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**+** 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 H2O2. 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

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 salina (HsNDK) are shown to be different only in an amino acid residue at position 30, Arg or Cys. NDKs are either in 0.154 M NaCl. NDK from HsNDK also shows a single peak in its ultracentrifugal analysis; HqNDK shows a hexameric structure, which is a trimer of dimers. Analytical ultracentrifugal data of HqNDK and HsNDK have shown that, in 1 M NaCl, both enzymes 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.

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 spots 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 assembly will be presented.

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