Skeletal muscle fibers consist of hexagonally packed actin and myosin filaments. According to x-ray diffraction and electron microscopy (EM), the structure is highly ordered both in hexagonal packing and along the filament axis. Globular actin monomers are organized in a double-stranded helix, while myosin molecules are stacked so that their elongated globular heads form a three-stranded helix. The attachment of these heads to actin filaments, followed by their rotation, is thought to cause sliding of the filaments past each other and thus provide the molecular basis of muscle contraction. This hypothesis is consistent with x-ray and EM evidence indicating a high degree of order in rigor, i.e., in the absence of ATP, with the heads forming cross-bridges between the myosin and actin filaments (Huxley and Brown, 1967) and the absence of these ordered cross-bridges in relaxing media (Heuser, 1983; Poulsen and Lowy, 1983). Unfortunately, x-ray diffraction does not provide direct information about crossbridge orientation, and EM cannot be used in functional muscle fibers. More specific measurements of cross-bridge orientation in functional fibers can be provided by EPR spectroscopy, which is primarily sensitive to both the orientation distribution of site-specific spin labels and also to their rotational motion. In previous EPR studies of spin labels attached to cross-bridges in glycinated muscle fibers, we have shown that in relaxed fibers the heads are dynamically disordered (executing large-amplitude microsecond motions) while in rigor the same heads are ordered and their motion is severely restricted (Thomas and Cooke, 1980; Thomas et al., 1980; Barnett and Thomas, 1984). The present work illustrates how the theory of conventional EPR lineshapes can be used quantitatively to determine the orientational distribution of spin labels attached to myosin heads.

**THEORY**

Conventional EPR spectra were simulated as described by Thomas and Cooke (1980). To a good approximation, in the absence of submicron-second rotational motion, the position at which a nitroxide spin label contributes to the absorption spectrum depends only on two variables: the nitrogen nuclear quantum number \( m_1 (\pm 1, 0, \pm 1) \) and the quantity \((\hat{\varepsilon} \cdot \hat{H}_0) \) where \( \varepsilon \) is the angle between the static magnetic field \( \hat{H}_0 \) and the principal axis \( \hat{z} \) of the spin label:

\[
H_{m_1}(\varepsilon, \theta) = \frac{g_1}{2} \hat{H}_0/g(\varepsilon) + m_1 T(\theta)
\]

\[
g(\varepsilon) = g_1 \cos^2 \theta + g_2 \sin^2 \theta
\]

\[
T(\theta) = \left[ T_\perp \cos^2 \theta + T_\parallel \sin^2 \theta \right]^{1/2}
\]

This orientation dependence is illustrated by the simulated conventional EPR \( V_1 \) spectra in the top row of Fig. 1. Thus for a uniformly oriented population of spin labels having a single value of \( \cos \theta \) (e.g., in a single crystal, in a stack of crystalline membranes oriented normal to \( H_0 \), or in a bundle of perfectly helical fibers oriented parallel to the magnetic field), one observes a three-line spectrum in which the position of the center line is determined by \( g(\theta) \) and the splitting between the three lines is determined by \( T(\theta) \). Fig. 1 shows that each of these lines is so narrow that spectra corresponding to only slightly different orientations are easily resolved.

If there is only partial orientational order of the spin labels within a sample, the EPR spectrum provides a direct and unambiguous readout of the orientational distribution. That is, in contrast to optical spectroscopy, EPR provides independent information about the average orientation, the orientational disorder, and the number of discrete preferred orientations in the population. For example, Fig. 1 shows spectra corresponding to a spherically weighted Gaussian distribution of orientations \( \rho(\theta) \) centered at \( \theta_0 \) and having a full width at half maximum of \( \Delta \theta \):

\[
\rho(\theta) \propto (\sin \theta) \exp \left(-\frac{1}{2}\left[\frac{\theta - \theta_0}{\Delta \theta/2}\right]^2\right)
\]

Each row in Fig. 1 demonstrates the sensitivity of the conventional EPR spectrum to changes in \( \theta_0 \). In each of the columns, the angular spread of orientations, \( \Delta \theta \), is varied from perfect orientation (\( \Delta \theta = 0^\circ \)) to a random distribution (\( \Delta \theta > 180^\circ \)). Following the spectra in each column, we note that the lines become progressively broader (and less equal in height) with increasing angular spread (\( \Delta \theta \)). The splitting, however, remains essentially constant in each column, i.e., is independent of \( \Delta \theta \). In

![Figure 1](image-url)

**FIGURE 1** Computer-simulated spectra showing the dependence of the conventional \( V_1 \) EPR spectra on \( \theta_0 \) (the center of the orientational distribution) and \( \Delta \theta \) (the full width at half maximum of a Gaussian distribution of spins about \( \theta_0 \); see Eq. 2). The columns represent different values of \( \theta_0 \) (0°, 45°, 90°), while \( \Delta \theta \) is varied along each row. The spectra are first derivatives of a Lorentzian line with half width at half maximum of 2.35 G, simulated using the following tensor values: \( g_1 = 2.00241; g_2 = 2.00274; T_\perp = 35.0 \text{ G; and } T_\parallel = 7.0 \text{ G.} \) The spectra have been normalized to the same total amplitude and are centered at 3,400 G with a scan range of 100 G.
contrast, variation of $\theta_i$ causes large changes in the splitting, as can be seen by comparing columns. Thus $\theta_i$ and $\Delta \theta$ can be determined independently.

RESULTS AND DISCUSSION

From the many possible ways to determine the spectral parameters in Fig. 1, we have chosen the distance between baseline crossing points of the derivative spectrum ($2T'$) to represent the splitting, and the relative peak amplitudes ($L/C$) to reflect broadening effects. (The amplitude is more sensitive to linewidth change than is a direct measurement of the peak-to-peak width.) These parameters are well defined in the experimental spectra of fibers oriented parallel to the magnetic field and minimize the

Figure 2  Dependence of the spectral splitting and amplitude ratio upon the orientational distribution of spins. The splitting parameter $2T'$ and the line height ratio $L/C$ are defined in the inset Fig. 2A. (A) $2T'$ vs $\theta_i$ for different values of $\Delta \theta$: 0° ($\triangle$), 30° (o), 60° ($\ast$). (B) $2T'$ vs $\Delta \theta$ for different values of $\theta_i$: 0° ($\Delta$), 15° (□), 30° (o), 45° ($\ast$), 60° (■), 75° (●), and 90° ($\ast$). (C) $L/C$ vs $\theta_i$ for different values of $\Delta \theta$: 0° ($\Delta$), 10° (□), 20° (o), 30° (■), 40° (□), 50° (●) and 60° ($\ast$). (D) $L/C$ vs $\Delta \theta$ for different values of $\theta_i$: 0° ($\Delta$), 15° (□), 30° (o), 45° ($\ast$), 60° (■), 75° (●), 90° ($\ast$), and for the best fit values of $\theta_i$ from Fig. 3. 67° (dotted line) and 80° (dashed line).
contribution of weakly immobilized or nonoriented spin populations arising from nonspecific labeling. For spectra with a large angular spread, the baseline crossing points become less well-defined, and analysis requires either the use of other spectral parameters or simulation of the entire spectrum.

Fig. 2 A shows the dependence of the splitting 2T° on the angle θν. The splitting 2T° is defined in the inset to the figure. This splitting is most sensitive to changes in θν at 45°, where a one degree change results in a one Gauss change (easily resolved) in the splitting 2T°. At angles smaller than 15°, the splitting dependence is only about a seventh of the dependence at 45°, but the measurement precision of this parameter still allows 4° resolution or better in θν. At large angles (θν ≥ 75°), there is a substantial loss of spectral sensitivity for the populations with angular distribution ∆θ > 20°. In general, however, Fig. 2 A provides a good estimate of the center of the orientational distribution independently of the distribution spread ∆θ. This finding is further substantiated by Fig. 2 B, which shows that there is a very weak dependence of the splitting on ∆θ (≈ 0.016 G per degree in most cases).

The second parameter, the line-height ratio of the low and central field peaks (L/C in the inset of Fig. 2 A) is a function of both θν and ∆θ. The latter dependence is used to determine the spread from experimental spectra. Fig. 2 D shows the line-height ratio (L/C) curves vs. ∆θ. The dependence is not unique, which is the direct result of the sensitivity to θν (Fig. 2 C), and which suggests that θν must be determined before determining ∆θ. The precision of the determination of ∆θ is somewhat lower than in the case of θν, partly because of lower sensitivity (compare the slopes in Fig. 2 A with those in Fig. 2 D), but mainly as a result of the line-height dependence on θν. This will be the case for θν near 90°.

We will now apply the theoretical results just presented to an experimental case, oriented muscle fibers (spin-labeled as in Thomas and Cooke, 1980) in which the fiber axis is parallel to the magnetic field. The experimental spectra for two different spin labels are presented in Fig. 3 (solid lines). In each case, the spectrum contains only three well-defined lines, suggesting that the analysis of Fig. 2 can be applied. The parameter 2T° was 29.6 G and 17.0 G for IASL- and MSL-labeled psoas fibers, and L/C was 0.62 and 0.54. From Fig. 2 A we first determined the range of possible values for θν to be 64°–68° for the IASL case (Fig. 3 A) and the corresponding range of values from Fig. 2 D for ∆θ to be 9° to 11°. We then simulated spectra at the four combinations of θν and ∆θ and by interpolation determined θν to be 67° and ∆θ to be 10°. A similar process gave θν of 80° and ∆θ of 13° for MSL fibers. These values are slightly different from those reported previously, because in the present study the spin-labeling was more specific and the fibers were better oriented, using the method of Cooke et al. (1982). Further, we then used these results to calculate the theoretical spectra (dotted lines in Fig. 3). The good fit between theory and experiment, especially for the IASL case (calculated 2T° of 30.0 G and L/C of 0.60), shows that the analysis given above can be successfully used to determine the orientation distribution of the nitroxide z axis in this oriented system. The shoulders in the experimental spectra, most easily detected in the spectrum from fibers labeled with MSL, may be due to a second conformation of the myosin head with respect to the fiber axis in rigor. This possibility has been suggested by an analysis of electron microscopy data (Taylor et al., 1984).

The quality of the computer fits to experimental spectra could be further improved by the use of nonaxial tensors (Polanszek and Freed, 1975). This would also introduce sensitivity to the azimuthal angular distribution, extending the model on which we have based our analysis. In addition, the orientational resolution can be improved by the use of deuterated spin labels (Polanszek and Freed, 1975).

Although the present analysis is sufficient to describe muscle in rigor with highly oriented heads, the analysis becomes more model-dependent at larger angular spreads. For example, a less ordered spectrum could be described by the sum of many Gaussian distributions of narrower angular spread as well as by a single distribution with larger spread. In these cases a promising approach is the analysis in terms of a complete set of spherical harmonics to describe the orientational distribution (T. Burghardt and N. Thompson, personal communication) as was done for the determination of the orientation distribution for spin labels in liquid crystals (Polanszek and Freed, 1975). The method of analysis described in the present study is also applicable to other macroscopically oriented systems, such as a stack of oriented membranes in which the membrane normal replaces the fiber axis in the analysis.

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EPR of Muscle Fibers
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