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Pericyclic Reactions Catalyzed by Chorismate-Utilizing Enzymes

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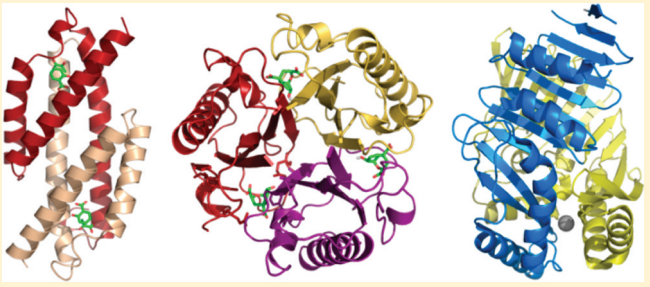
ABSTRACT: One of the fundamental questions of enzymology is how catalytic power is derived. This review focuses on recent developments in the structure−function relationships of chorismate-utilizing enzymes involved in siderophore biosynthesis to provide insight into the biocatalysis of pericyclic reactions. Specifically, salicylate synthesis by the two-enzyme pathway in *Pseudomonas aeruginosa* is examined. The isochorismate-pyruvate lyase is discussed in the context of its homologues, the chorismate mutases, and the isochorismate synthase is compared to its homologues in the MST family (menaquinone, siderophore, or tryptophan biosynthesis) of enzymes. The tentative conclusion is that the activities observed cannot be reconciled by inspection of the active site participants alone. Instead, individual activities must arise from unique dynamic properties of each enzyme that are tuned to promote specific chemistries.



Siderophores are low-molecular weight iron chelators produced by bacteria, fungi, and plants that scavenge iron from the environment and are frequently required for virulence in pathogenic bacteria.[1,2] The isochorismate synthase (PchA) and isochorismate-pyruvate lyase (PchB) from *Pseudomonas aeurginosa* are involved in the synthesis of the siderophore pyochelin.[3,4] PchA converts chorismate to isochorismate, which PchB in turn uses to produce salicylate by elimination of pyruvate. These enzymes will be used as the basis for a discussion of enzymatically catalyzed pericyclic reactions. It is useful to consider these enzymes in the reverse order of the biosynthetic pathway.

REACTIONS CATALYZED BY PCHB

PchB catalyzes chiefly the pericyclic reaction of Figure 1A. A pericyclic reaction is a concerted one in which a closed, cyclic flow of electrons (curved arrows) leads from bound reactants to bound products. For PchB, the reaction is a concerted but asynchronous [1,5]-sigmatropic shift with a quantitative transfer of hydrogen from C2 to C9 as detected by NMR[5] and confirmed computationally,[6] with an observed k_{cat}/K_m of 4.11×10^4 M^{-1} s^{-1}.[7] Interestingly, PchB can also perform a nonphysiological role as a chorismate mutase (Figure 1B), albeit with considerably lower catalytic efficiency[3] (k_{cat}/K_m = 1.96×10^2 M^{-1} s^{-1}).[7] This reaction, also pericyclic, is a Claisen rearrangement of chorismate to prephenate in which the concerted but asynchronous [3,3]-sigmatropic shift transfers the pyruvate tail from the C3 ether linkage to a C1−C9 linkage.[8] Therefore, PchB catalyzes two pericyclic reactions, which are unusual in biology.

Pericyclic reactions are attractive for study as examples of fundamental enzyme theory because the catalyzed reaction does not require a direct contribution from the enzyme. For example, no protons or electrons are donated or accepted from the enzyme or cofactors, nor are there covalent intermediates. Instead, the binding event leads to organization of the substrate into the transition state, thereby resulting in product formation. However, this also means that pericyclic reactions present problems for the use of many traditional ideas about enzyme catalysis, because the concepts from acid−base, metal ion, and covalent catalysis do not apply. Electrostatic stabilization is also problematic: the reactions are concerted in the sense that there are no intermediate compounds formed, but the cyclic electron distribution of the transition state is still an unsettled issue; i.e., the charge distribution in the transition state may be debatable. Nevertheless, the enzymes that perform these reactions, especially the chorismate mutases, have been studied in detail for the development of fundamental ideas of transition state theory and more generally catalysis.

PCHB IS A STRUCTURAL HOMOLOGUE OF THE AROQ CHORISMATE MUTASES

Sequence similarity (20%) between PchB and the chorismate mutases of the AroQ structural class led to the hypothesis that PchB is a structural homologue of *Escherichia coli* chorismate mutase (EcCM).[3] Three structures of wild-type PchB have been published,[9,10] and these indicate that PchB is indeed a structural homologue of EcCM (Figure 2). In the structure of EcCM, an oxabicyclic transition state analogue is held in place by interaction with a total of eight charged and/or polar amino acids.[11] In PchB, however, only five of those amino acids are conserved, and the decreased number of hydrogen bonds and ionic interactions may lead to known promiscuity of this enzyme and the ability to catalyze both the lyase and mutase reactions (Figure 3).[10] There is a major structural difference between the apo form and the pyruvate-bound or the pyruvate-

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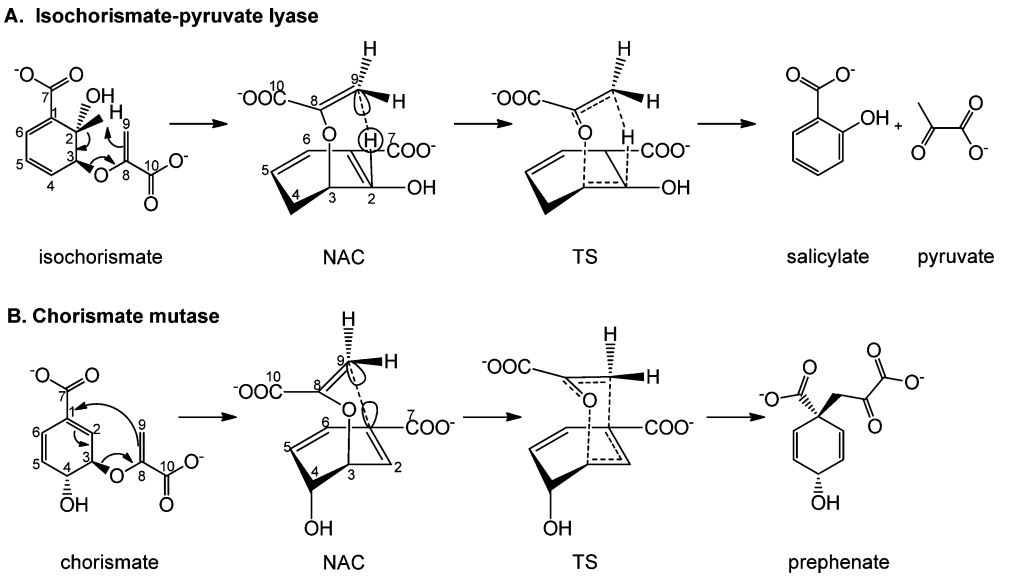

Figure 1. Pericyclic reactions catalyzed by PchB. (A) Isochorismate-pyruvate lyase: transfer of hydrogen from C2 to C9 concerted with pyruvate elimination. (B) Chorismate mutase: formation of C−C bonds between C1 and C9 concerted with fission of C−O bonds between C3 and the ether oxygen.

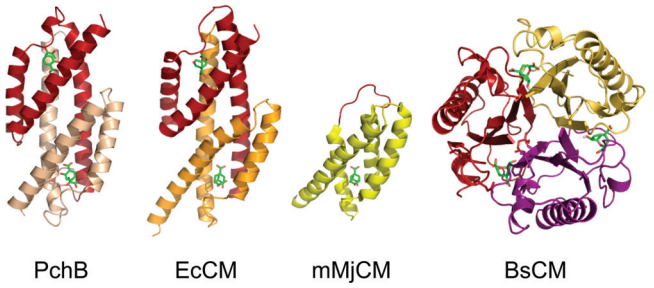

Figure 2. Structures of PchB, *E. coli* chorismate mutase, monomeric *Methanococcus jannaschii* chorismate mutase, and *Bacillus subtilis* chorismate mutase. The PchB dimer is colored red and tan with salicylate and pyruvate colored as green sticks (PDB entry 3REM). The EcCM dimer is colored red and gold with the oxabicyclic transition state analogue (TSA) shown as green sticks (PDB entry 1ECM). The mMjCM monomer is colored yellow with the inserted intrahelical turn colored red and the TSA colored green (PDB entry 2GTV). The BsCM trimer is colored red, yellow, and magenta with the TSA colored green (PDB entry 2CHS).

and salicylate-bound forms of PchB: the active site loop between helix 1 and helix 2 is disordered in the apo structure but fully ordered in the ligand-bound structures. A difference between the open and closed structures is due to a conserved active site lysine (residue 42, green in Figure 3) which hydrogen bonds to a bound pyruvate molecule. These structures may represent an open state for substrate or product entry and egress and a closed catalytic state.[10]

STRUCTURES OF THE CHORISMATE MUTASES

The chorismate mutases of the AroQ class, such as EcCM, comprise an intertwined dimer composed of three helices (Figure 2). The two equivalent active sites per dimer are buried and formed from amino acids from both monomers. In the only EcCM structure determined, a transition state analogue (TSA) is aligned in the active site by interaction of its carboxylates with arginines, one from each monomer.[11] A debate has arisen in the field about how EcCM (k_{cat}/K_m = 2.4 × 10^5 M^{-1} s^{-1})[12] derives its catalytic power, and this debate has consistently cited work on the chorismate mutase from *Bacillus subtilis* (BsCM;

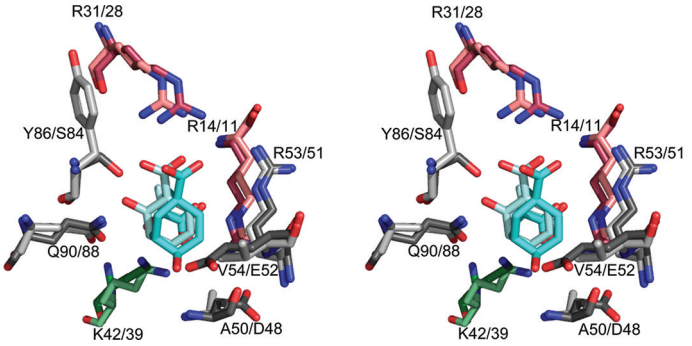

Figure 3. Stereo image comparing the active sites of PchB and EcCM. The arginines that align the carboxylates of the substrate are colored pink, and the lysine hypothesized to be important in the transition state stabilization theory is colored green. The ligands are colored cyan: salicylate and pyruvate in the PchB structure and the TSA in the EcCM structure. In all cases, the lighter shades are PchB and darker shades EcCM. Amino acids are labeled with PchB numbering/EcCM numbering.

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k_{cat}/K_m = 1 × 10^6 M^{-1} s^{-1}).[13] The data derived from EcCM and BsCM are sometimes compared directly even though the proteins are not structurally homologous. BsCM, an AroH chorismate mutase, is a trimer that forms a pseudo-α/β-barrel with active sites at the interfaces between the monomers (Figure 2). The structure of BsCM has been determined in apo, prephenate-bound, and TSA-bound forms;[14−16] however, there is little difference among the active sites of the three structures. In BsCM, the product or TSA is oriented in the active site by arginines comparable to those in the EcCM. Again, the active sites of EcCM and BsCM are usually considered comparable because of their shape and charge complementarity.[17]

Hilvert and colleagues have developed a monomeric AroQ chorismate mutase by inserting an eight-amino acid hinge−loop sequence into helix 1, which allows this helix to fold over to form a shortened coiled coil that is the structural basis of the protein and also allows for completion of the active site.[18−20] This engineered monomeric *Methanococcus jannaschii* chorismate mutase (mMjCM) has a kinetic parameter (k_{cat}/K_m = 1.9 × 10^4 M^{-1} s^{-1}) comparable to those of wild-type MjCM (k_{cat}/K_m = 6.4 × 10^4 M^{-1} s^{-1}) and the EcCM under similar conditions (k_{cat}/K_m = 3.0 × 10^4 M^{-1} s^{-1}),[18] yet mMjCM is a molten globule in the absence of ligand but forms the structure seen in Figure 2 in the presence of the oxabicyclic transition state analogue.[19,20]

CATALYTIC POWER OF CHORISMATE MUTASES

One hypothesis for explaining how chorismate mutases derive their catalytic power is electrostatic transition state stabilization (TSS). Considerable biochemical data support this hypothesis and indicate that a positively charged amino acid (Lys39 in EcCM and Arg90 in BsCM) stabilizes the developing negative charge during bond breaking at the ether oxygen.[8,12,13,21−23] Mutational analysis in EcCM (Lys39) and BsCM (Arg90) has determined this site to be critical for catalysis.[12,13] The conservative change in BsCM of Arg90 to a citrulline (isosteric but neutral) or to lysine (similarly positively charged) decreases the catalytic efficiency by at least 3 orders of magnitude, whereas deletion of the side chain (R90A) led to a complete abolition of activity.[13,23] Similar mutations in EcCM (for example, K39A) led to a decrease in k_{cat}/K_m of 5 orders of magnitude.[12] A critique of the mutagenesis work in the chorismate mutases is that mutagenesis at this lysine in EcCM alters the active site to an extent that the absence or presence of a positively charged amino acid at this site is immaterial.[24] The evidence of this comes from a shoulder at ∼210 nm in the circular dichroism spectra.[12]

A second hypothesis has a basis in quantum mechanical/molecular mechanical molecular dynamics simulations (QM/MM-MD), formation of a near attack conformation (NAC).[24−29] A NAC is defined as a structure in which the reacting atoms are within van der Waals contact distance and approach at an angle ±15° to the bond that is formed, or an orientation in which the π-orbitals overlap (Figure 1).[25,27] While a NAC is a "turnstile" through which reactants pass on the way to the transition state,[27] once a NAC is formed, the reaction occurs spontaneously without further electrostatic stabilization or steric strain.[24−27,29] Interestingly, the mutase reaction and elimination of the enolpyruvyl side chain are both observed in the uncatalyzed reaction.[30,31] However, the elimination reaction is not catalyzed by the chorismate mutase enzymes. Therefore, formation of the NAC for the Claisen rearrangement does not lead to the transition state for the elimination reaction.[27] In other words, in an aqueous environment, it is possible to form a variety of NACs that proceed through different transition states to produce different products. Chorismate mutases limit the type of NACs formed, and catalysis proceeds through a particular transition state to form the desired product. In this model, formation of the reactive conformation of the substrate is the prerequisite for the enzyme to be a catalyst.[24−27,29]

QM/MM calculations at very high levels of QM theory have found that catalysis is due to a combination of transition state stabilization and conformational effects for BsCM.[32] The substrate binds in the active site as a NAC according to all proposed definitions, but formation of this bound conformation is not the sole source of catalytic power. Instead, a combination of binding the reactive conformation and electrostatic transition state stabilization provides the decrease in the energy barrier for catalysis.[33−35] Recent benchmark calculations by Mulholland's group using high-level ab initio QM/MM methods give results in excellent agreement with experiment for the activation enthalpies and free energies of the BsCM reaction.[32]

CATALYSIS BY THE PCHB IS DEPENDENT ON POSITION 42

Whereas it is likely that PchB will perform the mutase reaction using a mechanism similar to that seen for its structural homologue, EcCM, it is unlikely that PchB will use the same mechanism to perform the physiologically important lyase reaction. More importantly, the two reactions must have differing near attack conformations and/or transition states (Figure 1), suggesting differing reaction mechanisms. Mutational analysis of PchB has led to insight into the alternative reaction mechanisms for the two reactions catalyzed by this enzyme.[7] Importantly, mutation of the lysine at position 42 (green in Figure 3) that is comparable to the lysine hypothesized to be important in electrostatic transition state stabilization in EcCM did not lead to structural perturbation of the active site. Evidence of this is provided in the form of circular dichroism data,[7] and also the X-ray structures of the K42A[7] and K42E[9] mutants. However, K42A-PchB showed 1% of WT mutase and lyase activities, whereas the K42E mutant had no detectable activity for either reaction.[7] Furthermore, examination of the effects of a variety of active site variants provides experimental evidence that agrees with the QM/MM calculations that suggest that neither a reactive substrate conformation nor electrostatic transition state stabilization completely accounts for k_{cat} of the rate-determining step for the two catalyzed reactions.[7]

An intriguing result comes from pH profiles of the K42H mutant: k_{cat} is titrated with changing pH, indicating that a positive charge at position 42 is necessary for efficient catalysis. It should be noted that this variant form retains the ability to catalyze the lyase reaction when the side chain is deprotonated (∼100-fold decrease in k_{cat}/K_M), indicating that the enzyme is a sufficient catalyst even without this side chain charge.[9] A calculation of an experimental $\Delta\Delta G^{\ddagger}$ by comparing the mutational effect at low and high pH gives a value of 2.4 kcal/mol for electrostatic transition state stabilization at position 42. The temperature dependence of k_{cat} experiments indicates the difference in $\Delta\Delta G^{\ddagger}$ between the uncatalyzed and enzyme-catalyzed lyase reaction is 7.43 kcal/mol, and there is a large entropic penalty for the transition from the enzyme−substrate

complex to the transition state.[36] One plausible explanation is that loop closing occurs after the initial binding event and may, with the positively charged amino acid at position 42, drive the formation of the transition state. This is in agreement with the pre-steady state kinetic experiments with mMjCM that indicate that conformational ordering occurs on the same time scale as catalysis, suggesting that conformational plasticity is linked to efficient enzymatic catalysis.[19]

PCHB IS UNUSUAL IN SIDEROPHORE BIOSYNTHESIS

Several bacterial species incorporate salicylate into their siderophores (Figure 4). The formation of yersiniabactin by

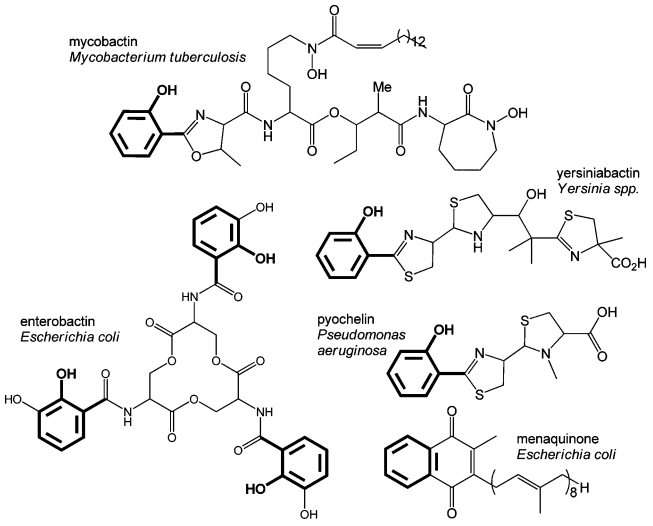

Figure 4. Biosynthetic products. All of the compounds described above require the conversion of chorismate to isochorismate as a part of their biosynthesis. The portion of the compound that is derived from the isochorismate is shown in bold. Mycobactin, yersiniabactin, and pyochelin are salicylate-capped siderophores. Enterobactin is also a siderophore, capped by dihydroxybenzoate. Menaquinone is an electron carrier and a form of vitamin K.

Yersinia enterocolitica and mycobactin by *Mycobacterium tuberculosis* requires the conversion of chorismate to salicylate, which is accomplished by a single enzyme (Irp9 and MbtI, respectively) in a single active site[37−40] (Figure 5). The

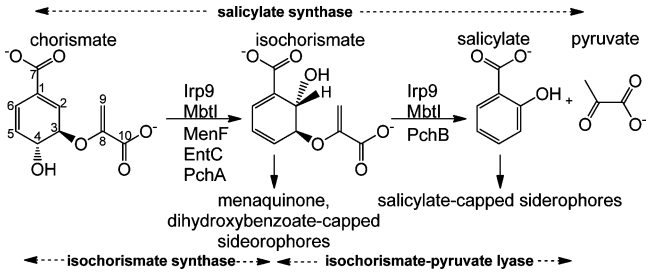

Figure 5. Reactions catalyzed by isochorismate and salicylate synthases.

salicylate synthases convert chorismate to salicylate via the formation of an isochorismate intermediate by general acid−general base catalysis, followed by a pericyclic reaction to generate salicylate, which is analogous to that catalyzed by PchB. The isochorismate synthases from *E. coli* (EntC and MenF) and *Pseudomonas aeruginosa* (PchA) are homologues of the salicylate synthases.[41−43] PchA produces isochorismate for conversion to salicylate by PchB and incorporation into the pyochelin siderophore. EntC is part of the enterobactin biosynthetic pathway, forming a dihydroxybenzoate-capped siderophore,[44,45] and MenF produces isochorismate as the first step in the biosynthesis of the electron carrier menaquinone.[46] Whereas these enzymes are hypothesized to perform analogous general acid−general base chemistry to produce isochorismate as observed for MbtI and Irp9, they do not perform the pericyclic reaction to produce salicylate. This means that PchA, MenF, and EntC cannot perform any pericyclic reactions despite homology to enzymes that can (Irp9 and MbtI). Most intriguingly, the two bifunctional enzymes do not have a PchB-like domain but are able to perform the lyase activity with the structurally and functionally homologous domain found in MenF and EntC.

STRUCTURES OF THE ENZYMES THAT SYNTHESIZE ISOCHORISMATE (MST ENZYMES)

The X-ray crystallographic structures of four enzymes that isomerize chorismate to isochorismate have been determined.[37,38,40−43] Irp9, MbtI, MenF, and EntC are structural homologues (PchA awaits structural determination for confirmation of this hypothesis), with an $\alpha-\beta$ fold first described for the TrpE subdomain of anthranilate synthase (Figure 6).[47] These enzymes belong to a family of enzymes

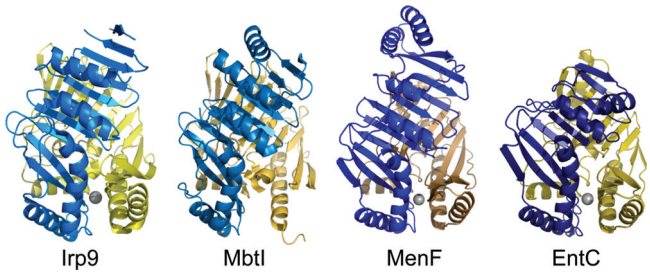

Figure 6. Structures of the salicylate synthases (Irp9 and MbtI) and isochorismate synthases (MenF and EntC). The domains of these monomeric proteins are colored blue and yellow. Mg^{2+} ions, if in determined structures, are shown as gray spheres (PDB entries 2FN1 for Irp9, 2I6Y for MbtI, 2BZM for MenF, and 3HWO for EntC).

involved in menaquinone, siderophore, or tryptophan biosynthesis and collectively have been named the MST family.[40] The catalytic mechanism for isomerization of the chorismate ring has been determined through the cumulative work of several groups by structural and mutational analyses.[38,40,41] A Mg^{2+} ion in the active site orients the C1 carboxyl group of chorismate. A lysine residue serves as a general base for the activation of water to attack at C2, and a glutamic acid is a general acid for elimination of the C4-OH (Table 1 and Figure 7). However, recent work by Ziebart and Toney indicates that activation of water as a nucleophile may not be solely attributable to Lys147 and -205 of EntC and MbtI, respectively, as K → Q mutations retained some activity.[48] The catalytic mechanism for conversion of isochorismate to salicylate by MbtI has been shown to be a sigmatropic, pericyclic mechanism in experiments analogous to those described above for PchB.[40] This reaction is pH-dependent, occurring only at pH ≥7.5.[40]

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Table 1. Function of Active Site Amino Acids in Isochorismate and Salicylate Synthases (residues chosen on the basis of the Irp9 structure[a] with Mg^{+2}, salicylate, and pyruvate bound)

	general base for ICS activity	general acid for ICS activity	chelate Mg^{2+} through H_2O	chelate Mg^{2+}		backbone carbonyl H-bonds to salicylate or isochorismate OH	orients pyruvyl carbonyl	orients pyruvyl carbonyl	chelate Mg^{2+} through H_2O	chelate Mg^{2+}	positive charge for pericyclic IPL activity?
Irp9[a]	K193	E240	E281	E284	H321	T348	Y372	R391	E417	E420	K424
Mbtl[b]	K205	E252	E294	E313	H334	T361	Y385	R405	E431	E434	K438
MenF[c]	K190	E240	N281	E284	H318	A344	Y368	R387	E413	E416	K420
EntC[d]	K147	E197	D238	E241	H276	A303	F327	R347	E373	E376	K380
PchA	K221	E269	D310	E313	H348	A375	Y399	R419	E445	E448	K452

[a]Irp9 data from ref 38. [b]MenF data from refs 41 and 42. [c]Mbtl data from refs 37 and 40. [d]EntC data from ref 43.

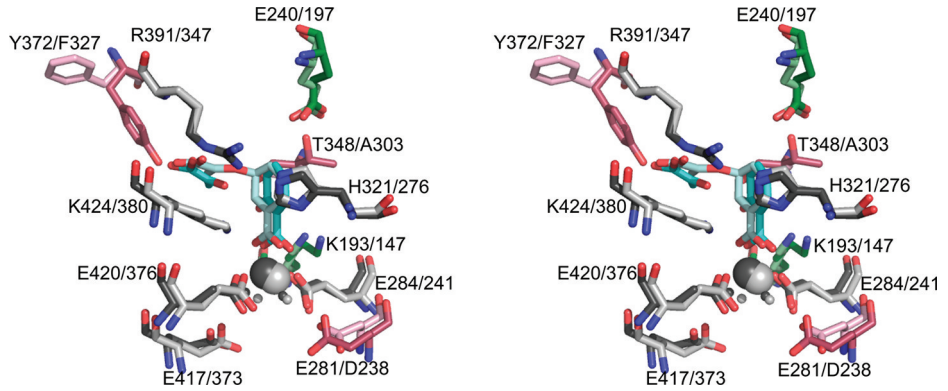

Figure 7. Stereoimage comparing the active sites of Irp9 and EntC. The general acid and general base residues are colored green, and Mg^{2+} is shown as a sphere. Nonconserved residues are colored pink. Ligands are colored cyan: salicylate and pyruvate in the Irp9 structure and isochorismate of the EntC structure. In all cases, the lighter shades are EntC and darker shades Irp9. Amino acids are labeled with Irp9 numbering/EntC numbering.

Chorismate mutase activity has also been detected in MbtI with a k_{cat}/K_m value approximately one-third of that of the salicylate synthase activity, using protein purified in two different ways, using a histidine tag or an intein-chitin tag.[40] In these experiments, whereas salicylate synthase activity is Mg^{2+}-dependent, the chorismate mutase activity was only detected when the Mg^{2+} was not present in the wild-type active site. MbtI has also been purified with a histidine tag and a second ion exchange step to produce a sample that has reduced or no detectable chorismate mutase activity, and the authors suggest that the mutase activity is the result of a contaminant.[48] With the contrary evidence in mind, MbtI may perform two pericyclic reactions. If so, the active site must be altered by the removal of the Mg^{2+} cofactor for the adventitious chorismate mutase catalysis, implying differing binding modes for the chorismate that lead to differing reactive substrate conformations and/or transition states and thus different products.

Presumably, the homologues that are lyase-active salicylate synthases must have structural features that the lyase-deficient isochorismate synthases do not. Whether this would be an amino acid for electrostatic stabilization of the transition state or an active site that selects for or arranges the substrate into a NAC is still an open question. A close examination of the active sites leads to the startling realization that all but three of the amino acids are strictly conserved (Table 1 and Figure 7). First, EntC has a phenylalanine in place of a tyrosine (all other homologues) at one location in the active site (residue 327). Mutation at this site decreased isochorismate synthase activity by more than 1 order of magnitude but did not lead to detectable salicylate production.[43] Second, the backbone carbonyl of T348 in Irp9 H-bonds to the salicylate-OH, and this amino acid is conserved in MbtI. The transition of this amino acid from Thr to Ala is a conserved change for the enzymes that are lyase-deficient (MenF, EntC, and PchA). It has been proposed that this amino acid is responsible for the differences in activities of the two groups of enzymes (lyase-active and lyase-deficient). However, mutation of A to T at this site in both MenF[41] and EntC[43] did not convert these enzymes to lyase-active: neither variant enzyme produced salicylate, and both exhibited significant decreases in their physiological isochorismate synthase activity. Finally, a trend can also be detected between lyase-active and lyase-deficient homologues at a residue that chelates the Mg^{2+} through a water molecule. In Irp9 and MbtI, the lyase-active enzymes, this amino acid is a glutamic acid [E281 and E294, respectively (see Table 1)]. However, the lyase-deficient homologues (MenF, EntC, and PchA) all have an amino acid that is one methylene group shorter, either an aspartic acid (EntC and PchA) or an asparagine (MenF). This site has largely been ignored in the literature, probably because of its distance from the site of chemistry. Nevertheless, as shown in the stereodiagram (Figure 7), this difference in side chain length may cause a shift in the binding of the Mg^{2+} and thus select for the products formed in the active site. It is tempting to propose that this shift may result in the inability to make a reactive substrate conformation in the lyase-deficient enzymes, but this hypothesis awaits testing.

■ ALTERNATE REACTION PATHWAYS AND THE FREE ENERGY LANDSCAPE

In this paper, we have discussed three of the folds that catalyze two pericyclic reactions, and the analysis illustrates that sequence and structural homology do not correlate to catalytic

predictability. (1) Structural homologues with strong sequence conservation at the active site may or may not have pyruvate-lyase activity (the salicylate and isochorismate synthases). (2) Structural homologues with a low degree of conservation of active site residues catalyze one or two pericyclic reactions (*E. coli* chorismate mutase or the isochorismate-pyruvate lyase, respectively). (3) Enzymes with differing folds perform the same chemistry (*E. coli* and *B. subtilis* chorismate mutases). The pericyclic reactions catalyzed in this discussion are single-substrate reactions and require no participation of the enzyme in the form of general acid−general base chemistry, metal ion catalysis (the Mg^{2+} ions of the MST enzymes are structural, orienting the substrate), or a covalent intermediate. Let us assume that the requirements for an active site that efficiently catalyzes one or more pericyclic reactions are the ability to force the substrate into a reactive conformation with the pyruvylenol tail over the ring and the ability to have a strategically placed positive amino acid to stabilize the developing negative charge of the transition state. In other words, the ability of the different enzymes to perform zero, one, or two pericyclic reactions may be conferred by simple positional interaction strategies. However, many changes to the first- and second-tier amino acids of the active sites of these enzymes have been made rationally and by directed evolution with minimal success,[43,49,50] strongly suggesting that simple structural correlations are insufficient to account for the observed chemistries.

The laboratory-evolved mMjCM that orders upon ligand binding[18−20] is suggestive of the idea that catalytic power may be derived from the ability of these enzyme active sites to develop a "catalytic network" as described by Benkovic, Hammes, and Hammes-Schiffer.[51] While these authors were not considering protein folding from a molten globule, they do suggest that catalysis must be considered in the context of protein motion, including large conformational modes such as changes elicited by binding of ligands and vibrational modes on the time scale of catalysis.[51] A three-dimensional free energy landscape with many possible alternate reaction paths that are occupied by multiple sequential intermediates allows for the generally similar yet subtly different active sites to be (or not to be) catalysts. The differing pathways traveled by the substrate−enzyme ensemble along the mountain ranges of the free energy landscape may require differing relative contributions of the NAC or electrostatic transition state stabilization.

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ABBREVIATIONS

AroH, protein family with sequence homology to chorismate mutases with an α/β-barrel fold; AroQ, protein family with sequence homology to chorismate mutases with all α-helical fold; BsCM, *B. subtilis* chorismate mutase; EcCM, *E. coli* chorismate mutase; EntC, isochorismate synthase from *E. coli*; Irp9, salicylate synthase from *Y. enterocolitica*; NAC, near attack conformation; MbtI, salicylate synthase from *M. tuberculosis*; MenF, isochorismate synthase from *E. coli*; mMjCM, laboratory derived monomeric chorismate mutase from *Me. jannaschii*; MST family, protein family containing proteins of menaquinone, siderophore, or tryptophan biosynthesis; PchA, isochorismate synthase from *P. aeruginosa*; PchB, isochorismate-pyruvate lyase from *P. aeruginosa*; PDB, Protein Data Bank; QM/MM-MD, quantum mechanical/molecular mechanical molecular dynamics; TS, transition state; TSA, transition state analogue; TSS, transition state stabilization; ICS, isochorismate synthase; IPL, isochorismate-pyruvate lyase.

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