THREE-DIMENSIONAL DISORDER OF DIPOLAR PROBES IN A HELICAL ARRAY

Application to Muscle Cross-Bridges

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ABSTRACT Fluorescence polarization and EPR experiments on azimuthally randomized helices bearing extrinsic (dipolar) probes yield information about the axial orientation and order of the probes. If the orientation of the probe on the structure bearing it is known and disorder is absent, the orientation of the structure may be ascertained. For cases where less probe orientation information is available and/or disorder is present, the available structural information is correspondingly reduced. Here we examine the available data on probes attached to cross-bridges in muscle fibers: four plausible cases of three-dimensional cross-bridge disorders are numerically modeled muscle in states of rigor and relaxation. In rigor, where the reported probe disorder is small (Thomas and Cooke, 1980), it was found that the cross-bridge disorder was also small. On the other hand, for the relaxed state where the probes are found to be completely disordered, the cross-bridges may have a considerable amount of order. This possibility is in concert with the results of x-ray diffraction, in which the presence of well-developed myosin-based layer lines indicates considerable order in relaxed muscle.

INTRODUCTION

The use of sulfhydryl-directed reagents containing certain spectroscopically active groups has provided new information about the disposition of muscle proteins in the contractile array under various conditions. Part of this work has involved the use of probe molecules under conditions such that the great majority of the introduced reagent forms covalent adducts with the fastest-reacting sulfhydryl group (SH_1) of myosin, which is known to be located in each of the globular head regions of the protein. Because this region forms the cross-bridge between myosin and actin filaments during muscle contraction, any information about the orientation of the probe molecules under a particular set of conditions can potentially yield knowledge concerning the orientations of the cross-bridges to which the probes are bound. Two spectroscopic techniques that are sensitive to the orientations of their respective probe molecules are those of fluorescence polarization and electron paramagnetic resonance (EPR). The principles and practice of fluorescence polarization spectroscopy have been reviewed extensively (Pesce et al., 1971; Mendelson, 1982), and the application of this technique to the helical array of extrinsically-labeled cross-bridges in muscle has also been set out (Tregear and Mendelson, 1975; Mendelson and Morales, 1977). Recently, Thomas and Cooke (1980), using the approach of McCalley et al. (1972), Gaffney and McConnell (1974), and Libertini et al., (1974) have shown how EPR spectra from labeled muscle fibers may be interpreted in terms of probe orientation and disorder.

These techniques do not require a knowledge of or assumption about the detailed shape of the cross-bridges; however, because the information they yield pertains to the probe molecules introduced rather than to the protein moieties, difficulties arise when orientational information about cross-bridges is desired. First and most straightforward, it is possible that the probe molecules may be free to move while bound to the cross-bridges; even if the macromolecules were essentially well-ordered and immobile, the spectroscopist might perceive substantial disorder in the axial arrangement of the probes. This possibility may be checked by the use of techniques that compare the rate of motion of the protein moieties and their attached probes, namely time-resolved fluorescence anisotropy decay (Mendelson et al., 1973) and saturation transfer EPR (Thomas et al., 1975). In nearly all of the cases discussed in this paper, it has been found by such means that the probe molecules are bound essentially rigidly to the cross-bridge.

A more serious problem is that, in the absence of information about the spatial relationship of the protein molecule and its attached probe molecule, no inferences about absolute cross-bridge orientation may be made from

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such spectroscopic measurements. Even changes in probe orientation cannot be interpreted unambiguously in terms of changes in cross-bridge attitude. To date, it has been usual to present the interpretation of such spectroscopic measurements on muscle in terms of the axial angles between the long axis of the muscle fiber and the absorption (and emission, for fluorescence polarization) transition moment vectors of the probe molecule, using the accepted dipolar treatment for the absorption and emission of radiation by the probes. Because the muscle fiber effectively possesses cylindrical symmetry, due to the relative azimuthal randomization of its many constituent myofibrils, only axial angular information about the probes is obtained.

It is known from x-ray diffraction measurements (Huxley and Brown, 1967 et seq.) that the cross-bridges in living (frog) striated muscle are helically arranged on the thick filaments. Although muscle in rigor, where a large fraction of cross-bridges are bound statically to actin sites, and contracting muscle give quite different patterns than resting muscle, where actin-myosin interaction is thought to be much more tenuous, a strong indication of a thick-filament–based helix is present in the resting-muscle layer line pattern. Fluorescence polarization and EPR measurements have not proved consistent with the presence of a helical array of probe molecules (dipoles) possessing a unique axial angle, in glycerinated striated muscle in either the relaxed or rigor state. In every case, some measure of disorder in the array is required to establish agreement between theory and experiment. So far, there have been three ways of characterizing and parameterizing this disorder. Tregear and Mendelson (1975) used a model in which a certain fraction of the dipoles were completely disordered; the remainder assumed a particular axial angle. A second approach used by Tregear and Mendelson (1975) was to consider dipoles distributed uniformly in a sector centered on a median axial angle, and to use the semi-angle of the sector as the "disorder paramenter." In both cases, appreciable amounts of disorder (random fraction at least 0.4, semi-angle at least 20°) were required to achieve satisfactory agreement with experiment. The third way of characterizing disorder (Thomas and Cooke, 1980) has been to allow the dipoles to be axially disordered in a sector, weighting the angular deviation $\Delta\theta$ from the mean axial angle by a Gaussian factor $[\exp(-\Delta\theta^2/2\sigma_A^2)]$ where σ is the standard deviation of the distribution. Yanagida (1981), using ethenonucleotides bound to the active site of myosin, has used this approach to fit fluorescence polarization data and finds, for example, a broad angular distribution ($\sigma_A \simeq 40°$) in glycerinated rabbit psoas muscle relaxed with etheno-ATP. An important independent affirmation of this type of model disorder is provided by the results of Thomas and Cooke (1980), because the type of EPR spectra produced by a mixture of random and ordered probes can be distinguished readily from those spectra due to angular spread around a mean angle. They find that the Gaussian-weighted model gives a very satisfactory fit to their data if the mean angles are chosen correctly and the standard deviation is ~6° in the rigor state. In relaxed muscle, or fibers extended until thick and thin filaments no longer overlap, the angular spread of the dipoles is much greater[1] (Thomas and Cooke, 1980; Thomas and Barnett, 1981) and this, together with Yanagida's (1981) results, is diagnostic of considerable dipolar disorder in the relaxed glycerinated muscle. The purpose of this paper, in addition to presenting a methodology relating cross-bridge orientation to spectroscopic observations, is to show that such extensive dipolar disorder is not necessarily indicative of large axial deviations of the cross-bridges themselves about their median positions. An alternative explanation to that proposed by Thomas and Cooke (1980) is then offered to try and reconcile such disorder of the probe molecules with the well-developed axial order of the cross-bridges seen in resting muscle (Huxley and Brown, 1967).

MODEL

We propose a general model of three-dimensional cross-bridge disorder, based around the vector reference system of Fig. 1, which illustrates the geometrical relationship of the cross-bridge orientation to be a laboratory axis system. As previously (Mendelson and Morales, 1977), the long axis of the muscle fiber lies in the direction specified by the unit vector $\hat{\mathbf{k}}$. In fluorescence polarization experiments the incident light propagates in the $-\hat{\mathbf{i}}$ direction and $\hat{\alpha}$ refers to the absorption dipole axis of the fluorophore. In electron paramagnetic resonance experiments the $\vec{\mathbf{H}}$ field vector is either in the $\hat{\mathbf{i}}$ or $\hat{\mathbf{k}}$ direction and the $\hat{\alpha}$ vector refers to the principle axis of the spin label. Unless otherwise stated in this paper $\vec{\mathbf{H}}$ is along the $\hat{\mathbf{k}}$ direction. For the present, $\hat{\chi}$ will not be specifically related to the cross-bridge structure. The unit vector $\hat{\chi}_0$ represents the mean cross-bridge orientation (before azimuthal averaging); any deviations of $\hat{\chi}$ from $\hat{\chi}_0$ may be pictured as a number-distribution of many cross-bridges about their median orientations, or as a time-dependent diffusion of a single cross-bridge around $\hat{\chi}_0$.

The relationship of a particular $\hat{\chi}$ to $\hat{\chi}_0$ may be specified by Euler angle transformations, in terms of the angles Λ and Γ; the relationship of the axial angle θ_{CB} $[= \cos^{-1}(\hat{\chi} \cdot \hat{\mathbf{k}})]$ to that of the mean orientation (θ_0) is given by

$$\cos\theta_{CB} = \cos\theta_0 \cos\Lambda + \sin\theta_0 \sin\Lambda \cos\Gamma. \quad (1)$$

Fig. 1 also shows, for a particular $\hat{\chi}$, the unit vector $\hat{\alpha}$, which is defined as lying parallel to the absorption transition moment of a dipole bound rigidly to the cross-bridge. The vector $\hat{\alpha}$ is related by the angles λ_A and γ_A to $\hat{\chi}$ in the same way that $\hat{\chi}$ was located with respect to $\hat{\chi}_0$. Thus

$$\cos\theta_A = \cos\theta_{CB} \cos\lambda_A + \sin\theta_{CB} \sin\lambda_A \cos\gamma_A \quad (2a)$$

$$\cos\theta_E = \cos\theta_{CB} \cos\lambda_E + \sin\theta_{CB} \sin\lambda_E \cos\gamma_E \quad (2b)$$

where we have defined an additional $\hat{\epsilon}$ vector for calculations of fluorescence polarization. This vector (not shown in Fig. 1), bears the same relationship as $\hat{\alpha}$ to $\hat{\chi}$.

[1]Fig. 3 of Thomas and Cooke (1980) suggests that a full width at half maximum of at least 90° ($\sigma_A > 38°$) is required to match closely a truly random population's spectrum, and we have found that in fact σ_A must be ~55° or more to achieve a fit to the "isotropic" case as good as that provided by a spectrum from relaxed muscle.

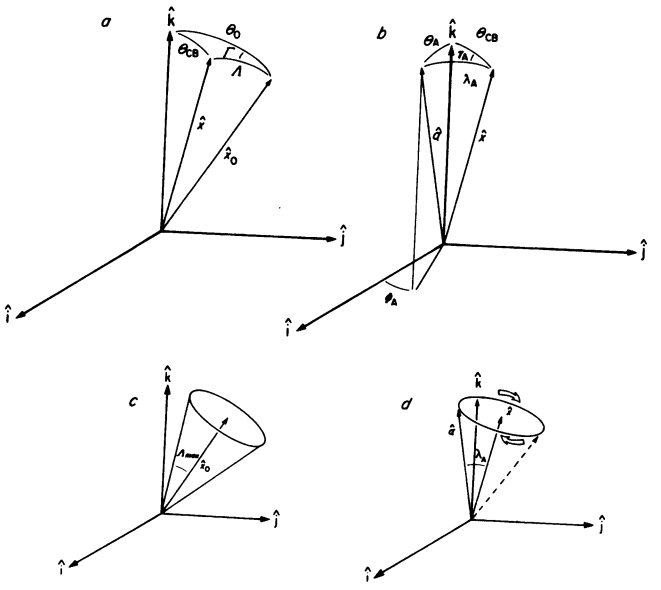

FIGURE 1 Definition of geometrical terms. (*a*) The vector $\hat{\chi}_0$ corresponds to the median of equilibrium cross-bridge orientation (before cylindrical averaging), and $\hat{\chi}$ to some instantaneous cross-bridge orientation. (*b*) The relation of the principal magnetic axis of the probe $\hat{a}$ to some cross-bridge orientation $\hat{\chi}$. (*c*) Distribution of cross-bridge orientations around $\hat{\chi}_0$. In models U and UT (see text), the vector $\hat{\chi}$ lies within a cone of semi-angle Λ_{max} centered on $\hat{\chi}_0$; in models G and GT (see text) the distribution of $\hat{\chi}$ and $\hat{\chi}_0$ is also axially symmetric but weighted with a Gaussian rather than a uniform term. (*d*) Torsional disorder of the probe aaxis around the cross-bridge axis (see text). In models UT and GT, $\hat{a}$ is allowed to rotate around $\hat{\chi}$, thus generating (cf. Fig. 1 *b*) a cone of semi-angle λ_A.

Having defined the appropriate relationships, we will now consider the form of the spatial distribution of cross-bridges. For a particular mean azimuth ϕ_0, the distribution of $\hat{\chi}$ around $\hat{\chi}_0$ depends on distributions in both angles Λ and Γ. In this paper, we consider two possible distribution functions in Λ. First, a uniform distribution with a boundary ($0 \le \Lambda \le \Lambda_{max}$), and second, a distribution weighted with a Gaussian factor exp $(-\Lambda^2/2\sigma^2)$ are used as models of disorder. These distributions will be represented by U (for uniform) and G (for Gaussian) respectively. Model U corresponds to free diffusion of the cross-bridge within a cone-shaped volume having reflecting barriers (see Mendelson and Cheung, 1978 and Fig. 1 *c*). The Gaussian case corresponds to inhibited diffusion that could arise because of steric or chemical interactions. Although it is known that the relaxation times of S-1 moieties are lengthened over the free myosin case (Mendelson et al., 1973, Thomas et al., 1975) the source of the alteration of rotational diffusion could be due to either of these causes.

The distribution in the angle Γ is considered to be uniform in the range $0 \le \Gamma \le 2\pi$. After multiplying by sin Λ to fulfill equal space-filling requirements, the two cases U and G described, respectively, a uniformly occupied conical volume of semi-angle Λ_{max} and a Gaussian-weighted three-dimensional distribution, both symmetric about $\hat{\chi}_0$ (Fig. 1 *c*). A further factor considered is the possibility of a distribution of the dipole vector(s) around the cross-bridge vector $\hat{\chi}$; this will be called "torsional" disorder (Fig. 1 *d*). In this case, γ_A (and γ_E) are either fixed (no torsional disorder), or γ_A lies with equal probability in the range $0 \le \gamma_A \le 2\pi$ (full torsional disorder); for fluorescence polarization maintaining the conditions $\hat{\alpha} \cdot \hat{\epsilon} = \cos \mu$, $\hat{\alpha} \cdot \hat{\chi}_0 = \cos \lambda_a$, $\hat{\epsilon} \cdot \hat{\chi} = \cos \lambda_E$ specifies the distribution in γ_E. Full torsional disorder of the dipolar probes around $\hat{\chi}$ is denoted by the letter T. This means that, in all, we consider four types of model disorder, labeled U, UT, G, and GT, corresponding to uniform or Gaussian weighting in Λ with or without averaging in γ_A.

Spectroscopic techniques yield intensities dependent on the axial and azimuthal angles of the dipolar probes, but in the muscle fiber, due to cylindrical averaging, the azimuthal dependence is lost and in general

$$\bar{I}(\theta_A) = \langle I(\theta_A, \phi_A) \rangle_{\phi_A} \tag{3}$$

where $I(\theta_A, \phi_A)$ is the intensity function and the brackets denote averaging over ϕ_A. For a pure one-dipole system, such as pertains approximately to the EPR measurements we shall consider, a single λ_A and γ_A characterize dipolar orientation with respect to the cross-bridge axis and the averaging in the various angles may be written as follows:

$$\bar{I}(\theta_0, \lambda_A, \gamma_A) = \frac{\int_0^{2\pi}\int_0^{2\pi}\int_0^{\Lambda_{max}} \rho(\Lambda, \Gamma, \gamma_A) \sin\Lambda \bar{I}[\theta_A(\theta_0, \lambda_A, \gamma_A, \Lambda, \Gamma)]\, d\Lambda d\Gamma d\gamma_A}{\int_0^{2\pi}\int_0^{2\pi}\int_0^{\Lambda_{max}} \sin\Lambda d\Lambda d\Gamma d\gamma_A}. \tag{4}$$

This is the most general form of the expression; in models without torsional disorder the integration over γ_A is omitted, and $\bar{I}$ is a function of γ_A. The ρ-function is the weighting in the appropriate angles and the form of $\bar{I}$ in the integral denoted that θ_A is itself a function of the angles in parenthesis which follow it. The final intensity will depend upon at least one more quantity, namely σ (model G or GT) or Λ_{max} (model U or UT). With a double-dipole system such as pertains to fluorescence polarization, and extra variable, namely λ_E, is required, and the expression is correspondingly more complicated. The original intensity functions $I(\theta_A, \phi_A)$ used the calculations of EPR spectra are as given by Thomas and Cooke (1980) after McCalley et al. (1972); for fluorescence polarization, the functions are as given by Mendelson and Morales (1977).

Specifically, the forms equation used in the four EPR cases considered are as follows:

GT,

$$\bar{I}(\theta_0, \lambda_A, \sigma) = \frac{\int_0^{2\pi}\int_0^{2\pi}\int_0^{\infty} e^{-\Lambda^2/2\sigma^2} \bar{I} \sin\Lambda d\Lambda d\Gamma d\gamma_A}{\int_0^{2\pi}\int_0^{2\pi}\int_0^{\infty} \sin\Lambda d\Lambda d\Gamma d\gamma_A} \tag{5a}$$

G,

$$\bar{I}(\theta_0, \lambda_A, \sigma, \gamma_A) = \frac{\int_0^{2\pi}\int_0^{\infty} e^{-\Lambda^2/2\sigma^2} \bar{I} \sin\Lambda d\Lambda d\Gamma}{\int_0^{2\pi}\int_0^{\infty} \sin\Lambda d\Lambda d\Gamma} \tag{5b}$$

UT,

$$\bar{I}(\theta_0, \lambda_A, \Lambda_{max}) = \frac{\int_0^{2\pi}\int_0^{2\pi}\int_0^{\Lambda_{max}} \bar{I} \sin\Lambda d\Lambda d\Gamma d\gamma_A}{\int_0^{2\pi}\int_0^{2\pi}\int_0^{\Lambda_{max}} \sin\Lambda d\Lambda d\Gamma d\gamma_A} \tag{5c}$$

U,

$$\bar{I}(\theta_0, \lambda_A, \Lambda_{max}, \gamma_A) = \frac{\int_0^{2\pi}\int_0^{\Lambda_{max}} \bar{I} \sin\Lambda d\Lambda d\Gamma}{\int_0^{2\pi}\int_0^{\Lambda_{max}} \sin\Lambda\, d\Lambda\, d\Gamma}, \tag{5d}$$

where

$$\bar{I} \propto -2\gamma^2 T_2^2 \sum_{m_I=-1}^{1} \frac{H_{res}(\theta_A, m_I)}{[1 + \gamma^2 T_2^2 H_{res}(\theta_A, m_I)]^2},$$

where

$$H_{res}(\theta_A, m_I) = H_{res}(0, 0)\left[1 - \frac{\Delta g}{g}\sin^2\theta_A\right] - m_I(T_{\parallel}^2\cos^2\theta_A + T_{\perp}^2\sin^2\theta_A)^{1/2}$$

with the definitions of constants as given Thomas and Cooke (1980) and θ_A related to the other angles by Eqs. 1 and 2.

The strategy is to use parameters relating to cross-bridge orientation (θ_0) and disorder (σ), as well as values of λ_A and λ_E ranging (with an appropriately fine mesh) from 0° to 90° (for λ) or 0° to 180° (for γ), to generate EPR spectra or fluorescence polarization ratios, and to compare the theoretical values with experimental observations. Most of the calculations have concerned EPR spectra, with the data of Thomas and Cooke (1980) being used for comparison. The results underline the need to distinguish between probe orientation and disorder and the orientation and disorder of the cross-bridges to which the probes are bound. The spectroscopic intensities were calculated using a program written in FORTRAN to run on an Eclipse S/230 minicomputer (Data General Corp., Southboro, MA). Actual integration of the expressions was carried out by the Gauss-Legendre quadrature technique, and the computed spectra were matched against experimental data by a least-squares minimization search procedure. For the EPR spectra we assumed [as did Thomas and Cooke (1980)] that the spin-label interactions could be treated as dipolar although this is known to be only approximately true (see McCalley et al., 1972) for nitroxide spin labels. This approximation causes small distortions of the central region of the spectrum when observing random or nearly random systems. Because these distortions were in many cases larger than differences occurring from the different angular parameters, the calculated spectra were fit against a computed pure dipole random spectrum (cf. Fig. 3*f*). The spectra distribution having uniform weighting. A satisfactory fit was considered to have been achieved when the χ-squared value was less than that obtained by fitting the data of Thomas and Cooke (1980) from relaxed fibers with that from their minced (random) myofibril data.

RESULTS

Rigor State

The rigor state occurs when a muscle fiber is deprived of ATP, and represents the simplest equilibrium state of the muscle that can be produced experimentally. In chemically skinned rabbit psoas muscle fibers, under conditions where full overlap of thick and thin filaments is allowed and ATP is excluded, there is evidence from biochemical (Cooke and Franks, 1980; Lovell and Harrington, 1981) and spectroscopic (Thomas et al., 1980) studies that the great majority, if not all, of the cross-bridges are bound to actin. The EPR measurements of Thomas and Cooke (1980) are consistent with a standard deviation of the dipole distribution of ~6°. Because some of this disorder may arise from sources not due to intrinsic cross-bridge disorder, namely axial skew of myofilaments, myofibrils, or fibers within the specimen, the observed value indicates a very high degree of axial order of dipoles in the rigor state. Using the model described above, several spectra were generated that gave a fit to the data that was considered satisfactory. One such spectrum is shown in Fig. 2, with the experimental spectrum. Because the cross-bridges are assumed to be bound rigidly to actin in rigor, no torsional freedom was concluded in the calculations. Solutions were found with

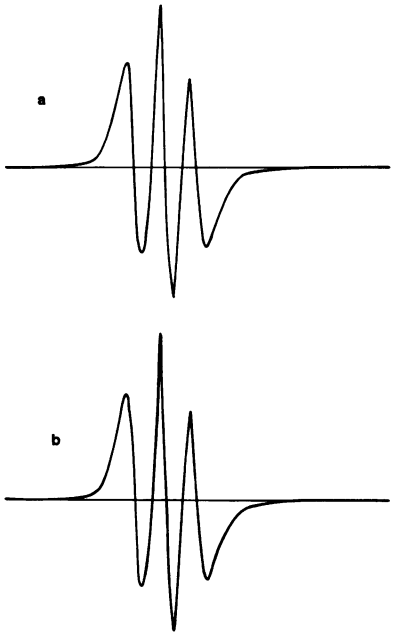

FIGURE 2 (*a*) Spectrum calculated using the values obtained by Thomas and Cooke (1980) as best fitting for muscle in rigor labeled with maleimide spin label, namely $\theta_0 = 82°$, $\sigma = 6.2°$ (and, implicitly, $\lambda = 0°$). Horizontal scale bar in this and all following spectra corresponds to a change in magnetic field (parallel to the fiber axis) of 100 gauss. (*b*) A spectrum calculated from the model in the text, using $\theta_0 = 30°$, $\sigma = 8°$, $\lambda_A = 60°$, $\gamma_A = 130°$. Other satisfactory fits with σ in the range 6°–8° were also found.

values of λ_A from 0° to 80°, the corresponding values of θ_0, the cross-bridge axial angle, tended to decrease with increasing λ_A from ~85° ($\lambda_A = 0°$) to ~20° ($\lambda_A = 80°$). The value of σ remained in the range 6°–8°. Thus we find that for the model considered the angular spread of the cross-bridges in rigor is comparable to the spread in dipole angle. This is consistent with the idea of a very high degree of specificity in the geometry of attachment of cross-bridges in rigor to thin filaments. We note however, that other less plausible models could produce these rather oriented dipole spectra while the cross-bridges themselves could have much greater disorder.

Relaxed State

The spectra obtained by Thomas and Cooke (1980) from relaxed glycerinated fibers are qualitatively very different from the spectra of those in rigor, as shown in Fig. 3. Thomas and Cooke (1980) noted a striking resemblance between experimental spectra such as that shown in Fig. 3, and those obtained from a suspension of homogenized labeled myofibrils, or—theoretically—from a population of dipoles directed randomly. The most straightforward interpretation of the results from relaxed fibers, and the one proposed by Thomas and Cooke (1980) is that the probe molecules in such fibers are arrayed essentially randomly.

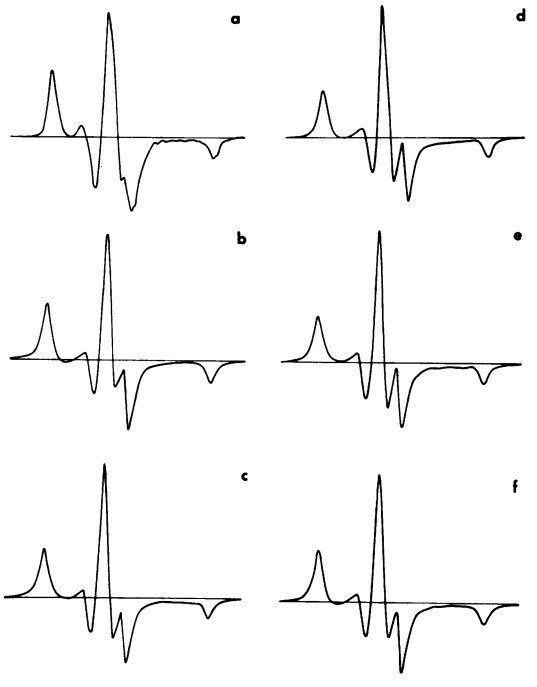

FIGURE 3 (*a*) Spectrum replotted from data of Thomas and Cooke (1980) obtained using maleimide spin label-treated relaxed muscle. (*b*) Model GT, $\theta_0 = 70°$; $\sigma = 21°$; $\lambda_A = 60°$. (*c*) Model G, $\theta_0 = 90°$; $\sigma = 30°$; $\lambda_A = 30°$; $\gamma_A = 10°$. (*d*) Model UT, $\theta_0 = 60°$; $\Lambda_{max} = 45°$; $\lambda_A = 45°$. (*e*) Model U, $\theta_0 = 75°$; $\Lambda_{max} = 75°$; $\lambda_A = 30°$; $\gamma_A = 180°$. (*f*) Isotropic; $\theta_0 = 90°$; $\sigma = 90°$; $\lambda_A = 0°$.

Using the model described above, it is possible to generate many partially-ordered cross-bridge/dipole arrays, and to compare the computed spectra produced by that with a completely isotropic distribution, with a view to minimizing the value of σ, the cross-bridge disorder parameter, while maintaining a satisfactory fit. An additional degree of freedom allowed for the relaxed state is that due to torsion around the cross-bridge axis $\hat{\chi}$. The effect of this rotation, for the cases where the dipole axis is not parallel to χ ($\lambda \neq 0°$), is to increase the axial spread of the dipoles at a given θ_0 and σ. It is not surprising, therefore, that we consistently have found that somewhat lower values of σ or Λ_{max} are possible with models GT and UT than with models G and U, when a satisfactory fit is found.

Also shown in Fig. 3 are theoretical spectra generated by the various models, with the condition that σ be minimized while an adequate fit is maintained. Model GT, with appropriately chosen θ_0 and λ_A, produces good agreement with experiment for values of σ as low as 21°. It is possible, therefore, to find model systems where nearly 70% of the cross-bridges lie within ±20° of the median axial angle, yet the spectrum that would be observed is effectively indistinguishable from the isotropic case and thus, from published measurements. Model G provided an estimate of the lower bound of σ of ~30°, while models UT and U yield disorder parameters Λ_{max} of at least 45° and 75°, respectively.

To test the possibility that varying the relative orientation of the magnetic field and the fiber axis might increase the amount of information available, we also computed the EPR spectrum with $\vec{H}$ perpendicular to the fiber axis ($\hat{k}$) for model GT with $\sigma = 21°$. The spectrum was virtually identical to the parallel field case, indicating that varying the orientation does not aid in choosing between possible models.

The central conclusion of this section is that we find that the angular spread of the cross-bridges in relaxed muscle may be substantially less than that suggested by the dipolar disorder, in contrast to the conclusion reached for the rigor state.

DISCUSSION

It is apparent from the results that considerable care must be exercised in inferring the orientation and distribution of macromolecules in an array on the basis of information about the disposition of bound probe molecules. An illustration of this principle is presented by the consideration of the change $\delta\theta_A$ in the axial angle of a dipolar probe rigidly bound to a macromolecule and related to the axis of the macromolecule by some λ_A and γ_A (Fig. 1). If the macromolecule undergoes a change in orientation it is apparent (see Fig. 1 *b* and Eq. 2a) that

$$\begin{aligned}\delta\theta_A &= \theta_A^{(1)} - \theta_A^{(2)} \\ &= \cos^{-1}\left[\cos\theta_{CB}^{(1)}\cos\lambda_A + \sin\theta_{CB}^{(1)}\sin\lambda_A\cos\gamma^{(1)}\right] \\ &\quad - \cos^{-1}\left[\cos\theta_{CB}^{(2)}\cos\lambda_A + \sin\theta_{CB}^{(2)}\sin\lambda_A\cos\gamma^{(2)}\right].\end{aligned} \tag{6}$$

In the absence of torsional change $\gamma_1 = \gamma_2$ and $\delta\theta_A \leq \delta\theta_{CB}$. An important special case occurs when $\gamma_1 = \gamma_2 = 0$ (or π) for then $\theta_{CB} = \theta_A + \lambda$ (or $\theta_{CB} = \theta_A - \lambda$) and $\delta\theta_A = \delta\theta_{CB}$. If torsion does occur and $\gamma_1 = 0$, then $\delta\theta_A \geq \delta\theta_{CB}$. At present it is impossible to ascertain the relative orientation of a dipole and cross-bridge axis. However, the $\gamma = 0$ situation occurs when $\theta_A = 0$; thus, the experimenter should strive to find probes oriented nearly along the fiber axis in the initial state to be certain of achieving the maximum sensitivity to subsequent change in cross-bridge declination. Similar considerations apply for a double dipole system as found in fluorescence. A recent experiment by Cooke (1982) found no change in dipole orientation upon stretching spin-labeled fibers in rigor. Although the dipole angles (68° and 83°) were far from zero for the two kinds of spin labels used, it seems improbable that both dipoles were oriented on S-1 so that the spectra were insensitive to $\delta\theta_{CB} \simeq 45°$.

A recent study of fluorescence polarization from ethenonucleotides bound to cross-bridges (Yanagida, 1981), assumes cylindrical symmetry of the angular distribution of the dipoles, but this represents a very special case of the general cross-bridge disorder model, i.e., case GT with $\theta_0 = 0$ (cross-bridge axis along fiber axis). This may explain why the calculated axial angles of the dipoles in Yanagida's (1981) analysis differ by at most 5°, irrespective of the

state of the fibers, whereas the dipolar separation under such conditions is much greater (32°). Thus, Yanagida's (1981) suggestion that the change in axial angle of cross-bridges during contraction may be small is not necessarily supported if a more general system of model disorder is considered.

If only axial disorder of the macromolecules is present, then Eq. 6 shows that the axial disorder of the dipoles will, in all probability, be less than that of the macromolecule. However, if torsional disorder of the macromolecules can occur about an axis nearly parallel to a direction in which the molecule is elongated, and the value of σ_A is substantial, a wide range of dipolar axial angles may be taken up while the mass of the macromolecule is distributed to only a small extent. This may provide a means of resolving the apparent disparity between the axial ordering of cross-bridge mass suggested by x-ray diffraction studies of resting, live (Huxley and Brown, 1967) and relaxed, detergent-skinned (Magid and Reedy, 1980) frog muscle, and the substantial axial disorder of probe molecules on cross-bridges as detected in relaxed glycerinated muscle fibers by EPR (Thomas and Cooke, 1980). There are other possibilities for the structural differences between living and glycerinated muscle. For example, it has been established (Rome, 1972) that the ordering of the myofilament array is partially disrupted by glycerination. In any case, it is likely that the major source of probe disorder in the relaxed glycerinated muscle fiber is due to disordering of the cross-bridge array, and the problem is to find plausible models of partial ordering that are consistent with a wide body of results obtained using different physical techniques.

One feature of the model that has not been discussed in detail is the identity of the cross-bridge axis, represented by the vector $\hat{\chi}$ in Fig. 1. It will be clear from our earlier discussion of the model that the general location of the axis within the cross-bridge must fulfill certain conditions. If torsional movement of the cross-bridge mass around the axis is to take place, it would be reasonable to place the axis close to a hydrodynamic axis of the myosin head cleaved from the rest of the myosin molecule. X-ray scattering measurements (Mendelson and Kretszchmar, 1980) are consistent with an elongated, asymmetric S-1 and generally agree with three-dimensional reconstructions of S-1 from electron micrographs (Seymour, 1980; Taylor and Amos, 1981). These show the molecule to be elongated roughly in the direction of attachment to actin, so to a first approximation the "cross-bridge axis" could be equated with this direction. Though the actual modes of motion of the cross-bridge may not be simply divisible into axial, azimuthal, and torsional diffusion of or about a cross-bridge axis, the model is nevertheless advanced as a starting point for further studies, in the absence of high-resolution structural and dynamic information about myosin.

H. E. Huxley's (1969) suggestion that the cross-bridge might attach to actin in a roughly perpendicular orientation and cause shortening by rotating to an acute axial angle (often abbreviated to the 90° and 45° states, respectively) has been a popular framework for models of the mechanism of muscle contraction. Reedy et al. (1965) used x-ray diffraction and electron microscopy of glycerinated insect flight muscle to establish the existence of distinct structural patterns in the cross-bridge lattice of the muscles relaxed and in rigor that correspond rather closely to the idealizations of the 90° and 45° states respectively, although no attachment to actin was seen in the relaxed muscle. Although it has been supposed that such states exist during contraction, no direct demonstration has yet been possible. It is therefore of interest to examine whether the EPR data of Thomas and Cooke (1980) are capable of providing model solutions consistent with $\theta_0 \sim 90°$ in the relaxed state, and $\theta_0 << 90°$ in rigor, without changing λ_A, the probe orientation angle on the cross-bridge. In fact, it can be seen from Figs. 2 *b* and 3 *b* that such a pair of cases may be found representing a decrease in θ_0 of 40° on passing from relaxation to rigor. To this extent, the data are consistent with such a model of the contractile process.

The model outlined in this paper has predictive power, particularly if torsional freedom occurs in the relaxed state. In such a case, probe molecules other than the maleimide spin label (MSL) used by Thomas and Cooke (1980) might well be bound such that the value of λ_A would be different, while cross-bridge–related parameters would be unchanged. This could lead to observation of much greater probe ordering than with MSL, under the same experimental conditions. This applies equally to fluorescence polarization, and work is currently in progress in this laboratory to re-examine the structural information available from muscle fibers labeled with fluorescent dyes, using the formalism presented here.

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