DEPOLARIZATION SPECTRUM OF DIFFRACTED LIGHT FROM MUSCLE FIBER

The Intrinsic Anisotropy Component

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ABSTRACT The depolarization signal of the diffraction patterns from muscle fibers includes information that differs from that of transmission birefringence experiments. Although both the birefringence studies and the phase shift studies of Yeh et al. (Yeh, Y, and G. Pinsky, 1983, *Biophys. J.*, 42:83–90; Yeh, Y., M. E. Corcoran, R. J. Baskin, and R. L. Lieber, 1983, *Biophys. J.*, 44:343–351) include inseparable intrinsic and form contributions, the present analysis shows that the magnitude of the E-field components of diffracted light is affected only by the intrinsic contribution. We have analyzed the amplitude portion of the data of which the phase shift portion had previously been reported (Yeh, Y., M. E. Corcoran, R. J. Baskin, and R. L. Lieber, 1983, *Biophys. J.*, 44:343–351). For the relaxed-to-rigor transition, these field amplitudes also exhibit changes when ATP concentration is decreased. The observed decrease in optical depolarization upon rigor is consistent with the idea that optically anisotropic elements move away from the myosin thick filament under such conditions.

In recent publications by our group the depolarization of light diffracted by skeletal muscle fibers has been shown to be sensitive to changes in the state of the fiber (1, 2). The most pronounced factor that indicates these changes has been the phase angle, δ, defined as the relative phase shift between the two mutually perpendicular electric field components upon its interaction with the fiber. Although exhibited on a diffraction order, this phase-angle signal carries information that is not exclusively for the diffraction process. In fact, unless the sarcomeric elements absorb radiation at the incident wavelength the information contained in the phase-angle measurement is very similar to that obtained in birefringence studies, particularly those conducted by Taylor (3, 4).

Two problems beset all birefringence experiments and indeed the same difficulties apply for our phase-angle measurements. One is the separation of the contributions to the overall birefringence signal: intrinsic and form. To separate form and intrinsic contributions to birefringence, often the fiber is immersed in some index matching fluid. Under those nonphysiological conditions, there is the critical question of whether the elicited intrinsic birefringence is of the same significance as native intrinsic birefringence. The other problem is that the measured phase angle and the magnitude of birefringence is related by the optical thickness or distance of light traversal. This thickness is not a trivial parameter to ascertain particularly for activated fibers. We show here that a complete characterization of the ellipsometry signal from the diffracted light can, to a major degree, overcome both of these difficulties.

To completely characterize the elliptically polarized light not only must the phase information be obtained but the amplitudes of the elliptically polarized electric field (in the laboratory frame of reference) must also be characterized. A measure of those quantities by reflectance ellipsometry was recently shown to be of value in the determination of intrinsic anisotropy of a crystalline system (5). Because amplitudes are sought in this experiment, not the phase retardation, there is no need to calculate the average path distance of beam traversal as was done by Eberstein and Rosenfalck (6). The purpose of this paper is to amplify these ideas and to point out indeed that field amplitude data exclusively contain intrinsic anisotropy information. Furthermore, the complete diffraction polarization spectra provide a unique optical tool to differentiate between the intrinsic contributions and the form contributions to ellipsometry measurements without resorting to other more invasive techniques (7, 8).

We start by examining the well-known Lorentz-Lorenz relationship for an isotropic dielectric (9). This equation

$$\frac{n^2 - 1}{n^2 + 2} = \frac{4\pi}{3} N\alpha \qquad (1)$$

provides the connection between the macroscopic index of refraction, n, and the microscopic molecular or atomic

system with polarizability, α, and number density, N. For such an isotropic system, either an increase in N or a change in α will lead to a change in the macroscopic quantity n, which affects the velocity of light propagation in the medium. If we now consider that a material has two distinctive principal axes, then each of the axes has its own distinctive spatial density and polarizability, $N_\perp$, $\alpha_\perp$, and $N_\parallel$, $\alpha_\parallel$. From Eq. 1, each component has its own macroscopic index of refraction, $n_\perp$ and $n_\parallel$. These quantities may be different due to (*a*) differences in $N_\perp$ and $N_\parallel$ alone but no differences in $\alpha_\perp$ or $\alpha_\parallel$ (form) or (*b*) differences in $\alpha_\perp$ and $\alpha_\parallel$ but no difference in $N_\perp$ and $N_\parallel$ (intrinsic). In a more general case, the changes in $n_\perp$ and $n_\parallel$ may result from changes in both the densities and the polarizabilities. The phase shift measured in our experiments or in a birefringence experiment contains all of this information. However, due to the small amount of energy loss by light scattering, passage of light through such a homogeneous anisotropic medium results in relative phase shifts with negligible change in the amplitudes of the transmitted electric-field vectors. Thus it is clear that the conventional birefringence experiment cannot optically separate the contributions from form effect and intrinsic effect.

A fiber, however, is spatially inhomogeneous in that it exhibits periodicity along the axis composed of both isotropic and anisotropic parts. Even though the measured phase shift on a diffracted order contains both intrinsic and form information as in the birefringence experiments, the **E**-field amplitude differences contain contributions only due to anisotropic polarization along the principal axes of the diffracting elements. This is because the **E**-field amplitude difference arises from the magnitude of the induced polarization along those principal axes due to molecular anisotropy. Thus measurement of these **E**-field amplitude differences in the diffracted order will provide information only about the degree of anisotropy exhibited by elements, which also have the spatial periodicity responsible for the diffraction pattern.

In characterizing the diffraction anisotropy fully, the initial step is to define a model that clearly exhibits the anisotropy of the significant element within the fiber. We assume in this model that the fiber is composed only of periodic arrangements of intrinsically anisotropic elements. Since the interacting wavelength of light, λ_0 is ~0.5 μm, it is appropriate to integrate the contribution of all the anisotropic elements over such a volume (λ_0^3). We consider the anisotropic element as the myosin rod: light meromyosin (LMM) and the S-2, possibly with a flexible joint at the LMM–S-2 junction (10). Assuming that these elements do not absorb visible light, the principal polarizabilities of each element may be given by real polarizabilities $\alpha_\perp$ and $\alpha_\parallel$, transverse and parallel, respectively, to the helical rod axis. Upon integrating over a symmetrical distribution of elements all of which have the same radial angle of declination, θ_t, from the thick filament axis, one finds that the sampled volume still has its principal optical anisotropy axes along the fiber axes.

$$\alpha_T = \begin{bmatrix} \alpha_1 & 0 & 0 \\ 0 & \alpha_2 & 0 \\ 0 & 0 & \alpha_1 \end{bmatrix}, \tag{2}$$

where

$$\alpha_1 = \alpha_\perp [1 - (\sin^2 \theta_t/2)] + \alpha_\parallel (\sin^2 \theta_t/2)$$
$$\alpha_2 = \alpha_\perp \sin^2 \theta_t + \alpha_\parallel \cos^2 \theta_t. \tag{3}$$

Note that if $\theta_t = 0$, then $\alpha_1 = \alpha_\perp$ and $\alpha_2 = \alpha_\parallel$, as expected.

In the most elementary model that exhibits optical depolarization, we consider diffraction as light coherently deflected into the various orders due to spatial periodicity of these anisotropic elements. If the light field incident upon the fiber is linearly polarized and oriented at 45° with respect to the fiber axis, which has an effective polarizability α_2, then the diffracted light will have its plane of polarization changed depending on the relative magnitude of α_1 and α_2. It is this change in the plane of polarization that measures the amount of intrinsic anisotropy. Within this dipole approximation where only single scattering processes are considered, the diffracted electric field at the angle of a diffraction maximum, θ_D, is given by

$$E_\perp = K E_0 e^{i\delta_1} \alpha_1$$
$$E_\parallel = K E_0 e^{i\delta_2} \alpha_2 \cos \theta_D, \tag{4}$$

where K is a constant, E_0 is the incident field magnitude, and $\delta = \delta_1 - \delta_2$ is the net phase shift due to birefringence.

In actual measurements, field amplitudes are not directly obtained. Instead, the Stoke's vectors (11) are the experimentally measurable quantities. If pure polarization states are assumed, then

$$I_M = K^2 E_0^2 [\alpha_1^2 + \alpha_2^2 \cos^2 \theta_D]/2$$
$$Q_M = K^2 E_0^2 [\alpha_1^2 \text{ M } \alpha_2^2 \cos^2 \theta_D]/2$$
$$U_M = K^2 E_0^2 \alpha_1 \alpha_2 \cos \theta_D \cos \delta$$
$$V_M = -K^2 E_0^2 \alpha_1 \alpha_2 \cos \theta_D \sin \delta. \tag{5}$$

The last two equations for U_M and V_M lead to the evaluation of δ, while from the first two equations, α_1 and α_2 can be determined to a constant K. The ratio

$$r \equiv |(\alpha_1 - \alpha_2)/(\alpha_1 + \alpha_2)|, \tag{6}$$

however, is independent of K and is a measure of intrinsic changes due to the state of the fiber. If the model had included isotropic contributions as well, the denominator would reflect the total contribution to the intensity. Note that in such a formulation, the significance of r is comparable to a measure of depolarization in studies of scattering of light by anisotropic macromolecules (12). The difference

between anisotropic macromolecules in solution and this situation is that here the basic scattering entity is a collection of well-ordered and oriented anisotropic molecules. Substitution of Eq. 2 into Eq. 6 yields an orientational dependence of r

$$r(\theta_t) = r_0 \frac{1 - \frac{3}{2}\sin\theta_t}{1 + \frac{r_0}{2}\sin\theta_t}, \quad (7)$$

where

$$r_0 \equiv |(\alpha_\perp - \alpha_\parallel)/(\alpha_\perp + \alpha_\parallel)| \quad (8)$$

measures the intrinsic polarizability changes, while $r(\theta_t)$ reflects both r_0 and θ_t dependencies. Note that for a fixed r_0 as the angle θ_t increases, r decreases. Thus in measuring changes in r, intrinsic anisotropy changes alone are obtained and, in principle, r and θ_t determinations can be combined to elicit the changes in the degree of form contribution.

We report here the experimental value of r for chemically skinned fibers taken at ATP concentrations that represents the relaxed-to-rigor transition. Data were obtained from fibers over three length ranges: 2.55, 3.05, and 3.75 μm. The basic data come from the fibers on which we had previously reported the phases shift information (2). Fewer samples are reported here because a more stringent set of criteria for data selection was used for these weaker signals. The basic criteria are that (*a*) data spread must be small, thus χ^2 values of <1 serve as one condition and (*b*) the degree of polarization uniformity must be good. This criterion ensures that we are not averaging over nonfiber related contributions (11). Here we used the condition that

$$R \equiv \frac{Q_M^2 + U_M^2 + V_M^2}{I_M^2} \geq 0.80. \quad (9)$$

Although this value is a much more relaxed requirement than set for truly statistically pure polarization states, ($R \rightarrow 1.00$), it provides an operational definition for relatively pure state.

In Fig. 1, those values of r for chemically skinned fibers are plotted against ATP concentration for the several sarcomere lengths indicated. Two features of the figure are of interest. First, there is a decrease in r value upon going from the relaxed state, 4 mM ATP, to the rigor state, O mM ATP. The decrease is very noticeable when the fibers are within overlap dimensions, while at sarcomere length (SL) of 3.75 μm, the change is much less pronounced. These results are similar to those reported in reference 2 and are consistent with the idea that as the fiber is placed into the rigor state, the intrinsically anisotropic elements are pulled away from the thick filament as S-1 makes its rigor complex with the f-actin. It is of interest to estimate

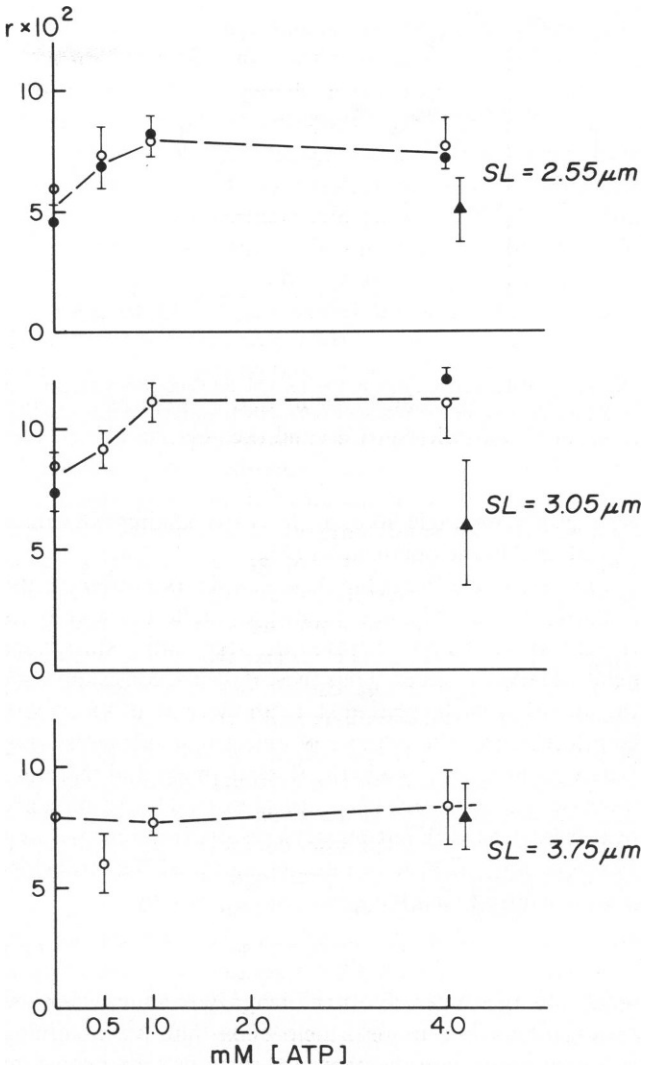

FIGURE 1 Polarization ratio, r, vs. ATP concentration at different sarcomere lengths. O and ● represent different experiments conducted at the same sarcomere length. ▲ values are from single fiber data of Z. Xu (unpublished results) at the corresponding conditions. Error bars represent maximum range of uncertainty from several runs at that condition.

the angle of declination of S-2 upon rigor if one assumes (*a*) that only the myosin rod is intrinsically anisotropic and (*b*) at $SL = 3.75$ μm, all S-2 are lying parallel to the thick filament, $\theta_t = 0°$. With these assumptions, the value of r at $SL = 3.75$ μm is simply r_0. We let $r_0 \sim 8.0 \times 10^{-2}$. At maximum overlap and in rigor, $r \sim 6.0 \times 10^{-2}$. Using Eq. 7, $\theta_t \sim 24°$. This value of θ_t is larger than other calculationed estimates based on established geometric constraints (3), but is still within a reasonable range.

More evidence that shows that the results are indicative of intrinsic anisotropy of the fiber is a comparison of single fiber data with these data from bundles of three fibers. Although we had shown that δ values varied with fiber thickness (2), these r values changed much less under identical conditions, as can be seen by the (▲) results from

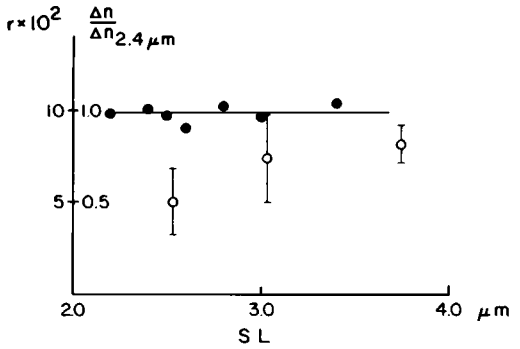

FIGURE 2 Polarization ratio, r, and relative birefringence ($\Delta n/\Delta n_{2.4\mu m}$) vs. SL for skinned fiber at rest state data shows constancy of ($\Delta n/\Delta n_{2.4\mu m}$) vs. SL, while r increases over the same increase in SL.

resting skinned single fibers at the corresponding SL values placed next to the bundle data (Fig. 1).

Furthermore at least for these few fibers examined, the relaxed fiber at 2.55 μm exhibits the smallest r value in its relaxed state. As SL is increased, the resting state fiber exhibits larger r values. Thus these data are consistent with the idea that in the resting state an increase of sarcomere length increases the number of anisotropic elements lying nearly parallel ($\theta_t = 0°$) to the thick filament and therefore increases the intrinsic anisotropy (Eq. 7). The SL data are next compared with our previous results (references 1 and 2) where only the phase angle δ is examined. The transformation from δ to birefringence, Δn, is given by

$$\delta = (2\pi/\lambda_0)\, d\Delta n, \qquad (10)$$

where d is the thickness of the fiber. Upon reanalyzing the resting fibers δ-value data, one finds that Δn, assuming isovolumic nature of the fiber (13) remains rather constant in value from SL of 2.2 to 3.4 μm. This difference between Δn vs. SL and r vs. SL (Fig. 2) further points to the fact that there is a difference between the information carried by the amplitudes (intrinsic alone) and that carried by the phase shift of the elliptically polarized diffraction signal (intrinsic and form contributions).

Finally we mention that a more complete analysis must take into account the relative proportions of axially aligned anisotropic elements, tilted elements, and isotropic elements that compose the remainder of the fiber. However, the fact that we have observed changes in r is a good indication that anisotropic elements provide a significant contribution to the total signal. Furthermore, multiple scattering, which will alter the diffraction ellipsometry signal, has not been included here. We have, however, demonstrated that the complete ellipticity signal can be used to separate intrinsic and form contributions to optical depolarization and birefringence.

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