s8a.m1.p9 **Crystal Structure of Phospholipase D from *Streptomyces antibioticus*.** A. Suzuki[1], K. Kakuno[1], R. Satio[1], Y. Iwasaki[2], T. Yamane[2] and T. Yamane[1]. [1]*Department of Biotechnology and Biomaterial Chemistry, Nagoya University, Japan* [2]*Department of Biological Mechanisms and Functions, Nagoya University, Japan.*
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Phospholipase D (PLD, E.C. 3.1.4.4.) hydrolyzes phospholipids to phosphatidic acid and alcohols, and also catalyzes transphosphatidylation. We have determined the crystal structure of PLD from *Streptomyces antibioticus* at 2.2A resolution using MIR methods.

Two orthorhombic forms of crystals were obtained by the hanging drop method at different temperature, 303K and 291K[1]. The high-temperature form is used for MIR method. Diffraction data from native, Hg-derivative and Pt-devivative crystals were collected on R-AXIS IV detector equipped on RU-300 rotating-anode generator. Diffraction images were processed by program *DENZO* and *SCALEPACK*. MIR phases were calculated using program *MLPHARE* and were improved by program *DM*. The molecular model was constracted by graphics program *TURBO-FRODO*, and refined by program *X-PLOR* to R=0.204 and free R=0.263 at 2.5A resolution. But five loops of about 40 residues were invisible in the density map of the high-temperature form. Therefore, futher analysis was done using low-temperature form of crystal. Starting model was prepared from high-temperature model by molecular replacement and refined against synchrotron data measured at beamline BL-41XU at SPring-8 to R=0.145 and free R=0.195 at 2.2A resolution.

PLD has two domains with same polypeptide topology. Each domain has a 8-stranded mixed β-sheet core with four α-helices on one side and one α-helix on another. The domains contacts each other on one side of the β-sheets as overall structure has quasi 2-fold symmetry. The catalytic site located at the intradomain contact region. Peptide loops with variable length stretching from both β-sheets form an catalytic site cleft. Two catalytic histidines, each of which belong to each domain, locate at the bottom of the cleft. Glycine-rich loop(G378 to G384) from C-terminal domain partially put the lid on the cleft. As the B-factor of the loop is relative large, the loop will move in the substrate binding.

[1] A. Suzuki et al. "Crystallization and preliminary X-ray diffraction studies of phospholipase D from *Streptomyces antibioticus*.", *Acta Cryst.* (1999), **D55**, 317-319.

s8a.m1.p10 **β-Glucosyltranferase: Substrate Binding and metal site.** L. Larivière[a], W. Rüger[b], P. Freemont[c], S. Moréra[a]. [a]*LEBS,UPR 9063 CNRS. Bât.34 , 91198-Gif-sur-Yvette, France* [b]*Arbeitsgruppe Molekulare Genetik, Ruhr Universität, Bochum, Germany* [c]*MSFL, ICRF, 44 Lincoln's Inn Field, London WC2A 3PX, UK.*
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β-Glucosyltransferase (BGT) is a DNA-modifying enzyme encoded by bacteriophage T4 which catalyses the transfer of glucose from uridine diphosphoglucose (UDPG) to 5-hydroxymethylcytosine (HMC) in double-stranded DNA. The glucosylation of T-4 phage DNA is part of a phage DNA protection system aimed at host nucleases. We previously reported the complete BGT co-crystal structure in the presence of UDPG[1] where the glucose is missing due to BGT cleavage. This BGT structure has provided us with a basis for detailed modelling of DNA bound to BGT. Furthermore, using the structural similarity between the catalytic core of glycogen phosphorylase and BGT, we have been able to model the position of the missing glucose moiety from UDPG.

We now report two BGT-UDP-Mg2+ structures from crystals grown in the same conditions except the concentration of magnesium ions. Crystal of BGT-UDP-Mg2+ at 20mM diffracts at 2.5Å resolution while crystal of BGT-UDP-Mg2+ at 40mM diffracts at 2Å resolution. Both crystals belong to P212121 space group but cell parameters are different. Both structures contain one magnesium ion in the UDPG binding site. The presence of a second Mg2+ ion far from the active site in the structure with 40mM Mg2+ could explain the difference of crystal packing between these two structures. Here, we present the metal site of BGT and from these two models, we propose a role for Glu163 in the catalytic mechanism for BGT.

[1] Moréra, S., Imberty, A., Aschke-Sonnenborn, U., Rüger, W. and Freemont, P. (1999). "T4 phage β-glucosyltransferase: substrate binding and proposed catalytic mechanism." *J. Mol. Biol.* **292**, 717-730.