of its time undergoing large-amplitude rotations on the microsecond time scale. There are two alternative interpretations of this result.

One possibility is that these dynamically disordered heads are all detached from actin. If so, then each head spends no more than 12–22% of its time actively participating in force generation. This would allow for a large reserve to replace the working heads when they have completed their power-strokes. Because our contracting fibers retain 70% of the stiffness of rigor fibers, consistent with previous observations (Goldman and Simmons, 1977), a small fraction of attached heads would imply that the stiffness is much greater for attached heads in an active fiber than for the average head in rigor. Evidence that some heads can bear more stiffness than others comes from observations that as many as half of the heads can be detached by AMPPNP (Fajer et al., 1988) or pyrophosphate (Pate and Cooke, 1988) without a significant decrease in stiffness.

An alternative interpretation is that the fraction of attached heads is high in contraction, as suggested by a simple interpretation of stiffness, but that many of the attached heads are in a dynamically disordered state (Huxley and Kress, 1985). This concept is consistent with the observation that ATP produces microsecond rotational motion of MSL-S1 either cross-linked to actin (Svensson and Thomas, 1986), or bound to actin noncovalently after the photolysis of caged-ATP (Berger et al., 1989). It is also consistent with the proposal that rapid rotational transitions of attached heads among several

angles could help explain force transients (Huxley and Simmons, 1971).

FUTURE STUDIES

These results would be greatly strengthened by data from probes attached to different sites on the myosin head. Virtually all of the spectroscopic information about myosin head motions during contraction comes from probes attached to SH1. Although fiber tension and stiffness are unaffected by labeling in most cases, ATPase rate constants are altered (Svensson and Thomas, 1986). Even if labeling does not alter the behavior of heads significantly, probes on other sites, such as the nucleotide binding site (Crowder and Cooke, 1987) or myosin light chains, are needed to determine whether this site is characteristic of the myosin head as a whole, or whether there are important internal motions within S1 (reviewed by Cooke, 1986; Thomas, 1987).

The present studies were made during steady-state conditions and thus can not resolve unambiguously the different motions that may occur in different phases of the cross-bridge cycle. The two components (rigor and relaxation spectra) used to construct the composite spectrum to fit the contraction spectrum (Fig. 4) are not unique; almost any two components having effective correlation times above and below 25 μs would give acceptable fits. Steady-state ST-EPR has very limited *motional resolution,* the ability to resolve distinct rotational correlation times in a heterogeneous mixture (Thomas et al., 1985; Thomas, 1986). In contrast, conventional EPR has very high *orientational resolution;* the ability to resolve distinct orientational distributions (Cooke et al., 1982, 1984; Barnett et al., 1986). The most direct evidence for a heterogeneous distribution of myosin head states during contraction comes from conventional EPR studies (Cooke et al., 1982, 1984), not from the present study. Thus, although the present study establishes unambiguously that most heads have substantial microsecond rotational mobility during contraction, it remains for future studies to describe the possible heterogeneity and anisotropy of these motions in detail. The principal requirement for this is time-resolution, achieved by pulsed excitation in EPR (Fajer et al., 1986) or phosphorescence (Stein et al., 1988), or achieved by the use of flash photolysis of caged ATP or caged calcium to produced transient chemical conditions during spectroscopic data acquisition (Berger et al., 1989).

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