

The photosynthetic process is initiated by the absorption of light energy by protein complexes called light harvesting antennas. In cyanobacteria and red algae the major antenna is called the phycobilisome (PBS). The PBS is an extremely large complex, with a molecular weight of 3-7MDa which is made up of pigmented proteins known as phycobiliproteins (PBPs) and unpigmented proteins known as linker proteins. Our goal is to obtain an atomic resolution structure of the entire PBS complex from the cyanobacterium *Thermosynechococcus vulcanus* using x-ray crystallography. Intact PBS was isolated in high phosphate buffer by sucrose gradient ultracentrifugation. Small blue crystals shaped like half moons were obtained in stabilization buffer in two to four weeks. Material obtained from the dissolving of extensively washed crystals was analyzed by fluorescence, SDS-PAGE and mass spectrometry (MS). The results of these experiments indicate that the crystals contain intact, functional PBS complex. Dynamic light scattering indicates a molecular weight of at least 2.8MDa. Preliminary diffraction experiments have indicated that the present crystals diffract poorly. A structure of phycocyanin (one of the PBP components) was determined as a half hexamer in the asymmetric unit. Within the phycocyanin disks, unstructured electron density could be identified in the position thought to be occupied by the linker. MS results for those crystals suggest that this is a phycocyanin rod structure contain the phycocyanin and three different linker proteins.

Keywords: light harvesting antenna, protein complex, energy transfer

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Crystal structure of the human GINS complex

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The GINS complex mediates the assembly of the MCM2-7 (minichromosome maintenance) complex with proteins in a replisome progression complex. The eukaryotic GINS complex is composed of Sld5, Psf1, Psf2, and Psf3, which must be assembled for cell proliferation. We determined the crystal structure of the human GINS complex: GINS forms an elliptical shape with a small central channel. The structures of Sld5 and Psf2 resemble those of Psf1 and Psf3, respectively. In addition, the N-terminal and C-terminal domains of Sld5/Psf1 are permuted in Psf2/Psf3, which suggests that the four proteins have evolved from a common ancestor. Using a structure-based mutational analysis, we identified the functionally critical surface regions of the GINS complex.

Keywords: GINS, MCM, replisome

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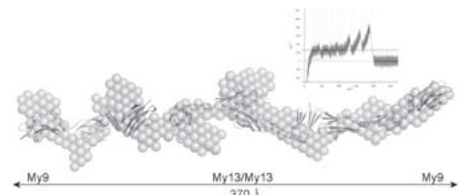
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Myomesin forms a 370 Å long two-chained, antiparallel filament across the muscle M-band region

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Sarcomeric filament proteins display extraordinary properties in terms of protein length and mechanical elasticity, requiring specific anchoring and assembly mechanisms. The M-band protein myomesin links the major sarcomeric filaments titin and myosin and is a unique filament model in terms of function and assembly. Our crystal structure of the domains My12 and My13 revealed a dimeric end-to-end filament of 143 Å. The two domains are connected by a six-turn alpha-helix, resembling a three body beads-on-the-string model with potentially elastic properties (Pinotsis et al, 2008 EMBO J.). We are currently presenting the entire C-terminal Ig domain array My9-My13 of myomesin. The crystal structures of the domains My9-My11 and My11-My13 combined with an overall low resolution model derived from small angle X-ray scattering data, reveal an unprecedented 370 Å long, two-chained filament composed of repetitive Ig-domain/alpha-helix motifs. AFM measurements confirm the elastic properties of the filament ascribed mainly to the helical linkers between the Ig-domains. Overall, our experiments provide a unique architectural insight for the tension-relaxation cycle of the muscle sarcomeres.



Keywords: muscle proteins, SAXS, AFM

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Electrostatic interaction explains D-staggered structure of collagen

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Single collagen molecules with ca 300 nm length self-assemble to form collagen fibrils in the extracellular matrix, which in turn form larger fibers. The details of the self-assemble process were unclear for many years, although it was known from electron microscopic and small angle X-ray diffraction observations that the fibers show a 67 nm (1 D) repeat pattern. As now known, this is because adjacent molecules are staggered in 67 nm intervals along the fiber axis. Though there were several preceding analyses to explain D-staggered structure in terms of electrostatic and hydrophobic interactions, none of them explained satisfactorily on the basis of the molecular interaction. Based on the single crystal structures of collagen-model peptides together with the fiber diffraction data from native collagen, we confirmed that the average molecular structure of collagen is not the prevailing Rich and Crick 10/3-helical conformation but the 7/2-helical conformation (Okuyama, et al., Biopolymers, 2006). Using this conformation, we examined electrostatic interactions between two collagen molecules arranged in the parallel fashion by changing relative offset along the molecular axis systematically. The amino acid sequences of alpha chain of human Type I, II and III collagen were used to form a triple-helical conformation with 7/2-helical symmetry. Charged residues were supposed to have +1 or -1 charge on their C(beta) atoms for convenience. The local minimum of Coulomb energy for homotrimers of Type I, II and III collagens clearly showed that the electrostatic interaction is one of important driving forces to form D-staggered structure.

Keywords: collagen, D-staggered, electrostatic interaction