47 amino acids repeat of connectin-like 4000K-protein in obliquely striated muscle of Annelida

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Connectin that exists in the striated muscle of vertebrates is an elastic protein with a molecular mass of approximately 3000 kDa. Invertebrates are also known to possess various connectin-like proteins, one of which is a connectin-like 4000 kDa protein that is present in the obliquely striated muscle of Annelida. We have determined the nucleotide sequence of the gene which encodes a connectin-like 4000 kDa protein using a cDNA library of the body wall muscle of Neanthes sp. In the deduced a.a. sequence from the nucleotide sequence, we discovered a sequence in which a unit of 47 amino acids was repeated 11 times. This 47-amino acid sequence repeat contained a high proportion of E and Q, and no significantly homologous protein was found in the database. After preparing a recombinant protein corresponding to this repeat sequence, we performed analytical ultracentrifugation and measured far-UV CD spectrum to estimate its secondary structure content. The sedimentation velocity experiment revealed that the protein had an extremely asymmetric shape, and 48% of the whole structure assumed beta-structure. It was thus demonstrated that this protein could cover a longer distance than other ordinary proteins with the same size. The fact that the connectin-like 4000 kDa protein of the obliquely striated muscle of Annelida has a distinct structure with the 47-amino acid repeat sequence may be related to the resting length of a sarcomere in the Annelida obliquely striated muscle, which is about twice as long as that in the vertebrate striated muscle.

Sequential analysis of Planarian connectin-like protein

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The striated muscle of vertebrates contains connectin, an elastic protein with a molecular weight of approximately 3,000,000 that functions to maintain the structure of sarcomeres. In addition to Ig, Fn3 and kinase domains, connectin has a PEVK elasticity-relating region. On the other hand, invertebrates possess connectin-like proteins some of whose domain structure are similar to that of vertebrate connectin.

Because connectin antibodies has reacted with 550 kDa band in planarian (Platyhelminth) electrophoresis sample, the planarian is suspected to possess connectin-like protein. Therefore, we searched for a possible candidate based on the genome information, thereby revealing a tentative sequence of the planarian (Platyhelminth) connectin-like protein. The total length of the tentative sequence was about 48 kb, and its molecular mass was estimated to be approximately 1820 kDa if the sequence was fully expressed. Based on the abundance of Ig and Fn3 domains and the presence of a kinase domain, this tentative sequence is considered to be that of connectin-like protein. In this tentative sequence, we observed a repeat sequence of 39-amino acids was repeated 34 times and a tandem Fn3 domain. Based on this tentative sequence, we performed RT-PCR and determined the partial sequence of the 8.4 kbp region, including the tandem Fn3 domain. The results of the analyses revealed that the tentative sequence is expressed as mRNA in planarian muscle.

in vitro 運動におけるアクチン束の自発的形成

Spontaneous formation of moving actin bundles in vitro

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Actin filaments are forming both static and dynamic structures fulfilling their cellular functions in vitro. Some ones are supported by many kinds of actin binding proteins but some others are spontaneously formed according just to their physical properties. Understanding the formation mechanism of dynamic structures of actin filament with respect to their dynamic property might be important for biophysical researches.

Frequently used assay for actin filament gliding under fluorescent microscope are usually composed of myosin-coated glass slide and buffer mediums sandwiched with another glass slide. The concentration of actin filament should be reduced to below 1.0 mg/mL in order to observe single filaments separately. We introduced un-labeled actin filament at the concentration of 0.1 mg/mL mixed with labeled filaments. After several ten minutes, bundles of moving actin filaments are spontaneously emerged. The width of bundle is about 0.1 mm winding to form a meshwork structures with the size of about 1.0 mm. Within the bundle of actin, filaments were moving bi-directionally with about 10 nm spacing. This mesh-like structure was formed when the actin concentration was over 0.1 mg/mL. At lower concentration, the structure was formed in the limited area but the shapes and the sizes are essentially the same in all cases. Therefore, these geometrical parameters of this structure should be determined by the physical properties of actin and myosin molecules on the glass side.

異なる滑り運動を持つ2種類のアクチン分子を独立したフィラメント形成する

Independent formation of actin filament with two actin species with different sliding velocities

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GI45V mutant of actin from dictyostyelium was reported to slide about half to its wild type along skeletal muscle myosin molecules on the glass slide. To understand why the velocity was slow even driven by the same myosin, we tried to co-polymerize these two actin molecules in the various ratios. Surprisingly, polymerized portion of filaments were composed of same actin species of either mutant or intact actin molecules. In addition, some filaments were randomly attached to each other to form a block copolymer. The block copolymers did not attached to skeletal myosin molecules on the glass slide in the presence of ATP in spite of the filament composed entirely from the same apecie did bind and slide along the glass surface at the same conditions.

The reason why these actin species polymerize independently might be caused from the difference in the velocity of polymerization. However, the difference in the binding constants between two molecules could not completely explain the reason why the block copolymers did not bind to myosin molecules. We are investigating the reason with respect to the difference of sliding velocity of each species of actin molecules. Sliding property of the block copolymers will be discussed in the meeting.

ヒト心筋SPOCに対する病態と治験の影響

The effects of disease and aging on human myocardial SPOC

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SPOC (Spontaneous Oscillatory Contraction) is a phenomenon observed in the contractile system of striated (skeletal and cardiac) muscle at an intermediate level of activation between contraction and relaxation. We previously reported that the period of the resting heart rate in various animal species is well correlated with that of sarcomere length oscillation under SPOC conditions (for review, see Ishiwata et al., Prog. Biophys. Mol. Biol. (2011) 105: 187-198). Recently, we reported the SPOC properties of human cardiac muscle fibers, such as SPOC period and propagation velocity of the SPOC wave, in various samples prepared from human hearts (47th Ann. Meeting of Biophys. Soc. Japan: S122). In the present study, we examined the SPOC properties by using single human cardiac myofilibrils prepared from non-failing hearts aged 19 to 65 years and failing hearts (dilated cardiomyopathy (DCM)) aged 15 to 63 years. The use of single myofilibrils allowed us to observe the movements of individual sarcomeres with no influence of connective tissues or intercalated disc. Our preliminary results show that the SPOC period in human cardiac myofilibrin is similar to that observed in myocardial fibers. At the meeting, we will discuss the effects of DCM and aging on human myocardial SPOC. This research has been approved by Human Ethics Committee at Waseda University.

拘束状態と環境媒体における骨格筋原線維の自動振動状態 (SPOC)

Characteristics of auto-oscillation (SPOC) for skeletal myofilibrils observed near the boundary region with relaxation conditions