Acta Cryst. (2002). A**58** (Supplement), C104

CRYSTAL STRUCTURE OF THE NADP-DEPENDENT GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE OF CYANOBACTERIA

T. Kitatani[1] T. Tada[1] Y. Nakamura[1] K. Wada[1] M. Tamoi[2] S. Shigeoka[2] K. Nishimura[1]

[1]Osaka Prefecture University Research Institute For Advanced Science And Technology Gakuen-Cho 1-2 SAKAI OSAKA 599-8570 JAPAN [2]Department of Food and Nutrition, Faculty of Agriculture, Kinki University, Nara 631-8505, Japan

Higher plants have two distinct glyceraldehyde-3-phosphate dehydrogenases (GAPDHs): cytosolic NAD-GAPDH and chloroplastic NADP-GAPDH. The cytosolic enzyme requires NAD as the sole coenzyme, while the chloroplastic enzyme utilizes both NADP(H) and NAD(H). The cyano*bacterium Synechococcus* PCC 7942 possesses only NADP-GAPDH as a tetramer. This NADP-GAPDH is not greatly activated by light irradiation or by addition of NADPH, being different from those of higher plants. We have determined the crystal structure of NADP-GAPDH originated from *S.* 7942 to elucidate the structure-function relationships of GAPDHs and to obtain some information about the activation mechanism. The recombinant NADP-GAPDH was expressed in *Escherichia coli* and the purified enzyme was crystallized using ammonium sulfate as a precipitant. The crystals were also soaked with NADP for 3 days. A complete data set was collected to a resolution of 2.5 Å using synchrotron radiation at KEK-PF. The crystals belonged to the monoclinic space group C2 with unit-cell parameters a = 149.4, b = 79.3 and c = 427.8 Å , and β = 101.3° . The structure was solved by the molecular replacement method. The electron density maps revealed the position of the cofactor in each subunit. Assignment of NADP improved the model to an R-factor of 20.7% with an R-free of 26.4%. The crystal structure revealed that one of a pair of Cys residues, both of which are involved in the light/dark regulation of the chloroplastic enzyme, is not found in the present enzyme.

Keywords: NADP-GAPDH HYDROGEN PEROXIDE CYANOBACTERIA

Acta Cryst. (2002). A**58** (Supplement), C104

ALTERNATE CONFORMATIONS OBSERVED IN CATALYTIC SERINE *OF BACILLUS SUBTILIS* LIPASE

H. Kondo[1] M. Suzuki[2] K. Kawasaki[1] S. Ohgiya[1] S. Tsuda[1]

[1]Research Institute of Biological Resources, Research Institute of Advanced Industrial Science and Technology (AIST) 2-17-2-1 Tsukisamu-Higashi, Toyohira SAPPORO 062-8517 JAPAN [2]Photon Factory, Institute of Materials Structure Science (IMSS), High Energy Accelerator Research Organization (KEK)

Bacillus subtilis extracellular lipase (BsL) has exceptionally small molecular weight (19.4 kDa) in lipase family, and is expected to be utilized for various industrial purposes. For the molecular design of mutant BsL more suitable for industrial usages, the detailed structural information is essential. Although the crystal structure of BsL was determined at 1.5 Å resolution [van Pouderoyen et al. (2001). J. Mol. Biol. 309, 215-226], in the present study a new crystal form of BsL, which provides diffraction data of higher resolution, was obtained, and the structure of BsL was determined at 1.3 Å. The crystal contains one BsL molecule in an asymmetric unit. MAD data of Pt derivative crystal were used for structure determination, and the refinement was carried out using native data collected at 1.3 Å resolution. It was newly found that one of the active site residues, Ser77, has alternate conformations in its side chain. The oxygen atom of the first conformer forms a hydrogen bond to the side chain of His155, a member of the catalytic triad. In contrast, the second conformer is constructed by another hydrogen bond to the side chain of adjacent His76. These two conformers presumably correspond to an active and inactive states, respectively. Alternate conformations in the catalytic serine have been also observed in *Fusarium solani* cutinase and *Penicillium purpurogenum acetylxylan* esterase. In addition, a glycerol molecule is found to be located in the active site, which probably occupies the binding site of a free glycerol that is produced by the enzymatic reaction.

Keywords: **LIPASE, αβ HYDROLASE FOLD, MAD**

Acta Cryst. (2002). A**58** (Supplement), C104

A STRUCTURAL CLUE ABOUT A STRINGENT RESPONSE RELATED PROTEIN FAMILY

O. Kristensen M. Gajhede

University of Copenhagen Protein Structure Group Department of Chemistry, Lab IV Universitetsparken 5 COPENHAGEN 2100 DENMARK

The Ppx/GppA phosphatase family contains enzymes of significant sequence homology with exopolyphosphatase and guanosine pentaphosphate phosphohydrolase activity. Here we report the first crystal structure determination of a member in this protein family with experimental MIRAS phases obtained from seleno methionine and ytterbium derivatives. The final phasing was extended to another crystal form through molecular replacement with a minimal model followed by ARP/wARP phase improvement and model building. Refinement has been performed at a resolution better than 2 Å. Despite very low sequence similarity to other proteins of known structure our study shows that the core structure of enzymes Ppx/Gpp family is based on a classical actin fold. The structure is divided in two domains separated by perpendicular α-helices. Examples of phosphate and nucleotide binding exist among other actin fold proteins and this may provide hints about active site location and the substrate binding environment until further information is available from crystallographic studies of complexes.

Keywords: **STRINGENT RESPONSE PPX/GPP FAMILY ACTIN FOLD**

Acta Cryst. (2002). A**58** (Supplement), C104

STRUCTURE OF THE ELECTRON TRANSFER COMPLEX BETWEEN FERREDOXIN AND FERREDOXIN-NADP+ REDUCTASE FROM MAIZE

G Kurisu [1] M Kusunoki[1] Y Onda[2] Y Kimata-Ariga[2] T Hase[2]

[1]Research Center for Structural Biology, Institute For Protein Research, Osaka University Research Center For Structural Biology 3-2 Yamada-Oka SUITA OSAKA 565-0871 JAPAN [2]Division of Enzymology, Institute for Protein Research, Osaka University

All oxygenic photosynthetically derived reducing equivalents are utilized by combinations of a single multifuctional electron carrier protein, ferredoxin (Fd), and several Fd-dependent oxidoreductases. Plant-type Fd is a small, soluble, acidic protein distributed in plants, algae and cyanobacteria. Each Fd-dependent enzyme is also distributed in same organism and functions in photosynthetic assimilation. About 20 years ago, the first structure of a plant-type Fd has been reported. After the crystal structure of Fd-NADP+ reductase (FNR) as a representative of Fd-dependent enzymes has been reported in 1991, biochemical and biophysical experiments have been carried out extensively in order to elucidate the protein-protein interactions. We report the crystal structure of the complex of maize leaf Fd and FNR from maize leaf at 2.59 Å resolution. The redox partners are in close contact at the prosthetic groups with the shortest distance being 6.0 Å. Interaction mainly occurs by electrostatic force, and the interface near the prosthetic groups is hydrophobic. Interestingly, the structures of Fd and FNR in the complex and in the free state alter in a number of ways. One such structural alteration is found at Glu 312 in the active site of FNR. We propose that this type of molecular communication not only determines optimal orientation of the two proteins, but also contributes to modulation of the enzymatic properties. The 3D structure of the photosynthetic electron transfer complex is important for further understanding of assimilatory reduction and molecular recognition mechanism closely related to the physiological conditions of higher plants.

Keywords: **ELECTRON TRANSFER FERREDOXIN COMPLEX**