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.

[1] Martins et al., *J. Biol. Chem*., 1999, **274**, 35407-35414. [2] Ramos et al., *Appl. Environ. Microbiol*., 1997, **63**, 4020-4025. [3] Borges et al., *J. Biol. Chem*., 2004, **279**, 9892-9898.

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