Structural Basis for Phosphorylated Autoinducer-2 Modulation of the Oligomerization State of the Global Transcription Regulator LsrR from Escherichia coli*

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Background: The Escherichia coli global transcription regulator LsrR regulates the expression of many genes in response to autoinducer-2.

Results: We determined the crystal structure of LsrR and demonstrated that phosphorylated autoinducer-2 triggers the disassembly of LsrR tetramers.

Conclusion: Our findings reveal a possible mechanism for the change in the oligomerization state of LsrR.

Significance: These observations may shed light on a gene transcription mechanism.

Quorum-sensing systems are widely used by bacteria to control behavior in response to fluctuations in cell density. Several small diffusible molecules called autoinducers act as signaling molecules in quorum-sensing processes through interplay with sensors. Autoinducers modulate vital physiological functions such as nutrient acquisition, gene transcription, and virulence factor production. In Escherichia coli, LsrR serves as a global transcription regulator that responds to autoinducer-2 to regulate the expression of a variety of genes, including the lsr operon and the lsrR gene. Here, we report the crystal structure of full-length LsrR from E. coli, which has an N-terminal DNA-binding domain and a C-terminal ligand-binding domain connected by a β-strand. Although only two molecules are found in one asymmetric unit, two neighboring dimers pack to form a tetramer that is consistent with the oligomerization state of LsrR in solution. Mutagenesis experiments and gel shift assays indicated that Gln-33 and Tyr-26 might be involved in interactions between LsrR and DNA. The LsrR-binding site for phosphorylated autoinducer-2 was predicted by structural comparisons of LsrR and DNA. The LsrR-binding site for phosphorylated autoinducer-2 triggers the oligomerization state of LsrR from the presence of phosphorylated autoinducer-2. Based on these observations, we propose that phosphorylated autoinducer-2 triggers the disassembly of the LsrR tetramer to activate the transcription of its target genes.

Physiological functions of bacteria such as nutrient acquisition, gene expression, motility formation, bioluminescence, and virulence factor production are vital for survival. These functions are efficiently modulated through a process called quorum sensing (QS), which is used by bacteria to communicate in response to fluctuations in cell density and species complexity (1). QS allows for changes in bacterial behavior when the cell density reaches a critical value. For example, QS was shown to repress virulence factor production and biofilm formation in Vibrio cholerae at high cell density (2). In Porphyromonas gingivalis, QS was reported to alter the expression of protein involved in hemin acquisition (3). In Clostridium perfringens, QS was discovered to be important for the regulation of virulence genes (4).

Generally, QS bacteria use small diffusible molecules called autoinducers as communication signals. Autoinducers are produced and secreted into the external environment by QS bacteria, and they accumulate with increased cell density. When the concentration of the autoinducer reaches a minimum threshold, it is detected by QS bacteria, which responds to the signal. The result is the population-wide alteration of the gene expression profile. QS enables bacteria in a population to adopt behaviors in unison (1, 5). Several classes of autoinducers have been reported to date, among which N-acylated homoserine lactones of Gram-negative bacteria and oligopeptides of Gram-positive bacteria are the most extensively studied (6). The autoinducer-2 (AI-2), whose precursor is 4,5-dihydroxy-2,3-pentanediol, is regarded as a “universal” signal shared by both Gram-positive and Gram-negative bacteria (7, 8).

AI-2 was originally discovered to be part of the QS pathway in V. cholerae, regulating bioluminescence (9). Subsequently, AI-2 was found to be synthesized by LuxS, an enzyme involved in the metabolism of S-adenosylmethionine (10). Genes homol...
ogous to LuxS have been identified in many species, and AI-2 is present in both Gram-negative and Gram-positive bacteria (7, 8). The well established AI-2-based QS systems have different mechanisms of AI-2 detection and signal transduction. In Vibrio harveyi, a two-component system senses the AI-2 signal and regulates the expression of the luciferase structural operon (luxCDABE) (1). In Escherichia coli, AI-2 must be imported into cells and phosphorylated by the AI-2 kinase LsrK, which triggers the shift of gene expression (11).

In E. coli, four genes, lsrACDB, encode the ATP-binding cassette transporter responsible for the internalization of AI-2. These four genes, together with two other genes, lsrFG, are the lsr operon (12). In the absence of p-AI-2, the expression of the lsr operon is repressed by LsrR, the key sensor of p-AI-2. LsrR binds a consensus sequence in the promoter region of the lsr operon (12–15). Further studies indicated that LsrR binds a four genes, together with two other genes, lsrFG, are the lsr operon. LsrR serves as a global regulator of a variety of genes in response to p-AI-2 (14, 16). Although LsrR is important in p-AI-2 sensing and gene transcription regulation, its detailed molecular mechanism remains unclear.

**EXPERIMENTAL PROCEDURES**

Cloning, Expression, and Purification of LsrR and Mutants—LsrR was cloned, expressed, and purified as described with minor modifications (17). In brief, genes encoding LsrR and its mutants were individually cloned into a pET28a expression vector with a C-terminal His tag for overexpression in E. coli BL21 (DE3) (Novagen). Target proteins were purified using a nickel-nitrilotriacetic acid column (GE Healthcare) followed by gel filtration. Purified LsrR protein was concentrated to 5 mg/ml for crystallization. For cross-linking assays, LsrR and mutants were concentrated to about 1 mg/ml in buffer (20 mM Tris, 1 M NaCl, 5% glycerol, pH 8.0). Protein purity was assessed by electrophoresis, and protein samples were stored at −80 °C.

Site-directed mutagenesis was performed using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) with wild-type LsrR plasmid as the template. Mutant proteins were expressed and purified as above.

Crystallization, Data Collection, and Processing—Crystals were grown at 22 °C using the hanging drop vapor diffusion method, initially mixing 1 μl of protein solution with an equal volume of reservoir solution (0.1 M Hepes, pH 8.0, 6% PEG 8000 (w/v), 8% ethylene glycol (v/v)). Crystals were soaked in cryo-protection solution containing 0.1 M Hepes, pH 8.0, 6% PEG 8000 (w/v), 8% ethylene glycol (v/v), and 10% glycerol (v/v) for several seconds and flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K at the synchrotron radiation beamline BL17U1 of SSRF (Shanghai, China). Data were integrated and scaled using the program HKL2000 (18).

Structure Determination and Refinement—Molecular replacement was carried out with the program Phaser (19) in the CCP4i suite (20), using the substrate-binding domain of SorC from Klebsiella pneumoniae (Protein Data Bank (PDB) code 2W48) as search model. After the initial phase was obtained, the LsrR structural model was automatically built using the program Buccaneer (21). After several rounds of refinement by REFMAC5 (22) and COOT (23) programs, the final model was refined to a 3.0-Å resolution with an R factor of 21.7% (R free was 26.7%). The N-terminal loop (residues 1–9) and the loop connecting β6–α11 (residues 215–224) in two molecules in the asymmetric unit are missing. The quality of the final model was analyzed with the program MolProbity (24). Data collection and model refinement statistics are in Table 1. All figures were prepared with PyMOL (25).

**Cross-linking Assay—**Disuccinimidyl suberate (Sigma) cross-linking reactions were carried out in 20 mM Tris, pH 8.0, 1 M NaCl, 5% glycerol. LsrR and mutants (at about 1 mg/ml) were incubated on ice with AI-2 (4,5-dihydroxy-2,3-pentanedione, 40 μM) for 1 h and subsequently boiled in SDS sample buffer (50 mM Tris, 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, pH 8.0). Samples were run on an 8% SDS-PAGE gel. Protein bands were visualized by Coomassie G-250 staining.

**Size Exclusion Chromatography Assays—**The purified wild-type or mutant (V153E and P173A, P175A) LsrR proteins in the presence or absence of AI-2 and p-AI-2 were loaded onto a Superdex 200 10/300 GL column (GE Healthcare) equilibrated with column buffer and eluted using the same buffer.

**Gel Shift Assays—**DNA fragment p-lsrR (119 bp) containing the lsrR promoter was prepared by PCR from E. coli genomic DNA using the primers 5’-AAATCCTGTTGTTGTGATGATGCCGCTGTTTGG-3’ (forward) and 5’-TATTATCTATTTATTTTTCAT-3’ (reverse). A digoxigenin (DIG) gel shift kit (second
generation, Roche Applied Science) was used to label DNA fragments and detect signals, according to the manufacturer’s instructions. Binding reactions were performed by incubating labeled DNA fragments with purified LsrR wild-type or mutant protein at 4 °C for 10 min in 10 μl of incubation buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 3 mM magnesium acetate, 0.1 mM EDTA, and 0.1 mM dithiothreitol). After incubation, 2.5 μl of gel loading buffer (0.25 M TBE; 34% glycerol) was added, and samples were electrophoresed in 4% native polyacrylamide gels in 0.5 M TBE buffer (45 mM Tris borate and 1 mM EDTA, pH 8.0).

Docking Experiments and Amino Acid Sequence Alignment—Model structures of p-Al-2 were prepared using the program PRODRG (26). Three-dimensional models of p-Al-2-LsrR complexes were simulated using the program HADDOCK (27). Docking was started with p-Al-2 and LsrR and driven by interaction restraints with active residues (Lys-288 and Asp-243). Amino acid sequence alignments of LsrRs used the programs ClustalW2 (28) and ESPript (29).

RESULTS

Overall Structure of LsrR—To determine the regulation mechanism of LsrR in response to p-Al-2, we overexpressed and purified full-length E. coli LsrR and crystallized it in the presence or absence of p-Al-2. However, we obtained LsrR crystals only in free form. LsrR crystals diffracted to 3.0 Å resolution, and the structure was solved by molecular replacement. The structure was refined to R\(_{\text{factor}}\)/R\(_{\text{free}}\) of 21.7/26.7% and showed good geometry as determined by the MolProbity program.

The LsrR protomer had two distinct domains, the N-terminal DNA-binding domain (DBD) and the C-terminal ligand-binding domain (LBD), connected by a linker region containing a β-strand (Fig. 1A). The N-terminal DBD had three α-helices (α1, α2, and α3) with α2 and α3 forming a helix-turn-helix motif, typical of DNA-binding proteins (Fig. 1B). After the DBD, a β-strand (β1) linker region connected the DBD and LDB (Fig. 1B). The C-terminal LBD adopted an α/β/α sandwich

Figure 1. Overall structure of LsrR. A, LsrR monomer. Left, schematic representation of LsrR monomer. Right, schematic drawing of LsrR topology. B, detailed view of the LsrR DBD. Residues that may be involved in DNA recognition are represented in stick mode. C, detailed view of the LsrR LBD. Dashes indicate residues 215–224 missing in the final model. D, schematic representation of the LsrR dimer in one asymmetric unit. E, superposition of subunit A (green) to subunit B (cyan). DBD domains of two molecules are related by a rotation of about 120°.
architecture. Seven β-strands formed a central core of twisted parallel β-sheets, flanked by helices α4, α10, α13, and α14 on the one side and α5, α6, α7, and α8 on the other side (Fig. 1C).

Two additional β-strands (β7 and β8) and four additional helices (α9, α11, α12, and α15) inserted in different loop regions connecting the elements in the α/β/α sandwich architecture (Fig. 1C).

Two LsrR subunits in one asymmetric unit form a dimer through interactions around the β1-strand from each subunit (Figs. 1D and 3C). Both the DBDs and LBDs of the two molecules were similar, with root mean square deviations (r.m.s.d.) of 0.526 and 0.635 Å over 42 and 220 aligned Ca atoms, respectively. Only small differences were observed in terminal loops of the two DBDs (Fig. 2A). In the two LBDs, helix α4 of subunit B was longer than in subunit A, and the loops connecting β2-α5 and α8-β5 adopted different conformations (Fig. 2B). Although the structures of the LBDs and DBDs in the two subunits were almost identical, the relative orientations of two domains in each subunit were different. As a consequence, subunit A folded into a compacted conformation different from the extended conformation of subunit B. When the LBDs of subunit A and B were superposed, the DBDs showed a rotation of about 120° (Fig. 1E).

Further analysis revealed that changes in dihedral angles of Gly-70 (ψA(70) = −166°; ψB(70) = −45°) and Cys-71 (ψA(71) = 92°; ψB(71) = −58°) were mainly responsible for the conformational differences between helices α4 of subunits A and B. Furthermore, the salt bridge between Arg-67 and Asp-95 in subunit B was replaced by a bridge between Glu-69 and Arg-88 in subunit A (Figs. 2C and 1E). These variations induced the observed conformational differences.

Assembly of LsrR Tetramers—LsrR was observed to be a tetramer in solution, consistent with PISA analysis (30) and a previous report (15). However, only two LsrR molecules (A and B) were found in one asymmetric unit in the crystals (Fig. 1D). The neighboring dimer CD, related by the crystallographic 2-fold rotation symmetry, packed tightly against the dimer AB (Fig. 3A). Two interaction interfaces between dimers AB and CD were formed by interactions between the LBDs of AD and BC (Fig. 3A).

Because each subunit of the LsrR tetramer interacted directly with two adjacent subunits in different conformations, two types of dimeric interfaces were created, interface I (AD/BC) and interface II (AB/CD) (Fig. 3A). Because dimers AD and BC or AB and CD were related by the crystallographic 2-fold rotation symmetry (Fig. 3A), there were two identical interface I and interface II in LsrR tetramer. For simplicity, we discuss only the interface I (AD) and interface II (AB).

The interface I had interactions between α8, α9, and the loops connecting β4-α8 and β5-α9 from each subunit (Fig. 3B). The interactions between subunit A and D were mainly hydrophobic. The large hydrophobic core architecture involved apolar residues Val-153, Met-157, Ile-160, Ile-171, Ala-174, Pro-175, Ile-183, and Leu-187 from each subunit. In addition, Glu-190 from subunit A formed a hydrogen bond with Ser-179 from subunit D and vice versa (Fig. 3B). The buried surface area of interface I, as analyzed by PISA (30), was about 1600 Å². Thus, the total buried surface area of the interfaces between dimers AB and CD was about 1600 Å², which was strong enough to tether these two dimers to form a stable tetramer in solution.

However, in interface II, the β1 strands from subunit A and B were antiparallel, pulling the two subunits together. Additional contacts surrounding the β1 strands reinforced interactions between subunit A and B. Interactions between A and B were mainly polar, including 11 hydrogen bonds and three salt bridges (Fig. 3C and Table 2). As a result, the buried surface area of interface II, analyzed with PISA (30), was estimated to be 1750 Å², much larger than the interface I.

The overall arrangement of the LsrR tetramer was different from the usual tetrameric assembly in which individual subunits were related either by a single 4-fold symmetry axis or by three mutually perpendicular 2-fold axes. In contrast, the
assembly of LsrR tetramer could be regarded as the dimer of two LsrR dimers, which has been observed in other transcriptional regulators. For example, the tetrameric assembly of the full-length sorbitol operon regulator SorC from *K. pneumoniae*, which can be viewed as the dimer of two dimers, is similar to the LsrR tetramer (31).

**Putative DNA-binding Site**—LsrR is reported to specifically bind to the *lsrR* promoter region (15). Our gel shift assay results confirmed the interaction of LsrR with the *lsrR* promoter (Fig. 4A). We hypothesized that LsrR bound the promoter region through its DNA-binding helix-turn-helix motif. To test our hypothesis, we mutated several amino acids in this region and monitored the DNA interaction. The DNA binding ability of mutants Q33A and Y26H was greatly reduced (Figs. 1B and 4C).

In addition, cross-linking assays revealed that these two mutants retained tetrameric assembly (Fig. 4B). Mutating Tyr-25 of *Salmonella typhimurium* LsrR (corresponding to

| Interaction amino acid residues of interface II | Distance (Å) |
|-----------------------------------------------|--------------|
| **Hydrogen bonds**                             |              |
| His-28 (NE2)                                   | Val-62 (O)   | 3.2 |
| Arg-61 (N)                                     | Gln-63 (O)   | 3.1 |
| Gln-63 (N)                                     | Arg-61 (O)   | 2.8 |
| Asn-65 (N)                                     | Ile-59 (O)   | 2.6 |
| Asn-65 (ND2)                                   | Gly-58 (O)   | 3.1 |
| Gly-58 (O)                                     | Asn-65 (ND2) | 3.6 |
| Ile-59 (O)                                     | Asn-65 (N)   | 2.8 |
| Arg-61 (O)                                     | Gln-63 (N)   | 3.0 |
| Val-62 (O)                                     | His-28 (NE2) | 3.4 |
| Gln-63 (O)                                     | Arg-61 (N)   | 2.9 |
| Gln-63 (OE1)                                   | Arg-61 (NH1) | 3.0 |
| **Salt bridges**                               |              |
| Arg-67 (NH₃)                                   | Glu-16 (OE1) | 3.2 |
| Asp-29 (OD2)                                   | Arg-101 (NH1)| 2.4 |
| Glu-75 (OE2)                                   | Arg-21 (NH₃)| 2.4 |
Tyr-26 of *E. coli* LsrR) to His resulted in a 10-fold derepression of the operon (13). Our results provide further evidence that Tyr-26 might be involved in the interaction of LsrR with DNA.

**Putative Ligand-Binding Site**—LsrR is a repressor in the large SorC/DeoR family of prokaryotic transcriptional regulators, and it contains an N-terminal DBD and a C-terminal LBD. The LBD is the sensor for specific effectors that modulate the DNA binding activity, triggering changes in target gene expression in response to the effector. Structural comparison of LBD with other structures in the PDB using the Dali server (32) gave many hits.

Among them structures in the PDB using the Dali server (32) gave many hits. The ligand-binding site in CggR complexed with the SorC and L-sorbose complex was not resolved groove about 15 Å long and 5 Å wide was identified (Fig. 2A). Although the residues that interacted with p-AI-2 were highly conserved (Fig. 5A), the corresponding residue in LsrR is Lys-288. In CggR, the corresponding residue is Lys-310, which forms a salt bridge with the phosphoryl group of dihydroxyacetone phosphate (DHAP) in a similar way (Fig. 5C). In addition, the phosphoryl group of p-AI-2 formed a hydrogen bond with the main-chain amide group of Glu-126. The position of the phosphoryl group was fixed by an ionic interaction and a hydrogen bond between p-AI-2 and LsrR residues Phe-124, Ile-210, Ile-244, and Leu-245, strengthening the interaction between p-AI-2 and LsrR. Sequence alignment of LsrR homologs from different species indicated that the residues that interacted with p-AI-2 were highly conserved (Fig. 5D), providing further evidence supporting the model of p-AI-2 binding to LsrR.

**p-AI-2 Induces Dissociation of LsrR Tetramer into Dimers**—LsrR interaction with DNA is impaired in the presence of p-AI-2 (15). Hence, we investigated the effect of p-AI-2 on the oligomerization of LsrR using chemical cross-linking. LsrR tetramers were captured in the absence of p-AI-2 and LsrR. Sequence alignment of LsrR homologs from different species indicated that the residues that interacted with p-AI-2 were highly conserved (Fig. 5D), providing further evidence supporting the model of p-AI-2 binding to LsrR.

For more insights into the ligand-binding mechanism, p-AI-2 was docked into the LBD structure of LsrR using the program HADDOCK. In this model, p-AI-2 was tethered in the putative ligand-binding site by a network of hydrogen bonds, salt bridges, and hydrophobic interactions (Fig. 5C). As expected, the phosphoryl group of p-AI-2 was located at the positively charged end of the groove, forming a salt bridge with Lys-288. In CggR, the corresponding residue is Lys-310, which forms a salt bridge with the phosphoryl group of dihydroxyacetone phosphate (DHAP) in a similar way (Fig. 5C). In addition, the phosphoryl group of p-AI-2 formed a hydrogen bond with the main-chain amide group of Glu-126. The position of the phosphoryl group was fixed by an ionic interaction and a hydrogen bond between p-AI-2 and LsrR. Additional hydrogen bonds formed between p-AI-2 and Leu-245 and Asp-243, guiding the accurate entrance of p-AI-2 into the groove. Glu-269 in CggR is at the position corresponding to Asp-243 in LsrR and is involved in orienting DHAP orientation (Fig. 5C). Moreover, p-AI-2 formed hydrophobic interactions with the LsrR residues Phe-124, Ile-210, Ile-244, and Leu-245, strengthening the interaction between p-AI-2 and LsrR. Sequence alignment of LsrR homologs from different species indicated that the residues that interacted with p-AI-2 were highly conserved (Fig. 5D), providing further evidence supporting the model of p-AI-2 binding to LsrR.

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Because the phosphoryl group of p-AI-2 was located at the positively charged end of the groove, forming a salt bridge with Lys-288. In CggR, the corresponding residue is Lys-310, which forms a salt bridge with the phosphoryl group of dihydroxyacetone phosphate (DHAP) in a similar way (Fig. 5C). In addition, the phosphoryl group of p-AI-2 formed a hydrogen bond with the main-chain amide group of Glu-126. The position of the phosphoryl group was fixed by an ionic interaction and a hydrogen bond between p-AI-2 and LsrR. Additional hydrogen bonds formed between p-AI-2 and Leu-245 and Asp-243, guiding the accurate entrance of p-AI-2 into the groove. Glu-269 in CggR is at the position corresponding to Asp-243 in LsrR and is involved in orienting DHAP orientation (Fig. 5C). Moreover, p-AI-2 formed hydrophobic interactions with the LsrR residues Phe-124, Ile-210, Ile-244, and Leu-245, strengthening the interaction between p-AI-2 and LsrR. Sequence alignment of LsrR homologs from different species indicated that the residues that interacted with p-AI-2 were highly conserved (Fig. 5D), providing further evidence supporting the model of p-AI-2 binding to LsrR.

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In addition, adding p-AI-2 reduced the formation of LsrR-DNA complexes dramatically (Fig. 6C), consistent with a previous report (15).

Lys-288 and Asp-243 in the binding groove, predicted to be important in p-AI-2 recognition, were mutated to alanine to monitor the effect of p-AI-2 on oligomerization. Mutants K288A and D243A formed tetramers in the presence or absence of p-AI-2 (Fig. 6D). Based on these observations, we proposed that p-AI-2 triggers the dissociation of LsrR tetramer into dimers.

FIGURE 5. Predicted p-AI-2-binding site of LsrR. A, comparison of the LBD structure of LsrR (green) with CggR (blue) and SorC (purple). Conserved grooves are indicated by arrows. Two corresponding loops (A and B) with conformational changes possibly triggered by ligand binding were indicated by an oval. B, surface representation of predicted LsrR p-AI-2 binding site. C, structural model of LsrR LBD complexed with p-AI-2 generated by HADDOCK. Enlarged view (right) shows detailed interactions between p-AI-2 and LsrR. Thin dashes indicate possible hydrogen bonds between LsrR and p-AI-2. Thick dashes indicate loop connecting β6-α11 (residues 215–224) missing in the final model. D, sequence alignment of LsrR from different species. Triangles, hydrophobic residues possibly involved in p-AI-2-LsrR interactions. Stars, residues that might form side-chain hydrogen bonds with p-AI-2.
Theoretically, the LsrR tetramer dissociates into AB and CD dimers. Because the buried surface area of the AB dimer was more than twice the area of the AD dimer (1750 Å² versus 800 Å²), the LsrR dimer in the presence of p-AI-2 is likely to be the AB form. We made mutants V153E and P173A,P75A in loops A and B in the interface I to obtain LsrR dimers in the AB form. Cross-linking and SEC assays showed that mutants V153E and P173A,P75A were dimers (Fig. 6, E and F). Next, we purified mutants V153E and P173A,P75A in dimer form by SEC (Fig. 6F) and examined their DNA binding ability. The DNA binding ability of mutant P173A,P75A in dimer form was nearly the same as wild-type LsrR. However, the binding of mutant V153E to DNA was much weaker (Fig. 6G). The elution peak of mutant V153E was much wider than that of mutant P173A,P75A and wild-type LsrR in SEC assay, indicating that mutant V153E in dimer form was not stable in solution (Fig. 6F). This might affect the interaction of mutant V153E with DNA.

DISCUSSION

To determine the molecular mechanism by which LsrR senses p-AI-2 and regulates gene transcription, the crystal structure of full-length LsrR was determined. An N-terminal DBD and a C-terminal LBD were observed in LsrR, connected by a linker region containing a β-strand (Fig. 1A). Two LsrR molecules formed a single asymmetric unit of a tight dimer (Fig. 1D). Two neighboring LsrR dimers packed against each other to form a tetramer (Fig. 3A). Mutagenesis and gel shift assays determined possible residues that could bind DNA (Fig. 4C). The p-AI-2-binding site was predicted by comparing LsrR with CggR and SorC (Fig. 5A). Several residues in the putative p-AI-2 binding groove of LsrR were predicted to be important for p-AI-2 recognition based on a docking model (Fig. 5C). Cross-linking, SEC, and gel shift assays showed that p-AI-2 induced dissociation of LsrR tetramers into dimers (Fig. 6, A and B) and reduced LsrR interaction with DNA (Fig. 6C). Based on structural and biochemical analysis, we propose that p-AI-2 disrupts the tetrameric assembly of LsrR, resulting in dissociation of LsrR from the promoter region and activation of target gene transcription.

**Structural Basis for Disassembly of LsrR Tetramer in the Presence of p-AI-2**—Binding of regulator molecules induces the conformational change of CggR and disrupts tetramer assembly (34). Based on structural and biochemical analysis, LBD is proposed to mediate regulation of CggR oligomerization (33). Comparison of the structure of CggR in free form to CggR complexed with ligands showed that the two loops of residues 174–184 and 203–211 shift toward the center of the ligand-binding site in the presence of ligand (Fig. 7). Conformational changes of these two loops disrupt interactions between CggR LBDs (33). Superposition of the structure of the LsrR LBD onto CggR demonstrated that two loops in LsrR connecting β4-α8 (residues 149–153, loop A) and β5-α9 (residues 171–180, loop B) were located at sites corresponding to the two loops of CggR structure (Fig. 7). Interestingly, loops A and B interact with loops A’ and B’ from the other subunit on the interface I, through hydrophobic interactions in the LsrR tetramer (Figs. 7 and 3B).
Loops A and B were adjacent to the LsrR ligand-binding pocket. Ligand binding to LsrR might induce conformational changes in loops A and B. No electron density could be assigned to residues 215–224 of the loop connecting β6-α11 in LBD of LsrR, indicating high flexibility. Loop β6-α11, at the entrance of the putative ligand-binding site, might participate in ligand binding (Fig. 7). We cannot exclude the possibility that upon p-AI-2 binding, the structure of loop β6-α11 is stabilized by ligand, contributing to the rearrangement of loops A and B. Because the conformational changes of the corresponding loops in CggR trigger tetramer dissociation, we propose that the interactions between loops A and B and A’ and B’ from neighboring subunits of LsrR are necessary to stabilize the tetramer. We propose that binding of p-AI-2 to LsrR leads to conformational changes in loops A and B, resulting in disassembly of the LsrR tetramer into dimers. Consistently, mutations in loops A and B disrupt the tetrameric assembly of LsrR (Fig. 6, E and F). This evidence further supports the important roles of loop A and B in regulating the oligomerization state of LsrR.

Mechanism of Transcriptional Regulation—Xue et al. (15) reported that LsrR binds a 30-bp DNA sequence at lsrA and the lsr gene promoter regions containing two conserved recognition sites for LsrR (Fig. 8). Deletion of either recognition site reduces LsrR binding to its target promoter regions (15). This previous work indicates that LsrR binds the two recognition sites cooperatively. Interestingly, we observed that the LsrR tetramer assembled as a dimer of two LsrR dimers. Each AB-type dimer had two DBDs, each of which might bind to one recognition site. The four DBDs of the LsrR tetramer were on the same surface with about 70 Å between the two DBDs of each AB-type dimer (Fig. 3A). The total reported length of the two recognition sites of LsrR and the linker between them is 22 bp, about 74 Å in length, and is compatible with the distance between the DBDs of each AB-type dimer. This supports that LsrR binds to target gene sites in tetramer form. Previous work demonstrated that CggR functions as a tetramer to recognize target DNA sites cooperatively. The two dimers of CggR differ in binding affinities for target DNA. High affinity binding of one dimer stabilizes the binding of the dimer with low affinity (35). In LsrR target promoters, the two recognition sites are not identical (Fig. 8) (15), resulting in different binding affinities for the two dimers in the LsrR tetramer. Similar to CggR, tetrameric LsrR might bind target gene sites cooperatively. We found that upon binding of p-AI-2, interactions between subunits were destabilized, leading to disassembly of LsrR tetramers into dimers.

In addition, the DNA binding ability of LsrR was greatly reduced by p-AI-2 (Fig. 4A). We hypothesize that p-AI-2 induces the disassembly of LsrR tetramers, reducing DNA binding ability. Based on the structure of the LsrR tetramer, an LsrR dimer could be the AB or AD form (Fig. 3A). Because the mutants of V153E and P173A, P175A in dimer form of AB LsrR might bind target gene sites cooperatively. We found that upon binding of p-AI-2, interactions between subunits were destabilized, leading to disassembly of LsrR tetramers into dimers.

Based on structural and biochemical analysis, we propose a hypothetical transcriptional regulation mechanism of LsrR in response to p-AI-2. In this model, in the absence of AI-2, LsrR behaves as functional tetramer binding to promoter regions and repressing transcription of target genes. AI-2 that is imported into E. coli cells is converted by the kinase LsrK to p-AI-2. Binding of p-AI-2 induces the conformational changes of LsrR and disrupts the tetrameric assembly, triggering the dissociation of LsrR from promoter regions and the activation of target genes.

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