OPTICAL ELLIPSOMETRY MEASUREMENTS ON THE DIFFRACTION PATTERNS FROM SINGLE FIBERS

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The technique of ellipsometry has been applied to the optical diffraction pattern to probe the structure and dynamics of the muscle sarcomere. The major advantage of this experiment over the technique of birefringence is that because the diffracted light is unencumbered by the strong forward unscattered beam, the amplitudes of the two electric field components and the phase between them can be measured (1). Because the electric field components of the diffracted light are a measure of the differential polarizabilities along the principal fiber axes, only the intrinsically anisotropic elements exhibiting sarcomeric periodicity contribute to this measurement. On the other hand, because phase shift comes from total optical path-length differences experienced by the two components of the electric field, both the intrinsic and the form anisotropic elements contribute to this effect. This total ellipsometry signal provides new insights on the molecular state of the sarcomeric elements, shown here to occur after changes in the state of the fiber.

Our model considers the sarcomere to be composed of isotropic and anisotropic elements. The principal anisotropic elements are the myosin rods (LMM + S-2); all other elements are considered isotropic. The fiber is assumed to exhibit axial symmetry: All cross-bridges (S-1 + S-2) are symmetrically arranged about the fiber axis. After all the elements are summed, the optical polarizability tensor maintains axial, α_1, and radial, α_2, principal polarizabilities that depend on the magnitude and orientation of these elements. When light at normal incidence and with its electric field 45° to the fiber axis is diffracted by the fiber, the intrinsic anisotropy yields a depolarization ratio

$$r = \left|\frac{\alpha_1 - \alpha_2}{\alpha_1 + \alpha_2}\right|. \quad (1)$$

The diffracted light also yields a phase shift given by

$$\delta = \frac{2\pi}{\lambda_0} d[\Delta n_F + \Delta n_I], \quad (2)$$

where d is the thickness of the fiber, Δn_F and Δn_I are the contributions to birefringence by form and intrinsic elements. The latter contribution is directly proportional to the depolarization ratio, r.

An ellipsometer is any device or series of optical devices that can fully characterize the state of the polarization of light. Here, the depolarization ratio and the phase difference as given by Eqs. 1 and 2 will fully describe the polarization properties of the diffracted light. Fig. 1 depicts two ellipsometer configurations that we have used successfully. The fundamental limitation on the usage of the quarter-wave plate device (Fig. 1 *A*) is the rate at which the quarter-wave plate can be rotated, which is limited to one rotation/30 s. Such rates cannot follow fast transients that develop where the fiber is stimulated by an electric field. Thus this instrument has been confined to the study of chemical-induced changes of skinned fibers. The second method (Fig. 1 *B*), separates the measurement of depolarization ratio by a Wollaston prism from the measurement of the phase angle by a fast photoelastic modulator (PEM). The limiting rate here is the rate at which the PEM can cycle through a period, which is 20 μs. The simultaneous recording of the two relevant parameters in real time allows for the use of this method to monitor truly dynamic events, such as tetanic contraction, fast twitch, and quick stretch or release studies.

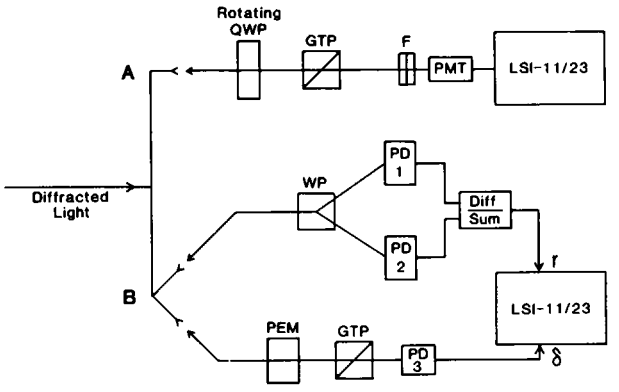

FIGURE 1 Schematic representations of the two types of ellipsometers. (*A*), rotating quarter-wave plate scheme. Light passes through the QWP and a Glan-Thompson polarizer (GTP) before being filtered (F) and detected by the photomultiplier (PMT). (*B*), the signal is initially split; one part goes through a Wollaston prism (WP) and is detected by the two photodiodes (PD1 and PD2); the difference and sum of these signals are ratioed and then fed to an on-line computer. The other part of the signal passes through the photoelastic modulator (PEM) and a GTP before being detected by PD3. Phase-shift signal is evaluated and sent to the computer.

BIOPHYS. J. © Biophysical Society · 0006-3495/86/01/43/02 $1.00
Volume 49 January 1986 43–44

APPLICATION TO TWO CASES

Relax-to-Rigor Transition

In the rigor state, S-1 heads of HMM are locked into an angle on the f-actin. Since the method used here is sensitive to anisotropic elements and their changes when they go into rigor, the measured quantities monitor the orientational changes of intrinsically anisotropic elements and packing changes of isotropic elements. Plotted in Fig. 2 are the relaxed (1 mM ATP) and rigor state data at several ionic concentrations, μ, of the bathing medium. It is apparent that at any μ condition, the rigor state has both a lower depolarization ratio, r, as well as a lower total birefringence, Δn (2), when compared with its corresponding relaxed state values. A decrease in the depolarization ratio upon rigor is consistent with the idea that as S-1 binds, the connecting S-2 element subtends a larger angle with respect to the fiber axis. These results together are consistent with the idea that when a fiber goes into rigor, intrinsic anisotropy changes dominate over form birefringence changes.

Low Ionic Strength Solutions

Brenner et al. (3) noted that a decrease of the total ionic strength of the relaxing solution causes an increase in the dynamic stiffness of the fiber. X-ray diffraction studies have indicated that an increase in the mass concentration at the actin occurred after the decrease of solution ionic strength (4). In our experiment, ionic strength was varied at both the relaxed (1.0 mM ATP) and the rigor (0.0 mM ATP) states. The trend of Δn and that of r with decreasing ionic strength differ. As the ionic strength is lowered from the normal 100 mM to 20 mM, there is a strong increase in Δn while the change in r is almost negligible. When the same experiment is conducted at rigor state, the trends are similar but the overall values of Δn and r are both smaller. The difference in our results between the rigor state and the low ionic strength state is consistent with the idea that the nature of movement and redistribution of matter are different for these two states.

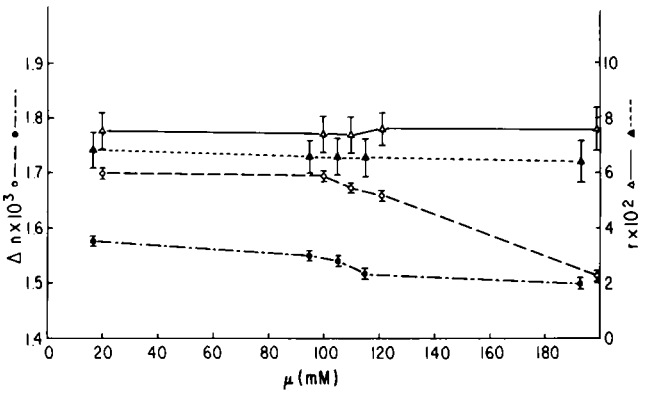

FIGURE 2 Ellipsometry parameters, Δn and r, plotted against the solution ionic strengths, μ. The relaxed fiber data, obtained at 1 mM ATP, is given by ○ for Δn, and △ for r; the rigor state fiber data is represented by ● for Δn, and ▲ for r. Straight line connections from point to point have been used to illustrate the trends.

We acknowledge the skillful assistance of Mrs. Quan You Li in the preparation of the single fibers.

This work is supported in part by grant AM-26817 from the National Institutes of Health to Y. Yeh, and by grant PCM-8300046 from the National Science Foundation to R. J. Baskin. J. S. Chen is a Visiting Scholar supported by the P.R.C.

Received for publication 11 May 1985.

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THREE-DIMENSIONAL RECONSTRUCTIONS OF OPTICALLY IMAGED SINGLE HEART CELL STRIATION PATTERNS

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INTRODUCTION

The quantification of sarcomere length in cardiac muscle is of critical importance for the unambiguous interpretation of contractile performance. Myocardial mechanics are routinely characterized by the interrelated parameters of length, force, velocity, and time. Any unknown variation in

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Volume 49 January 1986 44–46