The Crystal Structure of the *Escherichia coli* Autoinducer-2 Processing Protein LsrF

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**Abstract**

Many bacteria produce and respond to the quorum sensing signal autoinducer-2 (AI-2). *Escherichia coli* and *Salmonella typhimurium* are among the species with the *lsr* operon, an operon containing AI-2 transport and processing genes that are up regulated in response to AI-2. One of the Lsr proteins, LsrF, has been implicated in processing the phosphorylated form of AI-2: Here, we present the structure of LsrF, unliganded and in complex with two phospho-AI-2 analogues, ribose-5-phosphate and ribulose-5-phosphate. The crystal structure shows that LsrF is a decamer of βαβ barrels that exhibit a previously unseen N-terminal domain swap and have high structural homology with aldolases that process phosphorylated sugars. Ligand binding sites and key catalytic residues are structurally conserved, strongly implicating LsrF as a class I aldolase.

**Introduction**

Many bacterial species control expression of specific genes through the production, release, and detection of small signal molecules called autoinducers. This process, termed quorum sensing, allows bacteria to regulate behavior in a population-dependent manner, effectively coordinating their activity. Behaviors regulated by quorum sensing include bioluminescence, biofilm formation, and production of virulence factors [1].

While autoinducer production and recognition is generally species specific, autoinducer-2 (AI-2) has been shown to be produced and recognized by a variety of bacterial species, both Gram-positive and Gram-negative. First identified as a regulator of bioluminescence in *Vibrio harveyi* [2,3], AI-2 has been shown to control a wide variety of behaviors in different species, including motility in *Helicobacter pylori* [4], division, stress response, and biofilm formation in *Streptococcus mutans* [5,6], virulence and biofilms in *Vibrio cholerae* [7–9] and *Staphylococcus aureus* [10], and social and pluricellular behavior of *Erwinia carotovora* [12]. Since *V. harveyi* share an operon, named *lsr*, with *S. typhimurium* [13], have shown that these species recognize chemically distinct DPD adducts as AI-2: (2S, 4S)-methyl-2,3,3,4-tetrahydroxyterahydrofuran-borate in the case of *V. harveyi* and (2R, 4S)-methyl-2,3,3,4-tetrahydroxyterahydrofuran in the case of *S. typhimurium* and *S. meliloti*. The known forms of AI-2 are able to interconvert spontaneously in solution, suggesting that a mix of DPD-derived molecules exists in environments with LuxS-containing bacteria [16]; however, because the different forms of AI-2 can interconvert, bacteria that recognize chemically distinct forms of AI-2 can nonetheless communicate with each other [14].

While AI-2 has been shown to act as a signaling molecule in many bacterial species [17,18], the molecular details of AI-2 recognition and response have been studied in only a small number of species including *E. coli* [19,20], *S. typhimurium* [21,22], *Sinorhizobium meliloti* [13], *V. cholerae* [9,23–25], and *V. harveyi* [3,15,26,27]. *E. coli* and *S. typhimurium* share an operon, named *lsr* (for *LuxS* Regulated), that consists of *lsrA, lsrB, LsrC, lsrD, lsrF*, and *lsrG* (and, in the case of *S. typhimurium*, *lsrE*) and is responsible for the recognition and transport of AI-2. (Two additional genes involved in regulation of the *lsr* operon, *lsrR* and *lsrK*, are adjacent but are transcribed divergently.) These species internalize AI-2 via an ABC transporter complex comprised of LsrA, LsrB, LsrC, and LsrD [19,22]. Once internalized, AI-2 is phosphorylated at the C5 position by the kinase LsrK, giving rise to phospho-AI-2 (P-AI-2, Fig. 1) [20,21]. It is this phosphorylated form of AI-2 that binds to the repressor LsrR, inactivating repression and increasing transcription of the *lsr* operon; thus, the operon acts as a positive feedback loop, importing more AI-2 in response to detection of P-AI-2 [20]. Two additional genes in the *lsr* operon, *lsrF* and *lsrG*, are present in both *E. coli* and *S. typhimurium* and have been implicated...
in AI-2 processing while a final gene, lsrE, is found in the S. typhimurium lsr operon but not in E. coli [21]. lsrE has homology to epimerases, but deleting lsrE in S. typhimurium has no detectable impact on AI-2 uptake or transcription of the lsr operon.

Previous genetic studies demonstrated that the LsrF and LsrG proteins are involved in terminating the AI-2-dependent induction of the lsr operon. Mutants lacking LsrF or LsrG show increased transcription of the lsr operon, suggesting that these proteins process P-AI-2, thus reducing the concentration of P-AI-2 in the cell and restoring the repressor function of LsrR. Importantly, the increase in lsr transcription observed in the absence of LsrF or LsrG is AI-2 dependent, and over-production of LsrF or LsrG decreases the transcription of the operon to levels lower than in the wild type. The suggestion that LsrF plays a role in P-AI-2 processing is further supported by sequence homology with aldolase enzymes that process phosphorylated sugars [21]. Subsequent studies have shown that LsrG does, in fact, catalyze a reaction with P-AI-2 as a substrate, yielding 2-phosphoglycolic acid and an additional, as yet unidentified three-carbon fragment, raising the possibility that LsrG does, in fact, catalyze a reaction with P-AI-2 as a substrate, while class II aldolases require metal co-factors.

Class I aldolases act through the formation of a Schiff base with the substrate, while class II aldolases require metal co-factors. The best studied of the class I aldolases is fructose-1,6-bisphosphate aldolase (FBPA), which catalyzes the cleavage of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate in glycolysis. Crystal structures have been determined for FBPA [29,30] and a variety of other class I aldolases including 2-amino-3,7-dideoxy-D-threo-hept-6-uloseonic acid (ADH) synthase, which catalyzes a transaldol reaction of 6-deoxy-5-keto-8-fructose-1-phosphate with L-aspartate semialdehyde to yield ADH [31], and D-2-deoxyribose-5-phosphate aldolase (DERA), which catalyzes the reversible aldol reaction between acetaldehyde and D-glyceraldehyde-3-phosphate to generate D-2-deoxyribose-5-phosphate [32]. These structures reveal that the class I aldolases share a common fold, classified as a TIM α/β-barrel in SCOP [33], and a structurally conserved catalytic lysine responsible for Schiff base formation.

While sequence analysis suggests that LsrF will function as a class I aldolase and genetic data suggests LsrF is involved in P-AI-2 processing, the details of the role LsrF plays in processing P-AI-2 are not known [21]. To begin addressing this question, we have determined the crystal structure of LsrF, alone and in complex with the P-AI-2 analogues ribose-5-phosphate and ribulose-5-phosphate (Fig. 1). The structure reveals a decameric complex of TIM α/β-barrels. Despite strong structural homology to FBPA from Thermoproteus tenax and ADH synthase from Methanothermobacter jannaschii, the subunits participate in a form of domain swapping previously unseen in aldolase complexes. Key catalytic residues in these class I aldolases are structurally conserved in LsrF, and both P-AI-2 analogues bind LsrF in the canonical aldolase active site, strongly implicating LsrF as a class I aldolase.

**Results**

**LsrF Structure**

LsrF crystallizes as a decamer with each monomer having an αβ8-barrel fold (Fig. 2a), a ubiquitous fold commonly seen in proteins catalyzing aldolase reactions [28]. In a departure from the typical αβ8-barrel fold, the first β-strand of the LsrF barrel does not start until residue 51. Instead, the first 25 residues of the chain extend away from the barrel and pack against other subunits in a form of domain swapping previously unseen in aldolases (Figs. 2b and 3b). There is no interpretable density for residues 1–9, but the orientation of adjacent residues make it impossible for these residues to pack against the αβ8-barrel of their own chain. After a short coil, residues 34–43 (α1) form an α-helix that both caps the bottom of the barrel and makes extensive interactions with neighboring monomers (Figs. 2a, 2c, and 4). Following the first β-strand, the αβ8-barrel fold is briefly interrupted by a small stretch of α-helix (resides 59–62, α1a) that packs against a neighboring monomer. A relatively large loop joins β3 to α3 and is bounded by two short β-strands (residues 109–110, β3a, and 122–123, β3b) that anchor this loop. The canonical αβ8-barrel then continues until a final interruption when residues 254–257 (α8b) form an α-helix prior to α8. After the final helix of the αβ8-barrel, the C-terminal residues form an α-helix (α8b) that largely lies in the groove between the seventh and eighth helices of the barrel.

The LsrF oligomer has a disk-like structure, with two rings of five monomers stacked on top of each other giving rise to a decamer with D5 symmetry (Fig. 3). Each ring has a diameter of approximately 110 Å and has a central pore of about 15 Å diameter. Monomers make extensive contacts with the two adjacent subunits in the pentamer (subunit A interfaces with B and E, Fig. 3a). These contacts are largely hydrophobic, containing only two salt bridges (between residues Asp128 and Arg89 from chain A and Lys165 and Asp161 from chain B, respectively) and eleven potential hydrogen bonds. Most of the interactions between chains occur through the α-helices of the αβ8-barrels; in the A-B interface, helices 1a, 2, and 3, and the large loop between β3 and α3 from subunit A interact with helices 4, 5, and 6 from subunit B, burying some 1200 Å² of surface area on each monomer. Since any given

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**Figure 1. Structures of phospho-AI-2 and two analogues, ribulose-5-phosphate and ribose-5-phosphate.**

doi:10.1371/journal.pone.0006820.g001
A monomer participates in two of these interactions, pentameric interactions bury 18% of the solvent accessible surface area.

A second ring is related to the first by 2-fold symmetry axes perpendicular to the 5-fold axis of the pentamer. The stacked rings have a total height of approximately 70 Å, and the central pore runs this full length. Monomers stacked on top of each other (A and F; Fig. 3b) have extensive interactions that are significantly enhanced by a ‘swapping’ of N-terminal residues. In this swap, residues 10–24 extend away from the (αβ)8-barrel formed by their chain and pack into the interface between two adjacent monomers in the other pentamer, burying 460 Å², or 21% of the accessible surface area of this swapped coil. The rest of the A-F interface is largely composed of contacts involving helix a0 and the loops after helices 2 and 3. In total, nearly 2100 Å² are buried in this interface, 15% of the total accessible surface area.

One final interaction is due to the interface of the type seen between monomers B and F in Fig. 3b and is largely caused by the swapped 34 N-terminal residues. While this swapped coil packs chiefly against its direct neighbor from the other ring (i.e. A and F, Fig. 3b), it also makes contacts with β6 from the other monomer. In this case, 900 Å² of surface area is buried, though the value may be even larger if residues 1–9 (disordered in the structure) also contribute to this interface. When all of the interfaces are considered, approximately 40% of the total surface area is buried in oligomer formation, suggesting that the decamer is likely the predominant form of LsrF in vivo. Consistent with this conclusion, only decamers were observed in gel filtration experiments (data not shown).

Structure of the Ligand Binding Site

To identify the catalytic site of LsrF, we determined the structure of the protein in complex with two P-AI-2 analogues: ribose-5-phosphate (R5P) and ribulose-5-phosphate (5RP). In both structures, the ligand electron density allowed definitive placement of the phosphate group and illustrated the general path of the carbon chain, but was of insufficient quality for unambiguous placement of all ligand atoms (Fig. 5a, b). As an independent confirmation of placement of the phosphate, the LigandFit module of PHENIX [34] was used as an automated means for placing the ligands. The automated procedure positioned the phosphates in the same location as was modeled manually.
R5P and 5RP bind LsrF in the same location, near the entrance to the (αβ)8-barrel with the phosphate group oriented towards the coils following β7 and β8 (Fig. 2a & c). The phosphate is located near the positively charged side chain of Arg254 and is positioned to form hydrogen bonds with the side chain of His58, the side chain and main chain of Arg254, and the main chain of three glycine residues.
The ligand then extends across the center of the (αβ)₈-barrel, away from strands 7 and 8, in a largely polar environment. In particular, Lys203 is adjacent to the ligand; this residue has potential significance for the mechanism, as equivalently positioned lysines are responsible for Schiff base formation in other aldolases (below).

No large conformational changes were observed upon ligand binding, though there were small movements in a few binding site residues, most notably Asp251 and Met252 (with α-carbons shifting by 0.5–2.0 Å). A single water was built in the binding site of unliganded LsrF; this water is displaced upon ligand binding.

**Discussion**

While AI-2 mediated quorum sensing has been identified in many bacterial species, the benefits bacteria gain from this
Figure 5. The LsrF ligand binding site and potential catalytic residues. A. Stereoview of ribulose-5-phosphate bound to LsrF showing 20-fold NCS averaged 2F_o-F_c electron density. Density was contoured at 4.0 (red) and 2.0 (blue) σ and truncated 2.0 Å from ligand atoms. The position of the phosphate is unambiguous, and the general path of the ligand is clear. B. Stereoview of ribose-5-phosphate bound to LsrF showing 20-fold NCS averaged 2F_o-F_c electron density. Density was contoured at 5.0 (red) and 2.0 (blue) σ and truncated 2.0 Å from the ligand. The position of the phosphate is unambiguous, and the general path of the ligand is clear. C. Structural alignment of key catalytic residues from rabbit (blue bonds; 1J4E) and T. tenax (red bonds; 1OK4) FBPA with LsrF (white bonds). Ribulose-5-phosphate from LsrF is shown in ball and stick form. Residue numbering follows LsrF.

doi:10.1371/journal.pone.0006820.g005
communication are not fully understood. The presence of genes not involved in AI-2 transport in the \textit{lsr} operon (\textit{lsrF} and \textit{lsrG}) raises questions about the eventual fate of internalized AI-2. Experiments studying regulation of the \textit{lsr} operon in \textit{A. typhimurium} have implicated \textit{LsrF} in P-AI-2 processing, though biochemical studies have also raised the possibility that \textit{LsrF} acts on a product of the reaction involving P-\textit{AI}-2 and \textit{LsrG} or another P-\textit{AI}-2 adduct \cite{20, 21}. The structure of \textit{LsrF} and complexes with P-\textit{AI}-2 analogues presented here strongly suggest that \textit{LsrF} is a class I aldolase.

**Fold Comparisons with other Aldolases**

NCBI-Blast identifies \textit{LsrF} as belonging to the TIM phosphate binding superfamily and, in searching for conserved domains, all e-values better than 1e-5 suggest it to be an aldolase (the strongest match, with an e-value of 5e-175, is with the aldolase cluster PRK08227). A DALI search using the \textit{LsrF} monomer structure presented here identified two very similar structures (Z-scores greater than 25 and RMSD less than 2.0 Å), along with a large number of more distantly related structures that share the ubiquitous TIM barrel fold. The top DALI hits are the \textit{M. jannaschii} ADH synthase, which catalyzes a transaldol reaction (PDB ID: 2QJG) \cite{31}, and the FBPA from \textit{Thermoproteus tenax} (PDB ID: 1QJX) \cite{30}. These proteins have low sequence identity with \textit{LsrF} (31% and 25% respectively), but nonetheless share essentially identical folds, with the exception of the domain swapping involving the N-terminus of \textit{LsrF}.

Domain swapping has been observed previously in the \(\beta\)8-barrel fold family, initially by Huang \textit{et al} in phosphoenolpyruvate mutase where C-terminal residues are swapped, giving rise to dimers that further assemble into a tetrramer \cite{35}. C-terminal domain swapping has also been observed in the \textit{E. coli} fructose-6-phosphate aldolase, \textit{FSA} \cite{36}. Like \textit{LsrF}, \textit{FSA} crystallizes as a dimer where C-terminal residues are swapped, giving rise to the ubiquitous TIM barrel fold family. The top DALI hits are the \textit{M. jannaschii} ADH synthase, which catalyzes a transaldol reaction (PDB ID: 2QJG) \cite{31}, and the FBPA from \textit{Thermoproteus tenax} (PDB ID: 1QJX) \cite{30}. These proteins have low sequence identity with \textit{LsrF} (31% and 25% respectively), but nonetheless share essentially identical folds, with the exception of the domain swapping involving the N-terminus of \textit{LsrF}.

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**Comparison of the \textit{LsrF} active site with other aldolases**

The P-\textit{AI}-2 analogues ribose-5-phosphate and ribulose-5-phosphate bind to \textit{LsrF} in the same general position that other aldolases bind phosphorylated substrates (e.g. \textit{E. coli} DERA and FBPA, \textit{T. tenax} FBPA and \textit{M. jannaschii} ADH synthase), suggesting that the canonical aldolase active site is conserved in \textit{LsrF}. Structural-based sequence alignments with \textit{T. tenax} FBPA aldolase and \textit{M. jannaschii} ADH synthase, the two most closely related aldolase structures, and \textit{E. coli} FBPA aldolase show that residues in the ligand binding site are more highly conserved than one would expect based on overall sequence identities (Figs. 4 and 5c), supporting the premise that these residues are important for \textit{LsrF} substrate binding and activity. Notably, most of residues that hydrogen bond with the phosphate of the ligand in \textit{LsrF} (Arg254 and Gly 226, 227, and 253) are structurally conserved in all four structures, and the remaining residue (His34) is conserved in \textit{T. tenax} FBPA and \textit{M. jannaschii} ADH synthase, but not \textit{E. coli} FBPA, where it is replaced by a Glu. Examination of crystal structures of complexes of these proteins with phosphorylated ligands (1OK4, 2QJG, and 1J4E) shows that the conserved residues are positioned to form hydrogen bonds with the phosphoryl group of the ligand just as they are in \textit{LsrF}.

The defining catalytic residue for a type I aldolase is a lysine that forms a Schiff base with the substrate. Structural alignments of \textit{LsrF} with a variety of type I aldolases, including FBP aldolase from rabbit and \textit{T. tenax} ADH synthase from \textit{M. jannaschii}, and transaldolase B, DERA, and 2-keto-3-deoxy-6-phosphogluconate (KDPG) from \textit{E. coli} show structural conservation of the catalytic lysine in \textit{LsrF} K203. Other catalytically significant residues vary across different aldolases, but nonetheless potential catalytic residues in \textit{LsrF} can be identified from structural comparisons with FBP aldolase from rabbit and \textit{T. tenax} and ADH synthase from \textit{M. jannaschii}. These aldolases have an aspartate residue that acts as a general base, facilitating the carbon-carbon bond cleavage (or formation) by deprotonating an adjacent hydroxyl. The aspartic acid is then thought to donate the proton back during the reforming of the imine \cite{29, 31, 37}. This aspartate is structurally conserved in \textit{LsrF} (Asp57; Fig. 5c) and is well positioned to participate in catalysis as a general acid/base.

The identity of the catalytic residue that participates in the dehydration of the carbinalamine during formation of the Schiff base differs in the various species. In most aldolases, including rabbit FBP aldolase, the residue is a glutamate adjacent to the catalytic lysine \cite{29}. In \textit{T. tenax} FBP aldolase, and \textit{M. jannaschii} ADH synthase the catalytic glutamate is not conserved; instead, a tyrosine is positioned to act as a proton donor \cite{30, 31}. Neither of these residues is structurally conserved in \textit{LsrF}. Although a tyrosine (205) is adjacent to the position occupied by the catalytic Tyr in the other enzymes, it is too distant from the catalytic lysine (6.8 Å) to reasonably participate in catalysis. Rather, there is an aspartate (251) located only 2.9 Å from the K203, though on the other side of the lysine from the catalytic glutamate in rabbit FBP aldolase (Fig. 5c). This location, occupied by a serine in rabbit FBP aldolase and alanine in the other close homologues, makes Asp251 a very plausible replacement for the catalytic glutamate/tyrosine in other aldolases.

**Conclusion**

The structures presented here strongly support the classification of \textit{LsrF} as a class I aldolase, due to overall structural homology, the conservation of key catalytic residues, and conservation of the ligand binding site. Thus far, we have been unable to detect the products of the \textit{LsrF} reaction in vitro, either by NMR or TLC using radiolabeled substrate, in the presence or absence of the other P-\textit{AI}-2 processing enzyme \textit{LsrG} (results not shown). While previous work has implicated P-\textit{AI}-2 \cite{21} or an adduct of P-\textit{AI}-2 \cite{20} as the likely substrate for \textit{LsrF}, it is possible that additional enzymatic processing of P-\textit{AI}-2 or an additional co-factor is necessary for activity, and we are conducting genetic and biochemical experiments to address these possibilities.

If, as working model, we consider \textit{LsrF} to act directly on P-\textit{AI}-2 via a FBPA-like mechanism, we would expect the highly conserved catalytic K203 to form a Schiff base through nucleophilic attack on the carbonyl carbon one position away from the phosphate of the substrate (C4 of P-\textit{AI}-2), leading to the breaking of the C2-C3 bond and the formation of acetate and dihydroxyacetone phosphate (DHAP). (It should be noted that hydration and keto-aldol isomerization would be necessary to make P-\textit{AI}-2 an appropriate substrate for this reaction.) Intriguingly, prior work has shown that DHAP represses \textit{lsr} transcription in an LsrR-dependent manner \cite{19}. Thus, \textit{LsrF} could function to reduce \textit{lsr} transcription not only by reducing the amount of P-\textit{AI}-2 present in the cell as previously suggested \cite{21}, but also by catalyzing the formation of an inhibitor of \textit{lsr} transcription.

Further biochemical characterization of the \textit{LsrF} reaction will be necessary to fully understand the role \textit{LsrF} plays in AI-2
mediated quorum sensing, and the structures presented here provide details that will be of utility in the design of these experiments.

Materials and Methods

Overexpression and purification of LsrF

*E. coli* LsrF was cloned into plasmids pGEX-4T1 and pDEST-HisMBP for overexpression as glutathione-S-transferase and dual His₆-maltose-binding-protein fusions, respectively. Plasmids were transformed into *E. coli* strain BL21, and cultures were grown in Luria broth (Sigma-Aldrich) at 37°C to an OD₆₀₀ of 0.3. The temperature was then changed to 22°C, and when the culture reached an OD₆₀₀ of 0.9, protein expression was induced by the addition of 0.1 mM isopropyl β-D-thiogalactopyranoside. After induction, the bacteria were grown for 15 hours at 22°C before harvesting by centrifugation.

Cells producing the GST-LsrF fusion were resuspended in 25 mM Tris, pH 8.0, 150 mM sodium chloride, 5 mM DTT, 2.5 μg mL⁻¹ DNase, and protease inhibitors (2.5 μg mL⁻¹ aprotinin, 2.5 μg mL⁻¹ leupeptin, 1 mM Pefablock (Roche)), while cells producing the His₆-MBP-LsrF fusion were resuspended in 25 mM HEPES, pH 8.0, 200 mM sodium chloride, 25 mM imidazole, 1 mM β-mercaptoethanol, 2.5 μg mL⁻¹ DNase, and protease inhibitors (2.5 μg mL⁻¹ aprotinin, 2.5 μg mL⁻¹ leupeptin, 1 mM Pefablock). In both cases, the cells were lysed using a M-110Y Microfluidizer (Microfluidics) and the lysates clarified by centrifugation.

The GST-LsrF fusion was purified by affinity chromatography using glutathione agarose (Sigma-Aldrich). The fusion protein was digested with thrombin for 12 hours at 4°C while still bound to the glutathione agarose. LsrF was eluted from the agarose column in 25 mM Tris, pH 8.0, 1 mM DTT, to a NaCl concentration of 75 mM. LsrF was cloned into plasmids pGEX-4T1 and pDEST-MBP constructs. Plasmids were transformed into *E. coli* strain BL21, and cultures were grown in Luria broth at 37°C to an OD₆₀₀ of 0.3. The temperature was then changed to 22°C, and when the culture reached an OD₆₀₀ of 0.9, protein expression was induced by the addition of 0.1 mM isopropyl β-D-thiogalactopyranoside. After induction, the bacteria were grown for 15 hours at 22°C before harvesting by centrifugation.

The resulting protein solution was diluted with 25 mM Tris, pH 8.0, 1 mM DTT, 150 mM NaCl. The fusion protein was eluted from the column using a gradient from 25 mM Tris, pH 8.0, 150 mM sodium chloride, and 1 mM DTT. The resulting protein solution was diluted with 25 mM Tris, pH 8.0, 1 mM DTT, to a NaCl concentration of 75 mM. LsrF was then further purified by ion exchange chromatography using a SourceQ column (GE Healthcare) with a gradient from 0 to 1 M NaCl. As a final purification step, the protein was subjected to size exclusion chromatography on a Superdex 200 column (GE Healthcare), eluting in 25 mM Tris pH 8.0, 1 mM DTT, and 150 mM NaCl. The protein was concentrated to 9.6 mg ml⁻¹ for crystallization.

The His₆-MBP-LsrF fusion was also purified by affinity chromatography, but in this case using NINTA agarose (Qiagen). The fusion protein was eluted from the column using a gradient from the resuspension conditions to 25 mM HEPES pH 8.0, 200 mM sodium chloride, 250 mM imidazole as described in Tropea et al [38]. Protein containing fractions were pooled, and the concentration of imidazole was reduced to 25 mM by diluting with 25 mM HEPES, pH 8.0, 200 mM NaCl. The His₆-MBP tag was then digested from the LsrF using His₆-TEV protease [38]. The tag and protease were removed by passing the solution over NINTA resin, and the resulting LsrF solution was diluted to 50 mM NaCl with a 25 mM HEPES, pH 8.0. The LsrF was purified by ion exchange and size exclusion chromatography as described above, and the resulting LsrF was concentrated to 8.2 mg ml⁻¹ for crystallization.

Crystallization and Structure Determination

Crystals of LsrF were grown via the hanging drop method with a well solution of 22% PEG 400, 200 mM MgCl₂, 100 mM Tris pH 8.0. Unliganded crystals were grown from the pGEX-4T1 derived protein while protein for the ligand-soaked crystals came from the pDEST-MBP construct.

Unliganded crystals were soaked in 100 mM Tris pH 8.0, 25 mM MgCl₂, 27.5% PEG 400 for one minute and flash frozen in the diffractometer’s cryostream. Data were collected at 100K using an R-Axis-IV image plate detector mounted on a Rigaku 200HB generator. The crystals (P₁, a = 78.33 Å, b = 105.45 Å, c = 171.67 Å, α = 89.80°, β = 79.31°, γ = 89.61°) diffracted to 2.9 Å resolution. Ligand was introduced to LsrF crystals by soaking the crystals in 100 mM Tris pH 8.0, 100 mM MgCl₂, 27.5% PEG 400, 100 mM ligand (either ribulose-5-phosphate or ribose-5-phosphate, Sigma-Aldrich) for five minutes. Crystals were flash frozen in liquid nitrogen and data were collected at 100K at NSLS beamline X26C. The ribose-5-phosphate crystal (P₁, a = 78.33 Å, b = 105.45 Å, c = 173.42 Å, α = 89.51°, β = 79.79°, γ = 90.34°) diffracted to 2.5 Å resolution while the ribulose-5-phosphate crystal (P₁, a = 78.74 Å, b = 107.10 Å, c = 169.52 Å, α = 90.00°, β = 102.62°, γ = 90.00°) diffracted to 2.9 Å resolution. Data were processed using Denzo, Scalepack [39], and CCP4 [40]. It should be noted that while the unit cell of the ribulose-5-phosphate crystals is, in appearance, potentially monoclinic, the data does not scale well as monoclinic at higher resolutions. Moreover, the apparent large variation in the β angle for the ribulose-5-phosphate crystal is due to an alternative convention selected by Denzo rather than a significantly different cell.

The structure of unliganded LsrF was determined via molecular replacement with PHENIX [34], using ADH synthase from *M. jannaschii* (PDB ID: 2QIQ, 31% sequence identity) as the search model. A 20-fold NCS averaged map was calculated and the model built using Coot [41]. Because of the high degree of NCS, reflections were selected for the R-free set in thin resolution shells using DATAMAN [42]. The structure was refined to 2.9 Å using PHENIX and REFMAC [43], using NCS constraints. The model contains 2 copies of the LsrF decamer, though weak density made it impossible to model the N-terminal 9 residues, the C-terminal 2 residues, and residues 177–180, an apparent surface loop. The model exhibits good geometry (Table 1), with only eleven of 5440 residues outside the allowed region of the Ramachandran plot (calculated by Coot). The final model also includes 241 water molecules, and has a final R cryst of 0.209 and R free of 0.229.

The liganded structures were determined by molecular replacement via PHENIX, though in these cases the unliganded LsrF structure was used as the molecular replacement model and reflections for the R free set were selected randomly rather than in resolution shells. Refinement parameters for the ligands were calculated using the eLBOW module of PHENIX. The ribose-5-phosphate structure was refined via PHENIX and REFMAC, using NCS constraints, to 2.5 Å resolution, with R cryst = 0.205 and R free = 0.235. The size of the unit cell made it difficult to collect a complete data set at high resolution, and only 39% of the possible reflections were measured in the highest resolution shell. However, the lack of completeness is offset by the high degree of NCS (20-fold), making it reasonable to include data to this resolution. The final model included one ribose-5-phosphate per chain and 334 water molecules. The ribulose-5-phosphate structure was refined to 2.9 Å resolution via PHENIX and REFMAC, with NCS constraints, and the final model (R cryst = 0.195, R free = 0.228) includes one ribose-5-phosphate per chain and 376 water molecules. Both models are missing the same residues as the unliganded model (1–9, 177–180, and 290–291) and have good geometry (Table 1), with either one (LsrF/ribulose-5-phosphate) or zero (LsrF/ribose-5-phosphate) of 5440 residues outside allowed regions of the Ramachandran plot. The position of the electron-rich phosphate is clear for both ligands, but the density of the carbon backbone was relatively poor, revealing the general path of the ligand but not specific details and leading to high B-factors.
The Structure of E. coli LsrF

Table 1. Crystallographic data and refinement statistics.

| Data (highest resolution shell in parenthesis) | Unliganded | Ribose-5-phosphate | Ribulose-5-phosphate |
|-----------------------------------------------|------------|---------------------|----------------------|
| Resolution (Å)                               | 2.9 (2.900–2.975) | 2.5 (2.500–2.565) | 2.9 (2.900–2.975) |
| Unique reflections                            | 113374 (7444) | 121116 (5138) | 107205 (7028) |
| Rmerge                                        | 0.092 (0.361) | 0.086 (0.380) | 0.090 (0.436) |
| Mean I/σ                                      | 9.1 (1.9)  | 7.5 (1.8)  | 8.7 (2.0)  |
| Completeness (%)                              | 95.3 (90.5) | 81.7 (38.6) | 94.2 (83.7) | 94.2 (83.7) |
| Multiplicity                                  | 1.8 (1.7)  | 2.1 (1.7)  | 1.7 (1.6)  |

**Refinement**

|                           |           |           |           |
|---------------------------|-----------|-----------|-----------|
| Rmerge/Rmax                | 0.209/0.229 | 0.205/0.235 | 0.195/0.228 |
| RMSD bond length (Å)       | 0.013      | 0.014      | 0.013      |
| RMSD bond angle (°)        | 1.317      | 1.358      | 1.178      |
| Number of atoms per ASU   | 42541      | 42874      | 42916      |
| Average B factor (Å²)      | 37.04      | 35.77      | 25.42      |
| Protein                    | 23.87      | 25.07      | 16.89      |
| Water                      | 80.09      | 54.18      |            |
| Ligands                    |            |            |            |
| Ramachandran Plot          |            |            |            |
| Most favored (%)           | 97.1       | 96.0       | 93.8       |
| Allowed (%)                 | 2.7        | 4.0        | 6.2        |
| Disallowed (%)              | 0.2        | 0.0        | 0.0        |

Acknowledgments

We gratefully acknowledge the staff of the National Synchrotron Light Source X26C beamline for assistance with X-ray data collection. Data was also collected at the Princeton University X-ray Facility with the gracious assistance of Phil Jeffrey, who also provided valuable assistance in data collection at X26C.

Author Contributions

Conceived and designed the experiments: KBX STM. Performed the experiments: ZD KBX STM. Analyzed the data: ZD KBX STM. Wrote the paper: KBX STM.

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