

## Department of Molecular Physiology

---

Yoshiki Umazume, *Professor*  
Maki Yamaguchi, *Assistant Professor*

Shigeru Takemori, *Associate Professor*

### General Summary

Our efforts have been concentrated on clarifying the mechanism of muscle contraction in connection with the role of intracellular water.

### Research Activities

#### *Activity of intracellular water*

Magnetic resonance images are produced from differential transverse relaxation of water proton nuclear magnetic resonance signals from cells and tissues. With nuclear magnetic resonance measurements, water in skeletal muscle tissue can be classified into several components characterized by the transverse relaxation rate ( $T_2$ ). Measurements at various humidity levels allow us to directly measure the restriction energy imposed on each water component by the sarcomeric structural organization of skeletal muscle. Energy on the order of kT is stored in each water molecule surrounding the contractile proteins of the muscle. This evidence strongly supports our hypothesis that water serves as a heat sink for the contractile interaction of myoproteins.

#### *Protrusion of myosin heads with the increase of longitudinal strain in the sarcomere of striated muscle*

The liquid-crystalline structure of striated muscle is a great advantage for studying the linkage between structure and function with the X-ray diffraction technique. However, only the equatorial reflections had been studied in cardiac muscles because of the poor quality of the diffraction patterns. With improved specimens, we succeeded in analyzing layer lines of cardiac muscles even after the removal of actin filaments with gelsolin. With this technique we addressed the mechanism of the Frank-Starling law of the heart, which indicates that longitudinal strain strongly modulates the activation levels of cardiac muscle (known as stretch activation in skeletal muscle). Because sarcomere elongation had little effect on the lattice spacing of myofilaments at short sarcomere lengths where stretch activation is prominent in cardiac muscle, stiff connectin/titin filaments might not be involved in the mechanism of stretch activation. As another candidate, we propose that the protrusion of myosin heads from their backbone would modulate activation levels with sarcomere elongation. The intensity profiles of the layer lines in our X-ray diffraction patterns, obtained at BL-45XU at the Super Photon Ring 8 GeV (Large-scale Synchrotron Radiation Facility), allowed us to deduce head distribution at various sarcomere lengths with and without actin filaments. In cardiac muscle without actin filaments, but not in skeletal muscle, the protrusion of myosin heads from their back bone increased with sarcomere lengths. This result supports our hypothesis.

*Structure of the cardiac cell to which mutant troponin was introduced*

Several single mutations of troponin molecules have been reported to cause familial cardiomyopathies through the altered contractility of cardiac cells. From our molecular dynamics study, tropomyosin of mutant muscle fiber was predicted to be shifted compared with that of the wild type on muscle activation. To verify this prediction of our simulation, we performed an X-ray diffraction experiment in which wild-type or mutant troponin subunit T was introduced into skinned muscle specimens at beam line 15A at the Photon Factory, Tsukuba. No difference was detected in troponin reflections obtained from mutant fibers compared with that from wild-type fibers. However, the intensity of the myosin layer line that indirectly reflects the tropomyosin position was larger in the mutant fiber than in the wild-type fiber. These results indirectly support the prediction of the molecular dynamics simulation.

*Viscosity of the myofibril suspension*

Polyethyleneglycol (PEG) narrows the lattice spacing of skinned skeletal muscle sarcomeres. Because the diameter of PEG (molecular weight, 3350) seems to be several nanometers, the lattice spacing of 40 nanometers was suspected to be large enough for penetration of PEG. To determine whether PEG penetrates the sarcomere, we measured the specific gravity of myofibril suspensions from rabbit psoas muscle in the presence or absence of PEG. If PEG does not penetrate the sarcomere, the specific gravity of the supernatant after centrifugation of the myofibril suspension would be larger than that of the myofibril suspension. Our measurements indicated that PEG diffuses into the sarcomere to reach half of the external concentration.

*Accelerometry during exercise*

With minute accelerometers attached to various parts of the body during kendo and badminton play, subtle differences in the timing of movements between skilled and unskilled players were detected. Because acceleration directly reflects acting force and because its data can be fed instantaneously back to the practicing players, accelerometry is expected to powerfully support effective training in the field.

**Publications**

**Kimura M, Takemori S.** CH<sub>2</sub>-units on (poly)-ethylene glycol radially dehydrate cytoplasm of resting skinned skeletal muscle. *J Biochem* 2008; **43**: 841-7.

**Watanabe Y<sup>1</sup>, Takemori S, Tatsumi N<sup>2</sup> (<sup>1</sup>Seijyo Univ Economics, <sup>2</sup>Ibaragi Univ Educ).** Develop-

ment of wearable body-temperature recorders that cause minimal interference with kendo practice: The virtue of body-temperature measurements (in Japanese). *Budougaku Kenkyu* 2008; **41**: 17-23.