cell-cell or cell-extracellular matrix interactions by bidirectional signaling. They are involved in a number of physiological processes such as platelet aggregation, inflammation, tumor metastasis and other diseases. The αβ heterodimeric glycoproteins are composed from 19 different α subunits and 8 different β subunits [1]. The collagen binding integrin family consists of four collagen receptors that have a common β1 subunit non-covalently bound either to α1, α2, α10 or α11 subunit. They all have a 200 amino acid inserted domain (I-domain) in the N-terminal region of the αsubunit, which is responsible for recognition of the ligand [2]. The αI domain folds into a "Rossman fold", which forms a metal ion-dependent adhesion site, referred to as MIDAS [3].

The crystal structure of α1I and α2I, and also the complex structure of α2I bound to a collagen-like peptide, have been solved [4,5,6]. Comparison of the α2I in complex with a collagen-like peptide (open conformation) and α2I without ligand (closed conformation) showed that conformational changes occur, when the ligand is bound [6].

We have shown that two peptides, CTRKKHDC and CARKKHDC, bind to α1I and competitively inhibit collagen binding. We have modeled the open conformation of α1I in complex with a collagen-like peptide and characterized the binding of the ligand and the structural changes that are caused [7]. Our aims are to further study the binding of collagen-like peptides to α1I and to characterize the conformational changes that might occur.

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Keywords: integrin, I-domain, collagen-binding

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The Structure of the ParC Subunit of Topoisomerase IV from *Streptococcus pneumoniae*

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Topoisomerases relieve torsional stress in DNA within cells by breaking one or both strands of DNA, then either winding or unwinding the DNA helix, followed by strand closure.

TopoIV (whose subunits are encoded by *ParC* and *ParE*) is a decatenating enzyme that resolves interlinked daughter chromosomes following DNA replication. TopoIV uses a double-strand passage mode as does Gyrase (which controls DNA supercoiling by relieving topological stress arising from the translocation of transcription and replication complexes bound to DNA) by a different mechanism: Gyrase wraps DNA around itself, while TopoIV does not. The difference in DNA-wrapping between Gyrase and Topo IV contributes to their different functional roles within cells.

The ParC subunit of TopoIV has been crystallised in tetragonal and hexagonal forms. Analysis of the tetragonal crystals (diffracted significantly better than hexagonal ones) showed that they were internally twinned. Present in the crystal are two *I222* crystal lattices aligned in opposite directions and forming pseudo 4-fold symmetry.

The structure of ParC subunit was solved by Molecular Replacement using a model build on the basis of the homologous GyrA subunit from *E.coli*[1] (1AB4 deposited in the PDB) as a model. Analysis of packing of the protein molecules in the unit cell shows screw axes || to ***c***, going in opposite directions.

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Keywords: topoisomerase IV, ParC, twinning

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A Proposal to Engineer Protein Crystallization through Metal Ions

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A topic of current interest is engineering surface mutations in order to improve the success rate of protein crystallization. This report explores the possibility of using metal-ion-mediated crystal-packing interactions to facilitate rational design. *Escherichia coli* apo acyl carrier protein was chosen as a test case because of its high content of negatively charged carboxylates suitable for metal binding with moderate affinity. The protein was successfully crystallized in the presence of zinc ions. The crystal structure was determined to 1.1 Å resolution with MAD phasing using anomalous signals from the co-crystallized Zn(II) ions. The case study suggested an integrated strategy for crystallization and structure solution of proteins via engineering surface Asp and Glu mutants, crystallizing them in the presence of metal ions such as Zn(II) and solving the structures using anomalous signals [1].

[1] Qiu X., Janson, C.A., *Acta Crystallogr D*, 2004, **60**, 1545.

Keywords: crystallization, crystal packing, anomalous signals

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The Crystal Structure of Human CDK7 and Its Protein Recognition Properties

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CDK7, a member of the cyclin-dependent protein kinase family, regulates the activities of other CDKs through phosphorylation on their activation segment and hence contributes to control of the eukaryotic cell cycle. CDK7 also assists in the regulation of transcription as part of the transcription factor TFIIH complex. For maximum activity and stability, CDK7 requires phosphorylation, association with cyclin H, and association with a third protein, MAT1.

We have determined the crystal structure of human CDK7 in complex with ATP at 3 Å resolution. The kinase is in the inactive conformation, similar to that observed for inactive CDK2. The activation segment is phosphorylated at Thr170 and is in a defined conformation that differs from that in phospho-CDK2 and phospho-CDK2/cyclin A. The functional properties of the enzyme against CDK2 and CTD as substrates are characterized through kinase assays. Experiments confirm that CDK7 is not a substrate for kinase-associated phosphatase.

Keywords: cyclin-dependent kinase, cell cycle, structure

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Novel Vapour Diffusion Method

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We present a new method for the crystallization of biological macromolecules, combining advantages of the vapour diffusion method with advantages of the micro-batch method.

The classic vapour-diffusion, either in its hanging-drop or sitting-drop configuration is still the preferred method by many crystallographers. This is mainly due to its inherent dynamics and the final endpoint of the diffusion. The new method uses a combination of two oils. As in the micro batch method, the total system is protected from evaporation by paraffin oil (first phase). However, the protein/precipitant mixture (fourth phase) is able to equilibrate with a reservoir solution (third phase) via a second layer of oil (second phase), present under the upper layer of paraffin oil. The second oil is not miscible either with the paraffin oil, nor does it interact and influence the properties of both phases three and four. Procedures for

identifying suitable oils for the second phase and their influence on the crystallization dynamics are presented.

The system can be set-up in several ways. In one approach, the protein/precipitant and the reservoir can be applied under the paraffin oil, resulting in a classic micro-batch experiment. Successively, vapour diffusion can be turned on by the application of the second oil, allowing the diffusion of water molecules from the protein droplet to the reservoir solution. Since the system is directly protected from evaporation by the paraffin oil, smaller volumes of protein solution can be applied without any hassle. A suitable micro-plate for the novel method is presented.

Keywords: crystallography of proteins and nucleic acids, statistical analysis experimental data, light scattering

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The Crystal Structure of Mannosylglycerate Synthase from *Rhodothermus marinus* at 2.5 Å Resolution

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Mannosylglycerate (MG) is a compatible solute widely distributed among thermophilic and hyperthermophilic organisms. It accumulates in response to salt and heat stress in *R. marinus* and was found to protect several enzymes against heat inactivation [1,2]. The pathways for the synthesis of MG in *R. marinus* have been characterized in detail [1]. Mannosylglycerate synthase (MGS) is involved in the single-step pathway converting GDP-mannose and D-glycerate to MG, similarly to the GDP-mannose:α-mannosyltransferase GT55 family of Glycosyltransferases (EC 2.4.1.-), which retain the anomeric configuration of the substrate [1,3]. To date no 3D structure is known for any enzyme belonging to this family.

A Se-Met derivative of MGS has been recently crystallized and a Se K-edge MAD experiment was carried out on ESRF beamline ID29. The crystals belong to trigonal space group $P3_221$, with unit cell parameters $a = b = 148$, $c = 155$ Å. Preliminary structural analysis suggests that MGS may function as a dimer and that each monomer has two distinct domains: one mainly α-helical and the other predominantly β-sheet.

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Keywords: mannosylglycerate synthase, multi-wavelength anomalous X-ray dispersion, seleno-methionine derivative

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Local Conformational Similarity between Native and Denatured States

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Traditionally, the denatured state of a protein has been viewed as unfolded, having little to no secondary or tertiary structure. Recently, however, residual dipolar couplings have been used to demonstrate the presence of residual structure under denaturing conditions in a number of protein systems [1]. Furthermore, recent computational work has demonstrated that random coil statistics can be produced by allowing only a small fraction of a protein's phi-psi angles to vary [2].

In the present study we seek to experimentally validate this notion that on a local scale (8-12 residues) primary conformations of the native state are represented as significant conformations in the denatured state. The denatured state of staphylococcal nuclease is modeled as an 11 residue peptide corresponding in sequence to a loop region in the parent protein suggested to adopt multiple conformations in the native state. Monoclonal antibodies raised against the peptide and screened for tight binding to the parent protein serve as conformational probes. Isothermal titration calorimetry yields thermodynamic binding parameters from which the relative populations of the bound conformation in the native and denatured states can be obtained. Crystal structures of the peptide and protein bound complexes serve to verify the bound conformations and inform the analysis of the ITC data. Here, we report the structure of the 11-mer peptide in complex with an Fab fragment.

[1] Shortle D., Ackerman M.S., *Sci.*, 2001, **293**, 487. [2] Fitzkee N.C., Rose G.D., *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 12497.

Keywords: conformational analysis, denatured state, calorimetry

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Ubiquitin Binding Mechanism of Hrs-UIM

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Mono-ubiquitination plays an important role in degradation of growth factor receptors. Monoubiquitinated receptors are sorted into multivesicular bodies, which then fuse with lysosomes. Hepatocyte growth factor-regulated trypsine kinase substrate (Hrs) is one of the essential proteins for the sorting mechanism. Hrs can interact with ubiquitin by its ubiquitin interaction motif (UIM). The ability to bind ubiquitin is essential for the function of Hrs in sorting of ubiquitinated proteins. We present a crystal structure of an Hrs-UIM/ubiquitin complex. Data sets were collected to 1.7Å resolution with good statistics (R_{merge} = 5.1%) using synchrotron radiation (1.0 Å wavelength) at beamline PF-AR NW12 of Photon Factory, Tsukuba, Japan. Using the molecular replacement method, we have determined and refined the complex structure. It consists of two ubiquitin molecules and one UIM peptide, suggesting that Hrs-UIM can interact with two ubiquitin molecules simultaneously. Together with a binding assay using surface plasmon resonance, the crystal structure sheds light on the molecular mechanism of double-side ubiquitin recognition by Hrs-UIM, which facilitates efficient binding of multi-mono-ubiquitinated protein complexes. We propose the double-sided UIM as a new sub-class of UIM based on a sequence search which yielded a number of putative double-sided UIMs.

Keywords: transport, ubiquitin system, X-ray crystallography of proteins

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Crystal Structures of the Carbohydrate Recognition Domain of Emp46p and Emp47p

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Emp46p and Emp47p are type-I membrane proteins that cycle between the endoplasmic reticulum (ER) and the Golgi apparatus in vesicles coated with coat protein complex II (COPII). They are considered to function in pairs as cargo receptors for exporting soluble *N*-linked glycoproteins from the ER. To investigate the structural basis for the glycoprotein transport by Emp46p and Emp47p, we have determined crystal structures of the carbohydrate recognition domains (CRD) of Emp46p and Emp47p, in the absence and presence of metal ions. Both proteins fold as a beta-sandwich, and resemble that of the mammalian ortholog, p58/ERGIC-53. However, the nature of metal binding is different from that of Ca^{2+}-dependent p58/ERGIC-53. The CRD of Emp46p does not bind Ca^{2+} but instead contains K^+ near the putative ligand binding site. To our surprise, the CRD of Emp47p binds no metal ions at all. We suggest that the carbohydrate recognition by the hetero oligomeric complex of Emp46p and Emp47p is different from that of Ca^{2+}-dependent p58/ERGIC-53. Details of binding assay using surface plasmon resonance will be presented and compared with the crystallographic results.

Keywords: lectins, protein X-ray crystallography, protein transport