

P04.14.349*Acta Cryst.* (2008). **A64**, C340**X-ray crystallographic studies of the human ceruloplasmin-myeloperoxidase complex at low resolution**Valeriya Samygina¹, Alexey Sokolov², Elena Zakharova², Gleb Bourenkov³, Vadim Vasilyev², Hans Bartunik⁴¹Institute of Crystallography RAS, Laboratory of protein crystallography, Leninsky pr. 59, Moscow, Moscow, 119333, Russia, ²Institute for Experimental Medicine RAMS, Acad. Pavlov street 12, Saint-Petersburg, 197376, Russia, ³EMBL c/o DESY, Notkestrasse 85, Hamburg, 22603, Germany, ⁴MPG-ASMB c/o DESY, Notkestrasse 85, Hamburg, 22603, Germany, E-mail: lera@ns.crys.ras.ru

Ceruloplasmin (CP) is a 132 kDa copper-containing oxidase of mammalian blood plasma. It is capable of oxidizing Fe²⁺ 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

P04.14.350*Acta Cryst.* (2008). **A64**, C340**IPR beamline for macromolecular assemblies at SPring-8**Eiki Yamashita¹, Masato Yoshimura¹, Mamoru Suzuki¹, Takashi Kumasaka², Masaki Yamamoto³, Shinya Yoshikawa⁴, Tomitake Tsukihara¹, Atsushi Nakagawa¹¹Osaka University, Institute for Protein Research, 3-2 Yamadaoka, Suita, Osaka, 5650871, Japan, ²JASRI/SPring-8, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo 6795198, Japan, ³RIKEN/SPring-8, 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo 6795148, Japan, ⁴University of Hyogo, 3-2-1 Kouto, Kamigori-cho, Ako-gun, Hyogo 6781297, Japan, E-mail: eiki@protein.osaka-u.ac.jp

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 assemble will be presented.

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

P04.14.351*Acta Cryst.* (2008). **A64**, C340-341**X-ray crystallographic and ultracentrifugal analyses of haloarchaeal nucleoside-diphosphate kinases**Koji Nagata¹, Akihiro Yamamura¹, Takefumi Ichimura¹, Jun Ohtsuka¹, Ken-ichi Miyazono¹, Tsukasa Makino¹, Masahiko Okai¹, Toru Mizuki², Masahiro Kamekura³, Masaru Tanokura¹¹The University of Tokyo, Graduate School of Agricultural and Life Sciences, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan, ²Toyo University, 2100 Kujirai, Kawagoe-shi, Saitama 350-8585, Japan, ³Noda Institute for Scientific Research, 399 Noda, Noda-shi, Chiba 278-0037, Japan, E-mail: aknagata@mail.ecc.u-tokyo.ac.jp

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 sinaiensis* (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 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.