Microsecond rotational dynamics of phosphorescent-labeled muscle cross-bridges

ARTICLE *in* BIOCHEMISTRY · JUNE 1988

Impact Factor: 3.02 · DOI: 10.1021/bi00409a034 · Source: PubMed

2 AUTHORS, INCLUDING:

Richard D Ludescher

Rutgers, The State University of New Jersey

96 PUBLICATIONS **1,428** CITATIONS

Microsecond Rotational Dynamics of Phosphorescent-Labeled Muscle Cross-Bridges†

Richard D. Ludescher‡ and David D. Thomas*

Department of Biochemistry, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Received July 23, 1987; Revised Manuscript Received January 6, 1988

ABSTRACT: We have measured the microsecond rotational motions of myosin heads in muscle cross-bridges under physiological ionic conditions at 4 °C, by detecting the time-resolved phosphorescence of eosin–maleimide covalently attached to heads in skeletal muscle myofibrils. The anisotropy decay of heads in rigor (no ATP) is constant over the time range from 0.5 to 200 μs, indicating that they do not undergo rotational motion in this time range. In the presence of 5 mM MgATP, however, heads undergo complex rotational motion with correlation times of about 5 and 40 μs. The motion of heads in relaxed myofibrils is restricted out to 1 ms, as indicated by a nonzero value of the residual anisotropy. The anisotropy decay of eosin-labeled myosin, extracted from labeled myofibrils, also exhibits complex decay on the 200-μs time scale when assembled into synthetic thick filaments. The correlation times and amplitudes of heads in filaments (under the same ionic conditions as the myofibril experiments) are unaffected by MgATP and very similar to the values for heads in relaxed myofibrils. The larger residual anisotropy and longer correlation times seen in myofibrils are consistent with a restriction of rotational motion in the confines of the myofibril protein lattice. These are the first time-resolved measurements under physiological conditions of the rotational motions of cross-bridges in the microsecond time range.

Models of contractility correlate the structure of the protein lattice in the myofibril with kinetic schemes for the hydrolysis of ATP [reviewed by Cooke (1986)]. Most models associate force generation with some molecular displacement of the myosin cross-bridge, consisting of two globular S1 domains (heads) attached to a single S2 segment (part of the myosin rod), when it is bound to actin. A likely candidate for this displacement is a rotation of some or all of the myosin head (Huxley, 1969). Spectroscopic techniques that are sensitive to molecular rotation, such as EPR[1] and optical depolarization, may therefore provide specific tests of these models [reviewed by Thomas (1987)]. Since these measurements employ extrinsic labels, they are also specific for the motion of particular protein sites. We report here measurements of the microsecond rotational motion of labeled cross-bridges in myofibrils.

Myosin in solution is flexible [for reviews, see Harvey and Cheung (1982) and Ludescher et al. (1987a)]. Probe studies using fluorescence depolarization (Mendelson et al., 1973) and EPR (Thomas et al., 1975) as well as electric birefringence measurements (Kobayashi & Totsuka, 1975) have established that the myosin head is free to rotate nearly independently of the coiled-coil tail. It is also clear from electric birefringence (Highsmith et al., 1977), intrinsic viscosity (Morimoto & Harrington, 1974), and viscoelasticity (Hvidt et al., 1982) studies that the tail is flexible. Whether this motion involves rotation about a hinge or the flexing of a semirigid rod is a matter of interpretation. Both of these motional modes are also directly detected by optical anisotropy of a covalent phosphorescent probe attached to the fast-reacting sulfhydryl (SH1) on the myosin head (Eads et al., 1984; Kinosita et al., 1984). The microsecond time scale of these measurements is well suited for the detection of these large-scale protein motions.

Myosin cross-bridges are also flexible in synthetic thick filaments. Early studies using fluorescence (Mendelson et al., 1973) and EPR (Thomas et al., 1975) indicated that head rotational mobility decreases when myosin aggregates into filaments. The interpretation of this mobility decrease was ambiguous, however, because of the short time scale of fluorescence and the lack of time resolution in EPR. Anisotropy studies using phosphorescent probes (Eads et al., 1984; Kinosita et al., 1984) showed that filament formation not only limits the amplitude of the rotational motion of the head but also slows its rate of motion.

Solution studies of myosin motion, however, only provide background on possible cross-bridge motions in muscle fibers. Direct measurement, by saturation transfer EPR (ST-EPR), of cross-bridge motions in spin-labeled myofibrils (Thomas et al., 1980) and in fibers (Barnett & Thomas, 1984; Fajer et al., 1987) showed that ATP induces microsecond motions in myofibrils and that the motion in contraction is intermediate between that seen in rigor and relaxation, but closer to relaxation. However, ST-EPR's lack of time resolution prevented detailed interpretation in terms of rates and amplitudes. Time-resolved fluorescence studies of cross-bridge dynamics in muscle fibers have also provided information on motions in the nanosecond time scale (Burghardt & Thompson, 1985; Burghardt & Ajtai, 1985). These studies are limited to the range of about 100 ns by the short lifetime of the fluorophore. A recent phosphorescent study of cross-bridge motion in myofibrils does provide rate and amplitude information in the

† This work was supported by grants from the National Institutes of Health (GM 27906, AM 32961, and RR01439), the American Heart Association, the National Science Foundation (PCM 8004612), and the Muscular Dystrophy Association of America. D.D.T. is supported by an Established Investigatorship from the American Heart Association.

* Author to whom correspondence should be addressed.

‡ Present address: Department of Chemistry, The Wichita State University, Wichita, KS 67208.

[1] Abbreviations: BSA, bovine serum albumin; DTT, dithiothreitol; E5M, eosin-5-maleimide; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EPR, electron paramagnetic resonance; MOPS, 4-morpholinepropanesulfonic acid; NEM, *N*-ethylmaleimide; PMT, photomultiplier tube; RC, resistance × capacitance.

0006-2960/88/0427-3343$01.50/0

appropriate time range (Ishiwata et al., 1987), but the physiological significance of this study is limited by the use of a 60% sucrose media to slow the motion to measureable rates.

We report here time-resolved measurements under physiological conditions of phosphorescence depolarization from an eosin probe on cross-bridges in skeletal muscle myofibrils at 4 °C. We show that the probe is specifically attached to a single site on the myosin head and therefore selectively reports on the motion of this site. Anisotropy decays in the time range from 0.5 to 1000 μs indicate that there is no motion in rigor and that ATP induces complex dynamic behavior. The anisotropy decay of relaxed myofibrils is also similar to that observed for synthetic filaments formed from eosin-labeled myosin extracted from labeled myofibrils.

Materials and Methods

Reagents and Buffers. Eosin-5-maleimide (E5M) was obtained from Molecular Probes, Inc. (Eugene, OR), and eosin Y was obtained from Kodak. Glucose oxidase type IX, catalase, and ATP were obtained from Sigma. Coumarins were obtained from Exciton (Dayton, OH). E5M was stored in liquid nitrogen as a 2 mM solution in dimethylformamide.

The following solutions were used in our experiments: (A) 60 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM NaN_3, 10 mM imidazole, pH 6.8; (R) 60 mM KCl, 6 mM $MgCl_2$, 1 mM EGTA, 1 mM NaN_3, 10 mM MOPS, pH 7.0; (R_{150}/Pr) 120 mM potassium propionate, 1 mM $MgCl_2$, 1 mM EGTA, 25 mM MOPS, 1 mM NaN_3, pH 7.0; (R^5_{150}) 124 mM potassium acetate, 5 mM ATP, 6 mM $MgCl_2$, 1 mM EGTA, 25 mM MOPS, pH 7.0; (K/EDTA) 600 mM KCl, 5 mM ATP, 10 mM EDTA, 50 mM MOPS, pH 7.5.

Preparations. All operations on fibers, myofibrils, and myosin were carried out at 4 °C on ice except where noted. Rabbit skeletal muscle (psoas) fibers were prepared and glycerinated as described by Fajer et al. (1988). Fibers were stored in buffer R with 50% glycerol (R/glycerol) at −20 °C for 4–12 weeks before use. Myofibrils were prepared from fibers by grinding with a tissumizer (Tekmar, Cincinnati, OH) with eight cycles of 15 s on followed by 15 s off. Myosin was extracted from myofibrils prepared from labeled fibers as described by Thomas et al. (1980); synthetic myosin filaments were prepared by dialysis against R_{150}/Pr solution.

Myosin heads were labeled with eosin by reacting muscle fibers with E5M, according to a modification of the procedures used previously to label heads with maleimide derivatives (Thomas et al., 1980; Ishiwata et al., 1987). Small bundles of glycerinated fibers about 0.2–0.4 mm thick were tied to glass rods and washed with buffer A to remove glycerol. Membrane debris was removed by soaking fibers for 15 min in buffer A plus 0.5% Triton X-100 and 0.1 mM DTT. The fibers were then washed 3 times, treated with 0.2 mM NEM for 30 min, and washed again to remove NEM (all in buffer A). Under these rigor conditions, where the reactivity of SH1 on myosin is suppressed due to formation of rigor cross-bridges with actin (Duke et al., 1976), NEM will react with many other SH groups in the fiber. The fibers were then treated with 5 mM PP_i in buffer A for 15 min, E5M was added to a final concentration of 4 μM, and the reaction was quenched after 90 min by transferring the fibers to a solution of buffer A plus 4 mM DTT and 5 mM PP_i for 30 min. Unreacted dye was removed by soaking the fibers in solutions of 2 mg/mL BSA in buffer A for at least 8 h. Labeled fibers were stored in R/glycerol with 0.1 mM DTT at −20 °C.

Biochemical Assays. The protein concentration of myofibrils was determined by the biuret method, with BSA as a standard, and of myosin by the absorbance at 280 nm (Eads et al., 1984). The dye/head ratio was determined by measurement of the absorbance of dye at 530 nm adjusted for background scatter at 600 nm and by use of an extinction coefficient of bound dye of 83 000 M^{-1} cm^{-1} (Eads et al., 1984). For this assay, myofibrils at about 1 mg/mL were first brought to 0.5 M KCl, diluted 1:1 with 8 M urea, and centrifuged at 10000*g* for 5 min. The concentration of myosin was assumed to be half of the total protein concentration, and the eosin concentration was adjusted for the estimated 10% of dye attached to other proteins. Myofibril proteins were separated by polyacrylamide gel electrophoresis (6–12% gradient) as described in Eads et al. (1984). The presence of eosin in protein bands was determined by observation of eosin fluorescence under UV excitation. We found it difficult to observe non-myosin fluorescent bands even on overloaded gels of myofibrils labeled after NEM treatment. By this criteria, greater than 90% of the eosin fluorescence was located on myosin. Without NEM treatment, however, non-myosin fluorescent bands are readily visible, indicating significant eosin labeling of other muscle proteins. About 3–4% of the eosin in labeled myofibrils was present as noncovalent dye, determined by assaying the amount of dye removed from myofibrils after being mixed with 10 volumes of 95% ethanol with an extinction coefficient for free dye of 95 000 M^{-1} cm^{-1}. The ATPase activity in K/EDTA and R^5_{150} buffers was determined by monitoring the release of inorganic phosphate as a function of time as described by Eads et al. (1984).

Spectroscopic Methods. The apparatus used was a modification of the emission spectrometer described by Eads et al. (1984). Pulsed excitation at 488 nm was provided by a dye laser (containing 0.1% coumarin 152 in 95% dioxane–5% methanol) pumped by a Lambda-Physik XeCl pulsed excimer laser (Model EMG 53 MSC). The excitation light was vertically polarized with a Glan-Thompson crystal polarizer, and room light was excluded by a 488 ± 1 nm interference filter at the sample box aperture. The excitation light intensity was adjusted with neutral-density filters. The sample was contained in a box equipped for temperature and atmosphere control. Emitted light was collected with a lens focused on the sample, and the excitation light and fluorescence were rejected with a 1-cm solution filter containing 4 mM I_2 in CCl_4 and a Schott glass RG 665-nm long-pass filter. We found that it was essential to place the solution filter in front of the glass filter to eliminate a spike of filter phosphorescence excited by scattered laser light.

It was not possible to optically filter out all of the prompt fluorescence light, so we gated the PMT off during the laser flash and fluorescence emission by using prepulse information from the laser power supply to initiate a gate that shorts out the dynode chain in the PMT for about 1 μs. The amplitude and position of the gate was adjustable; the minimum dead time with our homebuilt apparatus was about 0.5 μs. The gate electronics also contained an electronic RC filter that averages the analog signal with an exponential time constant that is adjustable from 0.1 to 1 μs. We used a time constant of 0.44 μs for filtering of our anisotropy decays.

The emission decays polarized parallel and perpendicular to the excitation polarization were collected through a sheet polarizer. Data transients were acquired with two different CAMAC modules run by a LeCroy 3500 multichannel analyzer. Data were digitized from the filtered voltage transient by a DSP 2108 module with resolution from 0.05 to 5 μs per channel. Data were also collected in a photon-counting mode on a LeCroy multichannel scalar that has time resolution of

1 μs or more per channel. All decays from either instrument were collected with a full width of 1024 channels. A typical anisotropy emission experiment consisted of collecting decays from 2000 laser shots with the emission polarizer first vertical and then horizontal (a single loop) and repeating this for 20–30 loops. Each experiment thus represents 40 000–60 000 laser shots. Repeat experiments on the same sample were very similar, indicating that the small amount of bleaching that occurs does not affect the anisotropy decay. In the unpolarized emission intensity experiments (used to obtain excited-state lifetimes), the output polarizer was placed at the magic angle (54.7°) with respect to the vertical. The emission decays represent an average over 20 000 laser shots.

The phosphorescence samples were degassed prior to data collection according to an enzymatic procedure (Eads et al., 1984). The enzymes and glucose were added to the sample (0.1 mL in a 3 × 3 mm quartz cuvette) from concentrated stocks, and the sample was deoxygenated within 5 min. The sample was diluted about 1% by this addition. In the myofibril experiments, labeled fibers (usually four to five bundles) were ground in R_{150}/Pr buffer to give a final concentration of about 1 mg/mL. The concentration of sample (myofibrils or myosin) for anisotropy experiments was usually 0.5 mg/mL or less and about 0.05 mg/mL for the lifetime experiments. When called for, 100 mM MgATP was added directly to the cuvette to a final concentration of 5 mM.

Data Analysis. The anisotropy decay curves were analyzed by a nonlinear least-squares fit of the data to a one- or two-exponential function:

$$r(t)/r_0 = A_1 \exp(-t/\phi_1) + A_3 \quad (1)$$

$$r(t)/r_0 = A_1 \exp(-t/\phi_1) + A_2 \exp(-t/\phi_2) + A_3 \quad (2)$$

In these equations, $r(t)$ is the polarization anisotropy (calculated from the emission transients polarized parallel and perpendicular to the excitation light), r_0 is the initial experimental anisotropy at zero time, and A_i is the normalized amplitude and ϕ_i is the rotational correlation time for component i. The use of more complex functions (e.g., three exponentials) was not justified by the signal/noise. All of the anisotropy decays were better fit by a two-exponential than by a one-exponential function. Goodness of fit was evaluated by comparing χ^2 values for each fit and by evaluating plots of modified residuals (the difference between the measured and the fit values divided by the square root of the measured value). Residuals plots for two-exponential fits to anisotropy decays for both myosin filaments and relaxed myofibrils were flat and random about zero. The anisotropy decays collected with the DSP transient digitizer contain a base-line measurement in the first 128 channels. The average of this base line (over channels 1–80) was subtracted from the data prior to fitting and plotting.

Lifetime decays were fit to two- or three-exponential functions plus a constant background. χ^2 was generally in the range from 3 to 5 for a two-exponential fit and in the range from 1.1 to 1.8 for a three-exponential fit. For any particular data set, χ^2 always dropped by more than half on going from a two- to a three-exponential fit. We are not presently able to analyze for more than three exponentials. We conclude, therefore, that the total emission decays as at least a three-exponential function.

The fit parameters from the least-squares analyses of the anisotropy decays were further analyzed in a model-dependent fashion in terms of the wobbling-in-a-cone model (Kinosita et al., 1977). In this analysis the residual anisotropy is related to the semiangle of a cone (θ_c) in which the probe transition dipole can freely rotate. The normalized residual anisotropy A_3 and average correlation time $\langle\phi\rangle$ (equivalent to ϕ_1 in eq 1) are related to the semiangle and the wobbling diffusion coefficient $\langle D_w\rangle$ by (Kinosita et al., 1977; Lipari & Szabo, 1980)

$$A_3 = [x(1 + x)/2]^2 \quad (3)$$

$$D_w\langle\theta\rangle(1 - A_3) = -x^2(1 + x)^2\{\log[(1 + x)/2] + (1 - x)/2\}/[2(1 - x)] + (1 + x)(6 + 8x - x^2 - 12x^3 - 7x^4)/24 \quad (4)$$

where $x = \cos\theta_c$. A calculation using $\langle\phi\rangle$ is an interpretation of the motion in terms of a single cone. Since our observed anisotropy decays all required two well-separated exponential terms for an adequate fit (discussed below), we actually analyzed the decays in terms of a two-cone model (Eads et al., 1984; Ludescher et al., 1987a). In this analysis, the anisotropy decay is described as the product of two terms, each of which is a single exponential decay to a constant:

$$r(t)/r_0 \cong [(1 - \gamma_1)\exp(-t/\phi_1) + \gamma_1] \times [(1 - \gamma_2)\exp(-t/\phi_2) + \gamma_2]$$

where γ_i is r_∞/r_0 and θ_i is the rotational correlation time for component i. If $\theta_1 \ll \theta_2$, then

$$r(t)/r_0 \cong (1 - \gamma_1)\exp(-t/\phi_1) + \gamma_1(1 - \gamma_2)\exp(-t/\phi_2) + \gamma_1\gamma_2 \quad (5)$$

and the anisotropy decay is well approximated as a double exponential plus a constant. Since our measured correlation times for each component differ by a factor of 5–10 (shown below), this approximation seems justified. In this approach, the γ_i terms are calculated from the experimental A_i terms (see below); the corresponding D_i and θ_i model parameters are calculated from eq 3 and 4, by setting $A_3 = \gamma_i$ in eq 3 to calculate θ_c for component i (θ_i) and then by setting $\langle\phi\rangle = \phi_i$ (with $A_3 = \gamma_i$ and $x = \cos\phi_i$) in eq 4 to calculate D_w for component i (D_i):

$$A_1 = 1 - \gamma_1 \qquad A_2 = \gamma_1(1 - \gamma_2) \qquad A_3 = \gamma_1\gamma_2$$

Table I: Characterization of E5M-Labeled Myofibrils[a]

sample	ATPase activity (IU) K/EDTA	ATPase activity (IU) R^5_{150}	dye/head	specificity
untreated	0.50 (0.02)	0.058 (0.001)		
unlabeled	0.38 (0.01)	0.12 (0.01)		
labeled	0.19 (0.01)	0.064 (0.001)	0.61 (0.01)	0.82

[a] All ATPase activities were measured at 25 °C in the media described under Materials and Methods. IU for ATPase activities is micromoles of P_i released per minute per milligram of protein. Untreated myofibrils went through the labeling procedure as described under Materials and Methods without treatment with NEM, unlabeled myofibrils went through the labeling procedure including treatment with NEM, and labeled myofibrils went through the entire labeling procedure with both NEM and E5M. The values are an average of duplicate experiments, and the errors in parentheses are the range of these measurements.

Results

Specificity and Extent of Labeling. The procedure for labeling muscle fibers results in a labeling stoichiometry of 0.6–0.7 eosin molecule per head, with greater than 90% of the label on myosin (see Materials and Methods; Ishiwata et al., 1987). We summarize the ATPase activities of a representative preparation of labeled myofibrils in Table I. The K/EDTA activity is inhibited in the labeled myofibrils compared with that of both the untreated and NEM-treated controls. The K/EDTA activity is known to be stoichiometrically in-

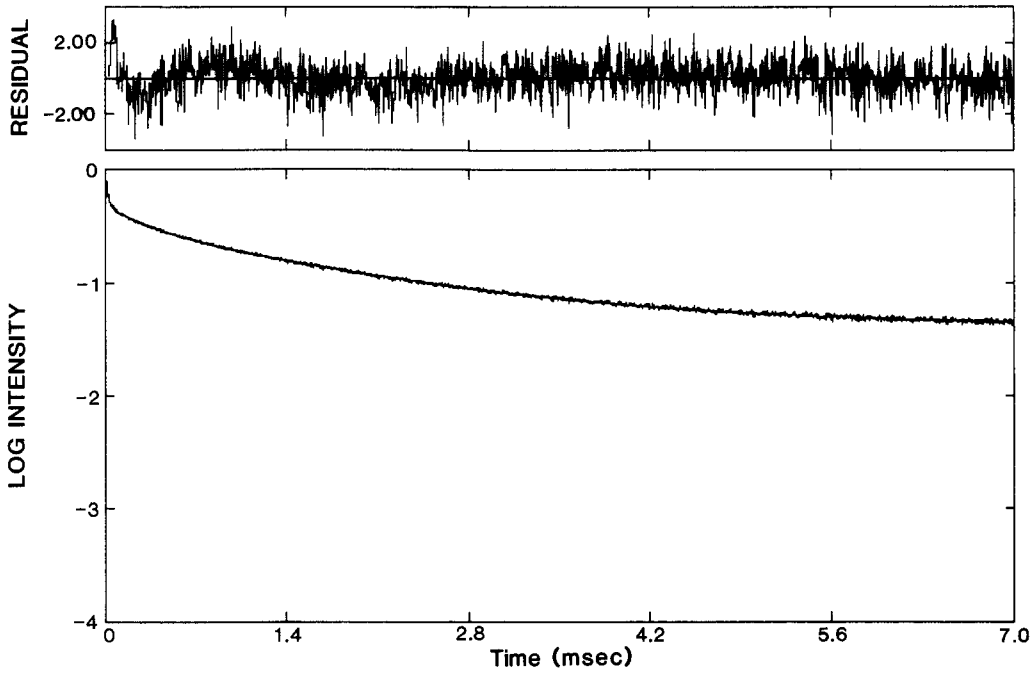

FIGURE 1: Transient phosphorescence emission intensity at 4 °C of E5M-labeled myofibrils in rigor (R_{150}/Pr solution). (Lower plot) The log decay of the raw data with the calculated fit to a sum of three exponentials superimposed on it. (Upper plot) The modified residual for this fit. See Materials and Methods for details of the analysis. Data collected at 7 μs per channel with the multichannel scaler and plotted as the log of the normalized intensity with the peak equal to 15 528.

Table II: Total Phosphorescence Emission Decay Parameters for Eosin-Labeled Myofibrils and Purified Myosin at 4 °C[a]

state	a_1	τ_1 (μs)	a_2	τ_2 (μs)	a_3	τ_3 (μs)
filaments						
-MgATP	0.419 (0.018)	68.7 (4.3)	0.400 (0.027)	378 (10)	0.181 (0.008)	1856 (26)
+MgATP	0.362 (0.010)	64.8 (6.0)	0.434 (0.014)	379 (17)	0.205 (0.004)	1809 (9)
myofibrils						
-MgATP	0.365 (0.014)	24.1 (2.7)	0.222 (0.006)	338 (15)	0.413 (0.008)	1613 (13)
+MgATP	0.420 (0.010)	20.9 (1.4)	0.348 (0.004)	393 (4)	0.232 (0.008)	2102 (4)

[a] Nonlinear least-squares fit parameters for total phosphorescence emission intensity decays collected at 7 μs per channel (7-ms total decay) with the multichannel scaler (see Materials and Methods). The data (e.g., Figure 1) were fit to $I(t) = \sum a_i \tau_i$. The values are averages over four measurements at 4 °C in R_{150}/Pr buffer (see Materials and Methods) with or without the addition of 5 mM MgATP. The values in parentheses are the standard errors of the mean (SEM) for the four measurements.

hibited by labeling of the fast-reacting thiol SH1 on the myosin head (Sekine & Kielley, 1964). The ratio of the fractional decrease in K/EDTA activity, relative to the NEM-treated control, compared with the labeling stoichiometry (see Materials and Methods) provides a measure of the specificity of SH1 labeling (see Table I). In summary, for this myofibril preparation about 90% of the label is on myosin with about 80% of this label on SH1.

The physiological ATPase activities in relaxation (R^5_{150}) of untreated and labeled myofibrils are similar and less than the activity of the NEM-treated control (Table I). This similarity appears to reflect an activation due to NEM treatment and an inhibition due to eosin labeling. The rate constants for ATP hydrolysis by S1 under low-salt conditions are known to decrease when SH1 is modified with iodoacetamide (Sleep et al., 1981). Ishiwata et al. (1987) on the other hand report that their labeling procedure, which includes an NEM treatment, activates the myofibril ATPase activity. Unfortunately, the reasons for this activation by NEM are unknown. The similarity in ATPase activities, however, encourages us to expect a similarity in rotational mobility between labeled and unlabeled myosin heads in myofibrils.

Total Intensity Decay of Eosin Phosphorescence. The decay of the total phosphorescence emission intensity (collected with magic angle polarization) of eosin-labeled myofibrils at 40 °C in rigor (R_{150}/Pr solution) is shown in Figure 1. The intensity transient decays in a complex manner, and the residuals plot indicates that these data require at least three, and possibly more, exponentials for an adequate description (see Materials and Methods). The fits of lifetimes and amplitudes for three-exponential fits to these data for myofibrils in rigor and relaxation buffer are listed in Table II. The intensity decay is partially sensitive to the presence of MgATP (Table II). In relaxing solution (containing 5 mM MgATP), the amplitude (preexponential) of the short-lifetime component increases slightly, and the values of the two longer lifetime components also increase, compared with those of myofibrils in rigor solution.

Complex decay behavior is also seen for synthetic filaments made from eosin-labeled myosin extracted from labeled myofibrils (Table II). For myosin filaments in rigor solution, the value of the short-lifetime component is longer than that seen in myofibrils, while the two longer components are closer to the myofibril values. MgATP also affects the distribution of lifetime amplitudes for eosin-labeled myosin filaments, but the values of the fit lifetimes are unaffected.

The multiexponential character of the eosin total intensity decay in myofibrils does not imply site heterogeneity. Extracted myosin shows the same complex distribution of lifetimes as the myofibrils, and eosin appears to display complex decay behavior in numerous other protein systems. Such behavior occurs when the linkage involves an iodoacetamide

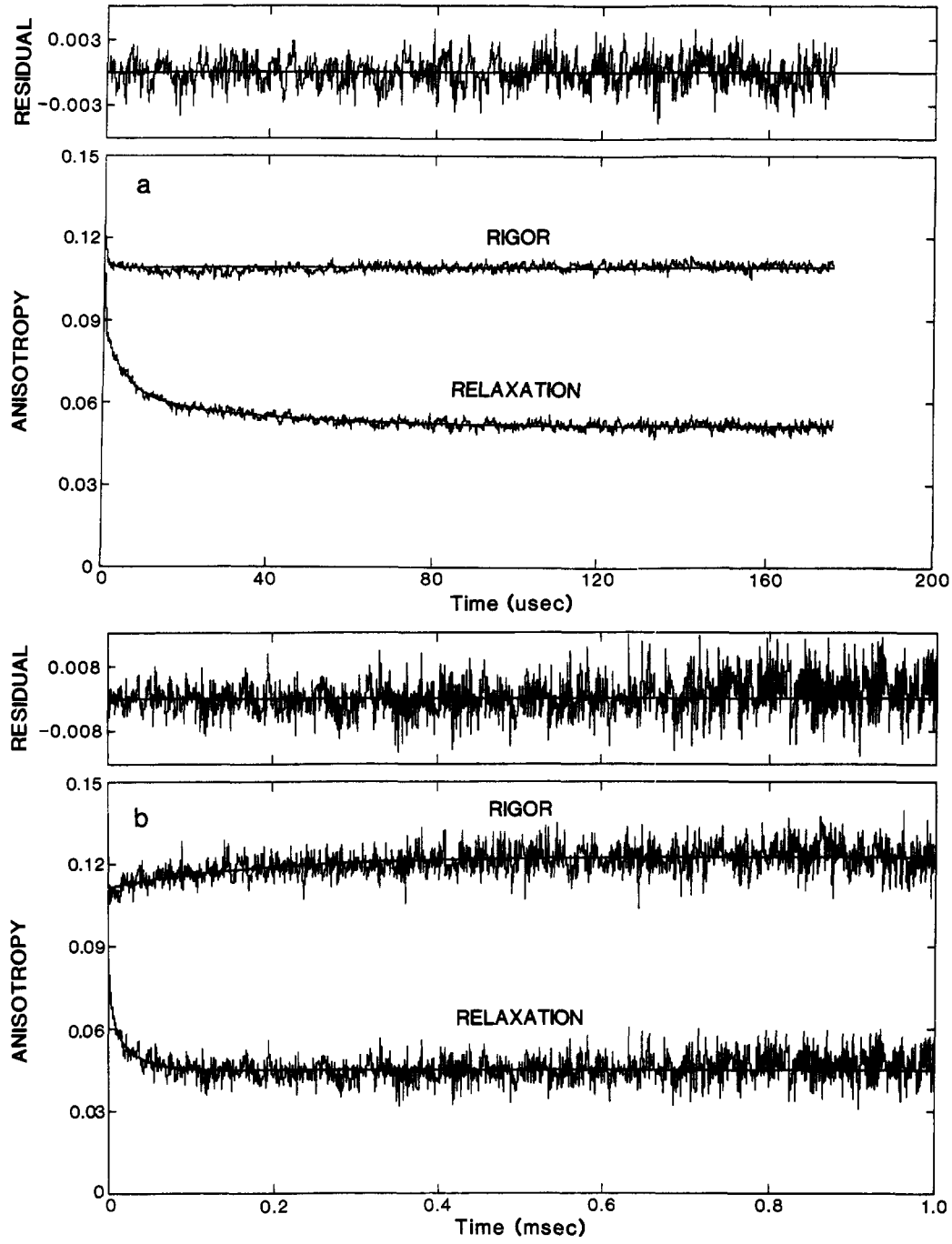

FIGURE 2: Transient phosphorescence anisotropy at 4 °C of E5M-labeled myofibrils in rigor (R_{150}/Pr solution) and in relaxation (R_{150}/Pr solution plus 5 mM MgATP), for (a) 0.2 μs per channel (DSP digitizer) and (b) 1 μs per channel (multichannel scaler). The calculated fits to eq 2 are superimposed on the data, and the residuals are shown for relaxation only.

(Eads et al., 1984), a maleimide (Jovin et al., 1981), or an isothiocyanate (Austin et al., 1979) moiety and when the labeled protein is water soluble (Cherry et al., 1976) or membrane bound (Burkli & Cherry, 1981). The case of a single-exponential decay for eosin bound to a protein is quite rare (Garland & Moore, 1979).

Anisotropy Decay of Eosin Phosphorescence. The anisotropy decays of myofibrils in rigor and relaxation are shown in Figure 2. In rigor the anisotropy is essentially constant over 180 μs (0.2 μs/channel, Figure 2a). When data are collected out to 1 ms (Figure 2b), a rise in the anisotropy is apparent in rigor and, to a smaller extent, in relaxation. This rise is small (<10%) but qualitatively reproducible between experiments and preparations. It is probably a consequence of the heterogeneous lifetimes of eosin observed in this system. If a short-lifetime component has a slightly smaller constant value for the anisotropy, for example, than a longer lifetime component, then the changing weighted average of the two components will give a rising anisotropy (Ludescher, 1984; Ludescher et al., 1987b). We interpret the anisotropy of the rigor myofibrils to mean that the myosin cross-bridges are immobile on the time scale from 1 to 1000 μs.

Relaxation, by the addition of 5 mM MgATP, induces complex rotational motion of the cross-bridges. The anisotropy behavior over 180 μs (Figure 2a) exhibits a biexponential decay to a constant. Analysis of data collected out to 1 ms (Figure 2b) does not reveal any additional decay components between 180 and 1000 μs. The residuals plots of fits using two exponentials plus a constant for data at both 0.2 and 1 μs per channel (Figure 2, upper plots) are flat and random about zero, indicating that these functions represent excellent fits to our data. The presence of a nonzero value of the residual an-

Table III: Phosphorescence Anisotropy Decay Parameters for Eosin-Labeled Myofibrils and Purified Myosin at 4 °C[a]

state	A_1	ϕ_1 (μs)	A_2	ϕ_2 (μs)	A_3	r_0
filaments						
-MgATP	0.458 (0.016)	3.3 (0.3)	0.160 (0.005)	27.5 (3.1)	0.382 (0.017)	0.124 (0.003)
+MgATP	0.456 (0.020)	2.8 (0.3)	0.146 (0.006)	21.6 (3.4)	0.398 (0.019)	0.132 (0.005)
myofibrils						
-MgATP			-0.081 (0.015)	208 (34)	1.081 (0.015)	0.106 (0.002)
+MgATP	0.341 (0.015)	4.9 (0.3)	0.186 (0.012)	38.4 (3.0)	0.474 (0.014)	0.088 (0.004)

[a] Nonlinear least-squares fit parameters for emission anisotropy decays collected at 0.1 or 0.2 μs per channel with the DSP digitizer (see Materials and Methods). All experiments were done in R_{150}/Pr buffer at 4 °C with or without the addition of 5 mM MgATP. A_i is the fractional amplitude, and ϕ_i is the correlation time for the ith component (eq 2). r_0 is the fit value of the anisotropy extrapolated to zero time. The values are averages and standard errors of the mean (SEM) for n experiments, where n = 9 for filaments (e.g., Figure 3) and n = 11 for myofibrils (e.g., Figure 2a).

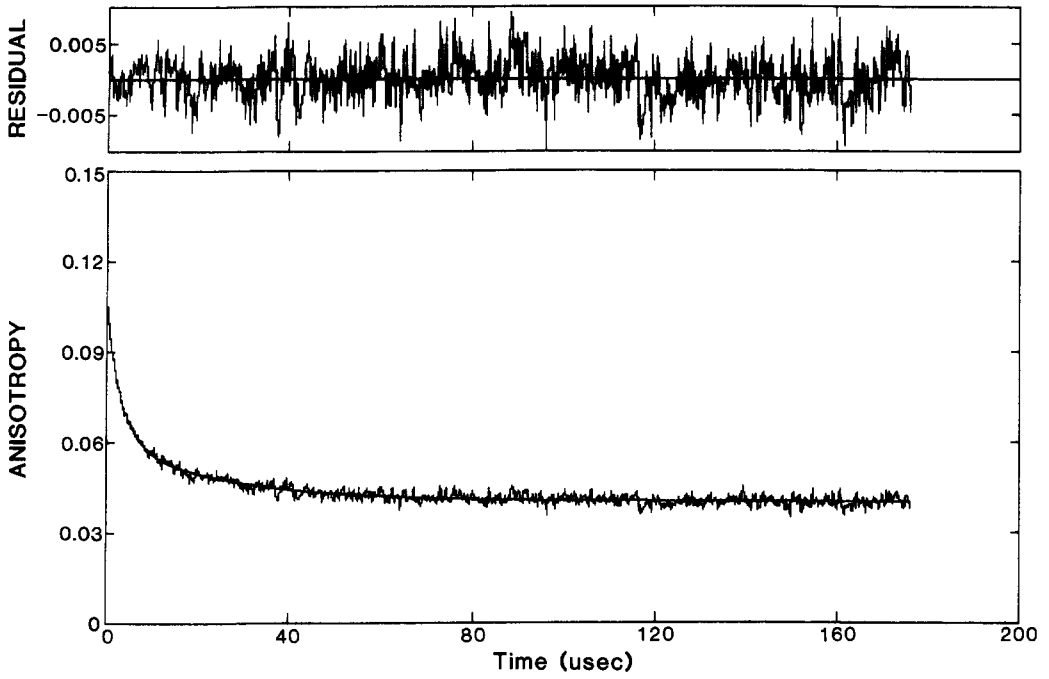

FIGURE 3: Transient phosphorescence anisotropy at 4 °C of synthetic filaments made from myosin extracted from E5M-labeled myofibrils. (Lower plot) The anisotropy decay of filaments in R_{150}/Pr solution. The raw data and the calculated fit to a sum of two exponentials plus a constant are plotted. (Upper plot) The residuals for this fit. Data collected at 0.2 μs per channel with the DSP digitizer.

isotropy (A_3) shows that the rotational motion of cross-bridges in myofibrils in the presence of MgATP is restricted over 1 ms. The anisotropy decay is unaffected by changes in [MgATP] over the range from 1 to 10 mM (data not shown).

The fit parameters for relaxed myofibrils at 0.2 μs per channel are listed in Table III. The fit correlation times for data collected at 1 μs per channel (decays out to 1 ms) are 4.4 ± 0.3 and 30.6 ± 2.5 μs, which are only slightly smaller than the high-resolution values. The value of r_0 for relaxed myofibrils is less than that for rigor myofibrils. Since the addition of MgATP to purified myosin does not decrease the value of r_0 (Table III and below), this difference may indicate that we are missing some faster motion. We also find that the fit value of r_0 is larger at 0.2 than at 1 μs per channel (0.088 ± 0.004 for 0.2-μs data and 0.075 ± 0.001 for 1-μs data), which is consistent with this interpretation. Our instrument has a dead time of about 0.5 μs due to transient gating off of the PMT during the intense laser flash and subsequent prompt fluorescence. For this reason we can not resolve any submicrosecond motions that may be present.

The emission anisotropy in rigor solution of synthetic filaments made from eosin-labeled myosin extracted from labeled myofibrils is shown in Figure 3. The anisotropy decays to a constant value over 180 μs and appears biexponential over this time range. The values of the fit correlation times and amplitudes are listed in Table III. The correlation times for myosin filaments in the presence of 5 mM MgATP (relaxing solution) are also listed. Other than a slight increase in the initial anisotropy, r_0, there is no significant dynamic effect of nucleotide binding. The dynamics of cross-bridges in myosin filaments are very similar to that seen in relaxed myofibrils. In myofibrils the correlation times are slightly longer, and the amplitude of the residual anisotropy content (A_3) is larger. Both of these dynamic differences are consistent with more restricted motion of cross-bridges in the complex environment of the myofibril than in solution. The similarities in the two sets of data, however, strongly suggest that the motion we observe in myofibrils is due to random fluctuations of detached cross-bridges.

Discussion

We have measured the anisotropy decay of phosphorescent-labeled myosin heads in myofibrils, under conditions corresponding to rigor and relaxation, and have compared these decays with those obtained for synthetic filaments made from myosin extracted from labeled myofibrils. In rigor (no ATP), the myosin heads in myofibrils are immobile over the time scale from 1 to 1000 μs (Figure 2b). In the presence of 5 mM MgATP, however, the heads are quite mobile, and the anisotropy decay is biexponential over 180 μs (Figure 2a). Data collected out to 1 ms do not reveal any additional motion. The value of the anisotropy at times greater than 500 μs is a nonzero constant, implying that the rotational motions of the cross-bridges are restricted in amplitude. The anisotropy decay

of relaxed myofibrils is similar to that of synthetic filaments formed from myosin extracted from labeled myofibrils (Figure 3). The rates of motion in the myofibrils are slower than those in myosin filaments, however, and the total angular amplitude of motion is smaller.

Analysis of Rotational Dynamics. Lifetime heterogeneity does not appear to complicate the interpretation of our anisotropy decays. As mentioned under Results, the rise in anisotropy over 1 ms seen in rigor and relaxed myofibrils can be explained as the result of different lifetime species with slightly different constant anisotropy values. If two species exist with the different initial anisotropy values (due to slight differences in the conformation of the dye at the protein surface, for example), then, in rigor, these initial values will remain constant while, in relaxation, the two species will undergo identical depolarization which will give different values of the final constant anisotropy. The changing weighted averages of these species can give rising anisotropy curves. The time course of this experimental rise, however, is much longer than the measured correlation times of relaxed myofibrils. The dramatic changes seen in the anisotropy of myofibrils upon addition of MgATP, moreover, are not accompanied by similar dramatic changes in the total intensity decay. The value of the short-lifetime component does not change significantly, and the amplitude increases only slightly upon addition of MgATP. The changes in the long-lifetime components are on a time scale too long to influence the measured correlation times. In addition, the dynamic similarities between relaxed myofibrils and myosin filaments imply that the measured correlation times are not artifacts. We conclude, therefore, that these correlation times accurately reflect dynamic behavior rather than photophysical artifacts (Ludescher et al., 1987b).

The anisotropy decays indicate that cross-bridges in rigor myofibrils are immobile on the time range from 1 to 1000 μs. This is essentially the same conclusion reached in a saturation-transfer EPR (ST-EPR) study of nitroxide spin-labeled myofibrils (Thomas et al., 1980), in which it was concluded that the effective correlation time is greater than 1 ms in rigor. However, because ST-EPR lacks time resolution, that study could not rule out the possibility of a submillisecond correlation time with a restricted amplitude (Thomas et al., 1985). The present study indicates that there is no correlation time in the range of 0.5 μs to 1 ms having any amplitude greater than about 5° and that there is no large-amplitude motion having a correlation time less than about 10 ms.

The anisotropy data indicate that cross-bridges in the presence of 5 mM MgATP exhibit complex decay behavior over 180 μs. We feel that this motion reflects the behavior of truly relaxed myofibrils for two reasons. First, the physiological ATPase (R^5_{150}, Table I) of labeled myofibrils, in which about 60% of the heads are labeled, is essentially the same as that of unlabeled myofibrils. Second, the anisotropy decay is unaffected by changes in the MgATP concentration from 1 to 10 mM (data not shown). The dynamics of relaxed cross-bridges in myofibrils (with bound MgATP) are very similar to the dynamics of extracted myosin. The differences, slightly longer correlation times and smaller preexponentials in myofibrils, are presumably due to the steric constraints of the myofibril protein lattice.

A submicrosecond correlation time has been directly detected in myosin filaments at 4 °C by transient absorption anisotropy (Eads et al., 1984). This technique was not applicable in the present study, due to light scattering of myofibril solutions. Although we cannot detect submicrosecond correlation times directly using the phosphorescence instrument

Table IV: Analysis of Anisotropy Data According to Motion in a Cone Model

	mode 1		mode 2		mode 3	
state	θ_1 (deg)	D_1 (s^{-1})	θ_2 (deg)	D_2 (s^{-1})	θ_3 (deg)	D_3 (s^{-1})
filaments[a]						
−MgATP	34	1.8×10^5	40	2.6×10^4		
filaments						
−MgATP			36	3.1×10^4	27	2.3×10^3
+MgATP			36	3.5×10^4	26	2.6×10^3
myofibrils						
+MgATP			29	1.5×10^4	26	1.5×10^3

[a] Parameters from transient absorption anisotropy decay data at 50-ns resolution (Eads et al., 1984). The remaining parameters are calculated from the data in Table III according to the procedure outlined under Materials and Methods.

in the present study, the low value of r_0 in relaxation, compared with that of rigor (Table III), probably reflects this fast motion. In order to test this supposition, we measured the anisotropy decay of myofibrils in 60% sucrose and found that the value of r_0 in the presence of 5 mM MgATP is the same as that seen in rigor (data not shown). Thus a submicrosecond cross-bridge rotational motion, similar to that occurring in myosin filaments (Eads et al., 1984), probably also occurs in relaxed myofibrils.

Physical Interpretation of Cross-Bridge Motion. We have analyzed the motion of cross-bridges in relaxed myofibrils and myosin in terms of a two-cone model for wobbling in a cone (Eads et al., 1984). In this interpretation each correlation time and amplitude corresponds to an independent dynamic mode of the molecule. The results of this analysis are listed in Table IV. We have also listed the cone angles and rotational diffusion coefficients calculated from TAA data on eosin-5-iodoacetamide-labeled purified myosin (Eads et al., 1984). As discussed above, it is clear that the dynamics of cross-bridges in synthetic thick filaments and in myofibrils are described by at least three motional modes over the time scale from 0.05 to 1000 μs. The slower of these two modes is analyzed in the present study.

The assignment of a physical interpretation to these motional modes is somewhat speculative. We can conclude, however, that the similarity in the rates and amplitudes of motion for heads in synthetic thick filaments and in myofibrils is strong evidence that the motions observed, in particular the slow motion (mode 3), are due to cross-bridge motions and not motion of the entire filament (such as long axis rotation). Motional mode 1 has been assigned to the wobble of the S1 portion of myosin, independent of the coiled-coil tail. This assignment, based on a comparison of the motion of purified S1 with the motion of heavy meromyosin, myosin monomer, and filaments, has been discussed elsewhere (Eads et al., 1984). Motional mode 2 has been assigned to motion of S1 plus some part of S2 (Eads et al., 1984), possibly corresponding to rotation about a hinge within the coiled-coil rod. Regions of increased proteolytic sensitivity, located at positions about 43 and 65 nm from the head/rod junction, are considered possible sites for a hinge. Using the formalism for rotational motion of a rigid rod (Broersma, 1960) as applied to myosin (Wegener, 1982), we calculate that the rotational diffusion coefficient of a rod the length of S1 plus either long S2 (65 nm) or short S2 (43 nm) is about (4–9) $\times 10^3$ s^{-1} (Ludescher et al., 1988). Using an alternative dynamic formalism in which the motion is modeled as the flexing of a semirigid rod that is fixed at one end (Hvidt et al., 1982), the comparable diffusion coefficients are (2–7) $\times 10^3$ s^{-1}. These calculations, which assume that S1 is continuous with S2, probably overestimate the diffusion coefficient. For this reason, we conclude

that motional mode 3 is consistent with restricted rotation of the entire cross-bridge, while mode 2 may correspond to motion of S1 plus only part of S2. In this interpretation, the rotational motion of the cross-bridge is quite complex. The globular head is free to wobble, while the S2 portion of the coiled-coil rod is internally flexible and able to rotate independently of the thick filament. It is also possible that the motions that we observe arise from motion entirely within S1 (Huxley, 1969; Huxley & Kress, 1985; Highsmith & Eden, 1986, 1987). Although we feel that this is unlikely for the reasons stated above and because the motion that we detect is similar in rate to that observed for myosin rod and LMM (Hvidt et al., 1984; Cardinaud & Benengo, 1985), the present experiments cannot rule out that interpretation.

The use of the wobbling-in-a-cone formalism assumes that the measured correlation times describe the dynamics of a single population. There are indications, however, that cross-bridges in both synthetic thick filaments and in myofibrils may exist in multiple conformations. Studies of chemical cross-linking (Chiao & Harrington, 1979), susceptibility to proteolysis (Ueno & Harrington, 1981), sedimentation velocity (Persechini & Rowe, 1984), and electron microscopy (Knight & Trinick, 1984) have all been interpreted in terms of multiple conformational states. The equilibrium between these states is influenced by $[Mg^{2+}]$, pH, and [ATP]. The states are usually described in terms of the average orientation of the cross-bridge on the surface of the thick filament. At low $[Mg^{2+}]$ or $[H^+]$ the cross-bridges are considered to be in a "spread" state, radially displaced from the thick filament surface, while at high concentrations of cations the cross-bridges are considered to be in a "compact" state, close to the filament surface. We have made measurements of head dynamics under conditions corresponding to the spread and compact states (Ludescher et al., 1988) and find that, although the changes in dynamics are qualitatively in the direction predicted by the model, the heads (or a large fraction of them) are still able to undergo large amplitude fluctuations in the putative compact state. The data we report here may also be interpreted in terms of a two-state model. The fast motions we observe (modes 1 and 2) may represent the motion of cross-bridges in the spread state while the slow mode (mode 3) may arise from less mobile cross-bridges (in the compact state). Alternatively, mode 3 may represent the transition between the two states. See Ludescher et al. (1988) for a more detailed discussion.

Relationship to Other Work. Previous fluorescence anisotropy measurements on relaxed myofibrils concluded that the correlation time is greater than 3 μs, consistent with the present results, but the limited time range (150 ns) prevented an accurate study of the microsecond motions (Mendelson & Cheung, 1976). ST-EPR studies of spin-labeled myofibrils (Thomas et al., 1980) yielded results consistent with the present study, but that technique did not provide the time-resolution needed to resolve the multiple modes of motion detected in the present study. Conventional EPR studies of spin-labeled muscle fibers (Thomas & Cooke, 1980; Barnett & Thomas, 1984) showed that the amplitude of microsecond myosin head rotational motion in rigor is less than or equal to 15°; the present study decreases this upper bound to about 5°. The fiber EPR studies showed that the amplitude of head motion in relaxation was at least 90° (Barnett & Thomas, 1984), which is consistent with the amplitude of motion found in the present study. Our results are also qualitatively consistent with the phosphorescence anisotropy decay observed for myofibrils in 60% sucrose at 0 °C (Ishiwata et al., 1987). Due to the high viscosity of this solution, those rotational measurements cover only about 10–15 μs when normalized to our conditions (4 °C in water). Thus the 40-μs correlation time detected in the present study at physiological viscosity was not detected in that study. In addition, the effects of MgATP were not saturating at 5 mM in 60% sucrose, and the residual anisotropy was less than half of our value (Ishiwata et al., 1987). The sources of these discrepancies are not obvious but are probably related to the presence of 60% sucrose in their media.

Conclusions

The microsecond rotational dynamics of myosin heads in synthetic thick filaments and in myofibrils are complex. The anisotropy decays with at least three correlation times over the time range from 0.05 to 1000 μs. The fast, submicrosecond, motions probably reflect the independent wobble of S1. The slower motions probably reflect the motion of S1 plus some or all of the S2 region of the cross-bridge. These slower modes may reflect the complex rotational motion of a flexible cross-bridge, or they may reflect cross-bridge motion plus a transition between a mobile and an immobile conformational state. Deciding between these alternatives will require both additional spectroscopic studies and more sophisticated modeling of the possible motional modes of the myosin cross-bridge. In any case, these cross-bridge motions are probably important in the detached phase of the cross-bridge cycle, facilitating the formation of active cross-bridges. Studies on contracting fibers, coupled with studies of effects that modulate motions of cross-bridges under equilibrium conditions, will be essential in understanding the role of molecular dynamics in force generation.

Acknowledgments

We thank Dr. Piotr Fajer for many helpful discussions, Franz Nisswandt for help in computer programming, Peter Voss and Sandra Johnson for technical assistance, and Ky Pham and Richard Stein for help in preparing the manuscript. We are especially grateful to Robert Bennett, who designed, constructed, and maintained the spectrometer and assisted in software development.

Registry No. MgATP, 1476-84-2.

References

Austin, R. H., Chan, S. S., & Jovin, T. M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5650–5654.

Barnett, V. A., & Thomas, D. D. (1984) *J. Mol. Biol. 179*, 83–102.

Barnett, V. A., & Thomas, D. D. (1987) *Biochemistry 26*, 314–323.

Broersma, S. (1960) *J. Chem. Phys. 32*, 1626–1631.

Burghardt, T. P., & Ajtai, K. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8478–8482.

Burghardt, T. P., & Thompson, N. L. (1985) *Biochemistry 24*, 3731–3735.

Burkli, A., & Cherry, R. J. (1981) *Biochemistry 20*, 138–143.

Cherry, R. J., Cogoli, A., Oppliger, M., Schneider, G., & Semonza, G. (1976) *Biochemistry 15*, 3653–3656.

Chiao, Y. C., & Harrington, W. F. (1979) *Biochemistry 18*, 959–963.

Cooke, R. (1986) *CRC Crit. Rev. Biochem. 21*, 53–118.

Duke, J., Takashi, R., Ue, K., & Morales, M. L. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 302–306.

Eads, T. M., Thomas, D. D., & Austin, R. H. (1984) *J. Mol. Biol. 179*, 55–81.

Fajer, P., Fajer, L., Brunsvold, N., & Thomas, D. D. (1988) *Biophys. J.* (in press).
Garland, P. B., & Moore, C. H. (1979) *Biochem. J. 183*, 561–567.
Harvey, S. C., & Cheung, H. C. (1982) *Cell Muscle Motil. 2*, 279–302.
Highsmith, S., & Eden, D. (1986) *Biochemistry 25*, 2237–2242.
Highsmith, S., & Eden, D. (1987) *Biochemistry 26*, 2747–2750.
Highsmith, S., Kretzschmar, K. M., O'Kinski, C. T., & Morales, M. F. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4986–4990.
Huxley, H. E. (1969) *Science (Washington, D.C.) 164*, 1356–1366.
Huxley, H. E., & Kress, M. J. (1985) *J. Muscle Res. Cell Motil. 6*, 153–160.
Hvidt, S., Nestler, H. M., Greaser, M. L., & Ferry, J. D. (1982) *Biochemistry 21*, 4064–4073.
Ishiwata, S. I., Kinosita, K., Jr., Yoshimura, H., & Ikegami, A. (1987) *J. Biol. Chem. 262*, 8314–8317.
Jovin, T. M., Bartholdi, M., Vaz, W. L. C., & Austin, R. H. (1981) *Ann. N.Y. Acad. Sci. 366*, 176–185.
Kinosita, K., Kawato, S., & Ikegami, A. (1977) *Biophys. J. 20*, 289–305.
Kinosita, K., Ishiwata, S., Yoshimura, H., Asai, H., & Ikegami, A. (1984) *Biochemistry 23*, 5963–5975.
Knight, P., & Trinick, J. (1984) *J. Mol. Biol. 177*, 461–482.
Kobayashi, K., & Tutsuka, T. (1975) *Biochim. Biophys. Acta 376*, 375–385.
Lipari, G., & Szabo, A. (1980) *Biophys. J. 30*, 489–506.
Ludescher, R. D. (1984) Ph.D. Dissertation, Chapter 3, University of Oregon, Eugene, OR.
Ludescher, R. D., Eads, T. M., & Thomas, D. D. (1987a) in *Optical Studies of Muscle Cross-Bridges* (Baskin, R. J., & Yeh, Y., Eds.) pp 33–66, CRC, Boca Raton, FL.
Ludescher, R. D., Peting, L., Hudson, S., & Hudson, B. S. (1987b) *Biophys. Chem. 28*, 59–75.
Ludescher, R. D., Eads, T. M., & Thomas, D. D. (1988) *J. Mol. Biol.* (in press).
Mendelson, R. A., & Cheung, P. (1976) *Science (Washington, D.C.) 194*, 190–192.
Mendelson, R. A., Morales, M., & Botts, J. (1973) *Biochemistry 12*, 2250–2255.
Morimoto, K., & Harrington, W. F. (1974) *J. Mol. Biol. 88*, 693–709.
Persechini, A., & Rowe, A. J. (1984) *J. Mol. Biol. 172*, 23–39.
Sekine, T., & Keilley, W. W. (1964) *Biochim. Biophys. Acta 81*, 336–345.
Thomas, D. D. (1987) *Annu. Rev. Physiol. 49*, 691–709.
Thomas, D. D., Seidel, J. C., Hyde, J. S., & Gergely, J. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1729–1733.
Thomas, D. D., Ishiwata, S., Seidel, J. C., & Gergely, J. (1980) *Biophys. J. 32*, 873–890.
Thomas, D. D., Eads, T. M., Barnett, V. A., Lindahl, K. M., Momont, D. A., & Squier, T. C. (1985) in *Spectroscopy and the Dynamics of Molecular Biological Systems* (Bayley, P., & Dale, R., Eds.) pp 239–257, Academic, London.
Ueno, H., & Harrington, W. F. (1981) *J. Mol. Biol. 149*, 619–640.
Wegener, W. A. (1982) *Biopolymers 21*, 1049–1080.

Human and Chick α2(I) Collagen mRNA: Comparison of the 5′ End in Osteoblasts and Fibroblasts

Joan C. Marini,*,‡ Gary S. Gottesman,§ and Michael A. Zasloff‡

Human Genetics Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20892, and The Howard Hughes Medical Institute, Bethesda, Maryland 20817

Received October 16, 1987; Revised Manuscript Received December 14, 1987

ABSTRACT: Type I collagen, a heterotrimer of two α1(I) chains and one α2(I) chain, is the major structural protein of bone, skin, and tendon. The collagen of patients with bone diseases has been studied in skin fibroblasts instead of osteoblasts because the genes for type I collagen are single-copy genes. While these studies should detect structural changes in the gene, they might fail to detect defects in processes which are dependent on tissue-specific expression. The studies reported here sought to determine whether the expression of type I collagen in skin and bone was characterized by the use of alternate promoters or alternative splicing in the N-propeptide region. Primer extension and nuclease S1 protection experiments were used to analyze the structure of the α2(I) mRNA from the 5′ end of the gene through the N-telopeptide coding region (exons 1–6) in human and chick osteoblasts and fibroblasts. The protection and primer extension experiments using human and chick mRNA demonstrated identical routes of splicing in skin and bone at the first five splice junctions. These studies provide reassurance that information obtained from the study of type I collagen in fibroblasts may be extrapolated to bone.

Type I collagen is the major structural protein of skin, bone, and tendon [for reviews, see Bornstein and Sage (1980) and Prockop et al. (1979a,b)]. The two peptides which comprise the type I heterotrimer, α1(I) and α2(I), are encoded by single-copy genes residing on different chromosomes (Solomon et al., 1984; Hender et al., 1983). Single-copy eukaryotic genes frequently utilize alternate promoters or alternative splicing in different tissues (Young et al., 1981; Sabate et al., 1985). The role these mechanisms may play in achieving tissue-spe-

* Correspondence should be addressed to this author at the Human Genetics Branch, Building 10, Room 8C429, National Institute of Child Health and Human Development, Bethesda, MD 20892.
‡ National Institute of Child Health and Human Development.
§ The Howard Hughes Medical Institute.

0006-2960/88/0427-3351$01.50/0