P04.17.392

Acta Cryst. (2008). A**64**, C353

Structure of collagen-helix motif

Kenji Okuyama
Osaka University, Department of Macromolecular Science, 1-1 Machikaneyama, Toyonaka, Osaka, 560-0043, Japan, E-mail：okuyamak@chem.sci.osaka-u.ac.jp

The triple helix is a specialized protein motif found in all collagens. Although X-ray diffraction studies of collagen began in the 1920s, the small amount of data available from fiber diffraction of collagen made the molecular structure determination difficult. In the early 1950s, two plausible fiber periods of about 20 and 30 Å were proposed, together with corresponding single-strand models having 7/2- and 10/3-helical symmetry, respectively. The first framework of the triple helix was proposed by Ramachandran and Kartha in 1955. Rich and Crick proposed another structure with the same framework to avoid some of the steric problems of the first model. Their framework, which involved a triple-helical structure with a period of 28.6 Å and 10/3-helical symmetry, was the same as one of two single-strand models proposed at that time, except for the number of strands. On the other hand, Okuyama et al. detected the triple-strand model with the other framework, with a fiber period of 20 Å and 7/2-helical symmetry, in the single crystal of $(Pro\text{-}Pro\text{-}Gly)_{10}$ in 1972. Although they proposed this structure as a new structural model for collagen, it has not been acknowledged as such, but instead has been regarded only as a model for a collagen-like peptide. Recently, we showed that both 7/2- and 10/3-helical models could explain X-ray diffraction data from native collagen quantitatively. Furthermore, the helical symmetries of collagen-model peptides analyzed at high resolution are very close to the ideal 7/2-helical symmetry, whereas no supporting data was found for the 10/3-helical model. This evidence strongly suggests that an average molecular structure of native collagen is the 7/2-helical model rather than the prevailing Rich and Crick (10/3-helical) model.

Keywords: collagen structure, fibre diffraction, single crystal structure analysis

P04.17.393

Acta Cryst. (2008). A**64**, C353

The structure of *Epiphyas postvittana* Takeout 1 suggests a ligand-carrying role for Takeout proteins

Cyril Hamiaux[1,2], Duncan Stanley[1], Edward Baker[2], Richard Newcomb[1]
[1]HortResearch, Private Bag 92169, Auckland, Auckland, 1142, New Zealand, [2]Laboratory of Structural Biology, School of Biological Sciences, 3A Symonds Street, Auckland, New Zealand, E-mail：chamiaux@hortresearch.co.nz

Members of the Takeout / Juvenile Hormone Binding Protein (To/JHBP) superfamily are small proteins (~240 amino acids) found exclusively in insects, being present in all insect orders. JHBP bind juvenile hormones involved in the maintenance of the larval state and sexual development in adults. In contrast, very little information is available for the more diverse To proteins. Expression profiles and regulation patterns of some To proteins indicate that these proteins are ubiquitous, but their function(s) remain largely unknown. To and JHBP have similar predicted secondary structures, possess a conserved N-terminal disulfide bond and are likely to share a common fold. JHBP, however, have a second disulfide bond that is not present in To, while To contain two conserved C-terminal motifs that are not found in JHBP, suggesting distinctive roles for To and JHBP. Here we present the first crystal structure of a Takeout protein, To1 from the lightbrown apple moth *Epiphyas postvittana*, solved by in-house sulfur-SAD phasing and refined to 1.3 Å resolution. To1 adopts the unusual α/β fold recently described for JHBP, but major structural differences are observed i) for the takeout motifs and ii) for the internal cavity. To1 highlights a very large, purely hydrophobic, cavity buried inside the protein, in which a surrogate ubiquinone moiety from *Escherichia coli* is bound. This provides insights into the binding mode and chemical structure of To1 endogenous ligand(s) and suggests guidelines for the docking of potential ligands inside the cavity. Our structure represents the first experimental evidence that To proteins act as ligand carriers and confirms that the unusual α/β fold is shared by all members of the To/JHBP family.

Keywords: takeout, juvenile hormone binding protein, crystal Structure

P04.17.394

Acta Cryst. (2008). A**64**, C353

Structural basis for the RPEL motif interaction with G-actin

Stephane Mouilleron, Sebastian Guettler, Richard Treisman, Neil Q McDonald
Cancer Research UK, Structural Biology, 44 Lincoln's Inn Fields, London, Greater London, WC2A 3PX, UK, E-mail：stephane.mouilleron@cancer.org.uk

Actin polymerisation plays a critical role in many important cellular processes including: cell motility, intracellular transport, control of cell shape and polarity. Monomeric forms of actin also have functional roles. For example, levels of G-actin regulate nucleocytoplasmic shuttling of the transcription factor MAL, a co-factor for SRF (serum-response factor). The actin-sensing mechanism resides within three consecutive RPEL motifs of MAL that together comprise a functional and regulated G-actin binding site. In this poster I report the molecular basis for RPEL motif interaction with G-actin by determining structures of two independent RPEL peptide:G-actin complexes at 1.45 Å and 2.3 Å resolution. The RPEL:G-actin structures explain concisely the sequence conservation defining the motif with the invariant RPEL arginine. Using a fluorescent polarisation assay we quantify the different affinities of individual MAL RPEL motifs and selected mutants that validate the structural data. Affinity differences may reflect different functional roles of the RPEL peptides in MAL regulation.

Keywords: G-actin, RPEL, MAL

P04.17.395

Acta Cryst. (2008). A**64**, C353-354

Structural basis for regulatory interplays between EB1, CLIP-170 and p150Glued

Ikuko Hayashi[1], Mike J Plevin[2], Mitsuhiko Ikura[2]
[1]Yokohama-City Univ., 1-7-29, Suehiro, Tsurumi-ku, Yokohama, Kanagawa, 230-0045, Japan, [2]Ontario Cancer Institute, 101 College Street, Toronto, ON, M5G 1L7, CANADA, E-mail：ihay@tsurumi.yokohama-cu.ac.jp

Microtubule is essential for many cellular processes. Recent live-cell imaging studies have identified proteins that associate specifically with the growing-ends of microtubules, which are termed microtubule plus-end tracking proteins (+TIPs). +TIPs

have been shown to control MT dynamics by local influences on microtubule assembly and to be responsible for 'search-and-capture' of microtubule targets, such as the cell cortex and chromosomes. However, the molecular mechanisms by which +TIPs perform these functions are not clearly understood. +TIPs include structurally unrelated proteins such as EB1, cytoplasmic linker proteins (CLIPs), the dynein-dynactin complex. Here we report the crystal structure of the C-terminal zinc-binding motif of CLIP-170 in complex with the microtubule-binding (CAP-Gly) domain of p150 Glued solved by multiple-wavelength anomalous diffraction using the zinc atom. The structure reveals that CLIP-170 binds p150Glued via the same surface as another +TIP, EB1. Using biochemical and biophysical approaches, we investigated protein interactions involving CLIP-170 and found that an intramolecular interaction area overlaps with EB1- and microtubule-binding sites in an analogous manner to p150Glued.

Keywords: cytoskeleton, crystal structure, NMR

P04.17.396

Acta Cryst. (2008). A**64**, C354

Topological classification of protein

Masanori Yamanaka, Yuno Natsume, Yukihiro Miyazawa
Nihon University, Department of Physics, Kanda-Surugadai 1-8, Chiyoda-ku, Tokyo, 101-8308, Japan, E-mail：yamanaka@phys.cst.nihon-u.ac.jp

Classification of the protein crystal structure is one of the important studies in molecular biology. SCOP [1], CATH [2], and FSSP [3] are known as some of the data base where the classification is based on the amino acid sequence, the secondary structure, and the protein structure. The classification of the graph with respect to the genus obtained by embedding the graph into two dimensional surface is a fundamental problem in the topological combinatorics. The genus is a measure to distinguish the graph complexity. The method is applied to classify the crystal [4]. One of the most interesting applications is the classification of RNA structure by the pseudo-knot [5]. In this study we apply the method to protein classification. The diagram of genus versus hydrogen-bond plot shows a rich structure. The fine structure of the motif can be distinguished well. In the classification of the protein mutant, which is defined as single or several amino-acid structural polymorphism, even the "zero amino-acid structural polymorphism" can be classified in several categories. In the analysis of the disulfide-bond formation facilitator (human), the data can fit well by the straight line in the diagram of genus versus hydrogen-bond plot. The result shows that the mutant can be classified further families. The method opens to construct the periodic table of protein.

[1] SCOP, http://scop.mrc-lmb.cam.ac.uk/scop.
[2] CATH, http://www.cathdb.info/latest/index.html.
[3] FSSP, http://www2.ebi.ac.uk/dali/fssp/fssp.html.
[4] M. Yamanaka and A. Tanaka, cond-mat/0510161.
[5] M. Bon, G. Vernizzi, H. Orland, and A. Zee, q-bio/060732.

Keywords: protein structure and folding, topological aspects of structure, biological macromolecules

P04.18.397

Acta Cryst. (2008). A**64**, C354

Structural insights into the mitochondrial import complex, TIM9.10

Chaille T Webb[1,2], Michael Baker[3], Michael T Ryan[3], Michael A Gorman[1], Peter M Colman[1], Jacqueline M Gulbis[1]
[1]The Walter and Eliza Hall Institute of Medical Research, Structural Biology, 1G Royal Pde, Parkville, VIC, 3050, Australia, [2]Department of Medical Biology, The University of Melbourne, Melbourne, VIC, Australia, [3]Department of Biochemistry, Latrobe University, Melbourne, VIC, Australia, E-mail：cwebb@wehi.edu.au

Over 99% of human mitochondrial proteins are synthesised from nuclear DNA and must be imported as immature precursors *via* a coordinated series of specific, tightly regulated events. Encoded topological signals ensure nascent proteins are ushered to their correct mitochondrial destination. Proteins destined for the inner or outer mitochondrial membranes contain internal targeting information. After transfer through the outer membrane's general import pore, preproteins encounter TIM9.10, a hetero-hexamer of two homologous polypeptides, Tim9 and Tim10. TIM9.10 mediates preprotein passage across and within the intermembrane space. Inner membrane carrier proteins (*e.g.* metabolite carriers) are transferred to the inner membrane translocase, Tim22, for insertion, whereas β -barrel proteins of the outer membrane are transferred to the sorting and assembly machinery, SAM. We have determined the structure of the hTIM9.10 assembly to 3.5 Å resolution and can now verify key contacts in our 2.5 Å structure from *Saccharomyces cerevisiaea*. The molecular assembly has a unique α -propeller topology in which alternating Tim9 and Tim10 subunits alternate about a central pseudo-hexad. Each subunit of the hexamer has a helix-loop-helix topology, and a highly conserved twin CX3C motif present in both forms two intra-chain disulfides that define a central loop. The six loops come together to to form one face of the core assembly, below which tentacle-like helices emanate. Helical protrusions in the structures of other chaperones have been implicated in substrate binding so we are now combining structural and biochemical approaches to investigate chaperone-substrate interactions in our system.

Keywords: membrane trafficking, mitochondria, chaperone

P04.18.398

Acta Cryst. (2008). A**64**, C354−355

Structural basis for peroxisomal localization of tetrameric carbonyl reductase

Nobutada Tanaka[1], Ken-ichi Aoki[1], Shuhei Ishikura[2], Makoto Nagano[2], Yorishige Imamura[3], Akira Hara[2], Kazuo T Nakamura[1]
[1]Showa University, School of Pharmaceutical Sciences, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo, 142-8555, Japan, [2]Gifu Pharmaceutical University, Mitahora-higashi, Gifu 502-8585, Japan, [3]Kumamoto University, Oe-honmachi, Kumamoto 862-0973, Japan, E-mail：ntanaka@pharm.showa-u.ac.jp

Peroxisomal matrix proteins are nuclear-encoded and synthesized in the cytosol. There are two well-characterized classes of peroxisome targeting signals (PTS), known as PTS1 and PTS2. Proteins carrying one of these signals are recognized in the cytosol by soluble PTS receptors, Pex5p for PTS1 and Pex7p for PTS2. The PTS1 is a carboxy-terminal sequence ending in the tripeptide Ser-Lys-Leu (SKL) or some conservative variant. All proteins bearing the PTS1 do not undergo cleavage of the targeting sequence upon transport into the peroxisome. Pig heart peroxisomal carbonyl reductase (PerCR) is a 100 kDa homotetrameric enzyme and exhibits NADPH-linked reductase activity towards alkyl phenyl ketones, alpha-dicarbonyl compounds, and all-trans-retinal. It belongs to the short-chain dehydrogenase/reductase (SDR) family, and its sequence comprises a C-terminal SRL tripeptide, which is a variant of the PTS1, SKL. PerCR is imported into peroxisomes of HeLa cells when the cells are transfected with vectors expressing the enzyme.