Structural Changes in the Muscle Thin Filament during Contraction Caused by Single and Double Electrical Pulses

Skeletal muscle undergoes a transient contraction (twitch) when stimulated by a single electrical pulse. In frog muscle, the duration of the twitch is about 100 msec at 16 °C. Skeletal muscle produces more tension when stimulated with a second pulse while the tension is high. This is called the "summation" of tension. Although this phenomenon has been known for many years [1], its molecular mechanism has not been understood clearly.

Skeletal muscle has a hierarchical structure (Fig. 1). The minimal contractile unit is called the sarcomere that consists of two kinds of filament, namely, the thin and thick filaments. The thin filament consists mainly of three proteins, namely, actin, troponin and tropomyosin. Actin is a globular protein that forms a filament to make the helical backbone of the thin filament. Tropomyosin is a rod-shaped protein that winds around the actin filament, and troponin binds to tropomyosin. The thick filament mainly consists of myosin molecules. Myosin has two globular domains (called myosin heads) and each head can bind to actin (crossbridge formation) and produce force utilizing the energy of ATP hydrolysis.

Contraction is regulated by Ca^{2+}, which is stored in an organelle called the sarcoplasmic reticulum in a muscle cell. After a stimulus, Ca^{2+} is released into the sarcomere. Troponin and tropomyoin regulate the binding of myosin to actin depending on the intracellular Ca^{2+} concentration: they inhibit the actin-myosin interaction in the absence of Ca^{2+}, but the inhibition is removed when Ca^{2+} binds to troponin. Summation may be due to an increase in Ca^{2+} concentration by repeated stimulation. However, studies with calcium-sensitive dyes showed that the intracellular Ca^{2+} concentration is not increased by repeated stimulation [2]. To verify the relation between Ca^{2+} concentration and tension, we performed a time-resolved X-ray fiber diffraction experiment that can provide information on the behavior of muscle proteins during summation [3].

The experiment was carried out at beamline **BL40XU**. The X-ray energy was 10.5 keV. The X-ray detector used was an X-ray image intensifier (V5445P, Hamamatsu Photonics) with a high-speed CCD camera (C7770, Hamamatsu Photonics). The specimen was a sartorius muscle of bullfrog. X-ray diffraction patterns were recorded at a time resolution of 1 msec during contractions caused by either single (SP) or double (DP) electrical pulses at 16 °C. In the case of DP experiments, the interval of the pulses was set at 15 msec.

The X-ray diffraction patterns from skeletal

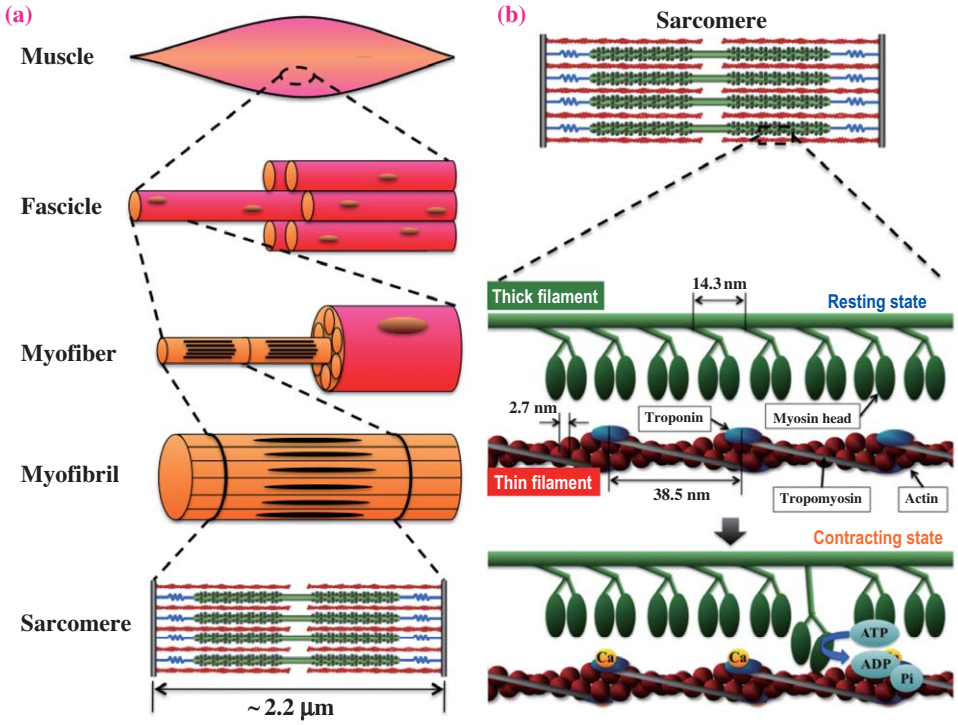

Fig. 1. **(a)** Hierarchical structure of skeletal muscle. **(b)** Schematic drawing of proteins within a sarcomere.

muscle consist of several diffraction peaks derived from the ordered array of muscle proteins in the sarcomere (Fig. 2). Figure 3 shows the time courses of the intensities of the (1,0) equatorial reflection ($I_{(1,0)}$) and the 38.5 nm meridional reflection ($I_{38.5}$). The (1,0) reflection arises from the hexagonal arrangement of the filaments and is related to the number of crossbridges. The 38.5 nm reflection arises from the regular axial arrangement of troponin molecules. In SP, $I_{(1,0)}$ decreased by 20% after the stimulus. In DP, it further decreased by 10% after the second stimulus, indicating that more crossbridges are formed, and $I_{38.5}$ increased by about 30% and then decreased as the tension developed. The initial intensity increase is considered to be due to the Ca^{2+}-induced structural change of troponin and the subsequent decrease due to crossbridge formation. In DP, $I_{38.5}$ decreased slowly after the second stimulus and dropped to about the same level as that observed in SP. This indicates that troponin molecules already undergo a maximal structural change in SP (twitch) and DP only prolongs it.

These results show that the conformational change in troponin is not enhanced by DP. Thus, the summation is not due to the increased Ca binding but due to the increased crossbridge formation. In SP, crossbridge formation takes longer than the dissociation of Ca^{2+} from troponin. Thus, only a small number of crossbridges are formed in SP. In DP, the second stimulus causes the release of Ca^{2+}, which prevents the dissociation of Ca^{2+} from troponin. Thus, more crossbridges are formed and tension looks summed. DP increases tension by maintaining troponin in the conformation that permits the actin-myosin interaction.

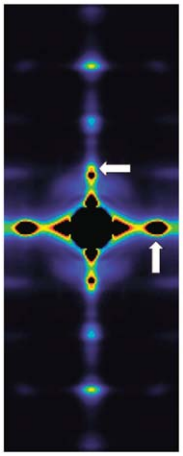

Fig. 2. X-ray diffraction pattern from frog skeletal muscle in a resting state. Muscle fibers are vertical. The horizontal arrow points the 38.5 nm meridional reflection and the vertical arrow the (1,0) equatorial reflection.

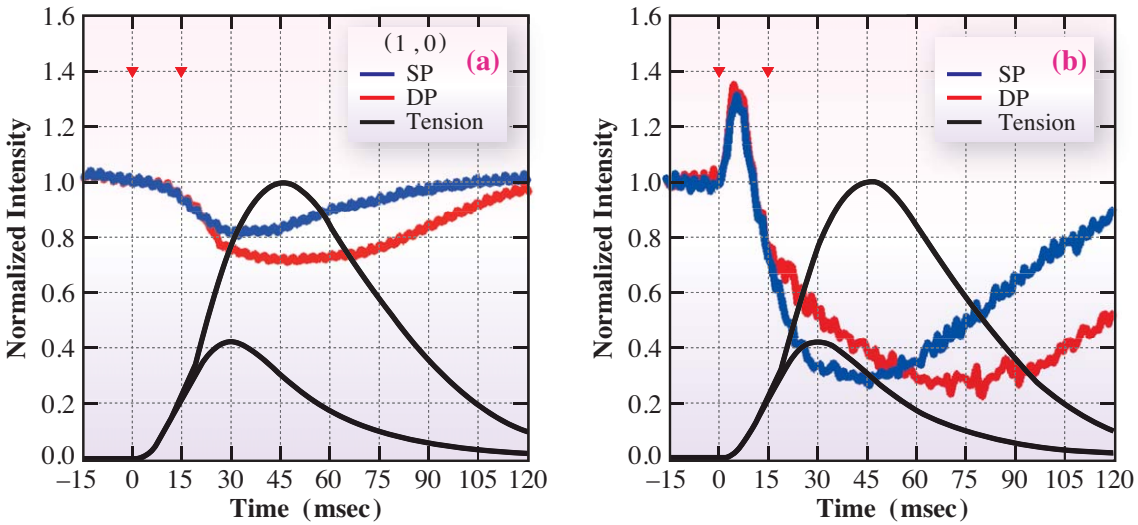

Fig. 3. Time courses of intensity changes of two diffraction peaks. The ordinate is the integrated intensity and the abscissa is time. The blue line denotes data obtained from SP experiments and the red line data obtained from DP experiments, and the black line shows tension. Red triangles indicate the onset time of the stimulus. **(a)** (1,0) reflection. **(b)** 38.5 nm reflection.

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