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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

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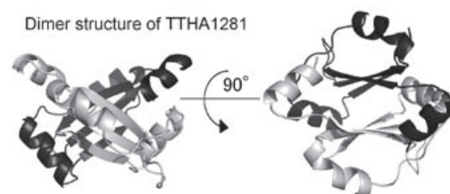
TTHA1281 from Thermus thermophilus HB8 is a small protein with 69 amino acids and its function is unknown. Primary structure analysis using the BLAST program showed that the homolog proteins with 50% of sequence identity are found in the radioresistant bacteria having a rapid DNA repair mechanism, Deinococcus 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

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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