Structural Transients of Contractile Proteins upon Sudden ATP Liberation in Skeletal Muscle Fibers

The molecular mechanism underlying muscle contraction remains one of the major unresolved problems in biology. A skeletal muscle is composed of bundles of muscle fibers (muscle cells). In a muscle fiber, myofibrils composed of a linear array of sarcomere running parallel to the fiber axis are packed (Fig. 1(a)). In each sarcomere, thin and thick filaments (contractile proteins) are regularly arranged in a hexagonal lattice (Fig. 1(b)). A thin filament is composed of helically polymerized actin monomers. The main constituent of a thick filament is myosin. Myosin is composed of two identical heads connected to a long tail (Fig. 1(c)). The myosin head contains all the components needed in the exertion of force, including the actin-binding site and ATP-binding pocket. The muscle contraction takes place by the interaction between actin and myosin utilizing the chemical energy produced by the hydrolysis of ATP. One of the major problems that need to be solved is to determine how force generation powered by the hydrolysis of ATP is associated with structural changes in the proteins actin and myosin when they interact with each other. Fiber X-ray diffraction is a useful technique for investigating structural events at appropriate spatial resolution (1-60 nm) and time resolution (milliseconds). Most of the X-ray diffraction studies have been done using either living muscle specimens or skinned muscle fibers. Usually, skinned muscle fibers, whose surface cell membrane has been removed or dissolved, have widely been used to study the effects of various reactants on the structural changes of contractile proteins. However, the use of skinned muscle fibers in the study of structural changes is open to the criticism that, due to the diffusion limit, the distributions of reactants are not uniform in muscle fibers because of their large diameter.

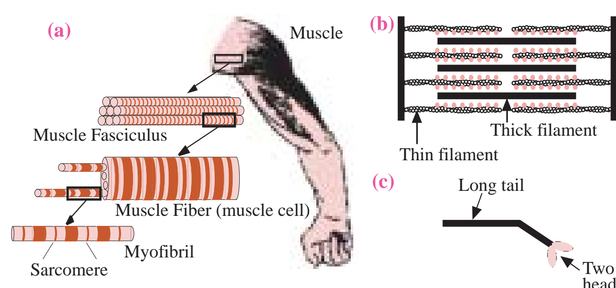

Fig. 1. (a) Schematic microanatomy of skeletal muscle. (b) Schematic drawing of sarcomere structure. (c) Schematic structure of myosin molecule.

One promising approach to overcome this difficulty is to use caged compounds. Caged compounds are inert when placed in a skinned fiber, but they can be split by an intense pulse of UV laser light to form biologically active substances, resulting in a sudden (within milliseconds) increase in their concentrations in the fiber. Some time-resolved X-ray diffraction studies have been carried out using caged compounds [1-3], but there have been limitations in both time resolution and the extent of reflections in X-ray diffraction pattern that can be analyzed. In these studies, only strong reflections (e.g., equatorial reflections and myosin meridional reflections) have been investigated, because the structural changes of contractile proteins caused by photolysis are fast (millisecond time scales) and the flux from X-ray source in these experiments was not high enough to measure intensities of weak reflections at millisecond time resolution. To understand fully the structural changes of contractile proteins induced by the flash of photolysis, one should investigate not only these strong reflections but also weak reflections (e.g. actin-based layer lines (ALL)), because they carry important information about the structure of the contractile proteins.

In the present study [4], we investigated the structural changes of contractile proteins in skeletal skinned muscle fibers during the relaxation induced by the flash photolysis of caged ATP by ultrahigh-speed time-resolved X-ray diffraction with a time resolution of 3.4 ms at high-flux **BL40XU** beamline. This beamline provides a photon flux of an order of 10^{15} s^{-1}, ~10^3 times higher than other undulator-based beamlines [5]. Thus, this beamline is suitable for time-resolved experiments at a high time resolution. As a material, overstretched skinned skeletal muscle fibers loaded with exogenous myosin heads were used.

Figure 2 shows the selected frame of the time series of diffraction patterns taken before and after the photolysis of caged ATP. Before photolysis, ATP was absent in the muscle fibers. This is the condition equivalent to the rigor state, and strong stereospecific interactions are known to occur between actin and myosin. In the diffraction pattern, all the ALL's between the first and sixth are strongly enhanced. The enhancement is caused by the myosin heads, which are bound to actin in a stereospecific manner. After photolysis, the concentration of ATP rapidly increases within the muscle fiber and the photorelease

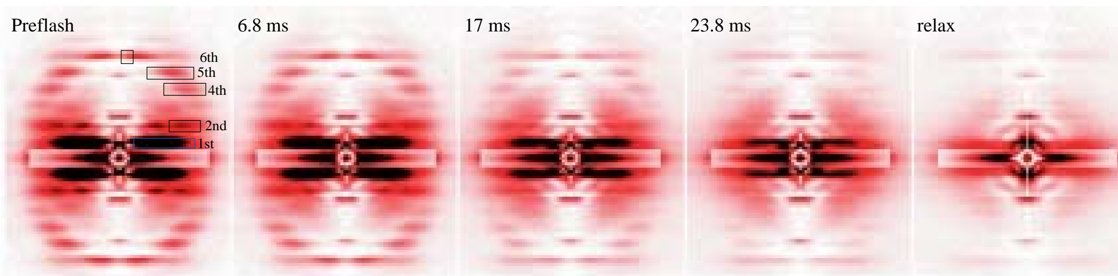

Fig. 2. Selected frames of time series of diffraction patterns taken during a caged ATP photolysis experiment (accumulation of multishots). The number in each pattern shows the time after flash. The boxes in the first pattern show the areas of intensity in Fig. 3. Time course of decay of integrated intensities of ALL's after photolysis.

of ATP allows the muscle fiber to enter into the relaxed state. In the diffraction patterns, these ALL's fade away, leaving the much weaker sixth ALL at the final stage of relaxation. The intensity of each ALL was integrated in the area indicated by a box shown in the first pattern in Fig. 2. The time courses of decay of the integrated intensities of ALL's are shown in Fig. 3. The decay of the lower-order ALL's (up to the fifth ALL) and the inner part of the sixth ALL was fast and was adequately fitted by a single exponential decay function. The rate constants for these ALL's were all similar. Also the changes in the intensity profiles of representative ALL's after photolysis, measured along the layer line, are shown in Fig. 4. It seems that the intensities of these ALL's decreased without changes in their profiles. These results suggest that after the photolysis of ATP (sudden ATP liberation) the myosin heads molecules simply detach from actin without passing through a state structurally distinct from the rigor state.

In this research, we found the structural changes of the contractile proteins during the course of relaxation caused by the photorelease of ATP.

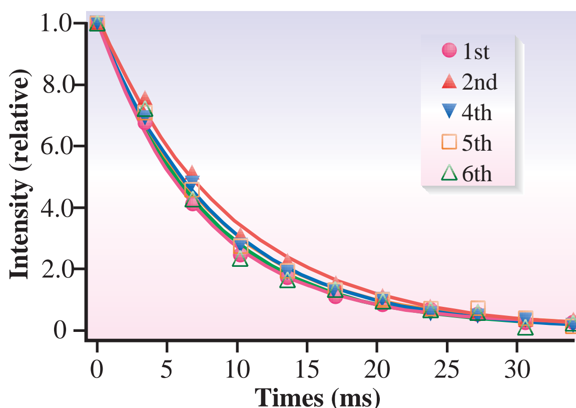

Fig. 3. Time course of decay of integrated intensities of ALL's after photolysis.

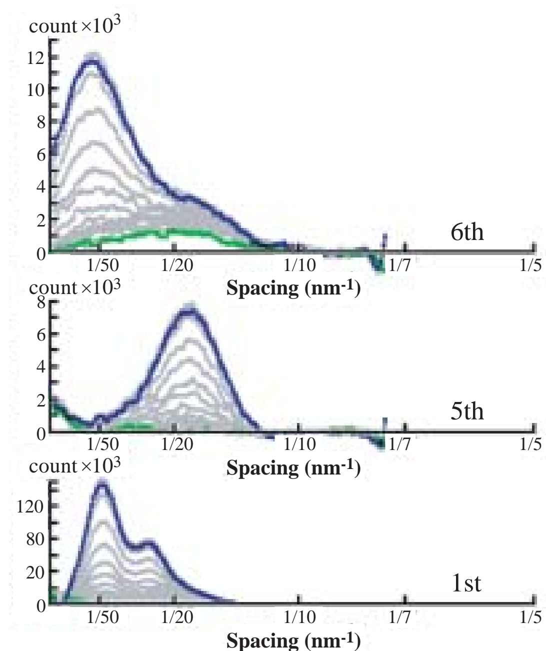

Fig. 4. Intensity profiles of representative ALL's before and after photolysis. The uppermost blue curve in each set of profiles represents the preflash level, and the lowermost green curve represents the relaxed level.

Jun'ichi Wakayama†

SPring-8 / RIKEN

E-mail: jwaka@nfri.affrc.go.jp

†Current address: Instrumentation Engineering Lab., National Food Research Institute

References

[1] K. J. V. Poole *et al.*: Adv. Biophys **27** (1991) 63.
[2] K. Horiuti *et al.*: J. Biochem. **115** (1994) 953.
[3] T. Yamada *et al.*: Biophys. J. **85** (2003) 1741.
[4] *J. Wakayama, T. Tamura, N. Yagi and H. Iwamoto: Biophys. J.* ***87*** *(2004) 430.*
[5] K. Inoue *et al.*: Nucl. Instrum. Meth. Phys. Res. A **467/8** (2001) 674.