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Human thioredoxin1 (hTRX1) is a small 12-kD oxidoreductase enzyme consisting of 105 amino acids and containing a dithiol/disulfide active site with multiple cellular functions. This enzyme has activity as a cellular reductase by a dithiol-disulfide exchange reaction using two cysteine residues (Cys32 and Cys35) in the conserved active site sequence. Apart from the two cysteines, there are three additional conserved cysteines, Cys62, Cys69, and Cys73 in the mammalian TRX, which have not been known to their biological functions. Although it has been identified that the Cys73 residue is involved in dimerization of hTRX via an intermolecular disulfide bond formation between Cys73 of each monomer in the oxidized state, biological function of the Cys62 and Cys69 residues in the non-active remain to be fully elucidated. In the previous paper, researchers proposed that the formation of a disulfide bond between Cys62 and Cys69 could give a way to transiently inhibit hTRX activity for redox signaling or oxidative stress. Furthermore, they proposed a model structure of the non-active site disulfide in the hTRX. Here, we present the high-resolution crystal structure of fully oxidized hTRX1, which shows an intramolecular disulfide bond between Cys62 and Cys69. The disulfide bond formation disengages a helix proximal to the active site and results in a conformational change of the hTRX enzyme, providing a structural basis for understanding the regulation mechanism of redox signaling or oxidative stress.

Keywords: human thioredoxin1, intramolecular disulfide, redox signaling

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Joint neutron and X-ray diffraction studies at 293 K of antifreeze protein

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Type III Antifreeze Proteins (AFPs) are small globular monomeric proteins (66 aa, M.W.=7kDa), which are highly homologous. Their shared antifreeze property is linked to a network of hydrogen bonding between a specific lattice plane on ice and several conserved, polar and solvent accessible amino acids located along a flat Ice-Binding Surface (IBS). We shall present our developments: 1) - Neutron Laue data collection on the new LADI III (ILL) on an ab-initio fully deuterated tiny crystal (volume = 0.12 mm^3, resolution = 2 Å), including production of fully deuterated protein, crystallization by macro-seeding in D_2O. The ratio resolution/volume is similar to the Human Aldose Reductase [1]. 2) - X-ray diffraction at Synchrotron ESRF beamline ID29 on a fully deuterated crystal of the same crystallization batch at a resolution of 1.05 Å, necessary to carry out a joint Neutron - X-ray refinement like for Human Aldose Reductase h AR [2]. 3) Specific H labelling on Leucine and Isoleucine of the fully deuterated protein, in order to create a contrast useful for specific phasing methods for neutron diffraction data. (Human Frontier Science Program).

Ref. 1. Hazemann, Blakeley et al., Acta Cryst, D61,1413,2005.
Ref. 2. Blakeley, Ruiz et al , PNAS, 105, 1844,2008.

Keywords: neutron diffraction, antifreeze protein, perdeuteraton

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X-ray induced perturbation in an ultra-high resolution protein structure

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Positions of hydrogen atoms and orientations of water molecules are important to functions of proteins. However, such information from protein crystals is easily disturbed by radiation damage. The damage can not be prevented completely even in the data collection at cryogenic temperatures. Therefore, influence of X-rays should be estimated exactly in order to bring out meaningful information from crystallographic results. Diffraction data from a single crystal of the high-potential iron-sulfur protein (HiPIP) from *Thermochromatium tepidum* were collected at SPring-8, and were merged into three data sets as exposure to X-rays. The maximum absorption doses were estimated to be 4.5×10^5, 9.0×10^5 and 1.4×10^6 Gy for the three data sets. Structures analyzed at 0.70 Å show detailed views of X-ray induced perturbation such as positional changes of hydrogen atoms of a water molecule. We will discuss about initial steps of radiation damage from the ultra-high resolution analysis.

Keywords: electron transfer, high-resolution protein structures, radiation damage

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High resolution crystals of human hematopoietic & lipocalin-type prostaglandin D synthases in space

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Hematopoietic and lipocalin-type prostaglandin (PG) D synthases (H-PGDS and L-PGDS) are responsible for production of PGD2, which acts as an allergic inflammatory mediator[1] and an endogenous sleep-promoting substance[2], respectively. The specific inhibitors of each enzyme are important for suppression of various diseases. To obtain high-quality crystals for structural analysis, we crystallized both H-PGDS and L-PGDS by using a counter-diffusion method under a microgravity environment on the International Space Station (ISS). The three-dimensional structure of human H-PGDS has already been determined in a complex with an H-PGDS inhibitor HQL-79 (PDB ID: 2CVD)[3]. Since novel H-PGDS inhibitors with affinities 100-fold higher than HQL-79 have recently been developed, we obtained high quality crystals of human H-PGDS in complexes with those novel inhibitors by using PEG 6000 as a precipitant in microgravity. The crystals showed diffraction up to 1.2