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Insights into TIM-barrel Prenyl Transferase Mechanisms: Crystal Structures of PcrB from *Bacillus subtilis* and *Staphylococcus aureus***

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Many enzymes involved in isoprenoid (or terpene/terpenoid) biosynthesis are prenyl transferases (PTs) that catalyze condensation reactions between prenyl diphosphates and various acceptors and structurally, most contain one of six main folds: α , β , γ , δ , ϵ , or ζ .^[1] Recently, several new PT structures that contain a triose phosphate isomerase (TIM) barrel fold have been discovered,^[2] and there is considerable interest in their mechanisms of action.^[2b, 2c] The first structure published was that of *Archaeoglobus fulgidus* geranylgeranylgeranyl phosphate synthase (AfGGGPS),^[2d] which catalyzes the reaction between geranylgeranyl diphosphate (GGPP) and glycerol-1-phosphate (G1P) to form geranylgeranylgeranyl phosphate (GGGP) (Scheme 1), a precursor of the (perhydro)geranylgeranyl-based phospholipids found in Archaea.^[3] A very similar reaction is carried out by the enzyme PcrB in several Bacilli but in this case the prenyl donor is heptaprenyl diphosphate (HepPP).^[4] The PcrB product, heptaprenylglyceryl phosphate (HepGP), is then dephosphorylated to heptaprenylglycerol, then acetylated, Scheme 1.^[4] The function of this ester product is unknown. However, PcrB forms part of an operon with PcrA, an essential DNA helicase,^[5] and a *pcrB* mutant (in *Bacillus subtilis*) has a deranged, “cloggy” pattern of growth,^[4] so it seems likely that PcrB is important for growth (or perhaps, virulence) in other Bacilli, such as the pathogen *Staphylococcus aureus*, and may be of interest as a drug target.

In GGGPS or PcrB, there is no isomerization of the isoprenoid chain during catalysis. However, in a third TIM-barrel PT, *Streptomyces ghanaensis* MoeO5 (SgMoeO5), involved

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in the first committed step in biosynthesis of the antibiotic moenomycin,^[2c] there is a remarkable *trans* → *cis* isomerization during the prenylation reaction, as shown in Scheme 1,^[2b, 2c] and we recently reported the structure of MoeO5 containing its bound product, FPG (2-(*Z*, *E*)-farnesyl-3-phosphoglycerate).^[2e] Here, we report the structures of PcrB from *B. subtilis* and *S. aureus* containing bound substrates as well as other ligand molecules which help define the catalytic mechanism, in addition to site-directed mutagenesis and bioinformatics results that point to the specific roles of several residues in PcrB catalysis.

BsPcrB structures were solved by using a previous BsPcrB structure (1VIZ)^[2a] as a search model, then the new BsPcrB structure was used as a search model for solving the SaPcrB structures by using the molecular replacement method (Table S1 and S2 in the Supporting Information). Detailed experimental procedures for protein expression, crystallization, data collection and structure determination are provided in the Supporting Information. The electron densities of the ligands were clear and are shown in Figure S1 in the Supporting Information. BsPcrB and SaPcrB both crystallize as dimers, and the dimer interface involves mainly hydrophobic residues in helices 4 and 5. This interface is different from that in the structure (1VIZ) reported previously,^[2a] but is the same as predicted by Peterhoff et al.^[6] (Figure S2 in the Supporting Information).

We show in Figure S3a the superimposed structures of the monomer subunits of BsPcrB (in green) and SaPcrB (in cyan) and in Figures S3b and S3c we show the BsPcrB structure (in green) superimposed on that of AfGGGPS (in yellow; Figure S3b) and SgMoeO5 (in orange; Figure S3c) in which it can be seen that there is high similarity between the structures of the TIM barrel folds in each system with C_α root mean square deviations (RMSD) in the range 0.98 – 1.51 Å over ~210 atoms, Figure S3. (The RMSD between BsPcrB and SaPcrB, GGGPS and MoeO5 are 0.98 Å, 1.06 Å and 1.51 Å, respectively, for 210, 204 and 167 matched atoms.)^[7] The major difference occurs in the region around loop 3, which replaces helix 3 in a canonical TIM barrel structure. This loop is rather open in PcrB and GGGPS, acting as a swinging gate to the hydrophobic substrate-binding tunnel, but it collapses inward in MoeO5, making a unique pocket for binding substrate in a bent conformation.^[2]

With BsPcrB we also obtained structures of the protein with bound G1P, FsPP (*S*-thiolofarnesyl diphosphate) and FsPP + G1P (Figure 1a), and with SaPcrB we obtained structures with a bound PEG (Figure 1b) and a sulfate ion. In BsPcrB-G1P/FsPP the G1P orientation is slightly different from that in the BsPcrB-G1P complex, suggesting flexibility. Consequently, BsPcrB-G1P and BsPcrB-FsPP are used for structural analyses of these two ligands. Detailed interactions of G1P and FsPP are shown in Figures 1c and 1d, respectively. The phosphate group of G1P is bound between two loops and forms hydrogen bonds to several peptide NH groups, as well as the side chain of N209. Both the 2-OH and 3-OH are hydrogen bonded to the side chain of E160 and Y158, Figure 1c. In the SaPcrB-SO₄ structure, a bound sulfate ion binds to the G1P binding site (Figure S4). In the BsPcrB-FsPP structure, the diphosphate group of FsPP is buried in a polar pocket with its prenyl moiety in a straight hydrophobic tunnel, and in the SaPcrB-PEG structure, the PEG polymer is seen in the same hydrophobic tunnel (Figure 1d). These structures are of interest since they show the positions of the reactive “head-groups” that condense to form the HepGP product. In addition, they help define experimentally the hydrophobic tunnel into which the C₃₅ side-chain can bind, in addition to confirming the nature of the dimer structure predicted by Peterhoff et al.^[6]

The reaction catalyzed by PcrB involves the attack of the nucleophile G1P via its C-3 OH group at C1 in HepPP. This reaction can reasonably be thought to require: 1) activation of the C-3 OH to facilitate nucleophilic attack; 2) removal of the product diphosphate and 3) a

route to removal of the C-3 OH proton that is lost during the reaction, from the active site region. To see which residues are most essential for catalysis and might thus be involved in these steps, we used the SCORECONS program^[8] which enables a rank-ordering of residues in terms of their conserved nature.

Many PTs such as farnesyl diphosphate synthase, undecaprenyl diphosphate synthase and squalene synthase, have an absolute requirement for a divalent cation, typically Mg^{2+} ,^[9] and in these systems Mg^{2+} is “anchored” by one or more Asp residues. We thus assumed that there would be a “most essential” Asp in PcrB. However, as can be seen in Table S3, the results of the SCORECONS analysis (using JPRED3^[10] alignments based on BsPcrB or SaPcrB) clearly indicate that the three most conserved residues in both species are Y and E: Y118, Y159 and E161 in *S. aureus* and Y118, Y158 and E160 in *B. subtilis*, Table S3. On inspection of the crystal structures of BsPcrB (+FsPP+G1P) it can clearly be seen that E160 is only ~2.7Å from the C-3 OH oxygen (Figure 2), which could facilitate removal of the OH proton, and two Tyr OH oxygens are also quite close by (both 4.2Å to the E160 carboxyl oxygens) and are poised to aid proton abstraction from the active site. We thus propose that these three residues are involved in C-3 OH activation and H^+ removal from the active site region. We also see, Table S3, that D14 is quite highly conserved, and based on an alignment with the MoeO5 structure (which contains an Mg^{2+} , Figure 2) we propose that D14 binds to Mg^{2+} which – as with other PTs, is important in removal of the diphosphate product of the reaction. We tested the activity of wild type protein in the presence of 50 μM EDTA as well as in the absence of $MgCl_2$. No product was found, indicating that Mg^{2+} is likely essential for catalysis. We also tested the activity of D14A mutants of both SaPcrB and BsPcrB and found no activity.

The reaction catalyzed by GGGPS is very similar to that catalyzed by PcrB - the only obvious chemical difference being that GGGPS uses a shorter prenyl diphosphate, and a SCORECONS analysis shows that Y124, Y165 and E167 are the three most essential residues and that D13 is also highly conserved, Table S3. Other highly conserved residues are all Glys that are presumably of structural importance. The role of the conserved D76 (or 77) is unknown. Based on these results then, the YYED set of residues is common to PcrB as well as GGGPS proteins and is involved in proton abstraction (YYE) and diphosphate removal (D).

Next, we consider the nature of the hydrophobic pocket that accommodates the C_{35} side-chain. In recent work, Guldán et al.^[4] have used computational docking (together with geometry optimization) of HepPP to a BsPcrB dimer model to propose a model for bound HepPP.^[4] This model (in yellow) is shown in Figure 3a superimposed on the FsPP substrate (in cyan) plus the PEG ligand (in green) and as can be seen in Figure 3a, there is very good accord between the disposition of HepPP in the computational model and the positions of the two ligands in the x-ray structures, albeit with some small differences in the head-group region, Figure 3a. Also of interest are the positions of residue A100 and Y104: A100 allows binding of substrates longer than GGPP (length defined by FsPP plus PEG), and Y104 locates near the terminus of HepPP and may play a role in chain length limitation. The residue corresponding to A100 in PcrB is W99 in GGGPS, and a previous study on a W99A mutant of GGGPS showed that mutation of this bulky residue to alanine allowed the protein to use substrates longer than GGPP.^[4] Interestingly, although Y104 is not highly conserved (Table S3), this position is either Y or F in essentially all sequences annotated as HepGPS, as highlighted in green, but is random in the GGGPS sequences. Correspondingly, W100 is conserved in the same GGGPS sequences (highlighted in yellow), but is Ala in the corresponding HepGPS sequences (highlighted in blue), enabling formation in long chain products.

To see to what extent there might be chain length discrimination in PcrB, we produced A100W and Y104A mutants of SaPcrB and tested the effects of these mutations on the conversion of C₂₀, C₂₅, C₃₀, C₃₅ and C₄₀ prenyl diphosphates to the corresponding glyceryl phosphate products. To investigate these effects, we used a radio-TLC assay with a distribution of polyprenyl diphosphate chain length substrates, obtained from the reaction of FPP with [¹⁴C]-IPP, catalyzed by *E. coli* octaprenyl diphosphate synthase.^[11] The results obtained, illustrated in Figure 3b and Figure S5 in the Supporting Information, show that conversion of A100 to W100 results in strong inhibition of the formation of the longer chain products, presumably because the bulky W100 side-chain inhibits binding of the longer chain substrates, while conversion of Y104 to A104 favors formation of longer-chain products. The observation that the Y104A mutant makes more C₄₀ products than does the wild-type protein implies, then, that Y104 is located at or near the terminus of the C₃₅ chain since, if it were not, there would be little effect on the chain length distribution. Of course, in cells, the actual product chain length distribution depends on the amounts of FPP, GGPP, HepPP and other prenyl diphosphates that are present, and this is likely to vary with growth conditions. *S. aureus* does, however, make quinones with C₄₀ side-chains[12] and it is possible that Y104 contributes to chain-length discrimination in the PcrB reaction since primarily C₃₅ products are found in the heptaprenylglycerol diester product,[4] but of course chain length discrimination can also occur in reactions occurring after the PcrB-catalyzed reaction.

Finally, we consider the structural differences between the BsPcrB and MoeO5 structures. As can be seen in Figure 4, in MoeO5 the prenyl side-chain in the FPG product (in yellow) is highly bent. The chain terminus in this bent structure would occupy the position of the Y118 side-chain in BsPcrB, and binding in this bent manner is only possible because the residue at this position in MoeO5 is an alanine, as shown in Figure 4. In addition, the conserved catalytic E160 (in BsPcrB) in MoeO5 is a tyrosine, Y201, which as with BsPcrB, is likely to be involved in H⁺ abstraction, this time from the 2-OH group of 3-phosphoglycerate, illustrated in Figure 4. The residue corresponding to D14 (BsPcrB) or D13 (GGPPS) in MoeO5 is D41, which interacts with Mg²⁺ in the active site.^[2e]

In summary: we report the structures of PcrBs from *B. subtilis* (4 structures) and *S. aureus* (2 structures), the first substrate (or other ligand) containing structure of these enzymes and the first structure of the *S. aureus* protein. The results clearly show the G1P and F(s)PP binding sites, plus, they map out much of the C₃₅ side-chain binding pocket, and there is good accord with earlier computational predictions as to the nature of this binding site, as well as the nature of the dimer structure. The most highly conserved residues are two Tyr and one Glu in the active site, proposed to be involved in 3-OH activation as well as H⁺ removal from the active site region, together with a conserved Asp, involved in Mg²⁺ binding and diphosphate removal. In addition, we propose that Y104 is involved in chain-length regulation. The overall structures are similar to those previously reported for MoeO5 but there, the bent FPG side-chain occupies the Y118 (BsPcrB) A124 (MoeO5) site, and E160 (BsPcrB) is replaced by Y201 (MoeO5), involved in 2-OH proton abstraction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Oldfield E, Lin FY. Angew Chem Int Ed Engl. 2012; 51:1124–1137. [PubMed: 22105807]
2. a) Badger J, Sauder JM, Adams JM, Antonysamy S, Bain K, Bergseid MG, Buchanan SG, Buchanan MD, Batiyenko Y, Christopher JA, Emtage S, Eroshkina A, Feil I, Furlong EB, Gajiwala

- KS, Gao X, He D, Hendle J, Huber A, Hoda K, Kearins P, Kissinger C, Laubert B, Lewis HA, Lin J, Loomis K, Lorimer D, Louie G, Maletic M, Marsh CD, Miller I, Molinari J, Muller-Dieckmann HJ, Newman JM, Noland BW, Pagarigan B, Park F, Peat TS, Post KW, Radojicic S, Ramos A, Romero R, Rutter ME, Sanderson WE, Schwinn KD, Tresser J, Winhoven J, Wright TA, Wu L, Xu J, Harris TJR. *Proteins-Structure Function and Bioinformatics*. 2005; 60:787–796.b) Doud EH, Perlstein DL, Wolpert M, Cane DE, Walker S. *Journal of the American Chemical Society*. 2011; 133:1270–1273. [PubMed: 21214173] c) Ostash B, Doud EH, Lin C, Ostash I, Perlstein DL, Fuse S, Wolpert M, Kahne D, Walker S. *Biochemistry*. 2009; 48:8830–8841. [PubMed: 19640006] d) Payandeh J, Fujihashi M, Gillon W, Pai EF. *Journal of Biological Chemistry*. 2006; 281:6070–6078. [PubMed: 16377641] e) Ren F, Ko TP, Feng X, Huang CH, Chan HC, Hu Y, Wang K, Ma Y, Liang PH, Wang AHJ, Oldfield E, Guo RT. *Angewandte Chemie-International Edition*. 2012; 51:4157–4160.
3. Matsumi R, Atomi H, Driessen AJM, van der Oost J. *Research in Microbiology*. 2011; 162:39–52. [PubMed: 21034816]
 4. Guldan H, Matysik FM, Bocola M, Sterner R, Babinger P. *Angewandte Chemie-International Edition*. 2011; 50:8188–8191.
 5. Petit MA, Dervyn E, Rose M, Entian KD, McGovern S, Ehrlich SD, Bruand C. *Molecular Microbiology*. 1998; 29:261–273. [PubMed: 9701819]
 6. Peterhoff D, Zellner H, Guldan H, Merkl R, Sterner R, Babinger P. *Chembiochem*. 2012; 13:1710–1710.
 7. Emsley P, Cowtan K. *Acta Crystallographica Section D-Biological Crystallography*. 2004; 60:2126–2132.
 8. Valdar WSJ. *Proteins-Structure Function and Bioinformatics*. 2002; 48:227–241.
 9. Christianson DW. *Chemical Reviews*. 2006; 106:3412–3442. [PubMed: 16895335]
 10. Cole C, Barber JD, Barton GJ. *Nucleic Acids Research*. 2008; 36:W197–W201. [PubMed: 18463136]
 11. Pan JJ, Kuo TH, Chen YK, Yang LW, Liang PH. *Biochimica Et Biophysica Acta-Protein Structure and Molecular Enzymology*. 2002; 1594:64–73.
 12. Nahaie MR, Goodfellow M, Minnikin DE, Hajek V. *Journal of General Microbiology*. 1984; 130:2427–2437. [PubMed: 6502136]
 13. Otwinowski Z, Minor W. *Methods in Enzymology*. 1997; 276:307–326.
 14. Brunger AT. *Acta Crystallogr D Biol Crystallogr*. 1993; 49:24–36. [PubMed: 15299543]
 15. Emsley P, Lohkamp B, Scott WG, Cowtan K. *Acta Crystallogr D Biol Crystallogr*. 2010; 66:486–501. [PubMed: 20383002]
 16. Brunger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. *Acta Crystallogr D Biol Crystallogr*. 1998; 54:905–921. [PubMed: 9757107]

Figure 1.

PcrB complex structures. A) BsPcrB-FsPP-G1P structure is shown with FsPP and G1P as a stick model and BsPcrB as a cartoon. The ligands in BsPcrB-FsPP and BsPcrB-G1P are very close to those in BsPcrB-FsPP-G1P. B) SaPcrB-PEG structure is shown with PEG as a stick model and SaPcrB as a cartoon. C) Detailed ligand-protein interactions in BsPcrB-G1P. D) Detailed ligand-protein interactions in BsPcrB-FsPP.

Figure 2. Most conserved residues in BsPcrB shown together with FsPP and G1P, as well as a catalytic Mg^{2+} from a superimposed MoeO5 structure.

Figure 3.

Hydrophobic tunnel in BsPcrB with TLC product distribution results. A) HepPP model (in yellow) by Guldan et al. is shown superimposed on FsPP (in cyan) from the BsPcrB-FsPP-G1P structure and PEG (in green) from the SaPcrB-PEG structure. B) TLC product distribution results for SaPcrB mutants with different chain length prenyl diphosphate substrates.

Figure 4. Structures of BsPcrB ligands (FsPP and G1P, in cyan), GGGPS ligands (G1P, in magenta) and MoeO5 ligands (FGP, in yellow). Conserved residues are also shown as stick models for the proteins.

Scheme 1.

Structures of molecules and reactions of interest. A) Reactions catalyzed by PcrB (n=5) and GGGPS (n=2) B) Reaction catalyzed by MoeO5.