Crystal Structure of Malonyl CoA-Acyl Carrier Protein Transacylase from \textit{Xanthomanous oryzae} pv. \textit{oryzae} and Its Proposed Binding with ACP

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\textit{Xanthomanous oryzae} pv. \textit{oryzae} (Xoo) is a plant bacterial pathogen that causes bacterial blight (BB) disease, resulting in serious production losses of rice. The crystal structure of malonyl CoA-acyl carrier protein transacylase (XoMCAT), encoded by the gene \textit{fabD} (Xoo0880) from Xoo, was determined at 2.3 Å resolution in complex with N-cyclohexyl-2-aminoethansulfonic acid. Malonyl CoA-acyl carrier protein transacylase transfers malonyl group from malonyl CoA to acyl carrier protein (ACP). The transacylation step is essential in fatty acid synthesis. Based on the rationale, XoMCAT has been considered as a target for antibacterial agents against BB. Protein-protein interaction between XoMCAT and ACP was also extensively investigated using computational docking, and the proposed model revealed that ACP bound to the cleft between two XoMCAT subdomains.

INTRODUCTION

Rice is one of the most common food staples throughout the world. Bacterial blight (BB) is a serious and destructive disease causing massive production losses of rice. BB is caused by \textit{Xanthomonas oryzae} pv. \textit{oryzae} (Ezuaka A, 2000), which is prevalent in tropical countries, particularly in Asia. However, no effective drugs against BB have been identified until now. Lee and coworkers reported the whole genomic sequence of Xoo (Lee et al., 2005), which has provided useful information for the selection of essential drug target enzymes from its 4538 putative genes. Xoo0880 (XoMCAT) is one of the target genes, which is related to the fatty acid synthesis (FAS) in bacteria (Payne et al., 2004; 2007; Yoon et al., 2011).

Biosynthesis of fatty acids is essential for all living organisms (Magnuson et al., 1993; White et al., 2005). The type I FAS system found in animals consists of a single polypeptide chain made up of eight distinct domains that is involved in all catalytic reactions. In contrast, bacteria have the type II FAS system, which involves a series of individual enzymes. Xoo0880 expresses malonyl CoA-acyl carrier protein transacylase (MCAT, EC2.3.1.39), which is responsible for transferring a malonyl group to acyl carrier protein (ACP). Resulting malonyl-ACP intermediate participates in type II FAS (Ruch and Vagelos, 1973). Thus MCAT takes part in FAS by extending the length of the growing chain by two carbon atoms via providing malonyl-ACP as a two carbon donor in the elongation step of FAS. MCAT also provides acyl-ACP thioesters, which are related with aromatic polyketide biosynthesis and secondary metabolites such as tetracyclines and erythromycins (Keating-Clay et al., 2003; Summers et al., 1995). MCAT has been considered as a target for antibacterial agents (Campbell and Cronan, 2001; Miesel et al., 2003), and our group selected XoMCAT as a potential drug target against BB. In this study, we determined the crystal structure of XoMCAT in complex with N-cyclohexyl-2-aminoethansulfonic acid (NHE) at the active site. To understand its interaction with ACP, protein-protein (P-P) docking of XoMCAT and ACP was carried out. The determined XoMCAT structure could be useful in developing antibacterial agents against BB.

MATERIALS AND METHODS

Cloning, expression and purification of XoMCAT

The strain of Xoo KACC10331 was obtained from the Rural Development of Administration (RDA), South Korea. The \textit{E. coli} host strain BL21 (DE3) was purchased from Novagen. Cloning, expression, and purification of XoMCAT were performed as described in the previous report (Jung et al., 2008). The purity of the enzyme was examined by SDS-PAGE analysis, and the protein concentration (7 mg ml\textsuperscript{-1}) was determined by Bradford (1976).

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Crystallization and data collection
Well-diffracted XoMCAT crystal was grown at 283K under the condition of 0.1 M CHES pH 9.0, 1.5 M (NH4)2SO4, and 0.2 M NaCl by the hanging drop vapor diffusion method in a week. Before collecting data, a crystal was frozen in liquid nitrogen with 25% (v/v) glycerol as a cryoprotectant. The entire data were collected at 2.3Å resolution using an ADSC quantum 270 CCD detector of the beam-line 17A at the photon factory (KEK), Japan. The data set was integrated and scaled using the DENZO and SCALEPACK programs (Otwinowski and Minor, 1997), respectively. All relevant crystallographic parameters and refinement details are summarized in Table 1.

Structure determination and refinement
Structure determination of XoMCAT was carried out by molecular replacement (MR) using the MOLREP (Vagin and Teplyakov, 2010) program using malonyl CoA-acyl carrier protein transacylase (MCAT; pdb id: 1mla) from E. coli (Serre et al., 1995) as a template model, which shares 52% sequence identity with XoMCAT. The MR solution structure showed a well fitted electron-density map (2Fo-Fc) for all of the residues, and structural modeling was carried out using the COOT (Emsley and Cowtan, 2004) graphics program. Complete structural refinement of XoMCAT was performed using the Refmac 5.1 (Murshudov et al., 1997) program from the CCP4 suite. Visualization and cartoon diagrams were drawn using PYMOL graphics program (Schrodinger, 2010). The pictorial representation of hydrogen bonding and non-bonded interactions for the complexes were derived using the program LIGPLOT (Wallace et al., 1995).

Protein-protein docking
The ligand protein ScACP (pdb id: 2a8b) from Streptomyces coelicolor (Schrodinger, 2010) was used for the protein-protein (P-P) docking study. The complex model of XoMCAT/ScACP was constructed using the program ICM MolSoft. The grid potential maps generated from the XoMCAT molecule were defined in a box around the hypothetical binding site, covering approximately half of the XoMCAT surface. The rigid-body docking was performed by sampling different positions and orientations of ScACP molecule with respect to XoMCAT molecule using a Pseudo-Brownian Monte Carlo procedure (Abagyan et al., 1994) implemented in the MolSoft ICM 3.6 program. After complete sampling over the XoMCAT surface was performed, total 23,779 conformations were generated and ranked based on their pairwise shape complementarity (PSC) score. All of the steps were performed using default parameters, which are given in the online manual (http://www.molsoft.com/gui/prot-prot.html). The top predictions from each cluster were then manually inspected and investigated based on several criteria, such as score, charge complementarity, hydrophobic interactions, and overall agreement with prior biological information.

RESULTS AND DISCUSSION
Overall structure of XoMCAT
The crystal structure of XoMCAT (Fig. 1A) was determined by MR, and the refined structure coordinate and structure factor was deposited in the Protein Data Bank (pdb id: 3R97). The final model structure was validated using the PROCHECK program (Laskowski et al., 1993), which showed that 91.7% of the residues were located in the allowed region. XoMCAT structure was composed of 14 α-helices and 11 β-strands, and the total accessible surface area was 12,585 Å². The overall structure of XoMCAT was folded into two subdomains. The large subdomain consisted of two non-contiguous segments formed from residues 1-129 and from 200-314. The remaining residues were located in the small subdomain. The large subdomain consisted of a short four-stranded parallel β-sheet surrounded by different lengths of 12 α-helices, a long loop with small anti-parallel β-strands at the edge, and another β9-strand located between the helices α11 and α12. The smaller subdomain contained four-stranded anti-parallel β-sheet capped by two α-helices.

Thus far, several MCAT structures from various species have been characterized, such as EcMCAT from Escherichia coli, pdb id: 1MLA (Serre et al., 1995), ScMCAT from Streptomyces, pdb id: 1n02 (Keating-Clay et al., 2003), MmMCAT from Mycobacterium tuberculosis, pdb id:2qc3 (Li et al., 2007), HpMCAT from Helicobacter pylori, pdb id: 2h1y (Zhang et al., 2007) and HmMCAT from human mitochondria, pdb id: 2c2n (unpublished). All MCATs including XoMCAT share the similar fold (Fig. 1B), and structural comparison of XoMCAT and other MCATs of EcMCAT, ScMCAT, MmMCAT, HpMCAT and HmMCAT showed the root mean square deviations (r.m.s.d) of 0.74, 1.52, 1.54, 1.37 and 1.35 Å, respectively. Although overall folds of the MCATs are similar, substrate binding pockets surrounding the active site are different. For examples, in HpMCAT,