03-Crystallography of Biological Macromolecules

PS–03.05.17
Crystal Structure of a NADH Oxidase from *Thermus thermophilus* Refined at 2.3 Å Resolution

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NADH oxidase (EC 1.6.99.3) from Thermus thermophilus is a flavoenzyme that catalyses the oxidation of NAD(P)H by molecular oxygen to NAD(P) and H_2O_2. The enzyme is a dimer with a molecular weight of 22000 D per monomer and accepts as cofactor FAD and FMN. The molecule crystallizes in spacegroup $P4_12_12$ with lattice dimensions a=94.8 Å and c=49.0 Å. The structure of the enzyme has been determined by isomorphous replacement using 3 derivatives and has been refined to 2.3 Å resolution. The present crystallographic R-value is 19.6 % for reflections between 8.0 and 2.3 Å resolution. The main part of the molecule is formed by a four stranded antiparallel ß-sheet covered by four helices. A C-terminal excursion of the second molecule of the dimer forms a fifth parallel strand to this ß-sheet. The interface between the monomers forms a deep cleft with contacts between the subunits mainly via a long bent helix. The FAD-binding site is situated at this interface between the monomers. As expected from the absence of the Gly-X-Gly-X-X-Gly consensus sequence for the nucleotide binding site this binding site is different from other FAD-binding proteins. The active site involves residues from both subunits of the dimer.

PS–03.05.18 CRYSTAL STRUCTURE OF PEROXIDASE FROM A FUNGUS *ARTHROMYCES RAMOSUS*. By N. Kunishima*, K. Fukuyama, H. Hatanaka†, Y. Shibano†, T. Amachi†, and H. Matsubara, Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan, and †Institute for Fundamental Research, Suntory Limited, Shimamoto-cho, Mishima-gun, Osaka 618, Japan.

Peroxidases (donor : H_2O_2 oxidoreductase [EC 1.11.1.7]) are a class of heme enzymes that catalyse the two-electron oxidation of a variety of compounds by hydrogen peroxide. The peroxidase isolated from a fungus, *Arthromyces ramosus*, (ARP) has higher activity than that of horseradish peroxidase. ARP consists of 344 amino acid residues and a non-covalently bound iron protoporphyrin IX. ARP has a sequence homology of about 20 % to other peroxidases. In order to elucidate the reaction mechanism of peroxidases as well as to compare the structure of ARP with that of cytochrome *c* peroxidase (CCP), X-ray analysis of ARP has been undertaken. ARP crystals belong to tetragonal, the space group of $P4_22_12$. The unit cell dimensions are a=b=74.57 Å, c=117.47 Å. Platinum, iodine, and mercury derivatives were prepared by soaking method. Native data to 1.9 Å and derivative data to 2.5 Å were collected with a screenless Weissenberg camera for macromolecular crystals using synchrotron radiation. The structure was solved by the multiple isomorphous replacement method. The overall figure of merit was 0.66 for the data to 2.5 Å resolution. The best map has shown that the spatial arrangement of α helices in CCP is basically conserved in ARP but the orientation and the length of some α helices are different. In the iodine derivative, an I_3^- is bound to ARP at the distal side of the heme. The proximal and distal ligands of the heme are His184 and His56, respectively. Crystallographic refinement is currently under way.

PS–03.05.19 STRUCTURE ANALYSIS ON CHIMERIC 3-ISOPROPYLMALATE DEHDROGENASE AND ITS THERMOSTABLE MUTANTS. by M. Sakurai, S. Kadono, K. Onodera, H. Moriyama, N. Tanaka, K. Imada*, Y. Katsube*, and T. Ooshima, Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, and Institute for Protein Research, Osaka University, Japan.

We are studying on chimeric 3-isopropylmalate dehydrogenase that are fusion of *Thermus thermophilus* IPMDH (10T) and *Bacillus subtilis* IPMDH (10M), and its thermostable mutants. The 2T2M6T is constructed by replacing 20-40% region from N-terminal of 10T to the corresponding mesophile. The 2T2M6T is sensitive to the heat. The mutants, S82R and I93L, recovered the thremostability. Crystallizations of the proteins were achieved by the hanging drop vapor diffusion method utilizing ammonium sulfate. The crystals of all proteins were isomorphous with that of the intact 10T that belong to space group $P3_221$ with the unit cell dimension of a=b=78.3A, c=158.6A. The diffraction data were collected by R-AXIS IIc. We successfully applied the molecular replacement method to solve the structure. The structural refinements were performed by the PROLSQ and X-PLOR. The final R-factors within 2.1 A resolution for 2T2M6T, S82R, and I93L are 19, 19, and 18 % respectively. The three compounds take almost the same structures to intact 10T with their r.m.s. displacement of Ca atoms are 0.16A. The distributions of temperature factors are also same, however I93L shows the more low values. In the detail, 2T2M6T have geometrical repulsion at Ile93 against Arg94. In the I93L the repulsion had removed. S82R have a new hydrogen bond between Arg82 and Glu87 through water molecule. These small but significant structural changes make the mutant compound thermostable.

PS–03.05.20 CRYSTALLOGRAPHIC AND SOLUTION STUDIES OF H-PROTEIN FROM THE GLYCINE DECARBOXYLASE COMPLEX IN PEA MITOCHONDRIA.
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The oxidation of glycine by plant mitochondria represents an important step in the metabolic pathway of photorespiration in leaf tissue and is catalyzed by the glycine decarboxylase complex. The complex, purified from pea leaf mitochondria, contains four proteins, P,T,H and L (Bourguignon, Neuburger & Douce, 1988). The pivotal enzyme is the 13.3kDA lipoamide-containing H-protein. Its sequence consists of 131 amino-acids (Macherel, Lebrun, Grignon, Neubürger and Douce, 1990). Its structure determination has been undertaken to further our understanding of the mechanisms involved in this multi-enzyme complex. This is the first report of the crystallization of a lipoic-dependent protein.
Crystals of H-protein were grown by vapour diffusion against 2M $(NH4)2SO4$ solution at pH 5.2. The space group is $P3_121$ (a=57.22, c=136.8 Å) with two independent molecules in the asymmetric unit and a crystal solvent content of about 46% (Sieker, Cohen-Addad, Neuburger & Douce, 1991). X-ray intensities were collected on a crystal of the native protein using a FAST/Enraf-Nonius area detector to 2.7Å resolution

A self rotation function calculation (Merlot program package) clearly revealed a non-crystallographic 2-fold axis which indicates the possibility of a dimeric association of the protein within the crystal. Multiple isomorphous replacement is being used to solve the structure. Three heavy atom derivatives were obtained (Eu nitrate,Gd nitrate, EtHgCl) and X-ray intensities were collected to 3.0 Å resolution on an Imaging plate system. Phasing is currently underway.

Small angle neutron scattering measurements were also undertaken in order to characterize the association state of the H protein in different solutions and also of H with the other proteins of the complex. The results will be discussed and related to biochemical studies of this complex (Oliver, Neuburger & Douce, 1990).

References

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PS–03.05.21 CRYSTALLOGRAPHIC STUDIES TOWARDS THE CATALYTIC MECHANISM OF GLYCOGEN PHOSPHORYLASE.

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Considerable time and effort has been devoted to determining the precise role of phosphate in the catalytic mechanism of glycogen phosphorylase (GP).

Previous time resolved crystallographic experiments with monochromatic radiation have allowed the reaction of heptenitol and phosphate to be followed in the crystal. One intermediate shot in this series of experiments indicated a possible attacking position of phosphate.

Binding studies in T state crystals with inorganic phosphate showed no binding at the catalytic site even at concentrations of 1M.

As part of a series of glycosidase inhibitors the nojirimycin tetrazole has been synthesised (Ph. Ermert & A.T. Vasella, 1991) and found to be a poor inhibitor of GP by itself (K_i=700μM), but in the presence of phosphate becomes one of the tightest known (K_i=50μM)).

A crystallographic binding study of the tetrazole/phosphate complex with T state GP showed phosphate to bind close to the postulated attacking position. These results have been confirmed with R state crystals which do exhibit a high affinity for dianions.

The rationale and implications for the catalytic mechanism will be discussed in the poster presentation.

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PS–03.05.22 TOWARDS THE STRUCTURE OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE BY SINGLE ISOMORPHOUS REPLACEMENT

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Leuconostoc mesenteroides glucose 6-phosphate dehydrogenase (G6PD) can utilise either NAD^+ or $NADP^+$ in the oxidation of glucose 6-phosphate, depending on the physiological conditions. This dual coenzyme specificity is unusual among dehydrogenases, most of which strongly prefer one over the other. In an attempt to understand the mechanism whereby the enzyme selects its coenzyme, and the protein structural basis for this, we are currently engaged in determining the crystal structure of the enzyme.

The enzyme is a dimer composed of two identical subunits of 485 amino acids and molecular weight ~ 54000. Crystals of the wild-type enzyme had been obtained and diffraction studies initiated, but the resulting crystals were very sensitive to radiation damage and only diffracted weakly. Furthermore, all attempts to prepare suitable heavy atom derivatives for multiple isomorphous replacement structure determination methods were unsuccessful, partly because of the absence of cysteine residues. The recent successful cloning and sequencing of the gene encoding *L.mesenteroides* G6PD, and the construction of a suitable strain of *E.coli* IH which this gene is expressed (Lee,W.T., Flynn,T.G., Lyons,C & Levy,H.R. (1991). *J. Biol. Chem.* **266**, 13028-13034.), prompted us to use site-directed mutagenesis to address this problem.

Six mutants of *L.mesenteroides* G6PD have been synthesised and characterised. In each of these either a serine or a glutamine residue has been changed to a cysteine, giving the potential for high occupancy, single site heavy atom binding.

Trigonal crystals for five of the six mutants have been grown ($P3_121$ or $P3_221$; a=b=105.7Å, c=224.3Å, α=β=90°, γ=120°), which are all isomorphous with the wild-type recombinant enzyme. These crystals are less sensitive to radiation damage, and diffract more strongly than those grom from the conventionally extracted enzyme. Co-crystallisation of the S215C mutant with sodium p-(hydroxymercuri)benzoate has yielded crystals isomorphous with the native wild-type. Crystals of this derivative have been used to collect X-ray diffraction data to 3.5Å resolution and subsequent difference Patterson analyses show that there are two mercury sites in the asymmetric unit, one in each monomer. To date, none of the other mutants have given any promise of heavy metal binding.

Refinement of the mercury sites, phasing, and then solvent flattening allowed the calculation of a 3.5Å resolution electron density map of sufficient quality to observe that our original choice for the enantiomorphic space group, $P3_121$, was wrong, as the map contained left-handed helices. Fitting some of the most easily interpretable areas of the $P3_221$ map allowed us to determine the non-crystallographic two-fold dimer axis. Using this axis, averaging techniques have further improved the map and it has now proved possible to trace a large portion of the protein density. Phase combination techniques are currently in progress and it is hoped to collect anomalous dispersion data to improve the phases further.

PS–03.05.23 CRYSTALLOGRAPHIC STUDIES ON 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM *TRYPANOSOMA BRUCEI*

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Sleeping sickness in man and a number of economically important veterinary diseases in sub-Saharan Africa are caused by sub-species of the parasitic protozoan *Typanosoma brucei* (*T.brucei*). The treatment of these conditions is unsatisfactory and less toxic, more effective drugs are being sought. The accelerated rate of glucose metabolism exhibited by the trypanosome while in the host's bloodstream suggests that its metabolic enzymes may prove viable targets for drug design.

We have recently crystallized recombinant *T.brucei* 6-phosphogluconate dehydrogenase (6-PGDH), the third enzyme of the oxidative branch of the pentose phosphate pathway. The crystals are trigonal, space group $P3_121$ or $P3_221$, with unit cell parameters a=b=135.1Å, c=116.7Å, α=β=90°, γ=120° and are stable in an X-ray beam . We have collected a 96% complete data set to 2.8Å resolution