Crystal Structure and Molecular Mechanism of Phosphotransbutyrylase from *Clostridium acetobutylicum*

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**Introduction**

The acetone-butanol-ethanol (ABE) fermentation by the anaerobic bacterium *Clostridium acetobutylicum* has been considered a promising process of industrial biofuel production. Phosphotransbutyrylase (phosphate butyryltransferase, PTB) plays a crucial role in butyrate metabolism by catalyzing the reversible conversion of butyryl-CoA into butyryl phosphate. Here, we report the crystal structure of PTB from the *Clostridial* host for ABE fermentation, *C. acetobutylicum* (CaPTB) at a 2.9 Å resolution. The overall structure of the CaPTB monomer is quite similar to those of other acyltransferases, with some regional structural differences. The monomeric structure of CaPTB consists of two distinct domains, the N- and C-terminal domains. The active site cleft was formed at the interface between the two domains. Interestingly, the crystal structure of CaPTB contained eight molecules per asymmetric unit, forming an octamer, and the size-exclusion chromatography experiment also suggested that the enzyme exists as an octamer in solution. The structural analysis of CaPTB identifies the substrate binding mode of the enzyme and comparisons with other acyltransferase structures lead us to speculate that the enzyme undergoes a conformational change upon binding of its substrate.

**Keywords:** *Clostridium acetobutylicum*, phosphotransbutyrylase, butyryl-CoA, butyrate metabolism

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monocytogenes, Enterococcus faecalis, Escherichia coli, Streptococcus pyogenes [17], Staphylococcus aureus, Bacillus subtilis [18], and Methanosarcina thermophila [19, 20]. However, the structures of PTB and BUK from C.
acetobutylicum, which is a well-known industrial host strain and biological producer of industrially important solvents, has not yet been reported. Therefore, we report the crystal structure of PTB from \textit{C. acetobutylicum} (CaPTB) to understand its molecular mechanism. Moreover, we reveal that the structure of CaPTB has a unique oligomeric status. The substrate binding mode of CaPTB and the conformational change upon binding of the substrate are also proposed.

**Materials and Methods**

**Cloning, Expression, and Purification**

The gene coding for phosphotransbutyrylase from \textit{Clostridium acetobutylicum} (CaPTB) was amplified using primers: forward, 5'- TATACATATGATTAAGAGTTTTAATGAAAT-3' and reverse, 5'- GGTGCTCGAGTTATTTATTGCCGCAACTA-3'. The amplified DNA fragment was cloned into pET28a vector. The CaPTB protein was expressed in the \textit{E. coli} BL21(DE3) strain. The cells were cultured in an LB medium with kanamycin at 37°C. At OD\textsubscript{600} = 0.7 at 600 nm, the cells were induced by adding 0.1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) and further cultured for 20 h at 18°C. The harvested cell pellet was resuspended in buffer A (40 mM Tris-HCl, pH 8.0, 5 mM β-mercaptoethanol) and the cell lysis was accompanied by ultrasonication. The cell lysate was centrifuged at 12,000 × g for 20 min and the cell debris was removed. The protein was purified using Ni-NTA agarose. To remove trace amounts of contaminants, size exclusion chromatography (Superdex200, GE Healthcare, USA) was applied. The purified protein was concentrated to 40 mg/ml for crystallization.

**Crystallization, Data Collection, and Structure Determination**

Initial crystallization screening was performed using commercial crystal screening kits such as Index, PEG/Ion, Crystal Screen (Hampton Research, USA), and Wizard I & II (Rigaku, Japan) and by employing the hanging-drop vapor-diffusion method at 20°C. Each drop was prepared by mixing 1.0 μl protein solution with 1.0 μl reservoir solution and then equilibrating against 50 μl of the reservoir solution. The CaPTB crystals were obtained from crystallization condition of 20% PEG3350, 0.2 M lithium sulfate, and 0.1 M HEPES (pH 7.5). For cryo-protection, 30% glycerol was added to the crystallization solution. The data were collected at 100 K at the 7A beamline of the Pohang Accelerator Laboratory (Korea), and the collected data were processed using the HKL2000 suite [21]. The CaPTB crystal belonged to monoclinic space group \textit{P}2\textsubscript{1}, with unit cell parameters of \(a=94.7\), \(b=143.4\), \(c=113.3\) Å, \(β=94°\). Assuming one molecule of CaPTB per asymmetric unit, the crystal volume per unit of protein mass was approximately 2.97 Å\textsuperscript{3} Da\textsuperscript{-1}, which corresponds to a solvent content of approximately 58.7% [22].

The crystal structure of CaPTB was solved by molecular replacement using MOLREP [23]. The structure of CaPTB from \textit{C. acetobutylicum} (CaPTB) to understand its molecular mechanism. Moreover, we reveal that the structure of CaPTB has a unique oligomeric status. The substrate binding mode of CaPTB and the conformational change upon binding of the substrate are also proposed.

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**Table 1. Data collection, phasing and refinement statistics.**

| CaPTB |
| --- |
| **PDB code** |
| **Data collection** |
| Space group | \textit{P}2\textsubscript{1} |
| Cell dimensions | \(a=94.7\), \(b=143.4\), \(c=113.3\) Å |
| \(α=90.0\), \(β=94.0\), \(γ=90.0\) |
| Resolution (Å) | 50.00-2.90 (2.95-2.90) |
| \(R_{\text{eq}} \) or \(R_{\text{merge}}\) | 33.5 (11.5) |
| \(I/σ(I)\) | 18.6 (4.4) |
| Completeness (%) | 95.9 (90.7) |
| Redundancy | 3.0 (2.3) |
| **Refinement** |
| Resolution (Å) | 50.00-2.90 |
| No. of reflections | 59813 |
| \(R_{\text{eq}} \) / \(R_{\text{free}}\) | 17.2 / 23.8 |
| No. of atoms | 18443 |
| Protein | 18035 |
| Sulfur ion | 240 |
| Water | 158 |
| \(B\)-factors | 37.9 |
| Protein | 40.6 |
| Sulfur ion | 77.1 |
| Water | 28.3 |
| R.m.s. deviations |
| Bond lengths (Å) | 0.013 |
| Bond angles (°) | 1.664 |
| Ramachandran plot |
| Most favored (%) | 98.2 |
| Additional allowed (%) | 1.8 |

*Values in parentheses are for highest-resolution shell.*
phosphotransacylase from *E. faecalis* (PDB code 1YCO, 42% sequence identity) was used as a search model. The final model building was performed using the program WinCoot [24] and the refinement was performed with REFMAC5 [25]. The geometric parameters of the final model were validated by using PROCHECK [26] and MolProbity [27]. The data statistics are summarized in Table 1. The refined model of CaPTB was deposited in the Protein Data Bank (PDB code 7VG9).

**Size-Exclusion Chromatography**

Analytical size-exclusion chromatography was performed using the Superdex 200 Increase 10/300 GL column (GE Healthcare Life Sciences). The column was equilibrated with a buffer containing 40 mM Tris–HCl, pH 8.0, and 150 mM NaCl. A protein sample of 0.5 ml with 1 mg/ml concentration was used for analysis. Standard samples, such as ferritin (440 kDa), conalbumin (75 kDa), carbonic anhydrate (29 kDa), and ribonuclease A (13.7 kDa), were used for calculation of the molecular weight.

**Results and Discussion**

**Overall Structure of CaPTB**

To understand the molecular mechanism of the butyrate biosynthesis of *C. acetobutylicum*, we determined the crystal structure of phosphotransbutyrylase from *C. acetobutylicum* (CaPTB, phosphate butyryltransferase) at a 2.9 Å resolution (Figs. 1B, 1C). It is assumed that the low resolution of the CaPTB structure is caused by the high solvent content percentage and the unique oligomeric state. The asymmetric unit contains eight CaPTB molecules forming an octamer (Fig. 1C). The CaPTB monomer consists of two α/β domains, which can be divided into the N-terminal domain (ND, residue Met1–Arg117 and Ser272–Lys301) and the C-terminal domain (CD, residue Thr118–Thr271) (Figs. 1B, 1D). These domains show a side-by-side arrangement and form a continuous β-sheet-like shape surrounded by α-helices. An active site cleft was formed between two domains that is known to show an open-close conformation change during substrate-binding [28]. The N-terminal domain consists of a four-stranded parallel β-sheet (β1–β4) with six α-helices (α1–α5 and α11) and one 3_10-helix (η1). The C-terminal domain is composed of a four-stranded parallel β-sheet (β7–β10) with one β-hairpin (β5–β6) and five helices (α6–α10).

The CaPTB dimer is formed by hydrogen bonds and salt bridge of the loop of β13–β14 and helix α8 and α10 between C-terminal domains in subunits A–B, including Lys132–Asp261 and Asp217–Lys254 (Fig. 2A).

![Fig. 2. Oligomeric status of CaPTB. (A) Interfaces for octamer formation of CaPTB. The residues for hydrogen bonding are presented with a stick model. The hydrogen bonds are shown as red-colored dotted lines. (B) Size-exclusion chromatography analysis of CaPTB. 1, 2, 3 and 4 indicate standard samples of ferritin (440 kDa), aldolase (158 kDa), ovalbumin (44 kDa), and ribonuclease A (13.7 kDa), respectively. (C) SDS-PAGE of purified CaPTB. The CaPTB monomer with a molecular weight of approximately 32 kDa is indicated.](image-url)
Furthermore, the dimer from the subunits A–B, C–D, E–F and G–H interact through helix-to-helix hydrophobic interactions using the hydrophobic residues including Val251, Met252 and Leu256. PISA software calculated the surface area at 6029 Å² [29].

The asymmetric unit of our CaPTB crystal contained an octameric structure (Figs. 1B, 2A). When the crystal structure was solved, we considered that the asymmetric unit of the CaPTB crystal contained four dimers, because the PTB enzymes are known to function as a dimer. However, previous studies suggested that CaPTB can form an octamer [30, 31], and the size-exclusion chromatography experiment confirmed that CaPTB exists as an octamer in solution (Figs. 2B, 2C). The octamer structure of the CaPTB consists of four dimers that are connected in a caterpillar track-like arrangement. The contacts for octamerization are mediated by hydrogen bonds and salt bridges between residues including Glu181–Arg202, Lys198–Asp211 and Asp211–Arg202. The hydrophobic interactions also heavily contribute to the octamerization using residues including Leu191, Ala194 and Met195, and these residues are located mainly in α7 of the C-terminal domains in each subunit (Fig. 2A). The octamer interface between each subunit (A–H, B–C, D–E, and F–G) buries a solvent-accessible area of 679, 721, 708, and 730 Å² as calculated by PISA [29]. The average solvation free energy gain (∆G) for the octamer formation of the interface was -7.9 kcal/mol with an average p-value of 0.165. This interface received a complex formation significance score (CSS) of 0.087. The results indicate that the interface plays an essential role in complex formation and the dimeric interface with specific interactions is not an artefact of crystal packing.

**Active Site of CaPTB**

To understand the reaction mechanism and substrate binding mode of CaPTB, we first attempted to determine the complex structure with its butyryl-CoA substrate. However, neither co-crystallization nor soaking with the substrate were successful. Instead, we observed four sulfate ions, which were added during the crystallization procedure, at the active site cleft formed between the two domains (Fig. 3A). The sulfate ions were stabilized by hydrogen bonds with the residues Lys254, Ser279, Ser283, Thr286, and Lys228. When we compared the structure of CaPTB with that of PTB from *L. monocytogenes* (*Lm*PTB, PDB code 3U9E, 39% amino acid identity, 1.8 Å...
Comparison of Similar Acyltransferases Proteins

We then attempted to compare the CaPTB structure with its structural homologues among phosphate acetyl/butyryltransferases, and the Dali server search showed that CaPTB was highly structurally similar to members of acyltransferases such as phosphotransbutyrylase from L. monocytogenes (LmPTB, PDB code 3U9E) and E. faecalis (EfPTB, PDB code 1YCO) and phosphotransacetylase (PTA, phosphate acetyltransferase) from M. thermophila (MtPTA, PDB code 2AF3) [19, 20] and P. gingivalis (PgPTA, PDB code 6IOX) [32] with RMSD for related elements ranging from 1.8 to 4.3 Å and 19-39% amino acid identity (Fig. 1B).

The superposition of the CaPTB structure with those of structurally similar enzymes revealed that the C-terminal domain of CaPTB was well superposed with other enzymes. However, α5 and α11 of the N-terminal domain of CaPTB was rotated by about 20 to 30 degrees from the other enzymes. Because the structure of CaPTB is an apo form, whereas other compared structures are complexed form with the substrate, the structural difference at the α5 and α11 might indicate that PTB undergoes an open-close conformational change upon the

Fig. 4. Structural comparison of CaPTB with other PTBs and PTAs. (A) Superimposition of CaPTB with other structural homologues. CaPTB, EfPTB, LmPTB, MtPTA, and PgPTA structures are presented with a ribbon diagram and distinguished with different colors. The regions with structural differences are highlighted with a cartoon diagram. (B) Surface conservation mapping of CaPTB. The CaPTB structure was shown with a surface conservation model. Highly conserved residues are shown as a stick model and labeled.
substrate binding (Fig. 4A). Moreover, we observed a local structural difference at the lid region, where CaPTB has an additional 20 residues compared with other enzymes. These additional residues form an helix-turn-helix structure. These helices of PTA show more of an open conformation compared with the corresponding helices of PTB, which seems to be caused by a structural change due to substrate specificity along with the lid region. A mapping of the conserved residues on the surface of CaPTB was performed with multisquence alignment of PTBs and PTAs using ClustalW [33] and ConSurf [34]. The CaPTB residues were located at the residues Lys186, Asp217, Lys228, Arg280, Ser283, and Glu285 (Fig. 4B). While the residues Asp217 and Lys254 contributed to the dimerization, other residues were involved in the enzyme catalysis and the substrate binding. The Lys254 residue was involved in both dimerization and substrate binding. Interestingly, residues involved in the octamer formation, such as Asp181, Arg202, and Asp211, were not conserved, which might be due to the fact that CaPTB possesses it own unique octameric oligomeric status.

In summary, we report the crystal structure of CaPTB, a crucial enzyme involved in butyrate biosynthesis. Unlike other PTBs, CaPTB forms an octamer, which is mediated by the tetramerization of four dimers. By comparing it with other structural homologues, we elucidated that CaPTB stabilizes its substrate conserved residues and undergoes a conformational change upon substrate binding. Finally, the conservation mapping analysis of PTB shows that, although residues involved in the enzyme catalysis and substrate binding are highly conserved among PTB enzymes, CaPTB has quite unique residues for octamerization, which is consistent with the fact that CaPTB forms a unique octameric oligomer.

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Conflict of Interest
The authors have no financial conflicts of interest to declare.

References
1. Jones DT, Woods DR. 1986. Acetone–butanol fermentation revisited. Microbiol. Rev. 50: 484-524.
2. Matta el-Ammouri G, Janati-Idrissi R, Jundles AM, Petideman D, Junelles AM, Jundles AM, Petideman D. 2018. Effects of butyric and acetic acids on acetone–butanol formation by Clostridium acetobutylicum. Biochimie 69: 109-115.
3. Amador-Noguez D, Brag IA, Feng X-J, Roquen N, Rabiniwitz JD. 2011. Metabolome remodeling during the aciogenic-solventogenic transition in Clostridium acetobutylicum. Appl. Environ. Microbiol. 77: 7984-7997.
4. Spekman HB. 1920. Gas production during the acetone and butyl alcohol fermentation of starch. J. Biol. Chem. 43: 401-411.
5. Millat T, Janssen H, Thorn JG, King JR, Bahl H, Fischer R J. et al. 2013. A shift in the dominant phenotype governs the pH-induced metabolic switch of Clostridium acetobutylicum phosphate-limited continuous cultures. Appl. Microbiol. Biotechnol. 97: 6451-6466.
6. Millat T, Janssen H, Bahl H, Fischer RJ, Wolkenhauer O. 2013. Integrative modelling of pH-dependent enzyme activity and transcriptomic regulation of the acetone–butanol–ethanol fermentation of Clostridium acetobutylicum in continuous culture. Microb. Biotechnol. 6: 526-539.
7. Jones SW, Paredes CJ, Tracy B, Cheng N, Sillers R, Senger RS, et al. 2008. The transcriptional program underlying the physiology of clostridial sporulation. Genome Biol. 9: R114.
8. Janssen H, Döring C, Ehrenreich A, Voigt B, Hecker M, Bahl H, et al. 2010. A proteomic and transcriptional view of aciogenic and solventogenic steady-state cells of Clostridium acetobutylicum in a chemostat culture. Appl. Microbiol. Biotechnol. 87: 2209-2226.
9. Grimmler C, Janssen H, Kraufj D, Fischer R J, Bahl H, Durée E, et al. 2011. Genome-wide gene expression analysis of the switch between aciogenic and solventogenic in continuous cultures of Clostridium acetobutylicum. J. Mol. Microbiol. Biotechnol. 20: 1-15.
10. Cary JW, Petersen DJ, Papoutsakis ET, Bennett GN. 1988. Cloning and expression of Clostridium acetobutylicum phosphotransbutyrylase and butyrate kinase genes in Escherichia coli. J. Bacteriol. 170: 4613-4618.
11. Papoutsakis E, Bennett G. 1997. Molecular regulation and metabolic engineering of solvent production by Clostridium acetobutylicum. Bioprocess Technol. 24: 253-280.
12. Steinbüchel A, Lütke-Eversloh T. 2003. Metabolic engineering and pathway construction for biotechnological production of relevant polyhydroxyalkanoates in microorganisms. Biochem. Eng. J. 16: 81-96.
13. Lütke-Eversloh T, Simon-Stieghaus H. 2004. Microbial polyhydroxyalkanoates. Macromol. Biosci. 4: 166-174.
14. Yu J-L, Xia X-X, Zhong J-J, Qian Z-G. 2014. Direct biosynthesis of adipic acid from a synthetic pathway in recombinant Escherichia coli. Biotechnol. Bioeng. 111: 2580-2586.
15. Saini M, Wang ZW, Chiang C-J, Chao Y-P. 2014. Metabolic engineering of Escherichia coli for production of butyric acid. J. Agric. Food Chem. 62: 4342-4348.
16. Lütke-Eversloh T, Fischer A, Remminger Holl, U, Kawada J, Marchesau Rh, Bögershausen A, et al. 2002. Biosynthesis of novel thermoplastic polyhydroxyalkanoates by engineered Escherichia coli. Nat. Mater. 1: 236-240.
17. Xu QS, Shirihai DH, Pufan R, Yokota H, Kim R, Kim SH. 2004. Crystal structure of a phosphotransacetylase from Streptococcus pyogenes. Proteins 55: 479-481.
18. Xu QS, Jancarik J, Lou Y, Kuznetsova K, Yekunin AF, Yokota H, et al. 2005. Crystal structures of a phosphotransacylase from Bacillus subtilis and its complex with acetyl phosphate. J. Struct. Funct. Genomics 6: 269-279.
19. Spekman HB. 1920. Gas production during the acetone and butyl alcohol fermentation of starch. J. Biol. Chem. 43: 401-411.
20. Lawrence SH, Luther KB, Schindelin H, Ferry JG. 2006. Structural and functional studies suggest a catalytic mechanism for the phosphotransacylase from Methanothermobacter thermautotrophicus. J. Bacteriol. 188: 1143-1154.
21. Thomsen J, Minor W. 1997. Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol. 276: 307-326.
22. Matthews BW. 1968. Solvent content of protein crystals. Acta Crystallogr. 26: 559-567.
23. Yagin A, Tepljakov A. 2010. Molecular replacement with MOLREP. Acta Crystallogr. D Biol. Crystallogr. 66: 22-25.
24. Emsley P, Cowtan K. 2004. Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60: 2126-2132.
25. Murshudov GN, Skubak P, Lebedev AA, Pannu NS, Steiner RA, Nicholls RA, et al. 2011. REFMAC5 for the refinement of macromolecular crystal structures. Acta Crystallogr. D Biol. Crystallogr. 67: 355-367.
26. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. 1993. PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.* **26**:283-291.
27. Chen VB, Arendall WB, 3rd, Headd JJ, Keedy DA, Immormino RM, Kapral GI, et al. 2010. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D Biol Crystallogr.* **66**:12-21.
28. Lawrence SH, Luther KB, Schindelin H, Ferry JG. 2006. Structural and Functional Studies Suggest a Catalytic Mechanism for the Phosphotransacetylase from *Methanosarcina thermophila*. *J. Bacteriol.* **188**:1143-1154.
29. Krissinel E, Henrick K. 2007. Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.* **372**:774-797.
30. Wiesenborn DP, Rudolph FB, Papoutsakis ET. 1989. Phosphotransbutyrylase from *Clostridium acetobutylicum* ATCC 824 and its role in acidogenesis. *Appl. Environ. Microbiol.* **55**:317-322.
31. Zhang Y, Yu M, Yang ST. 2012. Effects of ptb knockout on butyric acid fermentation by *Clostridium tyrobutyricum*. *Biotechnol. Progress* **28**:52-59.
32. Yoshida Y, Sato M, Nonaka T, Hasegawa Y, Kezuka Y. 2019. Characterization of the phosphotransacetylase-acetate kinase pathway for ATP production in *Porphyromonas gingivalis*. *J. Oral Microbiol.* **11**:1588086.
33. Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**:4673-4680.
34. Ashkenazy H, Abadi S, Marta E, Chay O, Mayrose I, Pupko T, et al. 2016. ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic Acids Res.* **44**:W344-350.