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## Loss of $\text{Ca}^{2+}$ -dependent Regulation in Glycerinated Skeletal Muscle Contraction\*

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It has been known that the contraction of skeletal muscle is regulated by  $\text{Ca}^{2+}$  concentration in myofibrils. Weber et al. found that  $\text{Ca}^{2+}$  is the most important factor to the contraction of skeletal muscle. Ebashi et al. also reported the same results on the contraction of skeletal muscle, and they found such the regulatory protein as TN-TM system, on the thin filament in myofibrils. The  $\text{Ca}^{2+}$  information in the myofibril is transmitted to actin and myosin interaction through this system.

However, we found the fact that the  $\text{Ca}^{2+}$  regulatory protein lost  $\text{Ca}^{2+}$  sensitivity by digestion of proteolytic enzymes, trypsin or  $\text{Ca}^{2+}$  activated neutral protease(CaANP), in the physiological condition of rabbit psoas glycerinated muscle. It was designated as  $\text{Ca}^{2+}$  in-sensitive fiber(CaIS-fiber). Moreover, we studied on the recovery of  $\text{Ca}^{2+}$  sensitivity in CaIS-fiber, and found that it recovered  $\text{Ca}^{2+}$  sensitivity by incubation in native TM or TN complex extracted from rabbit back muscle. Then, we proved these facts clearly by using techniques of tension mechanogram and SDS polyacrylamide gel electrophoresis. Normal glycerinated fiber has  $\text{Ca}^{2+}$  sensitivity(CaS-fiber) and developed tension by addition of  $\text{Ca}^{2+}$  in the presence of ATP, by contrary, it developed tension by addition of ATP in the presence of  $\text{Ca}^{2+}$ , too.

Electrophoretic pattern of CaS-fiber mainly contains ten bands from top to bottom, they are myosin heavy chain, actin, TN-T, TM  $\beta$  and  $\alpha$  myosin light chain 1, TN-I, TN-C, myosin light chain 2 and 3, respectively.

On the other hand, tryptic CaIS-fiber developed tension by only addition of ATP even in the absence of  $\text{Ca}^{2+}$ , and addition of  $\text{Ca}^{2+}$  after ATP has no effect to the more tension development. It was also the same results in  $\text{Ca}^{2+}$  treated fiber which was added as an activator of CaANP. The discrepancy on the electrophoretic pattern between CaS- and CaIS-fiber is the concentration of bands of TN-TM system. In CaIS-fiber, the concentration of TN-TM each band is becoming faint when it was compared with CaS-fiber. It means that a part of TN-TM system was digested with proteolytic enzymes, such as trypsin or CaANP. In particular, a new band appeared at 30 K daltons peptide on gel in CaIS-fiber. We assumed that this band may be one of the digested products of TN-T (Azanza et al. 1980). Moreover, CaIS-fiber recovered  $\text{Ca}^{2+}$  sensitivity with

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incubation in native TM or TN complex. When the tryptic or  $\text{Ca}^{2+}$  treated CaS-fiber was incubated in native TM or TN complex for 72 hrs, at  $4^{\circ}\text{C}$ , they recovered  $\text{Ca}^{2+}$  sensitivity in tension development. SDS polyacrylamide gel electrophoretic pattern of recovered fiber also recovered its concentration gradually to the original level of CaS-fiber on gel.

We had a conclusion from these results that  $\text{Ca}^{2+}$  in- and re-sensitization is due to exchanging of TN-TM system in myofibrils.