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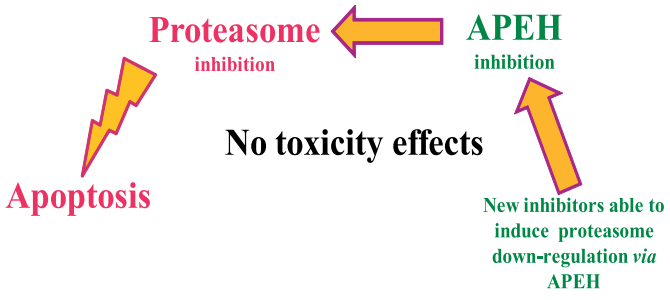
A Sulfolobus solfataricus acylpeptide hydrolase (APEH), named $APEH_{Ss}$ has been identified and characterized. The protein is the endogenous protease target of the already isolated inhibitor SsCEI [1]. APEH is one of the four members of the prolyl oligopeptidase family, which removes acylated amino 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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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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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

completely included in the dimerization surface.

Furthermore, we carried out a variety of physicochemical measurements (ITC, DLS and the analytical ultracentrifugation) to elucidate the functional aspects of the multimerization property of Rch1. As a result, we found out a valid correlation between the multimerization state of Rch1 and its NLS recognition property, where the IBB domain of Rch1 plays a role to control the multimerization. More detailed scenario about the NLS recognition mechanism of the Rch1 will be presented in the poster session.

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Keywords: importin-α

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Recent practices on sulfur SAD phasing using soft X-rays

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A single crystal X-ray diffraction data set contains critical three-dimensional structural information about the molecules which makeup the crystal. This information is the major direct experimental source for the subsequent elucidation of spatial structures of the crystallized molecules.

In recent years, single wavelength anomalous diffraction (SAD) phasing has become the major method used in macromolecular structure determination. Sulfur atoms are natively present in most protein molecules and their anomalous scattering signal when measured with soft X-rays makes them an ideal phasing probe. With the advances in methodology and diffraction data collection hardware, sulfur SAD phasing has contributed to many *de novo* crystal structure determinations. We have been actively trying this phasing method in routine crystal structure determinations. Several new structures have been determined by the S-SAD method with the data collected using either home lab Cr X-rays or synchrotron X-rays (Photon Factory beamlines 1A and 17A). Details of the data collection, data processing and phasing process will be presented.

Keywords: S-SAD, soft X-rays

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Multivariate methods for density modification of SAD phased maps

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Recently, we have derived and implemented a multivariate likelihood distribution for phase combination of density modified phases with initial SAD experimental phases [1] and a technique to reduce bias in the phase combination process [2].

Preliminary results suggest that the power of these methods can be further improved by incorporation of structure factors from a partially built model into the SAD multivariate function.

The new function is used for simultaneous refinement and density modification which is iterated with automated model building.

Many structures can be built automatically by the Crank software suite [3] that previously failed thanks to the combination of these new methods.

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Keywords: density modification, model building, refinement

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Phasing in the Home Laboratory

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Many examples of S-SAD and Se-SAD phasing have been reported with diffraction data collected away from absorption edges with copper radiation (1.54 Å) as well as at the K absorption edge of selenium with synchrotron radiation (0.979 Å). Chromium radiation (2.29 Å) has been available for in-house data collection for a few years now. As a result, a number of successful SAD experiments using Cr radiation have been performed by several groups [1], [2]. Furthermore, unpublished results from the PDB [3] indicate a higher utilization than the publication record shows. However, Cr radiation has not been fully utilized despite being ideally suited for measuring anomalous signals from weak anomalous scatterers such as sulfur, selenium, calcium, and other atoms commonly found in protein crystals.

We explore why softer X-rays generated by a Cr anode may be better than a synchrotron source. Beam stability, radiation damage, and mechanical issues are less of a problem with in-house experiments. The human psychology of travel and use of an unfamiliar experimental station may also be avoided with an in-house system. This is a cost effective method of single crystal X-ray diffraction data collection and structure determination that is especially useful when time is of the essence and synchrotron time is unavailable due to shutdowns and maintenance. In the end the tortoise may win the race.

With the addition of Cr radiation to the crystallographer's toolkit, in-house X-ray sources can routinely provide at least two wavelength options. This report also discusses the results of phasing by combining diffraction data from both Cu and Cr-collected data sets, as well as data sets collected with only Cu radiation.

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Keywords: sulfur, SAD, phasing

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Structural studies of serine acetyltransferase 1 from *Entamoeba histolytica*