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Crystal structure of the CDP-Diacylglycerol Synthetase

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1 Introduction

Glycerophospholipids are the main component of biological membranes. With different kinds of hydrophilic head groups attached to the glycerol-3-phosphate backbone, glycerophospholipids derivatize into phosphatidic acid (PA), phosphatidylglycerol (PG), cardiolipin (CL), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylinositol (PI).

Cytidine-diphosphate diacylglycerol (CDP-DAG) is a central intermediate liponucleotide product in glycerophospholipid biosynthesis pathways. It serves as the precursor for the synthesis of PI, PG, PS molecules. CDP-DAG synthetase (Cds) is an integral membrane enzyme catalyzing the formation of CDP-DAG from PA and CTP, while pyrophosphate is released as a byproduct [1]. Cds is essential for yeast cell growth [2] and plant photoautotrophic growth [3]. As an enzyme required for the regeneration of PIP2 (an important secondary messenger for signal transduction), Cds is a key regulator for phototransduction [4] and vascular morphogenesis [5].

To obtain an in-depth understanding on the fundamental mechanism of Cds, We have solved and characterized the first crystal structure of the Cds from *Thermotoga maritima* (TmCdsA) at 3.4 Å resolution [6].

2 Experiment

The TmCdsA protein was overexpressed in *E.coli* and purified by nickel affinity and size exclusion chromatography steps. Hanging-drop vapor diffusion method was used to crystallize TmCdsA. The X-ray diffraction data for phasing and structure refinement were collected on the BL-5A/BL-17A/BL-1A/NW12A beamlines at the Photon Factory (Tsukuba, Japan). The data were processed and scaled with HKL2000 or iMOSFLM. The crystal of TmCdsA belongs to $P6_522$ space group with unit cell dimensions $a = b$ =142.08 Å and c =198.37 Å. The structure was solved via single-wavelength anomalous dispersion (SAD) method with PHENIX program.

3 Results and Discussion

Overall sturcture

TmCdsA is a homodimer (Fig. 1A) and each monomer contains nine transmembrane helices named M1-M9. The C-terminus is at the periplasmic side and its N-terminus is facing the cytoplasmic side. Each monomer has three domains (Fig. 1B), namely the N-terminal domain (NTD), the middle domain (MD) and the C-terminal domain (CTD). The MD is located at the dimerization interface and flanked by the CTD and NTD at the peripheral region facing lipid bilayer. Sequence alignment results indicate that CTD is the most conserved domain while MD is the least conserved among all three domains.

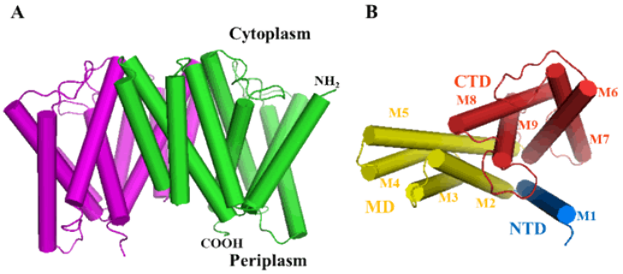

Fig. 1: Overall structure of TmCdsA. A. a TmCdsA homodimer viewed along membrane plane. B. a TmCdsA monomer viewed along membrane normal from cytoplasmic side.

Metal ions and the active site

Mg^{2+} and K^+ ions are both essential for TmCdsA to reach its maximal activity. By using Ba^{2+}/Mn^{2+} or Tl^+/Cs^+/Rb^+ ions as the heavy ionic surrogates for Mg^{2+} or K^+ ions, their binding sites were detected near the central region of CTD within each TmCdsA monomer (Fig. 2A). Mg^{2+} ion is coordinated by the carboxyl groups of Asp219 and Asp249. A coordination bond is also found between K^+ and Asp219. The distance between Mg^{2+} ion and K^+ ion is 5.9 Å. The pivotal roles of the charged residues for enzyme activity were further verified by mutagenesis studies. The activities of D219A and D249A mutants are almost completely lost. Some other charged residues around the Mg^{2+} and K^+ binding sites (e.g. Asp144, Lys167, Glu222, Lys226 and Asp246) are also crucial for the enzyme activity. Mutations of these residues to alanine lead to severe decrease of enzyme activity. The acidic residues form a patch of electro-negative surface necessary for the binding of metal ions and substrates molecules. Lys167 and Lys226 form ionic interactions with Asp219 and Asp246, respectively. These interactions may help to neutralize the negative charges on the surface of the active site and place the side chains of these two key Asp residues properly for catalysis.

Substrate-binding cavity with dual gateways

A potential substrate-binding cavity is located between the N-terminal and C-terminal domains (Fig. 2B). The Mg^{2+}-K^+ hetero-di-metal lies at the bottom of the cavity. The funnel-shaped cavity has two gateways that are open simultaneously to the cytoplasm and membrane respectively. Hence, the cavity can receive hydrophobic

substrate PA from membrane through the lateral opening and hydrophilic substrate CTP from cytosol through the cytoplasmic opening. After reaction, product CDP-DAG will be released back into the membrane through the lateral opening, and the by-product PPi returns into the cytosol using the cytoplasmic gateway (Fig. 2B).

In summary, our results not only reports for the first time the novel structure of a key membrane protein fundamental to phospholipid biosynthesis, but also suggests a two-metal ion catalytic mechanism for the synthesis of CDP-DAG within an amphiphilic cavity bearing dual gateways.

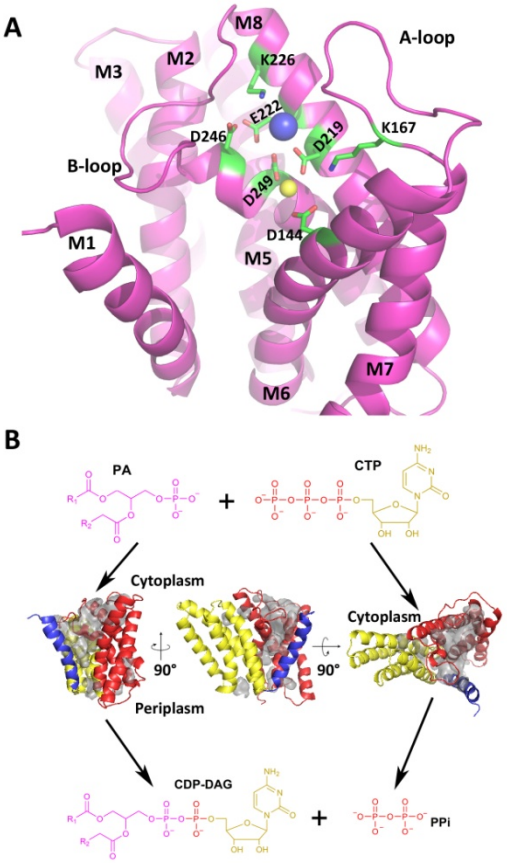

Fig. 2: The detailed structure of the active site (A) of TmCdsA and its substrate-binding cavity (B). Yellow sphere, Mg^{2+} ion; blue sphere, K^{+} ion; gray mask, substrate-binding cavity.

Acknowledgement

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