Fine structures in the light diffraction pattern of striated muscle

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Summary

Single skeletal muscle fibres of frog were illuminated with a He–Ne, argon-ion or rhodamine 6G dye laser. The fine structures lying within the diffraction columns moved parallel to the fibre axis without changing their pattern when either the wavelength or the incident angle of the laser beam was varied, or when the fibre was stretched slightly. However, their pattern remained nearly constant when the fibre was submerged in hypotonic or hypertonic solution. As the illumination of about 1 mm or 0.1 mm width scanned along the length of the fibre, new structures emerged while others faded away giving rise to the notion that the diffraction columns were moving in the direction of the scan. A decrease in the illumination width caused the structures lying on the periphery of the diffraction column to disappear and the width of the remaining structures to increase. Measurements rule out the existence of large diffraction planes in these muscles. In addition, they indicate that the fine structures come from the diffraction of the whole rather than independent components of the illuminated volume. The origin of the fine structures is explained by two diffraction models.

Introduction

The appearance of light and dark bands in striated muscle fibres is due to periodic variation in the refractive index along the fibre. As a result, the fibre has the appearance of an optical diffraction grating. Its diffraction of a monochromatic light beam displays a series of almost equally spaced columns. Each column contains many parallel and narrow discrete fine structures. The angular separation between the centroids of the diffraction columns has been interpreted to be directly related to the average striation spacing or sarcomere length. The determination of the centroid of the column or sarcomere length is dependent on the intensity distribution of the fine structures. Although light diffractometry is widely used to measure sarcomere length, the origin of the fine structure is still uncertain.

Attempts have been made to characterize the fine structures lying within the diffraction column. Cleworth & Edman (1972) suggested that the fine structures were...
due to the wavy A–I boundaries and waviness of the myofibrillar registers. Tameyasu et al. (1982) tabulated the separations of the fine structures lying within the first-order diffraction and found that certain separations were more probable than others. Leung (1983a) measured the positions of the fine structures when the muscle fibre was illuminated with a laser of variable wavelength and confirmed that the shift in the position of the fine structure due to a change in the wavelength of the incident light beam obeys the plane grating equation. Leung (1983b) also tracked the movements of the fine structures of electrically stimulated single myocardial cells and showed that each structure moved uniformly and independently of other structures during the contraction–relaxation cycle of the cell. Although there is no direct evidence that a fine structure is related to a specific component within the illuminated volume of the muscle fibre, each fine structure has been assumed to be the result of the scattering by a group of sarcomeres of nearly identical lengths (Cleworth & Edman, 1972; Tameyasu et al., 1982; Leung, 1983a,b,c).

Theoretical descriptions (Judy et al., 1982; Leung, 1982a) have some success in explaining the diffraction fine structures, but they are oversimplified. They describe the fine structures as the result of scattering of a plane wave by a sarcomere array within a homogeneous medium. In addition, they require that the sarcomeres of equal length are serially contiguous or clustered together. However, the output beam of a laser is not a plane wave (for example, Ruscio, 1966) and the muscle fibre is a heterogeneous medium. In striated muscle fibres, the fundamental diffractors are likely to be sarcomeres. Their dimensions and distribution determine the diffraction pattern. Accurate specification of the sarcomere array in living muscle fibre is probably impossible to achieve. Sarcomere length and myofibrillar diameter dispersion would lead to a noncrystalline or random sarcomere array (Leung, 1982b, 1983c; Leung et al., 1983) and to the expectation that the sarcomeres of equal length are dispersed throughout the illuminated section of the fibre rather than clustered within a small volume. The far-field diffraction of a plane wave by such a random sarcomere array bathing in a homogeneous medium could not fully explain the observed pattern of fine structures.

The diffraction pattern can provide structural information about the fibre. The diffraction fine structures could yield more information than just the sarcomere length once their origin is known. This investigation presents measurements which further characterize the diffraction fine structures and uses the results to evaluate different diffraction models in an attempt to explain the origin of these structures.

Materials and methods

The light source for diffraction was either a He–Ne (Spectra Physics, model 120), argon-ion (Spectra Physics, model 164–03) or rhodamine 6G dye laser (Spectra Physics, model 375). The dye laser output beam passed through an etalon to reduce its spectral line width to about 0.5 nm and its wavelength was measured with a monochromator (Spex, model 1702) to ±0.1 nm. The beam power of any laser was attenuated by a calcite crystal polarizer to about 5 mW.

Single fibres from the dorsal head of the semitendinosus muscle of Rana tigrina regulosa were