Enzymatic systems that exploit pericyclic reaction mechanisms are rare. A recent addition to this class is the enzyme PchB, an 11.4-kDa isochorismate pyruvate lyase from Pseudomonas aeruginosa. The apo and pyruvate-bound structures of PchB reveal that the enzyme is a structural homologue of chloramphenicol acetyltransferase from E. coli (20%). The enzyme is an intertwined dimer of three helices with connecting loops, and amino acids from each monomer participate in each of two active sites. The apo structure (2.35 Å resolution) has one dimer per asymmetric unit with nitrate bound in an open active site. The loop between the first and second helices is disordered, providing a gateway for substrate entry and product exit. The pyruvate-bound structure (1.95 Å resolution) has two dimers per asymmetric unit. One has two open active sites like the apo structure, and the other has two closed active sites with the loop between the first and second helices ordered for catalysis. Determining the structure of PchB is part of a larger effort to elucidate protein structures involved in siderophore biosynthesis, as these enzymes are crucial for bacterial iron uptake and virulence and have been identified as antimicrobial drug targets.

Many bacteria produce low molecular mass, high affinity iron chelators called siderophores in response to an iron-limiting environment, such as that found in a host organism. Siderophores are produced in a multistep biosynthetic process, secreted into the environment where they bind available iron (frequently out-competing host organism enzymes for the iron), and are then reinternalized for iron use by the pathogen (1–4). Salicylate serves as a building block for siderophores in response to an iron-limiting environment, such as that found in a host organism. Siderophores are produced in a multistep biosynthetic process, secreted into the environment where they bind available iron (frequently out-competing host organism enzymes for the iron), and are then reinternalized for iron use by the pathogen (1–4). Salicylate is then produced from isochorismate by a second enzyme, PchB. PchA has a putative structural homologue of salicylate-producing enzymes of the siderophore biosynthetic pathways from Y. enterocolitica and M. tuberculosis, Irp9 and Mbt1, respectively. Irp9, a 50-kDa enzyme with a complex α/β fold, is a structural homologue of the chorismate-utilizing enzymes anthranilate synthase (TrpE subunit) and 4-amino-4-deoxychorismate synthase (PabB subunit). Mbt1 is proposed to be a functional and structural homologue of Irp9 for which preliminary crystallographic information has been reported (6, 15). Both Irp9 and Mbt1 are bifunctional salicylate synthases that catalyze the production of salicylate from chorismate using a two-step process with an isochorismate intermediate (9). However, neither Irp9 nor Mbt1 shares any sequence similarity with PchB.

The physiological role of PchB is as an isochorismate pyruvate lyase (IPL)2 (Fig. 1a), but PchB shows no sequence similarity to other pyruvate lyases, which proceed through a general base mechanism (16–18). Instead, PchB shares 20% sequence identity with chorismate mutases of the AraQ family α subclass, which use a Claisen rearrangement mechanism (13, 19). Moreover, PchB has an additional catalytic activity as a chorismate mutase (CM) to produce prephenate (Fig. 1b) (13). In terms of $k_{cat}/K_m$, the catalytic efficiency of PchB performing the CM reaction is two orders of magnitude less than that of the physiological IPL reaction (13). To provide a structural framework for understanding the PchB catalytic mechanisms, we solved the structures of apo and pyruvate-bound PchB.

**EXPERIMENTAL PROCEDURES**

PchB Cloning and Protein Expression

PchB-His<sub>6</sub>—The pchB gene was cloned from P. aeruginosa PA01 genomic DNA by PCR. The forward primer (5’-TGG CGT ATC ATA TGA AAA CTC CGA A-3’) was designed to include a 5’ NdeI restriction enzyme site, and the reverse primer (5’-TTT AGA AAG CTT TGC GGC ACC CCG-3’) was designed to include a 3’ HindIII site. The PCR product was digested with NdeI/HindIII and inserted into the pET29b vector (Novagen). The resultant plasmid, which encodes the pchB gene with a C-terminal histidine tag, was then transformed into...
**P. aeruginosa Isochorismate Pyruvate Lyase**

![Chemical structures](image)

**FIGURE 1. Reaction schemes of enzymatic activities of PchB.** a, isochorismate pyruvate lyase activity. b, chorismate mutase activity. c, the oxabicyclic transition state analogue of the chorismate mutase activity is shown for comparison.

*Escherichia coli* BL21(DE3) cells. Protein was expressed in cells grown in Luria Bertani medium at 37 °C and 250 rpm. Protein expression was induced by the addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside at an optical density at 600 nm (A<sub>600</sub>) of ~0.7, and the cells were harvested after 3 h by centrifugation (5000 × g, 10 min, 4 °C). Cells were resuspended in ~10 ml 25 mM Tris-HCl, pH 8, 200 mM NaCl (buffer A) per liter of harvested cells and stored at −80 °C for purification.

**PchB**—A second overexpression system was generated to incorporate the naturally occurring stop codon and thus remove the C-terminal histidine tag. The QuickChange kit (Stratagene) and two complementary primers encoding the desired change (5'-GGG GTT GCC GCA TAA TAA AAG CTT GCG GCC-3', 5'-GGC CGC AAG CTT TTA TTA TGC GGC ACC CCG-3') were used according to the manufacturer’s instructions.

**Protein Purification**

**PchB-His<sub>6</sub>**—The cells were lysed by passage through a French Press three times. The lysate was clarified by centrifugation at 12,000 × g for 30 min at 4 °C. The cell extract was applied to a Chelating Sepharose Fast Flow (Amersham Biosciences) column charged with nickel chloride and pre-equilibrated with buffer A. The column was washed with buffer A supplemented with 5 mM imidazole. The protein was eluted with a 10-column volume 0–0.3 M imidazole gradient in buffer A. Fractions containing PchB-His<sub>6</sub> eluted at ~0.25 M imidazole. The collected fractions were concentrated, and the protein was further purified on a HiLoad 16/60 Superdex 75 (Amersham Biosciences) gel filtration column pre-equilibrated with 50 mM Tris-HCl, pH 8, 150 mM NaCl, 1 mM dithiothreitol, and 10% (v/v) glycerol. The purified PchB-His<sub>6</sub> was concentrated to 28.7 mg/ml (determined by the Bradford assay) and stored at −80 °C for crystallization.

**PchB**—The cells were lysed and the lysate clarified as described above for PchB-His<sub>6</sub>. The cell extract was applied to a Q-Sepharose Fast Flow (Amersham Biosciences) column pre-equilibrated in 25 mM Tris-HCl, pH 8.0. The protein was eluted with a 10-column volume 0–0.5 M NaCl gradient in the pre-equilibration buffer. Fractions containing PchB eluted at ~0.1 M NaCl. The collected fractions were concentrated, and the protein was further purified using the Superdex 75 gel filtration column as described above for PchB-His<sub>6</sub>. The purified PchB was concentrated to 68 mg/ml and stored at −80 °C for crystallization.

**TABLE 1**

| Protein sample | Amino acids in construct | Amino acids crystallized |
|----------------|--------------------------|--------------------------|
| Apo            | 1–101 + His tag          | 1–99                     |
| Pyruvate bound | 1                      | 1–101                    |

**PchB in structure**

| Ordered amino acids | Ions in active site |
|---------------------|---------------------|
| Monomer A           | 1–41; 50–96         | Nitrate                 |
| Monomer B           | 1–41; 49–92         | Nitrate                 |
| Pyruvate bound      | 1                   | 2 Pyruvates             |
| Monomer C           | 1–98                | 2 Pyruvates             |
| Monomer D           | 1–100               | 2 Pyruvates             |

**TABLE 2**

**Crystallographic statistics**

|                          | Apo                  | Pyruvate bound        |
|--------------------------|----------------------|-----------------------|
| Resolution Range (Å)     | 28.9-2.35            | 57.2-1.95             |
| Space Group              | P4<sub>1</sub>          | P2<sub>2</sub>          |
| Unit Cell (Å)            | a = b = 93.2, c = 60.2 | a = 54.5, b = 74.5, c = 88.9 |
| Observations             | 11,526               | 25,853                 |
| Total                    | 326,890              | 266,264                |
| Completeness (%)         | 99.9 (100.0)         | 95.0 (75.7)            |
| R<sub>free</sub>         | 0.067                | 0.066 (0.405)         |
| % > 3σ (/I)              | 84.3 (60.6)          | 80.7 (49.4)            |

**Refinement**

| Data collection          | Resolution Range (Å) | R<sub>free</sub> |
|--------------------------|----------------------|-------------------|
| Resolution Range (Å)     | 28.9-2.35            | 57.2-1.95         |
| Number of reflections    | 11,246               | 25,825            |
| R-factor<sup>*</sup>     | 0.224                | 0.210             |
| R<sub>free</sub><sup>**</sup> | 0.269                 | 0.252             |
| Number of atoms          |                      |                   |
| Protein, nonhydrogen     | 1397                 | 2984              |
| Nonprotein               | 75                   | 218               |
| Root mean square deviations |                   |                   |
| Length (Å)               | 0.006                | 0.005             |
| Angles (°)               | 1.08                 | 1.08              |
| Overall B factor (Å)     | 42.7                 | 28.6              |
| B factor for protein atoms | 42.5                 | 28.3              |
| B factor for water oxygens | 58.0                 | 34.9              |
| B factor for ligand      | 44.3                 | 44.3              |
| Wilson B factor (Å<sup>2</sup>) | 37.2                 | 26.8              |

<sup>*</sup> Values in parentheses are for the highest resolution shell: 2.43-2.35 Å (native), 2.02-1.95 Å (pyruvate bound).

<sup>**</sup> R<sub>free</sub> = <sup>Σ</sup>[Fo-F<sub>c</sub>]/<sup>Σ</sup>F<sub>c</sub> where the summation is over all reflections.

<sup>**</sup> R-factor = <sup>Σ</sup>[Fo-F<sub>c</sub>]/<sup>Σ</sup>F<sub>c</sub> for calculation of R<sub>free</sub> 10% of the reflections were reserved.
Isochorismate Pyruvate Lyase and Chorismate Mutase Activity—To ensure that purified PchB (both with and without the C-terminal histidine tag) was enzymatically active, the protein samples were subjected to assays for the known catalytic activities of PchB. Both His-tagged and untagged PchB demonstrated isochorismate pyruvate lyase activity using a coupled assay with PchA (isochorismate synthase) as previously described (data not shown) (13). Both forms of PchB also demonstrated chorismate mutase activity using a previously reported protocol (data not shown) (13).

Protein Crystallization

PchB-His6—Crystallization was carried out by the hanging drop method at 25 °C. Drops containing 1.5–3 μl of purified PchB-His6 protein were mixed with equal volumes of a reservoir solution composed of 0.1 M acetate buffer, pH 4.7, and 4.8–5.3 M ammonium nitrate. Large square pyramidal crystals formed after 48 h. These crystals and the structure determined from these crystals will henceforth be called “apo.”

PchB—Crystallization was carried out by the hanging drop method at 25 °C. Immediately prior to crystallization, the purified PchB protein was diluted to 20 mg/ml in the buffer used for gel filtration chromatography with 17 mM pyruvate and incubated on ice for 30 min. Drops containing 1 μl of this protein/pyruvate solution were mixed with a reservoir solution composed of 100 mM ADA (N-(2-acetamido)iminodiacetic acid), pH 7.0, 25% polyethylene glycol 3350, 0.15 M calcium acetate, and 10% glycerol. Large rod-shaped crystals appeared in 5–7 days and continued to grow to full size over 2–3 weeks. These crystals and the structure determined from these crystals will henceforth be called “pyruvate bound.”

Determination of PchB-His6 Fragment Size

Crystals of PchB-His6 dissolved in water and subjected to SDS-PAGE gave a single band corresponding to a molecular mass of 11 kDa, indicating that the crystals contained a fragment of the original PchB-His6 construct rather than the full-length protein with the C-terminal histidine tag. Further analysis of dissolved crystals by electrospray ionization mass spectrometry indicated a fragment with a mass of 11294.2 Da (experiments conducted by the University of Kansas Molecular Structures Group Mass Spectrometry Laboratory; www.msg.ku.edu/%7Emsg/mass.html). Dissolved crystals were analyzed by N-terminal sequencing, which indicated that the N terminus was intact (experiments conducted by the Kansas State University Bio-tech Core Facility; www.k-state.edu/bchem/biotech/). We conclude that PchB-His6 was proteolyzed in the crystallization drop, removing amino acids 100 and 101 and the C-terminal His tag and allowing amino acids 1–99 to crystallize (Table 1). These results are consistent with the refined structure.

Data Collection

Apo Protein—For data collection, apo crystals were transferred to the reservoir solution supplemented with 14% (v/v) glycerol as a cryosolvent and flash cooled at −160 °C. Diffraction data were collected at the Protein Structure Laboratory at the University of Kansas (www.psl.ku.edu) using an RaxisIV image plate system mounted on a Rigaku RUH3R rotating anode. The exposure time per frame was 20 min with a crystal to detector distance of 150 mm. The data were processed with DENZO and SCALEPACK (20). The crystals were initially assigned to the space group P422 with unit cell dimensions a = b =
93.2 Å and c = 60.2 Å. Data collection statistics are found in Table 2.

Pyruvate Bound—To ensure ligand binding, the drops containing pyruvate-bound crystals were supplemented with pyruvate to a final concentration of 100 mM (500 mM pyruvate in 50 mM Tris, pH 7.0, was added directly to the drop) and incubated for 5 days. For data collection, the crystals were transferred briefly to a cryosolvent composed of 30% polyethylene glycol 3350, 0.2 M calcium acetate, and 20% glycerol and then flashed cooled at −160 °C. These crystals were initially assigned to the space group P2221 with unit cell dimensions a = 54.5 Å, b = 74.5 Å, c = 88.9 Å. Data collection and processing were completed as described above for the apo crystals.

**Structure Solution and Refinement**

**Apo**—Molecular replacement calculations were performed using the program Phaser (21), the data from 25 to 2.5 Å, and all possible space groups of the P422 subgroup. The *E. coli* chorismate mutase structure (Protein Data Bank accession code 1ECM) (22) was used as a search model, yielding a clear solution in the space group P43212 with a log likelihood gain of 132.5 and no unrelated peaks higher than 75% of the difference between the top and the mean value. The map generated with this solution indicated extra density at the N terminus compared with the molecular replacement model and an area lacking density in the loop between the first and second helices. These two differences in electron density relative to the 1ECM molecular replacement model indicate a correct solution, because the map is not merely demonstrating the phase bias from the search model. After model building in the program XtalView (23), refinement was conducting by simulated annealing and individual temperature factor refinement using CNS (24). The final model contains two intertwined monomers per asymmetric unit.

**FIGURE 3.** Electron density maps for the apo and pyruvate-bound structures. *a* and *b*, comparison of the open and closed forms of PchB. *a*, the apo structure of PchB, with one monomer shown in red and the other in blue sticks. The yellow amino acids (Phe-41 and Pro-49) are the last ordered residues in the loop connecting the first and second α-helices. *b*, the closed, pyruvate-bound dimer with one monomer in purple and the other in pink sticks. To indicate the position of the analogous amino acids in panel *a*, Phe-41 and Pro-49 are again highlighted in yellow. Pyruvate 2 is depicted as green sticks with red oxygen atoms. *c*, stereo representation of the active site of the closed, pyruvate-bound dimer. Colors are as described in panel *b*. All electron density maps (cyan) are 2Fo − Fc maps contoured at 1σ. This figure was generated with PyMOL (30).
Monomer A comprises residues 1–41 and 50–96, whereas monomer B comprises residues 1–41 and 49–92. The model also includes 55 water molecules and 5 nitrate ions. A Ramachandran plot generated with PROCHECK (25) shows that the model exhibits good geometry, with 97.3% of the residues in the most favored regions and 2.7% of the residues in the additionally allowed regions. Refinement statistics are in Table 2.

Pyruvate Bound—Molecular replacement calculations were performed by AutoMolRep in the CCP4 program package using data from 50 to 3 Å resolution (26). The apo structure (above) was used as a search model, yielding a clear solution in the space group P2₁2₁2₁ with two dimers in the asymmetric unit. This solution had a correlation coefficient of 0.55 and an R<sub>factor</sub> of 0.45 (compared with the second unrelated peak with correlation coefficient of 0.27 and R<sub>factor</sub> of 0.57). The map generated with this solution indicated extra density in two of the monomers, corresponding to the ordering of the loop between the first and second helices. After model building in the program XtalView (23), refinement was conducted by simulated annealing and individual temperature factor refinement using CNS (24). The final model contains two dimers, each composed of two intertwined monomers. Monomer A, which is composed of residues 1–41 and 52–94, forms a dimer with Monomer B, composed of residues 2–41 and 48–94. Monomer C (residues 1–98) forms a dimer with monomer D (residues 1–100). Included in the model are 6 pyruvate molecules, 181 water molecules, and a Ca<sup>2+</sup> ion (at a crystal contact). A Ramachandran plot generated with PROCHECK (25) shows that the model exhibits good geometry, with 97.7% of the residues in the most favored regions and 2.2% of the residues in the additionally allowed regions.

Structural Analysis and Modeling

Structural Alignment—The structural alignments and calculations of root mean square deviations were all done using LSQMAN in the DEJAVU program package (27).

Modeling—Salicylate was docked by hand into each of the pyruvate 1 sites of the pyruvate-bound PchB structure using XtalView (23). The salicylate/pyruvate-bound model (with one salicylate and one pyruvate in each of the active sites of the closed dimer and one salicylate in each of the active sites of the open dimer) was refined using the wrong-ligand crystallographic refinement method (28), refining the complex against the 1.95 Å pyruvate-bound PchB crystallographic data with a slow cooling simulated annealing protocol starting at 2000 K in the CNS program package (24).

Figures—Figures were generated using MOLSCRIPT (29), PyMOL (30), and LIGPLOT (31).

RESULTS

Overall Structure—Crystals of apo PchB, grown using ammonium nitrate as a precipitant, belong to the space group P4<sub>3</sub>2<sub>1</sub>2. The structure was determined by molecular replacement using E. coli chorismate mutase (EcCM; Protein Data Bank accession code 1ECM) (22) as a search model. The asym-
The asymmetric unit contains two PchB monomers, each composed of three α-helices connected by loops to form an intertwined dimer (Fig. 2a). Several residues are disordered in both monomers, including eight to nine amino acids in the loop connecting the first and second helices and between three and seven amino acids at the C termini (Table 1).

The crystals of the pyruvate-bound structure were grown using polyethylene glycol 3350 as a precipitant and belong to a different space group, P2₁2₁2₁. The asymmetric unit for this crystal system contains two dimers of the same overall structure as the apo protein (Fig. 2b). One dimer (monomers A and B) has two open active sites as in the apo structure with disordered loops between the first and second helices and disordered C termini. In contrast, in the other dimer (monomers C and D), the corresponding loops are ordered and form a closed active site. Also, one additional turn of a C-terminal helix in each monomer is ordered.

Fig. 3, a and b, displays the disorder-to-order transition for the loop between helices 1 and 2, and Table 1 includes a summary of the ordered residues in the structures.

Superposition of the apo and both pyruvate-bound dimers yields root mean square deviations ranging from 0.60 to 0.72 Å for 168–172 α-carbons, indicating that overall these three structures are very similar, as expected. The only differences are found in the loop regions leading up to the regions of disorder. The overall root mean square difference for the superposition of the PchB structures with the E. coli chorismate mutase is 1.19 Å (for all three comparisons) for 154 to 155 Cα (for the apo and open pyruvate-bound structures, respectively) and 173 Cα for the closed pyruvate-bound structure. As evident in Fig. 4, a and b, the primary area of difference between the closed pyruvate-bound PchB structure and the inhibitor-bound EcCM structure is in the loop that closes over the active site (the same loop that is disordered in the open structures) and at the termini. A structure-based sequence alignment between the pyruvate-bound closed structure and EcCM is shown in Fig. 4c.

Interhelical Interactions—The N-terminal helices from each monomer twine together to form an anti-parallel coiled-coil. The interactions between these helices are driven by hydrophobic interactions, burying seven hydrophobic side chains. In EcCM, this interface is a seven-amino acid leucine and isoleucine zipper. In PchB, these amino acids are not strictly conserved, but the hydrophobic character of the helical face is maintained (Fig. 4c). The only amino acid that does not follow this hydrophobic repeat format in either protein is Arg-31 (PchB numbering system), which forms part of the active site.
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Active Site—The most obvious and important differences between the apo and the pyruvate-bound structures are in the ligands present and the loop connecting the first and second helices, which can be disordered (forming an “open” active site) or ordered (forming a “closed” active site). The apo structure has a nitrate ion deep in the open active site that is involved in an electrostatic interaction with Arg-31 (Fig. 5a). This nitrate ion is derived from the crystallization conditions (the precipitant is 6 M ammonium nitrate). Soaking experiments using the apo PchB crystals and a wide variety of ligands (substrates, products, and an oxabicyclic acid transition state analogue) did not result in replacement of the nitrate ion, most likely because of the huge molar excess of the nitrate ion. Nevertheless, we found that nitrate was not an effective inhibitor of PchB activity (data not shown).

We identified a new crystallization condition that does not rely on the nitrate precipitant. This condition led to the pyruvate-bound structure, which contains both open and closed forms of the active site in independent dimers within the same asymmetric unit. The open pyruvate-bound structure is analogous to the apo structure, with the loop between helices 1 and 2 disordered (Fig. 2b) and one pyruvate (called “pyruvate 1”) bound in the equivalent position as the nitrate. In the closed pyruvate-bound structure, two pyruvates are bound in each active site: pyruvate 1 and a second pyruvate (“pyruvate 2”), which interacts electrostatically with Arg-14 (Figs. 3c and 5b).

A closer inspection of these active sites was carried out in comparison with the E. coli chorismate mutase active site, the most similar structure known as determined using the DALI server (Fig. 2c) (32). Although PchB is not a physiological CM, it does have CM activity. Additionally, a single mutation (I87T) can abolish the physiological IPL activity without affecting the pyruvate 2 molecule. One amino acid, Tyr-86, in PchB is not conserved and corresponds to a serine in EcCM. Nevertheless, the side chain of the tyrosine in PchB is pointed such that the hydroxyl group is no longer part of the active site. The remaining two amino acids, Ala-50 and Val-54 (Asp and Glu, respectively, in EcCM), are not conserved at all and seem unimportant in binding the pyruvate molecules.

The physiological activity of PchB is to convert isochorismate into salicylate and pyruvate (IPL activity). In light of the structure in Fig. 5b, the question arises as to which, if either, of the two pyruvates bound in the closed active site correspond to the pyruvate generated by the lyase reaction. Previously, Gaille et al. (13) generated an in silico model of PchB based on the EcCM structure with TSA bound in which the TSA is computationally replaced with isochorismate. Their model is consistent with our electron density. Both suggest that pyruvate 1 of the pyruvate-bound structures (and the nitrate ion of the apo structure) binds in a position that would be occupied by the carboxylate group of the isochorismate substrate (position 7 in Fig. 1a) or the carboxylate of the salicylate product. The pyruvate 1 molecule (and the nitrate ion) is oriented by interaction with Arg-31 (Figs. 5 and 6a). Further, pyruvate 2 corresponds to the enolpyruvyl side chain that is cleaved during the reaction to form the pyruvate product. The carboxylate of this side chain (Fig. 1a, position 10) corresponds to the carboxylate of pyruvate 2, which is oriented by Arg-14 (Figs. 5b and 6a). A conservative model of salicylate and pyruvate (IPL products) bound to PchB generated using the wrong-ligand crystallographic refinement method (28) is displayed in Fig. 6b in a two-dimensional schematic representation to highlight the predicted hydrogen bonding contacts between the ligands and the enzyme. It stands to reason that the carboxylates of cho-
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isomerase (Fig. 1b) are analogously oriented for the secondary chorismate mutase activity to form prephenate. Finally, it seems clear that the movement of the loop between helices 1 and 2, including the Lys-42 residue that interacts with pyruvate 2, forms a gateway that opens and closes for substrate entry and product exit.

DISCUSSION

The catalytic mechanism of chorismate mutase enzymes, and especially that of the E. coli chorismate mutase, has been the subject of much speculation and debate (33, 34). Whereas most enzymes utilize covalent intermediates or acid-base catalysis to stabilize transition states and accelerate reactions, chorismate mutases catalyze an intramolecular (3, 3)-sigmatropic Claisen rearrangement without the formation of a covalent enzyme-substrate intermediate or the use of acid-base catalysis. Several hypotheses have been proposed to explain the origin of the catalytic power for conversion of chorismate to prephenate, which is accelerated relative to the non-catalyzed reaction by nearly two million-fold (35). These include 1) stabilizing the substrate in an energetically unfavorable pseudo-diaxial conformation that forces the reacting groups to be in van der Waals contact; 2) further exerting steric strain on the substrate bound in the reactive conformation; and 3) providing electrostatic stabilization of the transition state (33, 34).

PchB is an isochorismate pyruvate lyase. However, the amino acid sequence of PchB is unrelated to pyruvate lyses of other shikimic acid metabolites, all of which proceed by general base catalysis (16–18). We show herein that PchB has structural homology with chorismate mutases of the AroQα class, such as the E. coli chorismate mutase. Previously, PchB has been shown to have chorismate mutase activity (13), and recent studies have suggested that PchB uses a rare (1, 5)-pericyclic hydrogen transfer mechanism to produce salicylate from isochorismate (IPL activity) much more efficiently than the enzyme can produce prephenate from chorismate (CM activity) (Fig. 1) (36, 37). The confined active site of PchB provides an environment for the rearrangement of substrate noted for chorismate mutases (from pseudo-diequatorial in solution to pseudo-diaxial for catalysis) (38). The reduced charged/polar interactions between the substrate in PchB as compared with the transition state analogue in EcCM may produce the documented active site promiscuity that allows PchB to have both CM and IPL activities. The structures also support the electrostatic transition state hypothesis, because Lys-42 and Gln-90 are well positioned to stabilize the developing negative charge of the polar transition state in the chorismate mutase reaction (22) and the pyruvate byproduct in the isochorismate pyruvate lyase reaction. Therefore, the ordering of the active site loop suggests that the induced fit of the enzyme upon substrate binding locks Lys-42 into place for catalysis.

Further structural studies in which salicylate, prephenate, and the transition state analogue inhibitor of chorismate mutase activity are bound in the active site should help to further define the role of the active site amino acids in catalysis. Finally, co-crystallization of the 187T mutant form of the protein (which is IPL defective but CM competent) with a variety of substrates, products, and inhibitors bound may provide a method for dissecting the two activities of this bifunctional active site.

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