Acta Cryst. (2002). A**58** (Supplement), C114

CRYSTALLOGRAPHIC STUDIES OF THE LIPOPROTEIN LOCALIZATION FACTORS LoLA AND LoLB FROM *E. COLI*

K. Takeda[1] H. Miyatake[1] N. Yokota[2] S. Matsuyama[2] H. Tokuda[2] K. Miki[1,3]
[1]RIKEN Harima Institute/SPring-8 Theoretical Structural Biology Laboratory 1-1-1, Kouto, Mikazuki-Cho, SAYO-GUN HYOGO 679-5148 JAPAN
[2]Institute of Molecular and Cellular Biosciences, University of Tokyo
[3]Department of Chemistry, Graduate School of Science, Kyoto University

Lipoproteins modified by lipids are localized on the outer or inner membrane of Gram-negative bacteria depending on the sorting signal. Lol system (LolA, LolB and LolCDE complex) mediates translocation of the water-insoluble outer membrane lipoprotein across the periplasm. A periplasmic chaperone LolA and an outer membrane lipoprotein receptor LolB are members of Lol system. We determined crystal structures of LolA and LolB from *E. coli* at 1.65 and 1.90 Å resolution, respectively. Unexpected similarity on three-dimensional structure indicates that these proteins work as pouches wrapping the hydrophobic lipids of the lipoprotein in common binding site. Significant differences between LolA and LolB also reveal structural basis of the ATP independent translocation mechanism of lipoproteins from LolA to LolB, LolA is a closed pouch with a lid made by alpha-helices covering hydrophobic cavity of the pouch. The closed form of LolA is stabilized by hydrogen bonds between β-sheet and the α-helical lid. The lid is open to bind the lipoprotein, and tends to expel the associated lipoprotein. ATP hydrolysis energy released in cytosol by the LolCDE complex is converted another energy form as unstable LolA-lipoprotein complex in periplasm. LolB is an open-lid pouch that can associate with the lipoprotein easily. The Lol system utilizes slightly but significant structural differences structures in order to transfer hydrophobic lipoproteins across the periplasm without any additional ATP energy.

Keywords: LOL SYSTEM LOCALIZATION OF LIPOPROTEIN PROTEIN TRANSPORT

Acta Cryst. (2002). A**58** (Supplement), C114

CRYSTAL STRUCTURE OF (R)-HYDRATASE AND ITS MUTANT COMPLEXED WITH CROTONYL-CoA

H. Tamao[1] T. Tsuge[2] Y. Doi[2] K. Miki[1,3]
[1]RIKEN Harima Institute/ SPring- 1-1-1 Koto, Mikaduki SAYO HYOGO 679-5148 JAPAN [2]interdisciplinary Graduate School of Science and Engineering, Tokyo Insititute of Technology [3]Graduate School of Science, Kyoto University

(R)-hydratase from *aeromonas caviae* catalyzes the conversion of trans-2-enoyl-coa to (r)-3-hydroxyacyl-coa which is a monomer unit of polyhydroxyalkanoate. The preference of the enyzme for chain-length of substrates is restricted to four to six carbons. The enzyme is active in a dimeric form with a molecular weight of 14,000 for each subunit. Mammalian homologs have been found in peroxisomes and it is of interest to know the functional and evolutionary relationships between bacterial and eukaryotic enzymes. The aim of this work is to elucidate mechanisms of catalytic reaction and substrate recognition of the enzyme based on its three-dimensional structure.
Crystals of (R)-hydratase were obtained by vapor-diffusion equilibrated against a mother liquor containing polyethylene glycol as a precipitant. Structure analysis was conducted using the multiple isomorphous replacement method. Crystals of a D31A mutant complexed with crotonyl-CoA were also prepared and solved by the molecular replacement method. The structure of the enzyme contains a five-stranded β-sheet and two α-helices, and falls into a class of α+β-type.
In a dimeric form, two β-sheets are associated in an edge-to-edge fashion to form a ten-stranded β-sheet. Two of symmetry-related active sites are located at the subunit interface and sequestered from bulk solvent by long loop segments. In the D31A-crotonyl-CoA complex, the ADP- and pantetheine-halves of the ligand were bound at the surface and inside of the enzyme, respectively. Residues for specific binding to crotonyl-CoA are almost conserved among functionally related proteins.

Keywords: **POLYHYDROXYALKANOATE BIOSYNTHESIS HOT DOG FOLD SUBSTRATE RECOGNITION**

Acta Cryst. (2002). A**58** (Supplement), C114

THE CRYSTAL STRUCTURES OF SEMI-SYNTHETIC AEQUORINS

S. Toma[1] A. Nakagawa[1] T. Tukihara[1] S. Inouye[2] O. Shimomura[3]
[1]Institute For Protein Research Osaka University Crystallography Dept. 3-2 Yamadaoka SUITA OSAKA 565-0871 JAPAN [2]Yokohama Research Centre, Chisso Corporation [3]The Photoprotein Laboratory

The photoprotein aequorin obtained from the jellyfish *Aequorea aequorea* emits blue light in the presence of calcium ions. Because of its high sensitivity and harmlessness, the photoprotein is widely used as a calcium-indicator in various biological systems. Aequorin is a complex of apoaequorin (apoprotein), coelenterazine and oxygen. Replacement of the coelenterazine moiety in aequorin with the analogues of coelenterazine yields semi-synthetic aequorins having widely different light-emitting characteristics. We determined the crystal structures of four recombinant semi-synthetic aequorins (cp-, Br-, I- and n-aequorins) at resolutions of 1.6-1.8 Å, by molecular replacement method using the structure of aequorin as a search model. In cp-aequorin, the coelenterazine moiety has a cyclopentylmethyl group at C-8 instead of the benzyl group in native aequorin. The change of phenyl group into cyclopentyl group eliminates the π-π-interaction with Trp 108, making the protein structure less stable. Consequently, the reaction rate of calcium-triggered luminescence is increased. In Br-aequorin, I-aequorin and n-aequorin, the C-2 substituent of coelenterazine moiety is p-bromobenzyl group, *p*-iodobenzyl group and beta-naphtylmethyl group, respectively. These groups replace the *p*-hydroxybenzyl group of coelenterazine moiety in native aequorin that is stabilized by a hydrogen-bonding network involving one molecule of water. In these semi-synthetic aequorins, however, the water molecule found in native aequorin is absent. Bulky C-2 substituents are located between the helixes of EF-hands III and VI. The low calcium-sensitivities of Br-, I- and n-aequorins are possibly caused by the effects on the EF-hand structures of the bulky C-2 substituent and of the missing hydrogen-bonding network.

Keywords: **PHOTOPROTEIN CALCIUM BIOIMAGING**

Acta Cryst. (2002). A**58** (Supplement), C114

CRYSTAL STRUCTURE OF THE ADP-DEPENDENT GLUCOKINASE FROM *PYROCOCCUS HORIKOSHII*

H. Tsuge[1] H. Sakuraba[2] T. Koube[2] A. Kujime[2] N. Katunuma[1] T. Ohshima[2]
[1]Institute for Health Sciences, Tokushima Bunri University Institute For Health Sciences Yamashiro-Cho TOKUSHIMA-CITY 770-8514 JAPAN
[2]Department of Biological Sciences and Technology, Faculty of Engineering, the University of Tokushima, 2-1 Minamijosanjimacho, Tokushima 770-850

ATP is the most common phosphoryl group donor for kinases, however, certain hyperthermophilic archaea such as *Pyrococcus horikoshii* and *Thermococcus litoralis* utilize unusual ADP-dependent glucokinases and phosphofructokinases in their glycolytic pathway. We report the crystal structure of the apo ADP-dependent glucokinases from *Pyrococcus horikoshii*(phGK). The crystal belong to the orthorhombic space group $P2_12_12_1$with unit cell parameters a = 64.7, b = 74.7, c = 99.2 Å. Native and one mercury data were collected at Photon Factory BL6A. The structure of phGK was determined by single isomorphous replacement with anomalous scattering (SIRAS) method and refined at 2.0 Å resolution to an R-factor (Rfree) of 21.9% (28.0%). Current model contains ordered residues 7-156, 163-170, 175-430, 440-457 and 394 water molecules. Recently, the three dimensional structure of the ADP-dependent glucokinase from *Thermococcus litoralis* (tlGK) in a complex with ADP was revealed. The overall structure of two homologous enzymes (56.7%) are basically similar: it means that they consisted of the large α/β and small domains. However, a marked adjustment of the two domains can be seen between the apo phGK and holo tlGK structures. This kind of movement is also seen in ribokinase or adenosine kinase which they have similar topology, but only limited sequence homology and different structure to the ADP-dependent glucokinases. The substrate induced large conformational change appears to be crucial for the kinase reaction in ADP-dependent glucokinases, as predicted in other kinases as a general principle governing their catalytic mechanism.

Keywords: **KINASE ADP INDUCED FIT**