Insights into the Mechanism of Type I Dehydroquinate Dehydratases from Structures of Reaction Intermediates*

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The biosynthetic shikimate pathway consists of seven enzymes that catalyze sequential reactions to generate chorismate, a critical branch point in the synthesis of the aromatic amino acids. The third enzyme in the pathway, dehydroquinate dehydratase (DHQD), catalyzes the dehydration of 3-dehydroquinate to 3-dehydroshikimate. We present three crystal structures of the type I DHQD from the intestinal pathogens Clostridium difficile and Salmonella enterica. Structures of the enzyme with substrate and covalent pre- and post-dehydration reaction intermediates provide snapshots of successive steps along the type I DHQD-catalyzed reaction coordinate. These structures reveal that the position of the substrate within the active site does not appreciably change upon Schiff base formation. The intermediate state structures reveal a reaction state-dependent behavior of His-143 in which the residue adopts a conformation proximal to the site of catalytic dehydration only when the leaving group is present. We speculate that His-143 is likely to assume differing catalytic roles in each of its observed conformations. One conformation of His-143 positions the residue for the formation/hydrolysis of the covalent Schiff base intermediates, whereas the other conformation positions the residue for a role in the catalytic dehydration event. The fact that the shikimate pathway is absent from humans makes the enzymes of the pathway potential targets for the development of non-toxic antimicrobials. The structures and mechanistic insight presented here may inform the design of type I DHQD enzyme inhibitors.

Present in bacteria, fungi, and plants but absent in higher eukaryotes, the seven enzymes of the shikimate pathway catalyze sequential reactions to generate chorismate. Chorismate serves as a precursor of many biologically important aromatic compounds including the ubiquinones, folates, and aromatic amino acids (1). The essential nature of these proteins in a number of species, in combination with an absence of human homologs, makes the shikimate pathway an attractive target for the development of non-toxic antimicrobials, anti-fungals, and herbicides (2–4). Given the complexities inherent to inhibitor design, a comprehensive understanding of the structural and mechanistic framework underlying the function of the shikimate pathway enzymes should aid in the development of novel shikimate-targeting inhibitors.

The third step in the shikimate pathway, consisting of the dehydration of dehydroquinatinate to dehydroshikimate (Fig. 1), can be catalyzed by two unrelated enzymes, termed type I and type II dehydroquinatinate dehydratases (DHQDs). These two enzyme families lack sequence or structural homology and employ distinct reaction mechanisms (5–10). The type I DHQDs utilize a covalent Schiff base (imine) intermediate that results in a cis-elimination, whereas the type II reaction lacks a covalent intermediate and undergoes a trans-elimination (5–10). The phylogenetic distribution of the two enzyme types is somewhat disorderly, with closely related species often possessing different types. In general, the type I enzyme is found in plants and fungi as a domain within a multifunctional protein and in some bacteria as an ~29-kDa monofunctional protein that assembles into a homo-dimer. In contrast to the type I DHQD, the type II enzyme is found within a mostly non-overlapping subset of bacteria and exists as an ~17-kDa protein that assembles into a homo-dodecamer (8, 10–12).

Previously reported structures of the Salmonella typhi, Staphylococcus aureus, and Archaeoglobus fulgidus type I DHQD have characterized the enzyme in an apo and covalently bound post-dehydration intermediate state (8, 13–15). Here we present crystal structures of the type I DHQD from the two intestinal pathogens, the Gram-positive Clostridium difficile (cdDHQD) and Gram-negative Salmonella enterica (seDHQD), and characterize previously unobserved substrate and pre-dehydration reaction intermediate bound states of the enzyme. The similar mode of substrate and reaction intermediate binding and the reaction state-dependent behavior of a conserved active site histidine are identified, and their functional implications are discussed.

**EXPERIMENTAL PROCEDURES**

Cloning, Protein Overexpression, and Purification—Following published protocols (16), seDHQD and cdDHQD genes
Multiple Roles for Active Site Histidine in Type I DHQDs

from *C. difficile* strain 630 and *S. enterica* subspecies *enterica* serovar *typhimurium* strain LT2 genomic DNA were PCR-amplified and cloned into pMCSG7 and pMCSG19 vectors, respectively, each of which contains N-terminal hexahistidine tag followed by a tobacco etch virus protease cleavage sequence. Insert-containing plasmids were transformed into *Escherichia coli* BL21 (ADE3) strain from Agilent (Santa Clara, CA). Protein expression and purification were performed using standard Center for Structural Genomics of Infectious Diseases protocols (17, 18). Crystallization screens were set up immediately following purification, and the remaining enzyme was aliquoted, frozen in liquid nitrogen, and stored at −80 °C for future use.

**DHQD Assays**—Immediately before performing assays, protein was thawed and diluted to the appropriate concentration. Assays were performed at 37 °C in potassium phosphate buffer (100 mM, pH 7.5). Reaction was initiated by the addition of protein to a mixture of buffer and 3-dehydroquinic acid (Sigma-Aldrich). Formation of the conjugated enone carboxylate in dehydroshikimate was followed by measurement of protein to a mixture of buffer and 3-dehydroquinic acid. Data were fitted to the Michaelis-Menten equation using the enzyme kinetics module in SigmaPlot version 8.02.

**Protein Crystallization and Data Collection**—Protein concentrated to 7.5 mg/ml in a buffer containing 0.5 M NaCl and 10 mM Tris-HCl (pH 8.3) was used to set up sitting drops at a ratio of 1:1 protein to reservoir. Further detail regarding crystallization conditions is presented in Table 1. The substrate-bound structure was obtained by co-crystallization of the Lys-170→Met (K170M) mutant protein with 2 mM dehydroquinic acid. The pre-dehydration reaction intermediate bound structure was obtained by soaking a crystal in mother liquor containing 5 mM dehydroquinic acid for 15 min prior to freezing in liquid nitrogen. The post-dehydration reaction intermediate bound structure was obtained by co-crystallization with 1 mM dehydroshikimic acid. All crystals where immersed in mother liquor before being frozen in liquid nitrogen. Diffraction data were collected at 100° K at the Life Sciences Collaborative Access Team at the Advanced Photon Source, Argonne, IL.

**Structure Determination and Refinement**—Data were processed using HKL-3000 for indexing, integration, and scaling (20). Structures were solved with Phaser (21) using the *S. typhi* apo DHQD structure (Protein Data Bank (PDB) code 1GQN) as a starting model for the cdDHQD structure and the apo seDHQD structure (PDB code 3L2I) for determination of seDHQD structures. Structures were refined with Refmac (22) and manually corrected based on electron density maps displayed in Coot (23). All figures were prepared in the PyMOL Molecular Graphics System, Version 1.3 (Schrödinger, LLC). Atomic coordinates and structure factors were deposited in the PDB under codes 3M7W (pre-dehydration complex), 3NNT (K170M substrate complex), and 3JS3 (post-dehydration complex).

**RESULTS AND DISCUSSION**

*C. difficile* and *S. enterica* DHQDs Display a Similar Overall Structure—The cdDHQD and seDHQD share 56% sequence identity (Fig. 2A). Structures of the reaction intermediate bound seDHQD and cdDHQD protein were solved by molecular replacement and refined to a resolution of 1.95 and 2.20 Å, respectively. The DHQD monomer from each bacterium is characterized by an eight-stranded β-barrel motif and is observed to dimerize with helices 6, 7, 8, and 9, making anti-parallel intermolecular contacts, in a manner similar to previously reported DHQDs (8, 13–15). The overlay of cdDHQD and seDHQD shows a high degree of structural conservation (Fig. 2B).

Differing Side Chain Conformation of His-143 in Pre- and Post-dehydration States—To gain insight into the reaction mechanism of DHQD, we obtained structures of DHQD complexed with ligand. A soak of seDHQD crystals with the substrate, 3-dehydroquinic acid, produced a structure in which the substrate covalently linked at its 3-position to the Schiff base-forming Lys-170 was observed (Fig. 3, A and B). The bound ligand displays clear electron density corresponding to the 1-hydroxyl leaving group (Fig. 3A, arrow), and therefore this structure represents a pre-dehydration intermediate state of the reaction. Co-crystallization of the cdDHQD with the product, 3-dehydroshikimic acid, also produced a crystal structure in which a Lys-170⁵⁴⁸-bound covalent species is observed (Fig. 3, C and D). In this case, the bound ligand lacks electron density corresponding to the leaving group. Interestingly, an ordered water molecule is located directly above where the leaving group was observed in the pre-dehydration ligand (Fig. 3C, arrow) and is thus positioned to be the molecule dehydrated from the substrate. This structure, which is similar to previously reported type I DHQD intermediate complexes (8, 14), represents a post-dehydration intermediate state of the reaction. Together, these structures provide snapshots of successive steps in the reaction and thus allow for the identification of structural changes that occur over the course of the reaction.

A particularly striking reaction state-dependent structural behavior is revealed by an overlay of the pre- and post-dehydration reaction intermediate bound active sites. In the pre-
Multiple Roles for Active Site Histidine in Type I DHQDs

**TABLE 1**

| Species | Variant | PDB code | Color in figures | Active site ligand | Crystallization conditions | Space group | Unit cell dimensions | Completeness (%) | Resolution (Å) | I/|H9268 | Ligand | Water | Protein |
|---------|---------|----------|-----------------|-------------------|--------------------------|------------|---------------------|----------------|--------------|---------|--------|--------|--------|---------|
| S. enterica | Wild type | 3NNT | Cyan | Pre-dehydration covalent intermediate | 170 mM NH4OAc, pH 4.6, 25.5% (w/v) PEG 4000, 15% (v/v) glycerol | P1 | a = 184.33, b = 66.58, c = 128.23 | 99.9% | 29.69–1.95 (2.00–1.95) | 14.9 (2.8) | 14.5 | 35.9 | 11646 |
| C. difficile | Wild type | 3JS3 | Pink | Post-dehydration covalent intermediate | 100 mM Tris, pH 8.5, 30% (w/v) PEG 500 | P2 | a = 60.47, b = 139.62, c = 66.77 | 99.1% | 29.55–2.20 (2.26–2.20) | 3.8 (3.8) | 2 | 5.0 | 37.4 |

*asu, asymmetric unit.

*a.r.m.s., root mean square.

Dehydration complex, His-143 is proximal to both elements of the catalytic dehydration. The His-143 N\(^{\text{e2}}\) atom is 2.7 Å from the modeled pro-R C-2 hydrogen of the substrate and forms a 2.8 Å hydrogen bond with the 1-hydroxyl leaving group (Fig. 4A). In the post-dehydration covalent complex, the histidine side chain is displaced ~1.5 Å away from the site of catalytic dehydration to the position it adopts in all previously reported apo and post-dehydration reaction intermediate DHQD structures (8, 13–15). In this position, the His-143 N\(^{\text{e2}}\) atom forms a 2.9 Å hydrogen bond with the conserved residue Glu-86 (Fig. 4B).

To address the possibility that the displacement of His-143 observed in the pre-dehydration reaction intermediate bound structure is a non-biologically relevant artifact of the low pH of 4.6 of the crystallization condition, we used a similar crystal soaking protocol to test a crystal grown at neutral pH. A comparable behavior of His-143 was observed in this neutral pH crystal structure (data not shown), suggesting that the conformation of His-143 seen in the pre-dehydration structure is representative of the behavior of the residue over a wide range of physiological pH values.

Having ruled out pH in explaining the displacement of His-143, the only difference between the pre- and post-dehydration reaction intermediate bound structures is the presence of the leaving group in the pre-dehydration ligand. As such, the differential behavior of His-143 between the two structures can be attributed to the presence of the leaving group in the pre-dehydration reaction state. In particular, induction of the pre-dehydration conformation of His-143 is likely due to the potential for formation of a favorable hydrogen-bonding interaction between the N\(^{\text{e2}}\) atom of the histidine and the leaving hydroxyl group (Fig. 4). In the apo and post-dehydration reaction states, without the potential for His-143 to hydrogen-bond with the leaving group, adoption of the His-143 post-dehydration conformation is likely due to the potential for the N\(^{\text{e1}}\) atom of the histidine to hydrogen-bond with Glu-86 in this position. These structures define a never before characterized leaving group-dependent conformation of His-143 in which the residue is proximal to the site of dehydration only when the leaving group is present.

Intermediate State Crystal Structures Suggest Role for His-143 in Catalysis—Several lines of experimental evidence suggest that this conserved active site histidine may act to shuttle the proton from the C-2 position in the ring to the 1-hydroxyl leaving group. Hanson and Rose (9) used stereo-specific labeling experiments to demonstrate that type I dehydroquinate dehydrogenation results in cis-elimination. That is, the abstracted proton comes from the same side of the ring as the leaving group. This finding is consistent with a single residue both abstracting the proton from the ring and protonating the leaving group (9). Based on diethyl polycarbonate treatment resulting in an inactivation of the protein, Deka et al. (24) hypothesized that the conserved histidine might be the proton-transferring residue. Mutagenesis studies might be expected to conclusively address the role of this residue in proton shuttling. Although Leech et al. (25) showed that the E. coli His-143 → Ala (H143A) mutant had a profound loss of activity, potentially supportive of this histidine acting as the proton-shuttling entity, it was unclear whether or not the effect of the mutation was due solely to the role of this residue in Schiff base formation and hydrolysis. Based on the body of kinetic evidence, the proximity of the pre-dehydration conformation...
of His-143 to both the pro-\(R\) C-2 proton and the 1-hydroxyl leaving group, and the absence of another feasible proton-transferring residue, we propose a reaction mechanism consistent with previously hypothesized models (25–28) but in which His-143 catalyzes the dehydration event by its N\(^{\text{ε2}}\) atom abstracting the pro-\(R\) C-2 proton and donating it to the 1-hydroxyl leaving group (Fig. 5A).

Reaction State-dependent His-143 Behavior Suggests Conformation-dependent Catalytic Roles for the Residue—In addition to its role in the catalytic dehydration event, His-143 has a well established role in the formation and hydrolysis of the Schiff base intermediates. Leech et al. (25) found that the H143A mutant had a profoundly deficient reaction rate. They observed that the mutant enzyme slowly accumulated co-

FIGURE 2. Sequence and structure similarity between the cdDHQD and seDHQD type I DHQDs. A, sequence alignment of cdDHQD and seDHQD. Alignment was done in ClustalW2 version 2 using the default settings. Secondary features were defined using the cdDHQD structure in ESPript version 2.2. B, superposition of the biological dimer of the seDHQD pre-dehydration complex (cyan) and the cdDHQD post-dehydration complex (pink) structures (backbone root mean square deviation = 1.16 Å). Lys-170 and the reaction intermediates to which it is covalently bound are shown as sticks. Helices of the dimer interface are labeled.
valently bound reaction intermediate at the active site and interpreted this result to mean that the mutant enzyme has a diminished capacity both to form and later to hydrolyze the covalent Schiff base. Based on these findings, Leech et al. (25) proposed a model in which His-143 acts to facilitate the protonation or deprotonation of the carbinolamine reaction intermediate depending upon whether the Schiff base is being formed or hydrolyzed (Fig. 5B).

Considering that adoption of the His-143 pre-dehydration conformation appears to be leaving group-dependent, the hydrolysis of the Schiff base, which occurs post-dehydration, must initiate with His-143, adopting its post-dehydration conformation. As such, His-143 would appear to assume differing functionalities in each of its conformations. In its pre-dehydration conformation, the residue catalyzes the catalytic dehydration event (Fig. 5A), whereas in its post-dehydration conformation, the residue catalyzes Schiff base hydrolysis (Fig. 5B). Although the conformation His-143 adopts when it catalyzes Schiff base formation cannot be inferred from these structures, clearly, His-143 plays a complex and highly tuned role in which it shifts between two conformations to catalyze multiple aspects of the type I DHQD reaction coordinate.

Similar Positioning of Substrate and Reaction Intermediates within the Active Site Supports a Catalytic Role for the Schiff Base—Stereoelectronic principles dictate that the Lys-170 amine nucleophile will form the covalent Schiff base after approaching the carbonyl carbon of the substrate at the Bürgi-Dunitz (N=C\(\rightarrow\)O) angle of \(\sim 107^\circ\) (29, 30). According to this model of bond formation, the position of the Lys-170 amine relative to the 3-carbon of the substrate should differ markedly during its approach to the carbonyl when compared with after the Schiff base has formed. To meet these positional requirements, either Lys-170 or the substrate must undergo substantial movement as the substrate transitions from a non-covalent to Schiff base bound state. The simplest mechanism by which the enzyme might accommodate both the Bürgi-Dunitz approach and the corresponding active site rearrangements would be for the substrate to initially dock in a conformation that

**FIGURE 3.** Crystal structures of DHQD in pre- and post-dehydration covalent intermediate states. A, structure of seDHQD active site with covalent pre-dehydration reaction intermediate. Carbon atoms are depicted in cyan, oxygens are in red, nitrogens are in blue, and sulfurs are in yellow. Difference maps were calculated with the reaction intermediate omitted from the model. The \(F_o - F_c\) map is contoured at the 3.0σ level (red), and the \(2F_o - F_c\) map is contoured at the 1σ level (blue). An arrow indicates the 1-hydroxyl leaving group. B, schematic rendering of the pre-dehydration reaction intermediate shown in A. Distances between atoms are shown in angstroms. C, structure of the cdDHQD active site with covalent post-dehydration reaction intermediate. Model and maps are the same as A, except that carbons are shown in pink. The arrow points toward an ordered water molecule at a position consistent with that dehydrated from the substrate. D, schematic rendering of the post-dehydration reaction intermediate shown in C. Distances between atoms are shown in angstroms.
allows for a Bürgi-Dunitz approach before moving to the intermediate bound position in a process concurrent with bond formation. Whether this mode of substrate nucleophile approach is specific to these enzymes or is general to the Schiff base-forming enzymes is presently unknown. Based on the severe effect of the Lys-170 → Ala mutation on $k_{cat}$ but minimal effect on $K_m$, it has been argued that the Schiff base is likely to play a direct catalytic role in the reaction mechanism of DHQD (25). Suggestions on the mechanism by which the Schiff base might promote catalysis have focused on how it could distort the ring of the substrate in a manner that would stereoelectronically promote elimination or function to stabilize the carbanion reaction intermediate (25, 33, 34). However, in light of the stereoelectronic argumentation and experimental data describing substrate binding in other Schiff base-forming enzymes, in DHQD, it is also likely that prior to Schiff base formation, the substrate adopts an orientation that is different from the covalent reaction intermediate within the active site. In that case, it is conceivable that Schiff base formation is required to position the substrate in the proper orientation in which for catalysis to occur, presenting a scenario wherein the Schiff base plays only an indirect role in catalysis.

To gain insight into the initial substrate binding event within Schiff base-forming enzymes generally and to determine whether the Schiff base is directly involved in DHQD catalysis, we set out to capture a complex of DHQD with substrate. We reasoned that if the non-covalently bound substrate was similarly positioned relative to the covalently bound reaction intermediate, then formation of the Schiff base must not result in significant change in the position of the substrate within the active site and therefore cannot be promoting catalysis by positioning the substrate into the catalytically required orientation. In that case, having ruled out the proposed non-catalytic (orientation-determining) role of the Schiff base, it could be concluded that the Schiff base is likely to have only a direct involvement in catalysis.

To obtain a crystal structure of seDHQD in a non-covalent complex with substrate, site-directed mutagenesis was utilized to convert the reactive Schiff base-forming Lys-170 → Met (K170M). Methionine was chosen because it is the residue closest in shape to a lysine but cannot form a Schiff base. As previously reported (25), mutation of Lys-170 resulted in a dramatic loss in reactivity (Table 2). Co-crystallization of the K170M mutant enzyme with dehydroquinic acid produced a structure in which the substrate was observed non-covalently bound at the active site (Fig. 6A). A comparison of the pre-dehydration intermediate bound structure and K170M substrate-bound structure shows the ring core of the substrate to be similarly positioned but slightly shifted (~0.3 Å) within the active site, likely to avoid a clash with the Met-170 terminal methyl group (Fig. 6B). The similar orientation of substrate and reaction intermediate demonstrates a differential mode of substrate binding when compared with other Schiff base-forming enzymes. The dispensability of Lys-170 in substrate binding, as reasoned above, provides support for the Schiff base playing a direct catalytic role in the reaction mechanism of the protein.

$K_{170M}$ Substrate-bound Structure Provides Insight but Also Generates New Questions Regarding Schiff Base Formation—Although a previous study has shown a role for His-143 in Schiff base formation (25), the mechanism of His-143 involvement remains ill-defined. Based on mutagenesis studies, Leech et al. (25) proposed that His-143 catalyzes the conversion of the carbinolamine intermediate to the Schiff base intermediate (Fig. 5B). However, it is possible that His-143 affects other steps in Schiff base formation. The K170M substrate-bound structure reveals that His-143 adopts partial occupancies of both of the conformations observed in the wild type structures (Fig. 6A). Both positions of His-143 are proximal to the 3-carbonyl oxygen of the substrate (Fig. 6C). This structural observation positions the residue to protonate the carbonyl oxygen and/or to accentuate the dipole of the carbonyl by drawing charge away from the carbonyl carbon, which might promote the Lys-170 nucleophilic attack. The proximity of His-143 to the carbonyl oxygen prior to the formation of the covalent intermediate suggests that the residue may play a functional role in an earlier step of Schiff base formation than has previously been recognized.
Although providing new insight into the significance of the Schiff base in catalysis, the K170M substrate-bound structure presents new questions regarding how formation of the Schiff base occurs. The observed position of the substrate is inconsistent with the Lys-170 N\(^+\) atom approaching the carbonyl carbon of the substrate at an ideal stereoelectronic angle. Modeling Lys-170 into the K170M substrate-bound structure reveals that, without significant perturbations to the main chain, the maximal approach angle that the Lys-170 amine can achieve falls well short of the \(\sim 107°\) Bürgi-Dunitz angle (Fig. 6D). As such, the bond must form either by a non-Bürgi-Dunitz approach of the substrate in its observed position or by a Bürgi-Dunitz approach of the substrate in an unobserved position within the active site.

Possible exceptions to the Bürgi-Dunitz nucleophilic approach in the context of enzyme catalysis are not without precedent (35). In conjunction with previous observations, the mode of substrate binding observed here potentially supports a more widespread exception to the established mode of nucleophilic approach in enzyme catalysis. Alternatively, the approach of Lys-170 and formation of the covalent bond may occur when the substrate transiently adopts a conformation that places its carbonyl carbon at the Bürgi-Dunitz angle; this requirement may be met during the process of substrate docking into the active site. In that case, in the wild type enzyme, the covalent adduct may form before the substrate reaches its observed position in the K170M substrate-bound structure. The mechanism of Schiff base formation is the topic of ongoing study.

**Conclusions**—We report three crystal structures that address several issues about how type I DHQDs function. The substrate and pre-dehydration covalent intermediate bound structures provide the first view of these reaction states. The different conformation of His-143 in pre- and post-dehydration intermediate states defines a leaving group-dependent behavior of the residue. The proximity of His-143 to the C-2 proton and leaving group of the pre-dehydration reaction intermediate supports a role for this residue in the transport of the proton to the leaving group in the catalytic elimination and provides a rare example of the requirement of the leaving group for a residue to adopt the conformation consistent with its presumed catalytic role. Previous kinetic studies and the structural data presented here suggest a reaction mechanism in which His-143 moves between two conformations while undergoing a series of protonation/deprotonation events to

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**TABLE 2**

**Kinetic characterization of seDHQD and cdDHQD**

|               | \(k_{cat}\) | \(K_m\) | \(k_{cat}/K_m\) |
|---------------|------------|---------|-----------------|
| seDHQD wild type | \(210 \pm 5\) | \(21 \pm 3\) | \(10 \pm 1\) |
| seDHQD K170M | \(0.015 \pm 0.007\) | \(33 \pm 4\) | \(4.5 \times 10^{-4} \pm 2.3 \times 10^{-4}\) |
| cdDHQD wild type | \(125 \pm 4\) | \(36 \pm 9\) | \(3.5 \pm 0.9\) |

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**FIGURE 5. Proposed role of His-143 in type I DHQD-catalyzed reaction.** A, putative role of His-143 in the catalytic dehydration. Following formation of the covalent Schiff base linker between Lys-170 and the substrate 3-dehydroquinate 1, His-143 assumes its pre-dehydration position, where its N\(^+\) atom forms a key hydrogen-bonding interaction with the 1-hydroxy group of the reaction intermediate. In this position, the His-143 N\(^+\) atom abstracts the C-2 pro-\(R\) proton of the substrate to generate the carbanion intermediate 2, which gives rise to the enamine intermediate 3. The protonated His-143 then delivers its N\(^+\) proton to facilitate departure of the 1-hydroxyl group, which generates the ene-iminium intermediate 4. Because the H143 N\(^+\) atom can no longer form the critical interaction with the 1-hydroxyl leaving group, a shift to the post-dehydration position of the residue ensues. Finally, following Schiff base hydrolysis, the formally dehydrated product 3-dehydroshikimate is released. Boxed intermediates 1 and 4 represent likely states of the reaction captured by pre- and post-dehydration crystal structures. B, role of His-143 in Schiff base formation and hydrolysis based on Leech et al. (25). In the formation of the Schiff base, attack by the Lys-170 N\(^+\) atom on the 3-carbonyl carbon leads to formation of the carbaminolamine intermediate. His-143 then delivers a proton to facilitate departure of the hydroxyl leaving group to generate the Schiff base intermediate. Following the catalytic hydrolysis described in A, His-143 adopts its post-dehydration conformation and catalyzes the reverse reaction to hydrolyze the Schiff base and regenerate the active site.
catalyze multiple steps in the formation and hydrolysis of the Schiff base as well as the catalytic dehydration.

The K170M substrate-bound structure provides insight into the role of the Schiff base in catalysis. The similar mode of binding of substrate in the K170M variant and reaction intermediate in the wild type enzyme eliminates the possibility that the functional role of the Schiff base is to orient the substrate within the active site. Based on these results, we conclude that the Schiff base must have a direct role in catalysis. It is anticipated that the more detailed knowledge of the DHQD kinetic pathway provided by this work will aid in the design of novel anti-bacterials, which are particularly relevant for the emerging pathogens C. difficile and S. enterica.

Acknowledgments—We thank Dr. Elisabetta Sabini for facilitating the communications with the groups involved in this work and Dr. Scott Peterson and Dr. Keehwan Kwon for providing DHQD expression clones. Use of the Advanced Photon Source at Argonne National Laboratory was supported by the U. S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract DE-AC02-06CH11357. Use of the Life Sciences Collaborative Access Team (LS-CAT) Sector 21 was supported in part by the Michigan Economic Development Corporation and the Michigan Technology Tri-Corridor for the support of this research program (Grant 08SP1000817).

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