Phospholipase D (PLD, E.C. 3.1.4.4.) hydrolyzes phospholipids to phosphatic acid and alcohols, and also catalyzes transphosphatidylation. We have determined the crystal structure of PLD from *Streptomyces antibioticus* at 2.2Å 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-derivative 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 2.5Å resolution. But five loops of about 40 residues were invisible in the density map of the high-temperature form. Therefore, further 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 2.2Å 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.

---

**Keywords:** phospholipase D, *S. antibioticus*, intramolecular 2-fold symmetry.

---
