recessive juvenile parkinsonism (AR-JP). Parkin contains a unique ubiquitin-like domain in its N-terminus designated Uld which is assumed to be a interaction domain with the Rpn 10 subunit of 26S proteasome. To elucidate the structural and functional role of Uld in parkin at the atomic level, the X-ray crystal structure of murine Uld was determined and a molecular dynamics simulation of wild Uld and its five mutants (K27N, R33Q, R42P, K48A and V56E) identified from AR-JP patients were performed. Crystals of Uld were obtained by the hanging-drop vapor-diffusion method using NaCl as a precipitant. Diffraction data were collected to 1.65Å resolution. The structure of Uld was determined by the single-wavelength anomalous diffusion (SAD) method using an iodinated derivative. The final model gave the R-factor of 0.195 and R_{free}-factor of 0.244. Murine Uld consists of two α helices and five β strands, and its overall structure is essentially the same as that of human ubiquitin with a 1.22 Å rmsd for the backbone atoms. The MD simulations showed the K27N and R33Q mutations increase the structural fluctuation of these β strands including the α1 helix. Reversely, the V56E mutant restricted the spatial flexibility at the periphery of the short α2 helix by the interactions between the polar atoms of Glu56 and Ser19 residues.

Keywords: Parkin, ubiquitin-like domain, molecular dynamics simulation

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Structural and functional whole-cell project for the model organism, *Thermus thermophilus* HB8

Akio Ebihara[1], Akeo Shinkai[1], Mayumi Kanagawa[1], Yoshihiro Agari[1], Hitoshi Iino[1], Yoshiaki Kitamura[1], Keiko Sakamoto[1], Miho Manzoku[1], Kenji Fukui[1], Noriko Nakagawa[1,2], Ryoji Masui[1,2], Ken Hirotsu[1], Yoshitaka Bessho[1,3], Takaho Terada[3], Mikako Shirouzu[3], Shigeyuki Yokoyama[3,4], Seiki Kuramitsu[1,2,3]

[1]RIKEN SPring-8 Center, RIKEN Harima Institute, SR System Biology Research Group, 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, [2]Grad. Sch. of Sci., Osaka Univ., Toyonaka, Osaka 560-0043, Japan, [3]RIKEN Yokohama Inst., Tsurumi, Yokohama 230-0045, Japan, [4]Grad. Sch. of Sci., Univ. of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, E-mail：ebihara@spring8.or.jp

This research project aims to understand all fundamental biological phenomena at an atomic-resolution, on the basis of molecular structures and functions. Towards this aim, we selected the extremely thermophilic organism, *Thermus thermophilus* HB8, as a model organism, because many of the approximately 2,200 genes encoded in its genome have been selected during evolution and are common to many organisms. However, about 500 of the genes (proteins) are functionally-uncharacterized. As a first step to obtain functional clues about these proteins, we determined their three-dimensional structures. Based on the structures, we inferred the molecular functions of about 60% of them and intensively characterized several family proteins, such as the house-cleaning NUDIX hydrolases, metallo-beta-lactamases and DNA repair proteins. While we have continued to solve the structures of other uncharacterized proteins for their functional inference, we have also been exploring their functions by functional genomics analyses (mRNA, protein and metabolite) in combination with gene disruption and stress-perturbation. For example, we found that cyclic AMP receptor protein (CRP), which is known as a global transcriptional factor, regulates 22 genes, including ones presumably involved in host defense (1 characterized and 21 uncharacterized), whereas one of the CRP family proteins functions in stationary phase, and regulates 14 genes related to energy and redox metabolism (3 characterized and 11 uncharacterized). We also found that about 40 genes of unknown function display altered mRNA expression upon metal stress. All of the plasmids for protein expression and gene disruption prepared in our laboratory are now available from the RIKEN BioResource Center (see http://www.thermus.org/).

Keywords: structural genomics, functional genomics, systems biology

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X-ray crystal structure of a hypothetical Sua5 protein from *Sulfolobus tokodaii* strain 7

Yoshihiro Agari[1], Shinya Sato[1], Taisuke Wakamatsu[1,2], Yoshitaka Bessho[1,3], Akio Ebihara[1], Shigeyuki Yokoyama[1,3,4], Seiki Kuramitsu[1,2], Akeo Shinkai[1]

[1]RIKEN, Harima Inst. SPring-8 Center, SR System Biology Research Group, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, [2]Department of Biological Sciences, Graduate School of Science, Osaka University, [3]Systems and Structural Biology Center, Yokohama Institute, RIKEN, [4]Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, E-mail：y_agari@spring8.or.jp

The Sua5-yciO-yrdC domain proteins are widely distributed in prokaryotes and eukaryotes. One of the proteins in this family, *Escherichia coli* YrdC, preferentially binds to double-stranded RNA and DNA. It has been predicted to be a rRNA maturation factor. Sua5 consists of an N-terminal YrdC domain and a C-terminal Sua5 domain. The *sua5* gene was first identified in *Saccharomyces cerevisiae* as a suppressor of a translation initiation defect of the iso-1-cytochrome c (*cyc1*) gene. The function and 3D structure of Sua5 remain to be elucidated. In the present study, we determined the crystal structure of Sua5 (ST1526) from thermoacidophilic archaeon *Sulfolobus tokodaii* strain 7, which exhibits 49.7% similarity to *S. cerevisiae* Sua5. The overall fold of the N-terminal yrdC domain of *Sulfolobus* Sua5 is similar to that of *E. coli* YrdC, the *Z*-score being 21.3 and the r.m.s.d. value being 2.4 Å. A large concave surface exhibiting a positive electrostatic potential, which is similar to that in YrdC, was found in Sua5. Interestingly, excess electron density that might be due to an *E. coli*-derived nucleotide was observed on this concave surface. The C-terminal Sua5 domain consists of three α-helices and five β-strands, which adopt a Rossmann fold. A structure similarity database search using the DALI server revealed that the closest structure was that of *Methanocaldococcus jannascii* HypB, a GTP-binding protein that regulates metal binding. Thus, the three-dimensional structure of Sua5 showed that both the N- and C-terminal domains might be involved in nucleotide binding or metabolism, which is supported by the observation that Sua5 showed ATP hydrolysis activity, AMP being produced.

Keywords: nucleotide, Rossmann fold, translation factor

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Characterization of metal ions and protein oligomeric states in JCSG structures

Hsiu-Ju Chiu[1,2], Daniel McMullan[2,3], Mark W Knuth[2,3], Polat Abdubek[2,3], Ashley M Deacon[1,2]

[1]Joint Center for Structural Genomics, Stanford Synchrotron Radiation Laboratory, 2575 Sand Hill road, Menlo Park, CA, 94025, USA, [2]Joint

Center for Structural Genomics, [3]Genomics Institute of the Novartis Research Foundation, San Diego, California, E-mail：chiu@slac.stanford.edu

X-ray fluorescence spectra are routinely used to identify bound metals in our crystal structures. Using a standard setup available on all SSRL PX beamlines, a typical fluorescence scan can be collected in less than 1 minute. Since each metal has a characteristic emission energy, a single scan can be used to detect all possible metals in the crystal, except for light atoms such as S and Mg. When multiple metals are observed, X-ray diffraction datasets are collected above and below the relevant absorption edges. Anomalous difference Fourier maps are then used to unequivocally assign the metal sites. Of the 595 structures determined by JCSG, 163 contain metal ions (Zn^{2+} 38, Fe^{3+} 15, Ni^{2+} 23, Mg^{2+} 31, Ca^{2+} 23, Na^{+} 31 and the remaining are K^{+}, Mn^{2+} and Li^{+}). A sequence alignment against structural homologs, coupled with an examination of the metal binding environment suggests that nearly all the Fe^{3+} and Zn^{2+} ions found are related to protein function. In some cases, we identified sites with mixed metals, and in others, we unexpectedly found biologically relevant metal sites. Conversely, only ~50% of Mg^{2+} ions, ~10% of Ni^{2+} ions and almost none of the Ca^{2+} and Na^{+} ions are functionally relevant. These ions are typically introduced to the sample during protein purification or crystallization. A protein's oligomeric state often has functional implications. We use the crystal structure, in conjunction with size exclusion chromatography and static light scattering to assign the protein oligomeric state. Of the 270 proteins that have been analyzed, ~7% show a disprepancy between crystal packing and SEC/SLS. We will present a detailed analysis. The JCSG is funded by NIGMS/PSI, U54 GM074898. SSRL is funded by DOE BES, and the SSRL SMB program by DOE BER, NIH NCRR BTP and NIH NIGMS.

Keywords: structural genomics, metalloprotein, static light scattering

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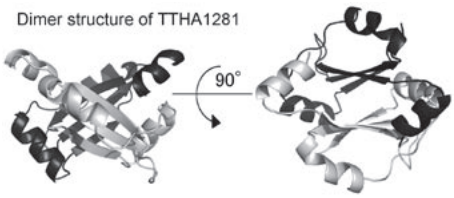
X-ray structure of TTHA1281 from *thermus thermophilus* HB8

Mitsugu Yamada[1,2], Hiromi Yoshida[2], Noriko Nakagawa[3,4], Yoshihiro Agari[3], Mayumi Kanagawa[3], Seiki Kuramitsu[3,4], Shigehiro Kamitori[2,3]

[1]Japan Atomic Energy Agency, Quantum Beam Science Directorate, 1233 Watanuki, takasaki, gunma, 370-1292, Japan, [2]Life Science Research Center and Faculty of Medicine, Kagawa University, 1750-1 , Kagawa 761-0793, Japan, [3]RIKEN, Harima Institute at SPring-8, Hyogo, Japan, [4]Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka 560-0043, Japan, E-mail：yamada.mitsugu@jaea.go.jp

TTHA1281 from Thermus thermophilus HB8 is a small protein with 69 amino acids and its function is unknown. Primary structure analysis using the BLAST program showed that the homolog proteins with 50% of sequence identity are found in the radioresistant bacteria having a rapid DNA repair mechanism, Deinococcus radiodurans and Deinococcus geothermalis, but the functions of these homolog proteins are also unknown. In this study, we determined the X-ray structure of TTHA1281 to obtain useful information about its functions. Initial phases were determined by a multiple-wavelength anomalous dispersion technique and the structure was refined to R = 0.214 using 2.0 Å resolution data (PDB code: 2E6X). The monomer TTHA1281 has three alpha-helices and a beta sheet consisting of three beta-strands. The DALI search revealed that structure of monomer TTHA1281 has a novel folding with no structure-similarity to other structure-solved proteins. In a crystal, TTHA1281 forms a tight dimer with 2-fold symmetry by stacking of beta-sheets, giving a large cavity covered by many of the basic amino acids, as shown in a figure. We supposed that this cavity is possibly related to the function of TTHA1281.



Keywords: protein crystallography, structural genomics, protein structure and folding

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Structural implications for ligand binding and thermostability of peptidyl-tRNA hydrolase 2

Katsumi Shimizu[1], Naoki Kunishima[2], Katsuhide Yutani[2]

[1]Kwansei Gakuin University, Advanced Research Center of Science, School of Science and Engineering, 2-1 Gakuen, Sanda, Hyogo, 669-1337, Japan, [2]RIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, E-mail：shimizu@kwansei.ac.jp

Peptidyl-tRNA hydrolase 2 (Pth2) catalyzes hydrolytic removal of the peptidyl moiety from peptidyl-tRNA molecule, allowing the reuse of the resultant free tRNA in protein biosynthesis. In order to investigate the structure-function and structure-thermostability relationships of Pth2, we carried out X-ray diffraction experiments and differential scanning calorimetry (DSC) measurements of two hyperthermophilic archaea, Pth2 from *Pyrococcus horikoshii* OT3 (*Ph*Pth2) and *Methanocaldococcus jannaschii* DSM 2661 (*Mj*Pth2) that grow at different optimum temperatures of 98 ºC and 85 ºC, respectively. The structures have been determined as similar biological dimers in different crystal forms: (1) $P4_12_12$, 1.2 Å and (2) $P4_322$, 1.9 Å in *Ph*Pth2, and (3) $C2$, 2.1 Å in *Mj*Pth2. In *Ph*Pth2, the structural difference between the two dimers was quantitatively evaluated by a multiple C_α-atom superposition. A significant structural difference was observed around the putative active site of this enzyme. A rigid-body rotation takes place so as to retain the dimeric twofold symmetry, suggesting positive cooperativity upon tRNA binding. The docking study suggests that the binding of tRNA requires its simultaneous interaction with both subunits of the *Ph*Pth2 dimer. In DSC measurement, the linear specific enthalpy change against denaturation temperatures at different pHs and excellent heat reversibility upon denaturation have been observed. The lower ΔC_p determined from DSC would be favorable in the temperature function of ΔG, comparing to the higher ΔC_p determined from structural analyses. The lower ΔC_p gives a higher ΔG in the wide temperature range and suggests that the denatured structures of these proteins are more compact than those of general proteins.

Keywords: oligomer, X-ray diffraction, differential scanning calorimetry