

## CONNECTIN FILAMENTS OF GTANT SARCOMERES OF CRAYFISH CLAW MUSCLE

T. Manabe<sup>1</sup>, H. Higuchi<sup>2</sup>, Y. Kawamura<sup>1</sup>, S. Kimura<sup>1</sup>, and K. Maruyama<sup>1</sup>. <sup>1</sup>Dept. of Biol., Fac. Sci., Chiba Univ., Chiba, <sup>2</sup>Dept. of Physiol., Jikei Univ. Sch. Med., Tokyo

In giant sarcomeres (10  $\mu$ m at rest of crayfish claw muscle), there is 3000 kDa protein in addition to 1200 kDa projectin. The rotary shadowed image of the isolated 3000 kDa protein was very thin filament, maximumly 0.9  $\mu$ m long.

When skinned opener fibers were stretched up to 14  $\mu$ m sarcomere length, resting tension developed, but on mild treatment with trypsin resulted in the decrease in tension development accompanied by splitting of the 3000 kDa protein.

Immunofluorescence microscopy using anti-3000 kDa protein antibodies revealed that the protein linked the Z line to the edge of the A band and it was movable depending on the sarcomere length.

From the present study, it is concluded that the 3000 kDa protein in the giant sarcomeres of crayfish claw muscle corresponds to connectin, elastic protein of vertebrate skeletal muscle.

## LOCALIZATION OF TWO MYOSIN ISOFORMS IN PERITONEAL NEUTROPHILS FROM GUINEA PIG. H. Takano-Ohmuro, M. Endo and K. Kohama\* Dept. of Pharmacol. Fac. of Med., Univ. of Tokyo, Tokyo, and \*Dept. of Pharmacol. Fac. of Med., Gunma Univ. Maebashi

We previously reported that peritoneal neutrophils of guinea pig has two isoforms of myosin II differed in the heavy chain (HC) (Zool. Sci., 1991).

To localize the isoforms, we subjected the cell lysate of the neutrophils to the centrifugation at 120,000 g for 70 min. The supernatant was used as cytosolic fraction (Cyt). The precipitate (ppt) was suspended in an isotonic solution and the suspension was centrifuged at 10,000 g for 30 min. The ppt after 10,000 g centrifugation was used as 10K fraction (10K) and the suspension was further subjected to the centrifugation at 120,000 g for 70 min. The ppt was used as 120K fraction (120K). When Cyt, 10K and 120K were subjected to native pyrophosphate gel electrophoresis, they showed only one band. The mobility of myosin in cytosol did not coincide with that in 10K. From peptide mapping and immunoreactivity, the difference could be explained by the difference in HC of both myosin. Myosin of Cyt comigrated with that of 120K. Our similar analyses showed that myosin of Cyt was identical in HC and light chains with myosin of 120K. The reason to explain the difference in the localization between Cyt and 120K remained to be examined.

MYOSIN FROM THE SEA SPONGE, *Halicondria okadai*

N. Kanzawa and K. Maruyama. Dept. Biol., Fac. Sci., Chiba Univ., Chiba.

We have attempted to purify myosin from the sea sponge, *Halicondria okadai*. There has been no report on sponge myosin.

Sea sponge myosin consisted of 220 kDa heavy chain and two species of light chains, 18 and 21 kDa. Two headed structure was observed under electron microscope. The K-EDTA activated ATPase activity was as high as 0.5  $\mu$ mole/mg/min, but the  $Mg^{2+}$ -ATPase activity was low and only slightly enhanced by rabbit F-actin.

Solubility of sea sponge myosin was lower than rabbit skeletal myosin: the former was only soluble by 40% at 0.3 M KCl as compared to the latter (by 80%). Sea sponge myosin formed thick filaments, 0.5-1  $\mu$ m long, at 0.3 M KCl, where rabbit skeletal myosin formed much smaller oligomers.

## ON THE TISSUE-SPECIFIC DISTRIBUTION OF TROPOMYOSIN ISOFORMS IN CRUSTACEAN MUSCLES T. Ishimoda-Takagi, S. Nakano, K. Hino and M. Itoh. Dept. of Biol., Tokyo Gakugei Univ., Tokyo.

We have previously shown that several species of tropomyosin (TM) isoforms were included in the spiny lobster and American lobster. Distribution of the TM isoforms was tissue specific, and tissue-specificity of the TM isoforms correlated considerably with the types of muscle fiber. In order to investigate generality of the tissue-specificity of TM isoforms in crustacean decapod, we examined tissue specificity of TM isoforms in the prawn, *Panaeus japonicus*, and the crab, *Erimacrus isenbeckii*. Four TM isoforms were present in the prawn muscles. Distribution pattern of TM isoforms was similar to that of spiny lobster. The TM component a was a main component of the cephalo-thoracic and abdominal muscles. The component b was mainly observed in the leg muscle. The component c was mostly involved in the dorsal muscles. Heart muscle contained a heart-specific TM isoform. The TM component corresponding to b was also observed in the crab leg muscle. Furthermore, crab heart muscle contained heart-specific TM isoforms. However, the TM isoforms corresponding to the components a and c, which were involved in the muscles to move abdomen, could not be identified in the crab of which abdomen is greatly reduced.