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X-ray structure of lysoplasmalogen-specific phospholipase D from *Thermocrispum* sp. RD004668

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1 Introduction

Lysoplasmalogen-specific phospholipase D (LyPls-PLD) from *Thermocrispum* sp. RD004668 is an enzyme that hydrolyzes choline lysoplasmalogen into choline and 1-(1-alkenyl)-sn-glycero-3-phosphate. Previous mutational studies revealed that the F211L altered its substrate specificity from lysoplasmalogen to 1-O-Hexadecyl-2-hydroxy-sn-glycero-3-phosphocholine (lysoPAF) [1]. Enzymes specific for lysoPAF are rare and could thus be used for clinical applications. In this study, to understand the mechanism of substrate binding and selectivity, we have determined the crystal structure of LyPls-PLD for the first time in substrate-free form [2].

2 Experiment

The tagless recombinant LyPls-PLD used in this study was provided from Asahi Kasei Pharma Corp. The enzyme solution was prepared at 10 mg/mL using a buffer containing 5 mM Tris-HCl pH 8.0 and 25 mM NaCl. The LyPls-PLD was crystallized with sitting-drop vapor diffusion method at 20 °C. Bipyramid-shaped crystals were obtained using the reservoir solution containing 0.1 M Ches pH 9.5, 50 mM NaCl and 12% PEG 3350. The crystals were transferred into a cryoprotectant solution containing 0.1 M Ches pH 9.5, 200 mM NaCl, 18% PEG 3350 and 20% glycerol, and then flash-cooled in the liquid nitrogen at 100 K. The X-ray diffraction data were collected at PF BL-1A, and the data were processed using the program XDS and AIMLESS from the CCP4 suite.

3 Results and Discussion

The structure of LyPls-PLD was determined by the molecular replacement method using the His-tagged LyPls-PLD structure (PDB ID, 7YMP) as a search model [2]. The model refinement was performed up to a resolution of 2.9 Å, with R-factor and R-free factor of 17.8% and 21.5%, respectively (Table 1). The Asymmetric unit contains two LyPls-PLD monomers, which are extensively associated with each other by 2-fold symmmetry. The strong electron density peak was detected at the active site pocket, which was assigned as Ca^{2+} ion in the course of model refinement. Overall structure of the LyPls-PLD represents a typical TIM barrel fold, and is structurally the most homologous to the glycerophosphodiester phosphodiesterase (GDPD) from *Thermoanaerobacter tengcongensis* (Fig. 1). Based on the structures obtained in this study, we expect to further elucidate the substrate specificity swiching mechanism by using molecular dymanics and docking simulatios.

Table 1: Data collection and refinement statistics

	LyPls-PLD with Ca^{2+}
Space group	$P4_32_12$
Unit cell (Å, deg)	a = b = 142.95, c = 126.13
Resolution (Å)	2.91 (3.09-2.91)
R_{meas}	0.111 (1.160)
I/σ	16.1 (2.6)
Completeness (%)	100.0 (100.0)
Multiplicity	13.4 (14.3)
R_{work}/R_{free}	0.178/0.215

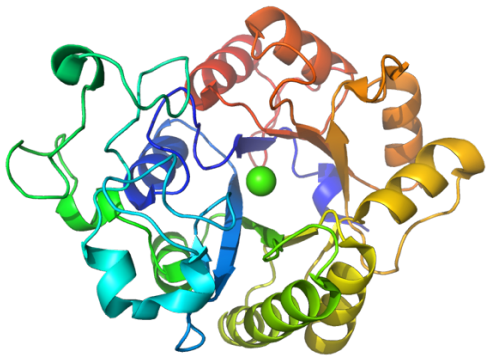

Fig. 1: Overall structure of LyPls-PLD. The bound Ca^{2+} is depicted as a ball model colored in green.

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References

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