Hdj1 by 50–90%. These data demonstrate that Hdj1 has two peptide-binding sites and both sites are essential for the cochaperone activity. In the initial stages of the refolding reaction, one protomer of the Hdj1 homodimer binds through site 2 to the hydrophobic polypeptide. The other protomer binds through site 1 to the Hsp70 C-terminal region.

Keywords: heat shock protein, molecular chaperone

MS22.P26

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The crystal structure of the autoinhibitory domain of talin

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Talin is a large cytoskeletal protein essential for activating integrin adhesion receptors and coupling them to actin cytoskeleton. In the cytosol, talin adopts a default autoinhibited conformation where a major integrin binding site on the N-terminal talin head (talin-H) is masked by a C-terminal rod segment (talin-RS). Here we report the high resolution crystal structure of talin-RS. In combination with previous mutagenesis data, the structure provides insight into how talin-RS inhibits the integrin binding on talin-H. Structural comparison of the crystal structure and a previously reported NMR structure will also be presented. In addition, the crystal structure of talin-RS shows its unexpected capability of tetramerization. Our results shed light upon the mechanism of the talin autoinhibition in regulating integrin activation and signaling during diverse cell adhesion and migration processes.

Kewywords: talin, autoinhibition, integrin

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Reduced nDsbD sheds light on protein-protein interactions in disulfide cascades

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Bacterial growth and pathogenicity depend on the correct formation of disulfide bridges, a process controlled by the Dsb system in the periplasm of Gram negative bacteria. Proteins with a thioredoxin fold play a central role in this process. A general feature of thiol:disulfide exchange reactions is the need to avoid a long-lived product complex between protein partners.

We have solved the structure of the N-terminal domain of the transmembrane reductant conductor DsbD (nDsbD) of *E.coli* in its reduced form, to a resolution of 1.8 Å (PDB code 3PFU) [1]. This structure complements NMR, surface plasmon resonance, mutagenesis and *in vivo* experiments, as well as already published structures of the oxidised form of nDsbD [2], of the oxidised and reduced forms of the C-terminal domain of DsbD (cDsbD) [3] and of the covalent complex of nDsbD with cDsbD [4], in investigating the interaction between these two soluble domains of the protein [1]. The oxidation-state-dependent affinity of this interaction is explained in terms of subtle differences in structure and electrostatics. nDsbD is a fairly rigid oxidoreductase with a unique immunoglobulin-like fold which ensures that the active-site cysteines are only accessible after specific protein-protein interactions with its thioredoxin-like partners. Small conformational differences in the active-site and in the protective cap-loop region of reduced and oxidised nDsbD affect the way it interacts with its partners. The oxidation-state-dependent affinities between the two domains ensure that nDsbD and cDsbD are not trapped in non-productive complexes that would block electron transfer from the cytoplasm to the periplasm.

Our observations have wider implications for the interactions of the ubiquitous thioredoxin-like proteins with their substrates and are of general importance for oxidative protein-folding pathways in all organisms.

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Keywords: thiol:disulfide oxidoreductases, oxidative folding

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Crystal structure of acap1 involved in endocytic recycing

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Endocytic recycling is critical for many cellular events, including nutrient uptake, cell polarity, cell motility and signal transduction, but little is known about how cargo proteins are sorted, and even cargo sorting has been predicted to be unlikely in the endocytic recycling pathway. ACAP1 is Arf6 GTPase-activating Protein, controlling the return of Arf6 to the inactive GDP-bound state. ACAP1 is not only as key negative regulator of Arf6, but also as its effector by being core components of clathrin coat complexes. ACAP1 is reported as an adaptor of a novel clathrin coat complex for endocytic recycling in key physiological settings, the recycling of TfR from the recycling endosome, stimulation-dependent recycling of integrin and insulin-stimulated recycling of glucose transporter type 4 (Glut4). The molecular mechanism of cargo sorting remains to be known.

The crystal structures of N-portion and C-portion of ACAP1 have been determined by x-ray crystallography, and give help to elucidate the mechanism of its interaction with membrane and cargo protiens in Endocytic recycling pathway.

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Keywords: crystal, clathrin, endocytic recycing

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APEH-mediated down-regulation of proteasome by potential anticancer molecules

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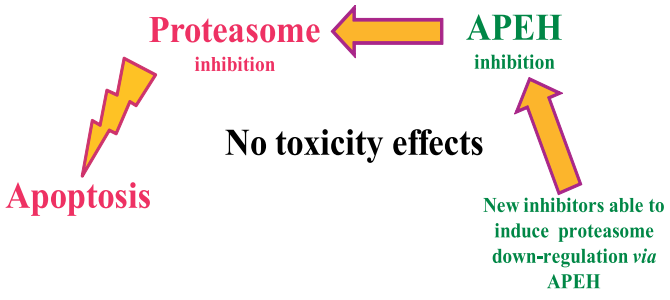
A Sulfolobus solfataricus acylpeptide hydrolase (APEH), named $APEH_{Ss}$ has been identified and characterized. The protein is the endogenous protease target of the already isolated inhibitor SsCEI [1]. APEH is one of the four members of the prolyl oligopeptidase family, which removes acylated amino acid residues from the N terminus of blocked oligopeptides. SsCEI is the first protein able to efficiently inhibit APEH from mammalian sources with IC_{50} values in the nanomolar range. The 3D model of $APEH_{Ss}$ alone or in complex with SsCEI has been proposed, suggesting an inhibition mechanism of steric blockage on substrate access to the active site or on product release [2]. Interestingly, the identified reactive site loop (RSL) of SsCEI includes an unusual amino acid sequence which cannot be classified in any of the canonical motifs of serine protease inhibitors so far characterized. Therefore, a number of small peptides has been designed and synthesized on the basis of the RSL sequence. They are surprisingly stable and highly structured in aqueous solutions and show inhibitory activity against *S. solfataricus*, and human APEHs with IC_{50} values in the order of low micromolar. In light of a recently proposed cooperative role for the APEH–proteasome enzymatic system in controlling protein turnover, we investigated the proteasome down-regulation *via* APEH inhibition, hypothesizing that APEH can be used as a new target to indirectly control/modulate proteasome functions in tumoral cells. We identified different compounds that induce proteasome down-regulation *via* APEH, in both cell-free and cell-based assays. These molecules efficiently down-regulated human APEH activity in a dose-dependent manner in the human colon carcinoma cell line (Caco-2 cells) or in the baby-hamster-kidney (BHK) cell line, without any toxic effects and inducing proapoptotic and antitumoral effects. Surprisingly, in both cell lines such molecules markedly reduced the proteasome activity with a concomitant accumulation of several known cytoplasmic proteasome substrates. Furthermore, a molecular docking analysis has been carried out to assess the potential enzyme binding sites involved in the APEH–inhibitors interactions.

These results represent a starting point for a promising strategy in cancer therapy involving a new class of molecules for proteasome down-regulation mediated by knock-out of APEH activity.



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Keywords: APEH inhibition, proteasome, molecular modeling

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Structural basis for floral induction by rice florigen Hd3a

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Florigen is a hypothetical leaf-produced signal that induces floral induction at the shoot apex. The nature of florigen has remained elusive for more than 70 years. But recent progress toward understanding the molecular mechanism for flowering in *Arabidopsis* has led to the suggestion that FLOWERING LOCUS T (FT) is the mobile flower-inducing signal. Actually, the protein encoded by Hd3a, a rice ortholog of FT, moves from the leaf to the shoot apex and induces flowering in rice [1]. The floral transition by the FT or Hd3a protein is achieved by transcriptional activation of floral genes at the shoot apex, however, the molecular mechanism remains unclear. To understand this mechanism, we performed a structural analysis of the rice florigen Hd3a.

The polypeptide encoding the rice florigen Hd3a was expressed in bacterial cells with a novel high-level bacterial expression system pCold-GST [2]. The crystal structure of Hd3a was determined. Hd3a-interacting proteins were identified by Yeast Two-Hybrid and evaluated interactions with Hd3a by NMR titration experiments, isothermal titration calorimetry (ITC) measurements and GST-pull down assay. From these results, the molecular mechanism of floral induction by florigen will be discussed.

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Keywords: floral induction, florigen, crystal structure

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Crystal structure of human importin-α (Rch1)

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Importin α is involved in a variety of nuclear transport processes of external viral proteins responsible for the infection. To elucidate structural basis of viral infection responsible for the transport mechanism of viral components across the nuclear membranes, we carried out structural study of the major variant of the human importin α, Rch1. We truncated the N-terminal of the Rch1, and then could crystallize the IBB (importin β binding domain) truncated mutant of Rch1 (ΔIBB-Rch1) by the vapor diffusion technique. We collected X-ray diffraction data of the ΔIBB-Rch1 using the mail-in system [1] operated in SPring-8, Japan. The ΔIBB-Rch1 crystallized in space group $P4_32_12$ with cell dimensions of $a=b=139.10$ Å $c=140.96$ Å. We solved the crystal structure by the molecular replacement technique using human importin α5 as a template structure. The resultant structure of ΔIBB-Rch1 formed a tightly bound homo-dimer in the right-handed super-helical manner. Each protomer of the dimer consists of ten ARM repeats, where the ARM1 and ARM5 is the major dimerization region. The region corresponding to the typical NLS binding sites are