X-ray Data Collection from Heavy-atom derivative crystals of *Streptomyces antibioticus* Phosholipase D

Atsuo. Suzuki, Masatake. Akita, Michiaki. Tanaka, *Takashi. Yamane

Department of Biotechnology and Biomaterial Chemistry, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603

Phospholipase D (PLD, E.C. 3.1.4.4.) hydrolyzes phospholipids to phosphatidic acid and alcohols. For elucidation of the structure-function relationship, we have been proceeding X-ray crystal analysis of a PLD from a bacterium, *Streptomyces antibioticus* . The analysis is in progress by the isomorphous replacement method because no three-dimensional structure of homologous proteins is not available. Here we report the x-ray diffraction study of the PLD crystals of two heavy-atom derivatives, one is a Hg-derivative, another is a Xe-derivative.

Crystals of *S. antibioticus* PLD were grown by the hanging drop method at 303 K by mixing 5μl of 1% enzyme solution with 5μl of a reservoir solution containing 20% (w/v) polyethylene glycol 6000 and 0.1M Tris hydroxymethyl aminomethane, pH = 7-8. The Hg-derivative crystal was provided by soaking a PLD crystal in 1mM Hg(CH3COO)2 solution for 3 days. The Xe-derivarive was prepared by pressuring the crystal in quartz capillary by Xe gas at 5 kg cm-2.

X-ray diffraction data from two derivative crystals were measured by the oscillation method using a Rigaku imaging-plate detector R-AXIS IV equipped on beamline BL41XU at the SPring-8. The wavelength of the X-ray was set to an absorption edge of the heavy-atoms containing in each derivative, 1.00 Å and 0.358 Å for the Hg-derivative and the Xe-derivative respectively. In data collection, the crystal of the Hg-derivative was frozen at 100 K, whereas data collection from Xe-derivative was done at room temperature.

Crystals diffracted to 1.8 Å resolution for the Hg-derivative and to 2.5 Å for the Xe-derivative. The diffraction spots of Xe-derivative split into multiple parts because the crystal cracked in data collection probably due to radiation damage. Therefore, the processing of diffraction data was possible only for the Hg-derivative. Data processing and ¬duction were done with DENZO and SCALEPACK programs. A summary of the data collection from Hg-derivative is given in Table 1. Searching of Hg-site and calculation of phases are now under way.

Table 1 Summary of data collection from Hg-derivative

Frame number	33
Oscillation range (°)	3.0
Exposure Time (s)	120
Resolution(Å)	1.8
Total reflections	138,384
Independent reflections	38,320
Rmerge (%) ($\Sigma |I - <I>| / \Sigma <I>$)	7.6
Completeness(%)	75.1