Structure of the Bacterial Deacetylase LpxC Bound to the Nucleotide Reaction Product Reveals Mechanisms of Oxyanion Stabilization and Proton Transfer

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**Background:** LpxC is a metal-dependent deacetylase essential for lipopolysaccharide biosynthesis.

**Results:** The LpxC reaction product binds an extensive, conserved groove with the 2-amino group positioned in the active site.

**Conclusion:** The product-bound LpxC structure reveals conserved ligand interactions and stabilization of a phosphate mimic of the oxyanion intermediate.

**Significance:** LpxC structures are critical to elucidate the catalytic mechanism and design of novel antibiotics.

The emergence of antibiotic-resistant strains of pathogenic bacteria is an increasing threat to global health that underscores an urgent need for an expanded antibacterial armamentarium. Gram-negative bacteria, such as *Escherichia coli*, have become increasingly important clinical pathogens with limited treatment options. This is due in part to their lipopolysaccharide (LPS) outer membrane components, which dually serve as endotoxins while also protecting Gram-negative bacteria from antibiotic entry. The LpxC enzyme catalyzes the committed step of LPS biosynthesis, making LpxC a promising target for new antibacterials. Here, we present the first structure of an LpxC enzyme in complex with the deacetylation reaction product, UDP-(3-O-(R-3-hydroxymyristoyl))-glucosamine. These studies provide valuable insight into recognition of substrates and products by LpxC and a platform for structure-guided drug discovery of broad spectrum Gram-negative antibiotics.

The increased prevalence of pathogenic bacteria with resistance to clinically useful antibiotics is a growing threat to public health. Despite this urgent, unmet medical need, few novel classes of antibiotics and antibiotic leads, e.g. linezolid, daptomycin, and platensimycin, have been discovered in the last 30 years (1–3). Antibiotic-resistant Gram-negative bacteria are of growing medical significance and consist of pathogens such as *Acinetobacter baumannii*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Neisseria meningitidis*, and *Pseudomonas aeruginosa*. *E. coli* and *P. aeruginosa* are commonly found in hospital-acquired infections such as pneumonia and sepsis.

Pathogenicity of Gram-negative bacteria derives in part from components of their cellular membrane. One component, lipopolysaccharide (LPS), constitutes a formidable barrier that protects Gram-negative bacteria from chemicals and antibiotics. The lipid A component of LPS is a potent immunogen that promotes a fatal hyperimmune response in infected hosts. Because LPS is essential for survival of many Gram-negative bacteria (4, 5), the LPS biosynthetic pathway represents an intriguing target for next generation antibiotics.

LPS has three structural components as follows: lipid A, O-antigen, and core oligosaccharide. Lipid A consists of fatty acids linked to a phosphorylated glucosamine disaccharide that anchors LPS to the outer membrane. Lipid A biosynthesis is accomplished by nine enzymes situated at the cytoplasmic face of the inner membrane (4). The enzyme catalyzing the committed step of lipid A biosynthesis is LpxC, a metal-dependent deacetylase that removes the acetyl group from the 2-amino group of the inner amino acid in lipid A. The enzyme catalyzing the committed step of lipid A biosynthesis is LpxC, a metal-dependent deacetylase that removes the acetyl group from the 2-amino group of the inner amino acid in lipid A. In contrast to lipid A, LPS can be produced by a variety of Gram-negative bacteria (4, 5), and the LPS biosynthetic pathway represents an intriguing target for next generation antibiotics.

LpxC is a validated target for small molecule antibacterial agents (9, 10). Hydroxamates, exemplified by BB-78485 and Chir-90 (Fig. 1B), bind and inhibit LpxC with nanomolar potency in vitro and exhibit antibacterial activity in vivo (11–13). Despite these features, hydroxamate groups confer relatively nonspecific metal binding that could limit clinical utility (14). Historically, hydroxamate-containing molecules have shown poor pharmacokinetic properties and the potential for adverse events (10, 15–17). Alternatives to the hydroxamate class are therefore desirable.

Crystallographic and NMR structures have been reported for LpxC from a number of species, including *Aquifex aeolicus*, *Aquifex aeolicus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The structures of LpxC from these species represent a new class of antibacterial targets.

The atomic coordinates and structure factors (code 4MDT) have been deposited in the Protein Data Bank (http://wwpdb.org/).

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[^3]: The abbreviations used are: myr-UDP-GlcNAc, UDP-(3-O-(R-3-hydroxymyristoyl))-N-acetylglucosamine; myr-UDP-GlcN, UDP-(3-O-(R-3-hydroxymyristoyl))-glucosamine; PDB, Protein Data Bank; CAPS, 3-(cyclohexylamino)propanesulfonic acid; IMCA, CAT, Industrial Macromolecular Crystallography Association Collaborative Access Team.
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*P. aeruginosa, E. coli,* and *Yersinia enterocolitica* (12, 18–30). These structures have captured the enzyme bound to a variety of small molecule ligands, including (i) isolated components and analogs of the myr-UDP-GlcNAc substrate, (ii) hydroxamate-based inhibitors, and (iii) other small molecule ligands such as imidazole and cacodylate (12, 18–30). These studies offer structural and mechanistic insight that can assist in the development of LpxC-targeted antibiotics. Despite these advances, structural information is currently lacking for LpxC bound to a natural substrate or product.

Here, we present the crystal structure of LpxC in complex with myr-UDP-GlcN, the natural product of the *in vivo* deacetylation reaction. The structure reveals key interactions with all four segments of the product as follows: uridine, pyrophosphate, glucosamine, and myristate. In addition, we identified an unexpected phosphate anion serendipitously coordinated to the catalytic Zn$^{2+}$/H11001 and the 2-amino leaving group. The bound phosphate is stabilized by an extensive network of hydrogen bonds to residues previously implicated in catalysis, suggesting it may approximate the tetrahedral oxyanion of the transition state. These analyses have mechanistic implications and suggest routes to obtain broad spectrum LpxC agents beyond the known hydroxamate classes.

**EXPERIMENTAL PROCEDURES**

**Protein Purification and Crystallization—** *E. coli* LpxC was cloned and purified as described previously (31) with the exception of a C125S mutation. Protein was concentrated in 20 mM Hepes, pH 7.0, 50 mM NaCl, and 0.5 mM zinc sulfate to 12 mg/ml (0.35 mM), as determined by absorbance at 280 nm using a calculated extinction coefficient of 22,920 M$^{-1}$ cm$^{-1}$. Crystals were grown by hanging-drop vapor diffusion with a reservoir solution of 0.4 M NaH$_2$PO$_4$, 0.8 M K$_2$H PO$_4$, 0.2 M CAPS, pH 10.5, 50 mM Li$_2$SO$_4$ at 293 K and appeared after 3 days. Crystals were cryo-protected in mother liquor supplemented with 20% ethylene glycol. Neither myr-UDP-GlcNAc nor myr-UDP-GlcN was added during purification and crystallization.

**TABLE 1**

| Data collection and refinement statistics | E. coli LpxC |
|-----------------------------------------|--------------|
| Data collection                         | C2           |
| Space group                             | C2           |
| Cell dimensions                         | a, b, c (Å)  |
|                                         | 168.97, 103.52, 103.97 |
|                                         | 90, 103.96, 90 |
| Resolution (Å)                          | 50 to 2.59   |
|                                         | (2.73 to 2.59) |
| R$_{int}$ or R$_{merge}$                 | 4.3 (42.4)   |
| I/σI                                     | 17.2 (2.3)   |
| Completeness (%)                        | 96 (79.3)    |
| Redundancy                               | 3.6 (2.8)    |
| Resolution range (Å)                    | 50 to 2.6    |
| No. of reflections                      | 51,830       |
| R$_{int}$/R$_{merge}$                    | 19.7/24.0    |
| No. of atoms                            | 9364         |
| Protein                                 | 385          |
| Ligand/ion                              | 76           |
| Water                                   | 50           |
| Average B-factors                       | 65           |
| Protein                                 | 65           |
| Ligand/ion                              | 76           |
| Water                                   | 50           |
| Root mean square deviations             | 0.01         |
| Bond lengths (Å)                        | 1.28         |
| Bond angles (°)                         | 2.31         |
| Molprobity score                        | 0.33         |
| Estimated coordinate error from Luzzati plot (Å) | 0.33 |

**Structure Determination—** Data were collected at beamline 17-ID of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source (Argonne, IL). Data were processed with AutoPROC (Table 1) (32) and phases determined by Molecular Replacement with PHASER (33) using the structure of *E. coli* LpxC (PDB code 3p3g) as the search model. Refinement (Table 1) was performed with BUSTER (34, 35) interspersed with successive rounds of manual rebuilding in Coot (36). Structural alignments were performed using LSQKAB as implemented in the CCP4 program suite (33). Surface area calculations were performed with Areaimol using a probe sphere with a radius of 1.4 Å (37).
Mass Spectrometry—For native state mass spectrometry, purified *E. coli* LpxC was exchanged into 50 mM ammonium acetate, pH 6.5 (Fluka), using a HiTrap desalting column (GE Healthcare). A 15 μM solution of protein was infused at 5 ml/min into either an LXQ linear ion trap mass spectrometer or an LTQ Orbitrap hybrid mass spectrometer, with a heated capillary temperature of 250 °C.

Mass measurements were made by binding samples (0.5–2 μg) to a reversed-phase protein trap column (Michrom), and desalted by washing with 2% acetonitrile, 0.01% trifluoroacetic acid (TFA) followed by elution with a solution of 64% acetonitrile, 0.01% TFA into an electrospray mass spectrometer (LTQ, Thermo). The resultant spectra were deconvoluted using ProMass (Novatia) to yield the whole protein mass.

For bound ligand analysis, protein was extracted with a solution of 80% (v/v) acetonitrile, centrifuged to remove precipitate, and diluted with water to a final concentration of 50% acetonitrile. Samples were injected onto a 2.1 × 50 mm Waters BEH C18 column at a flow rate of 200 μl/min, with a gradient from 85% water, 15% acetonitrile, 0.1% dimethylisopropylamine to 37% water, 63% acetonitrile, 0.1% dimethylisopropylamine over 5 min. The samples were eluted into the LXQ mass spectrometer and analyzed in negative ion mode, using the following transitions: 832.3 > 391 and 790.4 > 391, corresponding to the loss of UDP~ from product and substrate, respectively.

**RESULTS**

*E. coli* LpxC Structure and Identification of Bound myr-UDP-GlcN—The overall structure of *E. coli* LpxC presented here is similar to that described previously (30). The structure contains two tightly packed α + β domains of identical topology that are distinguished by unique structural elements, named insert I and insert II, located between their respective 4- and A-helices (Fig. 2A). The catalytic site containing a single bound Zn²⁺ lies in a cleft formed at the base of the domain interface. Inserts I and II juxtapose the catalytic site and form a conserved hydrophobic tunnel known to bind fatty acids. The average root-mean-square Cα deviations range from 0.8–1.4 Å after superposition of *E. coli* LpxC with available LpxC crystal structures from diverse bacteria; *A. aeolicus* LpxC (blue, PDB code 2ier) (25), *Y. enterocolitica* (green, PDB code 3nzk) (29), *E. coli* (red, PDB code 3p3g) (30), and *P. aeruginosa* (gray, PDB code 3uhm) (28).

![FIGURE 2. Overall structure of *E. coli* LpxC.](https://example.com/figure2)

*Mass Spectrometry*—For native state mass spectrometry, purified *E. coli* LpxC was exchanged into 50 mM ammonium acetate, pH 6.5 (Fluka), using a HiTrap desalting column (GE Healthcare). A 15 μM solution of protein was infused at 5 ml/min into either an LXQ linear ion trap mass spectrometer or an LTQ Orbitrap hybrid mass spectrometer, with a heated capillary temperature of 250 °C.

*Results*—The overall structure of *E. coli* LpxC presented here is similar to that described previously (30). The structure contains two tightly packed α + β domains of identical topology that are distinguished by unique structural elements, named insert I and insert II, located between their respective β4- and A-helices (Fig. 2A). The catalytic site containing a single bound Zn²⁺ lies in a cleft formed at the base of the domain interface. Inserts I and II juxtapose the catalytic site and form a conserved hydrophobic tunnel known to bind fatty acids. The average root-mean-square Cα deviations range from ~0.8–1.4 Å after superposition of *E. coli* LpxC with available LpxC crystal structures from divergent bacteria (Fig. 2C).

After modeling and refinement of four *E. coli* LpxC monomers in the asymmetric unit, significant electron density remained in *F*ₐ₋ₙ – *F*ₐ calc difference maps (Fig. 2, A and B). In contrast to previous serendipitous observations of co-purified

**FIGURE 2.** Overall structure of *E. coli* LpxC. A, three-dimensional fold and domain architecture of *E. coli* LpxC (yellow) bound to myr-UDP-GlcN. Unbiased residual *F*ₐ – *F*ₐ electron densities for myr-UDP-GlcN and phosphate are shown (4α, blue mesh). The catalytic Zn²⁺ is depicted as a silver sphere. B, close-up of the *F*ₐ – *F*ₐ electron density shown in A. C, backbone Ca superposition of myr-UDP-GlcN bound *E. coli* LpxC (yellow) with LpxC crystal structures from diverse bacteria; *A. aeolicus* LpxC (blue, PDB code 2ier) (25), *Y. enterocolitica* (green, PDB code 3nzk) (29), *E. coli* (red, PDB code 3p3g) (30), and *P. aeruginosa* (gray, PDB code 3uhm) (28). D, surface representation of *E. coli* LpxC with myr-UDP-GlcN. Purple corresponds to conserved regions among Gram-negative pathogens.
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The reaction product adopts an elongated conformation that spans ~25 Å and buries ~575 Å² of accessible protein surface area. All monomers of the asymmetric unit adopt the same conformation and reveal product binding in the same relative orientation. The product is ~75% buried by E. coli LpxC protein contacts, which mostly involve three well conserved regions, inserts I, II, and the basic patch. Of the 26 residues observed to contact myr-UDP-GlcN (<4 Å cutoff), 11 are conserved in medically relevant Gram-negative pathogens (Fig. 2D).

Phosphate-binding Site—We identified additional tetrahedron-shaped electron density adjacent to the catalytic Zn²⁺ that could not be explained by myr-UDP-GlcN (Figs. 2B and 4). Previous observations of a second inhibitory Zn²⁺ ion in the catalytic site of A. aeolicus LpxC led us to consider the possibility that multiple Zn²⁺ ions were present (24). However, anomalous difference Fourier maps confirmed binding of a single Zn²⁺ ion in the catalytic site (Fig. 4), thereby excluding the possibility of multiple Zn²⁺ ions in this crystal structure. Acetate, another reaction product of the enzyme, also failed to account for the observed density and resulted in low B-factors and residual peaks in difference maps. In contrast, either a phosphate or sulfate ion could account completely for the electron density and yield reasonable B-factors after refinement. Because our E. coli LpxC crystals were grown in the presence of 1.2 M phosphate buffer, the final refined structure contains a single phosphate anion at this position.

Phosphate binding is stabilized by the catalytic Zn²⁺ and an extensive network of hydrogen bonds to key active site residues and Zn(II), and the major species carrying an additional adduct of 792 Da (Fig. 3, A and B). To confirm that this species was myr-UDP-GlcN (791 Da), purified protein was extracted with acetonitrile, and the extracts were analyzed via LC-MS² using transitions for both myr-UDP-GlcN and myr-UDP-GlcNac. The product myr-UDP-GlcNwas observed at near stoichiometric levels, along with lower levels of substrate (Fig. 3C).

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Phosphate binding is stabilized by the catalytic Zn²⁺ and an extensive network of hydrogen bonds to key active site residues and myr-UDP-GlcN (Fig. 4). By directly coordinating to the phosphate, the catalytic Zn²⁺ exhibits tetrahedral coordination geometry with an average deviation of 8° from ideal (~109.5°). Putative hydrogen bonds between each of the remaining phosphate oxygens and the side chains of Thr-191, His-265, and Glu-78 are observed and likely stabilize the bound anion. Moreover, the 2-amino and myristoyl 3-hydroxyl groups of the reaction product also interact with the phosphate (Fig. 4). Electrostatic and geometric similarity to the tetrahedral oxyanion transition state may explain why phosphate binds the LpxC catalytic site in the presence of myr-UDP-GlcN.

UDP Binding Pocket—The nucleotide-binding pocket forms a complementary fit to the uridine moiety, which is further stabilized by direct hydrogen bonding, π stacking, and favorable nonpolar van der Waals contacts (Fig. 5A). The uridine O4...
and N3 groups make hydrogen bonds to the backbone amide and carbonyl groups, respectively, of Asp-160. These interactions are buttressed by hydrophobic and π-stacking interactions with Lys-262 and Phe-161. The ribose of UDP, which adopts a C2-endo pucker, is solvent-exposed and not specifically recognized by hydrogen bonding to the protein (Fig. 5A).

The α- and β-phosphate groups of the product are recognized directly by Lys-239. In addition, the N-terminal end of the B′-helix presents consecutive backbone amide groups that contact the pyrophosphate moiety directly or via ordered water molecules. Finally, two additional water molecules mediate contacts between the pyrophosphate and Lys-143 and Asp-242.

Structural superposition revealed that interactions to UDP observed in the *A. aeolicus* LpxC structure (25) are significantly different from those observed here in the product-bound *E. coli* LpxC structure. Although the β-phosphates are similarly positioned, the α-phosphate and ribose groups do not superimpose (Fig. 5B). Consequently, the uridine base of myr-UDP-GlcN is rotated by ~45° relative to that observed for isolated UDP, allowing for more optimal π-stacking with Phe-161 (Fig. 5B).

**Recognition of GlcN**—In the product-bound state captured here, the closest protein side chain to the GlcN 2-amino group is that of His-265, which is positioned ~4 Å away. Although too long for a direct hydrogen bond, the extended distance is likely influenced by the bound phosphate, which directly engages His-265. Instead, the 2-amino group hydrogen bonds to the bound phosphate and the backbone carbonyl of Leu-62. Additional recognition of the GlcN moiety is achieved by water-mediated contact to Asp-242 and direct interaction between the 6′-OH and Lys-239. Finally, a pair of conserved phenylalanines, Phe-192 and Phe-194, provides a hydrophobic patch upon which the GlcN moiety binds, with Phe-194 also contacting the 4′-OH.

**Structure of Inserts I and II**—Although interspecies conformational differences have been previously noted for inserts I and II (30), less is known about the inherent flexibility of these structural elements within a species when comparing functional and inhibited states. Superposition of *E. coli* LpxC in the product-bound state presented here with the previous inhibitor-bound structure (30) reveals key structural rearrangements of insert I (Fig. 6A). The conformation of insert I in the product-bound state agrees well with that observed in prior structures of *A. aeolicus* (24) and *P. aeruginosa* (23) LpxC, wherein the βa-strand of insert I contains a β-bulge characterized by a backbone kink at Asp-59 to Thr-60 (Fig. 6B). Notably, the β-bulge allows hydrogen bonding between the backbone carbonyl of Leu-62 and the 2-amino group of myr-UDP-GlcN (Fig. 6B). In contrast, a flip of the peptide backbone at Leu-62 was observed in previous *E. coli* LpxC structures with the diacetylene scaffold hydroxymate inhibitor (LPC-009) (Fig. 6C), which appears stabilized by interactions to the inhibitor as well as a hydrogen bond between Thr-60 and the peptide backbone of Leu-62 (30). These results demonstrate that insert I of *E. coli* LpxC is capable of adopting multiple ligand-dependent conformations.

Subtle movement is also observed within insert II when comparing the product and LPC-009 bound structures (Fig. 6A). There is an ~2.5 Å shift of the insert II helix beginning at Phe-194, which adopts different rotamers in the two structures (Fig. 6, D and E). In the product-bound structure, the side chain of Phe-194 is adjacent to the GlcN moiety and fills a portion of the active site cleft that is otherwise enlarged in the LPC-009-bound structure. As a result, the conformation of insert II in the product-bound state of *E. coli* LpxC results in a wider substrate passage when compared with the LPC-009-bound structure (Fig. 6, D and E).

**DISCUSSION**

**LpxC Substrate Binding and Recognition**—The extensive enzymatic, kinetic, and mutagenic characterizations of *E. coli* LpxC over the past decade can now be informed by the structure of the enzyme bound to the reaction product myr-UDP-GlcN. One of the most surprising aspects of this work is that the reaction product, acquired during expression in the native *E. coli* host, remained stably bound to the enzyme despite multiple purification steps and crystallization under high ionic strength and alkaline conditions. Co-purification of myristic and palmitic acid bound to the hydrophobic tunnel has been previously observed in crystal structures of *A. aeolicus* LpxC (24), although co-purification and crystallization of myr-UDP-GlcN bound to LpxC is unprecedented. If the product had sim-
Similarly co-purified with the *E. coli* LpxC used to generate previous crystal structures (30), it is likely to have been displaced by the inhibitors used for co-crystallization.

The identification of reaction product, as opposed to the N-acetylated substrate, confirms that the present structure represents a snapshot of the enzyme after catalysis yet before complete product dissociation. The liberated acetate product, which has a reported $K_D$ of 8 mM for *E. coli* LpxC (38), is not observed in our structure. However, myr-UDP-GlcN has been reported to bind wild type *E. coli* LpxC with a $K_D$ of 10 μM (38). Binding is sensitive to mutation of several conserved active site residues shown in the structure to be in close proximity to myr-UDP-GlcN. Phe-192 is known to contribute 3 kcal/mol of free energy to product binding, and mutation of this residue to alanine decreases catalytic efficiency ($k_{cat}/K_m$) by 700-fold (38). Asp-246 and His-265 also contribute significantly to product affinity (38). The structure reveals Phe-192 and His-265 making hydrophobic and van der Waals contact with the GlcN moiety, whereas Asp-246 likely contributes to product affinity indirectly by stabilizing the conformation or ionization state of His-265.

Although much has been learned from previous structures of small molecules that mimic parts of the natural LpxC ligands (e.g. UDP and TU-514), the structure of LpxC bound to an intact reaction product precisely defines the ligand interactions required for LpxC function. Importantly, the structure reveals conformations of the GlcN and UDP moieties that are distinct from those inferred based on previous crystal structures (18, 25). These differences are likely due to truncation of the ligand and possibly sequence divergence between *A. aeolicus* and *E. coli* LpxC. The structures will therefore assist design strategies to better exploit the GlcN and UDP binding pockets (39), both of which are known to contribute significantly to product binding affinity (38).

**LpxC Flexibility and Inhibitor Binding**—Hydroxamate inhibitors linked to a diacetylene scaffold motif, as exemplified by LPC-009, provide important leads for optimization of new LpxC inhibitors. Although these inhibitors display sub-micromolar potency *in vitro* and corresponding *in vivo* whole cell activity against wild type strains of *E. coli*, opportunity exists for further improvement by exploiting structure-based approaches. Toward this end, Zhou and co-workers determined crystal structures of four inhibitors of the LPC-009 series (30, 40), including one (LPC-009) that was solved with LpxC enzymes from *E. coli*, *A. aeolicus*, and *P. aeruginosa*. These structures revealed species differences in the conformation of inserts I and II, which led to recognition that the active site volume in *E. coli* LpxC was significantly larger than that of *A. aeolicus* and *P. aeruginosa* LpxC (30). However, it was unclear whether *E. coli* LpxC had the potential to sample different conformational states, including those with more constricted active site volume. The structure presented here demonstrates that inserts I and II of *E. coli* LpxC adopt an alternative conformation to accommodate binding of myr-UDP-GlcN. Additional studies addressing the energetics and kinetics of inhibitor binding in relation to the conformation of inserts I and II will be important for future drug design.

**Insights into the Catalytic Mechanism**—Structural and biochemical studies over the past decade have led to two proposed mechanisms for LpxC catalysis (18, 20, 24, 27, 38, 41, 42). One mechanism suggested Glu-78 and His-265 function as a general acid-base catalyst pair with the oxyanion intermediate.
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stabilized by Thr-191 and Zn\(^{2+}\) (27). A second mechanism suggested Glu-78 alone serves as a bifunctional general acid-base catalyst, whereas His-265 electrostatically stabilizes the oxyanion intermediate (41). The crystal structure presented here supports the former mechanism. Based on the product-bound structure, Glu-78 is too far (~6 Å) from the 2-amino leaving group to serve as a general acid. In contrast, His-265 is positioned closer (~4 Å) to the 2-amino group and makes a direct hydrogen bond to the phosphate oxygen (O3) that itself hydrogen bonds to the 2-amino group (Fig. 4). The position of the phosphate ion is significant; it overlaps with the position of a cacodylate molecule observed in a previous A. aeolicus LpxC structure, suggesting that phosphate structurally mimics the tetrahedral oxyanion intermediate. In addition to His-265, the phosphate contacts Glu-78, Thr-191, and Zn\(^{2+}\) (Fig. 4).

Based on the structure presented here, we constructed a model for the oxyanion intermediate by introducing minimal changes to the observed positions of myr-UDP-GlcNAc and the oxyanion group, the latter being determined by the phosphate position (Fig. 7A). The resulting model could be generated without conformational changes to active site residues, showed no steric clashes, and even showed a reasonable fit to the electron density map for the product and phosphate ligands (data not shown). The model shows direct interactions between Glu-78 and a Zn\(^{2+}\)-coordinated oxygen of the oxyanion, consistent with this oxygen originating from the Zn\(^{2+}\)-bound water that is activated by Glu-78 (Fig. 7A). Interestingly, His-265 is also close to this oxygen, consistent with the proposal that His-265 may substitute as the general base in E78A LpxC mutants that retain residual catalytic activity (27). However, the closest interaction involving His-265 is with the 2-amino group, suggesting that the primary function of His-265 is to serve as the catalytic acid. The remaining interactions involving the oxyanion are with the Zn\(^{2+}\) and Thr-191, consistent with a primary role for each in stabilization of the transition state (27). Taken together, the structure and model of the oxyanion intermediate presented here show how conserved amino acids essential for LpxC activity recognize and stabilize both the negatively charged oxyanion intermediate and the final deacetylated product. The structure supports an LpxC reaction mechanism involving a general acid base catalyst pair in which Glu-78 serves as the general base to deprotonate and activate the Zn\(^{2+}\)-bound nucleophilic water and His-265 serves as the general acid to protonate the 2-amino leaving group (Fig. 7B).

Among LpxC enzymes, E. coli LpxC is the best characterized biochemically and among the most clinically relevant. The new structure fills an important gap in our understanding of molecular recognition of ligands by LpxC. In addition to new insights into the mechanisms of substrate and product recognition and catalysis, the structure complements a large body of inhibitor-bound structures of LpxC. The results provide additional insights to improve the design of next generation antibiotics that target this essential enzyme.

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