PRIMARY STRUCTURE OF THE I BAND REGION (900 KDA) CONNECTIN/TITIN FROM CHIKEN BREAST MUSCLE

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Connectin/titin is a giant elastic protein of striated muscle. It extends from the Z line *via* the I band to the M line of the A band in a sarcomere. Its entire primary structure has been investigated in human skeletal muscles (3.4–3.7 MDa) and cardiac muscles (2.97–3.35 MDa). The differences in the primary structure of various isoforms are found in the elastic portion of the I band region. The present study on chicken breast muscle connectin/titin elucidated the structure of the primary sequence of the I band region. The number of the Ig domains was similar to those of the human cardiac muscle. On the other hand, the PEVK segments were constituted of 459 residues that were longer than that of human cardiac N2B (163 residues) but shorter than that of human skeletal muscle isoforms (1400–2174 residues). Furthermore, the I band region of chicken breast muscle connectin/titin contained a N2A region but not a N2B, and the identity of the residues ranged 70–80% compared to human skeletal connectin/titin. Therefore, the domain arrangement of chicken breast muscle connectin/titin resembled that of the human skeletal muscle isoforms.

DISASSEMBLY OF MYOFIBRILS IN MYOTUBES CULTURED ON POLYACRYLAMIDE-GEL BY MECHANICAL STRETCH

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Chick embryonic skeletal muscle was cultured on stretchable acrylamide sheets (10% acrylamide, 0.4% bis) on which collagen was covalently crosslinked by sulfo-SANPAH In an previous meeting, we have shown that ordered striation pattern of matured myofibrils completely corrupted by 10%-stretch while loosing of the acrylamide sheet did not affect the ordered array of myofibrils. Here, we examined the time course of the myofibril disassembly. It was shown that disassembly after the stretch occurred gradually in a stepwise manner. A-band disappeared from forming myofibrils first and then I-Z-I structure disintegrated. Immunofluorescence microscopy with anti-desmin indicated desmin IFs were dislodged from Z-lines and restored longitudinal orientation as detected before anchoring on Z-lines. Alpha-actinin also scattered in cytoplasm though fluorescence intensity was appeared to be higher at sub-membranous regions. Immunofluorescence against myosin and actin also detected uniformly in cytoplasm. SDS-gel electrophoresis of detergent soluble fraction of the stretch cells suggested about 50% of actin and myosin were released by stretch.

CONFORMATION CHANGE OF ACTIN FILAMENT ACCOUNTS FOR THE STAINING PATTERN OF FLUORESCENCE PHALLOIDIN ON MYOFIBRILS

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Fluorescence phalloidin (fl-PHD) has been used as a convenient tool to detect actin filaments. Interestingly, however, fl-PHD staining on skeletal I-filaments are time dependent and uniform staining is observed only after prolong incubation. We examined the localization of fl-PHD on the I-filament in detail and obtained a new staining pattern; the reported uniform staining is further followed by the reduction of the Z-line fluorescence. These results do not support the idea that PHD competes with nebulin for the binding sites. Rather we expect that this changing pattern of PHD staining in I-filament is induced by conformational change of actin molecules in itself. Therefore, the possibility that the PHD binding ability of actin subunit change with time were examined. Pelleting experiment showed that the amount of PHD bound to the I-filaments did not change with time. In addition, DNase I assay indicated that addition of 1/10 PHD to actin (mol/mol) could stabilize most of actin in I-filament from depolymerization by KI treatment. The observation indicated that PHD affected actin which did not attach to PHD in myofibrils.

CHICKHEN DIAPHANOUS PROTEIN CAN BIND TO F-ACTIN

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Diaphanous family proteins are involved in a number of actin-mediated cellular processes. In this study, we examined the direct binding of cDia with F-actin. Chicken Diaphanous (cDia) isolated from a cDNA library of gizzard has an apparent molecular mass of 145 kDa. Immunoblot analysis using the specific antibody against cDia235-983 showed the relatively stronger reaction with the 145kDa band in brain than in other organs including gizzard. Therefore, chicken brain was first washed with E buffer (0.05% NP-40, 0.5 mM EDTA, 0.5 mM EGTA, 0.2 mM PMSF, and HEPES-KOH, pH 7.5) and then extracted with a solution containing 1 mM EDTA, 0.1 mM PMSF, and 2 mM Tris, PH 9.0. After dialysis against E buffer, the extract was applied to an F-actin affinity column. cDia was mostly bound to the column and eluted from it with 0.1 M KCl. A recombinant protein of a full-length cDia carrying a histidine tag was expressed in an insect cell line (High Five) and purified with a Ni-NTA agarose column. The recombinant cDia was coprecipitated with the F-actin beads, while BSA was not. These results suggested that cDia had the direct binding ability to F-actin.

SEARCHING OF ACTIN-BINDING PROTEINS FROM CHICKEN BRAIN WITH F-ACTIN AFFINITY COLUMN CHROMATOGRAPHY

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We analyzed chicken brain with F-actin affinity columns that contained F-actin as a ligand to search novel actin-binding proteins. Brain extracts was overcharged to collect proteins that are low amounts but have high affinity to F-actin. The proteins which specifically bound to the column were eluted with salt solutions, separated with SDS-PAGE, cleaved with proteolytic enzymes or CNBr, and analyzed their amino acid sequences with PTH gas phase sequencer and mass spectromety (LC-Q). The eluents contained several previously identified actin-binding proteins such as Arp2/3 complex, cortactin, and drebrin. A few of known proteins that are related to the vesicle transport but have not been reported to have actin-binding activity also found in the column eluents. Some proteins have sequences similar to hypothetical proteins or putative homologues of other organisms whose functions have not been yet unclear. They might be novel actin-binding proteins.

A NOVEL PROTEIN CONSTITUTING CA^{2+}-INDUCED CONTRACTILE FILAMENTS

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Carchesium is a sessile peritrich ciliate that possesses a contractile organelle called spasmoneme. It can repeat the contraction-reelongation cycle by Ca^{2+} absorption and desorption. In this study, we modified proteins extracted from spasmoneme with DHNBS (a water-soluble equivalent of HNBB) and obtained a novel and hydrophobic 200 kDa protein by immunoblotting using anti-HNB antibody. This protein could be extracted from spasmoneme of *Carchesium polypinum* with 6 M guannidine HCl. In our previous study, the chemical modification with HNBB which modifies tryptophan residues specifically shows the loss of spasmoneme contraction. We concluded that 200 kDa hydrophobic protein is essential for spasmoneme contraction. Here we report an important and novel protein interacted with spasmin in spasmoneme.

THE PRIMARY STRUCTURE OF CONNECTIN-LIKE ELASTIC PROTEIN OF ANNELID OBLIQUELY STRIATED MUSCLE

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We heve previousely reported that a giant connectin-like elastic protein (4000 kDa) is present in obliquely striated muscle of a polychaete (Annelida) bodywall muscle (Kawamura *et al.* 1994). This 4000 kDa protein cross-reacts with only a few antibodies among a number of antibodies to vertebrate connectin/titin and therefore, its primary structure is predicted to be largely different from that of connectin/titin. In the present study, we have cloned from the cDNA library of polychaete (*Neanthes* sp.) bodywall muscle using the polyclonal antibodies to the 4000 kDa protein. Two positive clones, 2.6 and 2.1 kb, were obtained and sequenced. In both cDNAs there were PEVK segments, rich in P, E, V and K residues which correspond to the elastic segment of connectin/titin in vertebrate striated muscle. The PEVK content was 93%, more than in the PEVK segment of human striated muscle connectin/titin. Furthermore, there were immunoglobulin (Ig) domains in addition to the PEVK segments in the 2.6 kb cDNA. These results suggest that the annelid connectin has an elastic primary structure common to vertebrate connectin/titin.

STUDIES ON A MATRIX PROTEIN IN THE PRISMATIC LAYER OF JAPANESE PEARL OYSTER, *PINCTADA FUCATA*

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The mollusk shell is a hard tissue consisting of calcium carbonate and postal code organic matrices. The organic matrices are believed to play important roles in shell formation. In order to clarify the mechanism of shell formation, we tried to characterize the organic matrices from the prismatic layer of shells of Japanese pearl oyster, *Pinctada fucata*. The shell of this species consists of two layers, nacreous and prismatic layers. In this study, we focused on the matrix proteins of the prismatic layer. Shells were decalcified in 1 M aqueous acetic acid, and water-insoluble matrix derived from the original prismatic layer was separated. It was then treated with an SDS/dithiothreitol solution at 95°C for 10 min. The crude extract was loaded onto a C_{18} Sep-Pak cartridge and the eluate was applied to reverse-phase HPLC, which afforded a major protein with an apparent molecular mass of 14 kDa as estimated by SDS-PAGE. N-terminal sequence analysis of this protein indicated that the protein would be a novel one. It was also found that this protein had an anti-calcification activity *in vitro*.