16 of their cysteines. Although no clear evidence exists for a physiological function of mammalian CRISP found mainly in the epididymis and salivary glands, snake venom CRISP are known to inhibit smooth muscle contraction and cyclic nucleotide-gated (CNG) ion channels. The structure of CRISP-*a* from *Naja atra* is determined at 1.58-Å resolution using the sulfur-SAD method and consists of unique disulfide patterns and two distinct structural domains: a protease sandwich fold (N-terminal) and an ion channel-blocking BgK toxin fold (C-terminal). With one positively charged cluster found at water accessible helix regions next to the Ser-His-Glu triad of the protease domain, heparin binding plays a role in regulating CRISP-*a* activity. As important residues identified to block CNG and K^+ channels of other toxin homologues are located at one face of the ion channel-blocking domain, the structure provides a basis for rational design of a peptide blocker of the CNG channel. The ion channel-blocking domain and heparin-binding site of CRISP-*a* are suggested to play a chaperone role in leading it to the site of protease action for remodeling of the extracellular matrix in mammalian cells.

Keywords: sulfur-SAD phasing, toxin CRISP structure, heparin

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Structure of Parasporin-1, a Novel Bacterial Cytotoxin against Human Cancer Cells

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The crystal structure of parasporin-1 from *Bacillus thuringiensis* strain A1190 has been determined at 1.76 Å resolution. Parasporin-1 belongs to the Cry protein family, which includes insecticidal pore-forming toxins successfully utilized in agriculture; however, the protein is not insecticidal but specifically toxic to particular types of cultured human carcinoma cells. This strict selectivity suggests its potential use as an anti-cancer drug.

Parasporin-1 has a three-domain architecture common to available structures of other insecticidal Cry proteins; the main chain of each domain is superimposed reasonably well with their counterparts in spite of low sequence homology. Significant deviations are found in a few limited regions. Of particular interest is the N-terminal extension upstream of domain 1, which clamps the domain to domain 2 and which presumably disable the transformation of the domain necessary for pore formation. Among the available Cry protein structures, only the inactive Cry2Aa protoxin has an analogous structure. These observations along with biochemical results [1] suggest that parasporin-1 may act as a simple ligand to activate an unidentified signaling pathway leading to malfunction of membrane channels rather than as a pore-forming toxin.

[1] Katayama H., et al., *J. Biochem.*, 2005, **137**, 17.

Keywords: pore-forming toxins, anticancer biochemistry, receptor recognition

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Structure of Diol Dehydratase Reactivating Factor – A Novel Molecular Chaperone

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Diol dehydratase and glycerol dehydratase are adenosylcobalamin-dependent enzymes that catalyze the conversion of 1,2-propanediol, 1,2-ethanediol and glycerol to the corresponding aldehydes. Glycerol, a physiological substrate for the enzyme, inactivates the enzyme in an irreversible manner. Diol dehydratase reactivating factor is a molecular chaperone, reactivating the inactivated diol- and glycerol dehydratases in the presence of AdoCbl, ATP and Mg^{2+}. Here we report the crystal structures of ADP-bound and nucleotide-free forms of diol dehydratase reactivating factor.

Initial electron density map of the selenomethionine-substituted ADP-bound form was obtained from the MAD diffraction data collected at the BL38B1 beam line, SPring-8, Japan. Diffraction data sets for native ADP-bound and nucleotide-free crystals were collected at the BL41XU beam line, SPring-8, Japan.

Structure of nucleotide-free diol dehydratase reactivating factor is similar to that of nucleotide-free glycerol dehydratase reactivating factor reported by Liao *et al*. [1]. The ADP-bound form of diol dehydratase reactivating factor shows rearrangement of domains with respect to its nucleotide-free form.

[1] Liao, et al., *Structure*, 2003, **11**, 109.

Keywords: diol dehydratase reactivating factor, molecular chaperone, crystal structure

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Structural and Functional Analysis of PDI–related Proteins

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Protein Disulfide Isomerase[PDI]–related proteins are residents of the endoplasmic reticulum and are involved in several functions, some of which include redox and chaperone activities. Their function involves several non-covalent weak interactions with specific epitopes on substrate proteins. The molecular basis of these interactions has not been understood until recently [2].

We recently elucidated the first crystal structure of such a eukaryotic PDI–related chaperone, Wind from Drosophila [1]. It has been identified that Wind binds Pipe (a 2-*O*-sulfotransferase) *in vitro*. A putative peptide binding site has been mapped on the b'-domain for substrate binding with the requirement of the integrity of a surface on the d'-domain. Crystal structures of several Wind–mutants and their complexes with the peptides mimicking the Pipe binding site were elucidated giving some clues about the binding mechanism. Further, the structure of a mammalian orthologue of Wind, Erp28 has been solved, suggesting a functional role for the structural conservation between the proteins.

[1] Ma Q., Guo C., Barnewitz K., Sheldrick G. M., Söling H. D., Uson I., Ferrari D. M., *JBC*, 2003, **278**, 44600. [2] Barnewitz K., Guo C., Sevvana M., Ma Q., Sheldrick G. M., Söling H. D., Ferrari D. M., *JBC*, 2004, **279**, 39829.

Keywords: chaperone, protein disulfide isomerase, Wind

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TPR Repeat Domain of O-linked GlcNAc Transferase: Similarities to Importin Alpha

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Addition of N-acetylglucosamine (GlcNAc) is a ubiquitous form of intracellular glycosylation, catalyzed by the conserved O-linked GlcNAc transferase (OGT). OGT contains an N-terminal domain of tetratricopeptide (TPR) repeats that mediates the recognition of a broad range of target proteins. Nuclear pore complex components are major OGT targets, as OGT depletion by RNAi results in the loss of GlcNAc modification at the nuclear envelope. To gain insight into the mechanism of target recognition, we solved the crystal structure of the homodimeric TPR domain of human OGT, containing 11.5 TPR repeats[1]. The repeats form an elongated superhelix. The concave surface of the superhelix is lined by absolutely conserved asparagine residues, in a manner reminiscent of the peptide-binding site of importin α. Based on this structural similarity, we propose that OGT employs an analogous molecular mechanism to recognize its targets.