Sarcomere lengths of thick skeletal muscle specimens measured under an epi-illumination-type polarization microscope

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Abstract—The purpose of this study was to measure sarcomere lengths of thick muscle fiber bundles at resting and at isometric tetanic contractions. We developed a novel measurement system using an epi-illumination-type polarization microscope and an image processing algorithm using an ellipse-type Gabor filter. Images with striation patterns of frog skeletal muscle were obtained by the microscope and the image processing algorithm. Individual lengths of 10 consecutive sarcomeres of a single muscle fiber were measured by gauging each width of the striation pattern, which was proved to be derived from striation structures of the single fiber by performing experiments using different polarization lights and different focus depths. At the resting state, each sarcomere length was identical at the fixed muscle length and in proportion to the length ranging over 91–123% of the natural length. Each sarcomere length was unchanged at the steady state during isometric tetanic contractions. Individual sarcomere lengths in the central part of the skeletal muscle were identical at resting and at isometric tetanic contractions even in the thick muscle fiber specimen.

Key words: Sarcomere length; thick muscle fiber bundle; skeletal muscle; epi-illumination-type polarization microscope; ellipse-type Gabor filter; resting; isometric tetanic contraction.

1. INTRODUCTIONS

Skeletal muscles produce different tensions according to their lengths. The fundamental unit of a skeletal muscle structure is the sarcomere and the maximum tension is produced when the sarcomere length of the muscle is at a so-called natural length of 2.2 μm. It is well known that the relation between active tension and sarcomere length varies in two different ways — the ascending limb and the descending limb. In the ascending limb the tension increases as a muscle lengthens and in the
descending limb the tension decreases as a muscle lengthens. However, it has not been fully clarified whether individual muscles work in the ascending limb or in the descending *in vivo*. Soleus muscles of cats [1] and triceps muscles of monkeys [2] and humans [3] are demonstrated to work in the ascending limb by measuring the relation between joint angles and torques in joint movements, whereas human extensor carpi radialis brevis muscles are demonstrated to work in the descending limb [4]. It is said that muscles have elasticity because the characteristic of muscles in the ascending limb is similar to positive elasticity of normal springs. Meanwhile the characteristic of muscles in the descending limb seems strange from a control engineering point of view. The characteristic that the tension decreases as a muscle lengthens is regarded as negative elasticity. Such negative elasticity is expected to make the muscle dynamics unstable. However, even in the descending limb muscles work stably. Mechanisms of the stability have not been elucidated theoretically and experimentally yet.

To clarify the stabilization mechanisms in the descending limb, not only muscle lengths but also sarcomere lengths have been recently measured in experiments using isolated muscle specimens. Laser diffraction or transmission-type microscopes were used to measure the sarcomere length of muscle fiber specimens in previous reports [5–8]. Sarcomere lengths are uniform during isometric tetanic contractions in experiments using a single muscle fiber [5]. However, in these reports, investigators needed to prepare a single muscle fiber or a thin muscle sample because the amount of transmitted light should be sufficient even after passing through the specimen. Such methods are hardly applied to thick muscle fiber bundles and thus sarcomeres in thick muscle fiber bundles have not been investigated yet.

One of the aims of this study is to develop a new method for measuring sarcomere lengths of thick muscle fiber bundles. We developed a new measure using an epi-illumination-type polarization microscope and a novel image processing algorithm using an ellipse-type Gabor filter. It is also the aim of this study to measure sarcomere length in thick muscle specimens by applying our measure.

2. METHODS

2.1. Animal preparation

We obtained thick muscle fiber bundles from dorsal parts of semitendinosus muscles of *Rana nigromaculate* (leopard frog) and *Rana catesbeiana* (bullfrog). Using a stereoscopic microscope we resected about 20% of muscle fiber bundles, removed connective tissues from the muscle and obtained a thick muscle fiber bundle with a diameter of 1.0–1.5 mm.