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X-ray crystallographic studies of the human ceruloplasmin-myeloperoxidase complex at low resolution

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Ceruloplasmin (CP) is a 132 kDa copper-containing oxidase of mammalian blood plasma. It is capable of oxidizing Fe^{2+} and Cu^{+}. Besides, CP can oxidize biogenic (epinephrine, serotonin) and synthetic (p-phenylene diamine, o-dianisidine) amines. It also oxidizes glutathione in the presence of either NO or H_2O_2. Hereditary deficiencies connected with inhibited synthesis or altered activity of CP result in neurodegeneration and diabetes, both caused by oxidative stress due to accumulation of ferrous iron. CP is a marker of inflammation acute phase, possessing the features of a universal antioxidant. Myeloperoxidase (MPO) is a ~140kDa heme-containing protein, major constituent of neutrophils which produces a strong antibacterial agent hypochlorous acid (HOCl). HOCl play an important role in the cell defense against microbial infections. However, MPO can release into extracellular fluids, where HOCl became a powerful oxidant responsible for tissue damage and the initiation of acute and chronic vascular inflammatory disease. CP forms a complex with MPO under physiological conditions and inhibits its activity. We solved CP-MPO crystal structure at 4.7 Å which provide useful information despite low resolution. Comparing biochemical data on CP effect upon MPO activity with the results of a structure analysis reveals the likely mechanism of MPO inhibition. It seems to be realized via the contact of peptide loop linking domains 5 and 6 in CP with the heme pocket of MPO. This loop of CP is essentially susceptible to proteolysis with serine proteinases. We suggest that MPO protects the anti-oxidant potential of CP, which is particularly important in foci of inflammation where numerous serine proteinases can be found.

Keywords: protein complexes, macromolecular X-ray crystallography, macromolecular structure-function relationships

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IPR beamline for macromolecular assemblies at SPring-8

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Biological macromolecular assemblies play significant roles in many biological reaction systems, including energy transfer, protein synthesis, protein analysis and signal transduction. A detailed understanding of the functions of the macromolecular assemblies requires information derived from three-dimensional structure analysis. A beamline for crystal structure analysis of biological macromolecular assemblies at SPring-8 is operated by the Institute for Protein Research, Osaka University. Since features of crystals of biological macromolecular assemblies are extremely weak diffraction power, narrow space between the diffraction sports and x-ray radiation sensitive, it is essential to use high brilliance and high paralleled synchrotron radiation for diffraction data collection. This beamline is specially designed to collect high quality diffraction data from biological macromolecule assembly crystals with large unit cells. A newly designed high precision diffractometer, which has mu-axis, is installed. Diffraction data from crystals of biological macromolecular assemblies with large unit cell (two unique axes of over 500 angstrom) has been collected at 4.0 angstrom resolution. The present status of the beamline including the new diffractometer and detector systems and a recent result of crystal structure analysis of biological macromolecular assemble will be presented.

Keywords: X-ray crystallography of biological macromolecules, biological macromolecular assemblies, synchrotron structural biology research

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X-ray crystallographic and ultracentrifugal analyses of haloarchaeal nucleoside-diphosphate kinases

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Nucleoside-diphosphate kinase (NDK, EC 2.7.4.6) has been shown to be ubiquitous in living organisms, with a few exceptions. The main function of NDK is to exchange γ-phosphates between nucleoside triphosphates and nucleoside diphosphates, thus playing a key role in maintaining cellular pools of all nucleoside triphosphates. Haloarchaea, extremely halophilic aerobic archaea constitute a distinct group in the archaea. The majority of the strains grow best at NaCl concentrations of 3.5-4.5 M, and to compensate for the high salt in the environment, haloarchaea accumulate salts, mainly KCl, up to 5 M. Most of the haloarchaeal proteins are thus adapted to function in the presence of high salt concentrations, and lose their activities at low salt concentrations. NDKs from two haloarchaea *Haloarcula quadrata* (HqNDK) and *Haloarcula sinaiiensis* (HsNDK) are shown to be different only in an amino acid residue at position 30, Arg or Cys, out of 154 amino acid residues. They differ in optimal NaCl concentration for activity; HqNDK shows the optimal activity at 1 M NaCl, whereas HsNDK is the most active at 2 M NaCl and its activity diminishes 5-fold in 1 M NaCl. In this study, we have determined the crystal structure of HqNDK at 2.5 Å resolution, which shows a hexameric assembly (a trimer of dimers). Analytical ultracentrifugal data of HqNDK and HsNDK have shown that, in 2 M NaCl, both eyzymes are hexameric, whereas, in 0.5 M NaCl, HqNDK remains hexameric but HsNDK dissociates into dimers. These data demonstrate the structural basis of their different degrees of tolerance to low NaCl concentration; the amino-acid replacement at position 30, Arg to Cys, destabilizes the trimeric assembly of the dimeric NDK subunits in low NaCl concentration.

Keywords: nucleoside metabolism, halophilic enzymes, quaternary association of proteins

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Structure of vault purified from rat liver

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Vault is a 12.9-MDa ribonucleoprotein particle with a barrel-like shape that is highly conserved in a wide variety of eukaryotes. Multiple copies of two additional proteins, vault poly(ADP-ribose) polymerase and telomerase-associated protein 1 , as well as a small vault RNA are also associated with vaults. (Kedersha and Rome, Kickhoefer et al., Michael P. Kowalski et al.). The vault crystals belong to space group *C*2 with unit-cell parameters a = 708.0 Å, b = 385.0 Å, c = 602.9 Å, β = 124.8°. Rotational symmetry searches based on the R factor and correlation coefficient from non-crystallographic symmetry (NCS) averaging indicated that the particle has 39-fold dihedral symmetry (Kato et al.). Electron cryo-microscopy electron density was used as the starting model for phase improvement and phase extension. A model of the MVP was composed of 9 β-sheet domains, shoulder domain and cap long helix. C terminal regions form a intermolecular pseudo β-sheet ring and a intermolecular pseudo α helix ring, which may be initiation structures of oligomerization of MVPs.

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Keywords: X-ray crystallography of biological macromolecules, crystallography of biological macromolecules, large molecular assemblies

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Macromolecular X-ray powder diffraction from the *in vivo* arm photophore of firefly squid

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The firefly squid, Watasenia scintillans, emits brilliant flashes of light from three tiny luminous organs which are located at the tip of each of the fourth pair of arms. The notable feature of the histology of the brachial organs is that they contain numerous rod-like bodies, which are 2.5-5 micrometer long and 1-3 micrometer thick, of protein assembly. Previously, its X-ray diffraction pattern was determined by directly irradiating a photophore at the tip of the fourth arms and showed numerous sharp reflections. It suggested that the rod-like bodies were made by micron-sized macromolecular crystal. X-ray diffraction data of the rod-like bodies extracted from the photophore were collected at room temperature at BL40B2/SPring-8 using its SAXS system with camera distance of 2111 mm and imaging plate system. The powder diffraction pattern was also observed from the extracted rod-like bodies, up to the resolution of 15 Å spacing with 5 minutes exposure. Indexing of the diffraction rings was done by using software McMaille v.3.04 (A. Le Bail,2004) and suggested that the micro-crystal belonged to an orthorhombic space group $P2_12_12_1$ with unit cell dimensions a=348 Å, b=195 Å, c=214 Å. The microscopic observation showed that the micro-crystal emitted a greenish fluorescent light by the excitation with 400-410 nm light. Since the luciferase of Watasenia is membrane-bound, the micro-crystal may be the storage of the luciferin binding protein. These results show that there is a unique macromolecular assembly in intact organs with the crystallographic manner and it is expected to be determined its crystallographic structure in the future.

Keywords: powder X-ray diffraction, protein crystallography applications, macromolecular assemblies

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Designing selective inhibitors to target NagZ a family 3 glycoside hydrolase

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NagZ is a family 3 beta-glucosaminidase involved in remodeling of the bacterial cell wall. It removes terminal N-acetylglucosamine residues from internalized cell wall degradation intermediates that are subsequently recycled back into the cell wall during biosynthesis. In the presence of beta-lactam antibiotics however, the NagZ product 1,6-anhydroMurNAc-oligopeptide accumulates in the bacterial cytosol to levels sufficient to induce the expression of the ampC beta-lactamase gene through direct activation of the AmpR transcriptional activator. Previously, the crystal structure of Vibrio cholerae NagZ in complex with PUGNAc, a potent and fairly selective inhibitor was determined to 1.7Å. This structure revealed a large open pocket beneath the 2-acetamido methyl group of the inhibitor. This is in contrast with the architecture of the human family 20 hexosaminidases and family 84 O-GlcNAcases. Family 20 and 84 enzymes form a tight envelope around the 2-acetamido group, holding it in position to participate as a nucleophile during catalysis. This structural difference suggests that extensions off the 2-acetamido group of PUGNAc would confer high inhibitor selectivity toward NagZ. Subsequent synthesis of derivatives two N-butylPUGNAc and Valeryl-PUGNAc possessing modifications to this region have been chosen for crystallographic analysis. These derivatives are potent and selective for NagZ. We present a detailed structural comparison of the family 3 NagZ:N-butylPUGNAc complex (to 2.5Å) with human family 20 and 84 glycosidase crystal