The X-ray Structure of the PurR-Guanine-purF Operator Complex Reveals the Contributions of Complementary Electrostatic Surfaces and a Water-mediated Hydrogen Bond to Corepressor Specificity and Binding Affinity*

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The purine repressor, PurR, is the master regulatory protein of de novo purine nucleotide biosynthesis in Escherichia coli. This dimeric transcription factor is activated to bind to cognate DNA operator sites by initially binding either of its physiologically relevant, high affinity corepressors, hypoxanthine (\(K_d = 9.3 \mu M\)) or guanine (\(K_d = 1.5 \mu M\)). Here, we report the 2.5-Å crystal structure of the PurR-guanine-purF operator ternary complex and complete the atomic description of 6-oxopurine-induced repression by PurR. As anticipated, the structure of the PurR-guanine-purF operator complex is isomorphic to the PurR-hypoxanthine-purF operator complex, and their protein-DNA and protein-corepressor interactions are nearly identical. The former finding confirms the use of an identical allosteric DNA-binding mechanism whereby corepressor binding 40 Å from the DNA-binding domain juxtaposes the hinge regions of each monomer, thus favoring the formation and insertion of the critical minor groove-binding hinge helices. Strikingly, the higher binding affinity of guanine for PurR and the ability of PurR to discriminate against 2-oxopurines do not result from direct protein-ligand interactions, but rather from a water-mediated contact with the exocyclic N-2 of guanine, which dictates the presence of a donor group on the corepressor, and the better electrostatic complementarity of the guanine base and the corepressor-binding pocket.

The atomic coordinates and structure factors (code 1WET) have been deposited in the Protein Data Bank, Brookhaven National Laboratory, Upton, NY. To whom correspondence should be addressed. Tel.: 503-494-4427; Fax: 503-494-8393; E-mail: brennanr@ohsu.edu.

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1 The abbreviations used are: CBD, corepressor-binding domain; Wat, water.
binding specificity. The key PurR residue that specifies an oxygen at the purine exocyclic 6-position is Arg<sup>190</sup>. The guanidinium group of Arg<sup>190</sup> donates two hydrogen bonds to the O-6 acceptor of hypoxanthine (9). Thus, the side chain donors of Arg<sup>190</sup> dictate the requirement for hydrogen bond acceptors at this purine position. However, the mechanism of discrimination at the exocyclic 2-group was unclear, as was the stereochemical basis by which PurR binds guanine more tightly than hypoxanthine (8).

To begin the structural elucidation of the allosteric mechanism of PurR, which transduces the signal of corepressor binding by the CBD to effect DNA binding by the distal DNA-binding domain, the structure of the corepressor-free CBD was determined (13). From this and the hypoxanthine-bound PurR structure, the role of that purine corepressor binding was discerned. Upon binding hypoxanthine, there is a dramatic rearrangement in the tertiary and quaternary structures of the CBD N-terminal subdomain that results in the juxtaposition of the two hinge regions of PurR, which are over 40 Å away (13).

Corepressor binding is essential for this juxtaposition as it shifts the equilibrium from the open form of PurR, in which the N- and C-terminal subdomains of the CBD are rotated far apart, to the closed conformation. Although likely, it was not known if guanine binding causes the identical structural changes that result in this allosteric shift.

To address the issues of the corepressor specificity and the higher affinity of PurR for guanine and its possible consequences on the intramolecular signaling pathway and allosteroy of this protein, we have determined the structure of a PurR-guanine-purF operator complex at 2.5-Å resolution. As expected, the PurR-purF operator contacts and DNA bending are nearly identical to those seen in the PurR-hypoxanthine-purF operator complex (9). Strikingly, the structure reveals that the higher affinity of guanine for PurR (over hypoxanthine) and the discrimination against xanthine as a corepressor arise from 1) a corepressor–water–protein hydrogen bonding network, which involves the exocyclic N-2 hydrogen bond donor of guanine and the hydrogen bond acceptors, O– of Glu<sup>222</sup> and the backbone carbonyl oxygen of Phe<sup>221</sup>, and 2) the intrinsic negative electrostatic environment of the corepressor-binding pocket, which favors the more positive guanine as the corepressor.

### EXPERIMENTAL PROCEDURES

**Crystallization and Data Collection**—PurR was overexpressed in *E. coli* and purified by DE53 cellulose and heparin-agarose chromatography as described (14). Crystals of the PurR-guanine-purF operator complex were grown by the hanging-drop vapor diffusion method, which was used previously to crystallize the PurR-hypoxanthine-purF operator complex (15). Briefly, protein at a concentration of 0.25–0.50 mM was saturated with guanine and mixed 1:1 (v/v) with a 0.50 mM solution of the 16-base pair purF operator, which has an additional 5′-nucleoside overhang on both strands, the identity of which is irrelevant to crystallization. The protein/corepressor/DNA solution was mixed 1:1 with the reservoir solution, which was 25% polyethylene glycol 4000, 0.40 M ammonium sulfate, 25 mM hexamminecobalt chloride, and 0.10 M ammonium phosphate, pH 7.5. Crystals initially grow as thin two-dimensional plates and, within a period of 2 weeks to several months, melt and are replaced by large rhombohedral crystals (typically 0.6 × 0.2 × 0.3 mm). The PurR-guanine-purF operator crystals are isomorphous to the PurR-hypoxanthine-purF operator crystals and take the space group C222<sub>1</sub>, with unit cell dimensions of a = 176.0, b = 95.08, and c = 81.51 Å. There is one PurR monomer, one guanine molecule, and a purF operator half-site in the asymmetric unit.

X-ray intensity data were collected at room temperature with an Area Detector Systems Corp. multidetector area detector (16) and a Rigaku RU200-H rotating anode operating at 40 kV and 150 mA. The data from two crystals were processed and merged with the software provided by Area Detector Systems Corp. The R<sub>merge</sub> of the data to 2.5 Å is 6.29%, and the data are 87% complete from 10.0 to 2.5 Å and 78% from 2.7 to 2.5 Å. The redundancy of the data is 3-fold. The I/σ(I) is 7.4 for the entire data set and 1.4 for the data in the resolution shell between 2.7 and 2.5 Å.

**Refinement**—The previously determined structure of the PurR-hypoxanthine-purF operator complex minus the hypoxanthine and solvent molecules was used as the starting model for refinement (9). Rigid body refinement was carried out on the model followed by XYZ and B factor refinement as implemented in TNT (17). The contour level of the map is 3.5σ. The hydrogen bonds formed between the water molecule (Wat<sup>1</sup>) and the carbonyl oxygen of Phe<sup>221</sup> and the carbonyl side chain oxygen of Glu<sup>222</sup> are shown. Such a donor-acceptor arrangement leaves only hydrogen bond acceptors and results in a water-specific interaction with the exocyclic N-2 of guanine and discrimination against O-2 of xanthine.

**FIG. 1. Omit map (F<sub>o</sub> − F<sub>c</sub>) of guanine, Wat<sup>1</sup>, Phe<sup>221</sup>, and Glu<sup>222</sup>.**

The map was calculated using structure factors from a structure in which guanine, Wat<sup>1</sup>, Phe<sup>221</sup>, and Glu<sup>222</sup> were removed, and an additional 20 cycles of refinement were carried using TNT (17). The contour level of the map is 3.5σ. The hydrogen bonds formed between the water molecule (Wat<sup>1</sup>) and the carbonyl oxygen of Phe<sup>221</sup> and the carbonyl side chain oxygen of Glu<sup>222</sup> are shown. Such a donor-acceptor arrangement leaves only hydrogen bond acceptors and results in a water-specific interaction with the exocyclic N-2 of guanine and discrimination against O-2 of xanthine.

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<sup>2</sup> M. A. Schumacher and R. G. Brennan, unpublished data.
subdomain, which consists of a core of six β-strands (βA–βE and βJ) surrounded by four α-helices (I–III and IX), and the CBD C-terminal subdomain, which consists of a core of five β-strands (βF–βI and βK) surrounded by five α-helices (IV–VIII). Three crossover peptides connect the two subdomains and serve as a floor to the corepressor, which is bound in the cleft between the two CBD subdomains. As seen in the periplasmic binding proteins, these peptidic linkages act as a hinge to allow movements of subdomains upon ligand association and dissociation (9–12).

All contacts observed between PurR and the corepressor hypoxanthine in the PurR-hypoxanthine-purF operator complex are also present in the PurR-guanine-purF operator complex. Stacking interactions occur between guanine and the side chains of Tyr73 and Phe221, which sandwich the corepressor, and Phe74. Hydrogen bonds are provided by Thr192, Asp275, and Arg190. O-γ of Thr192 hydrogen bonds with N-7 of the guanine ring (2.73 Å, O-γ–N-7) and also makes van der Waals contact with the corepressor C-8 via its side chain methyl group. Asp275 makes a hydrogen bond with N-9 of the purine (2.92 Å, O-92–N-9). The importance of Arg190 in corepressor binding specificity at the exocyclic 6-position was revealed in the PurR-hypoxanthine-purF operator complex (9). In the PurR-guanine-purF operator complex, Arg190 also specifies an oxygen at the exocyclic 6-position by providing two hydrogen bonds to this exocyclic amine from its side chain N-7 and NH-1 atoms (3.03 Å, N-7–O-6; and 2.72 Å, NH-1–O-6). Arg190 contributes further to corepressor binding affinity by hydrogen bonding to a water molecule that is simultaneously hydrogen-bonded to N-1 of the guanine ring (3.15 Å, H₂O–O-1; and 2.72 Å, H₂O–N̈H₃). The essentially identical binding observed between PurR and hypoxanthine and guanine in the PurR-hypoxanthine-purF operator and PurR-guanine-purF operator complexes and the identical corepressor-mediated conformations of both complexes reveal how PurR utilizes two related molecules as corepressors. However, these interactions do not explain the nearly 7-fold higher affinity of guanine for PurR or the mechanism by which PurR discriminates at the purine exocyclic 2-position, i.e. against xanthine.

On the basis of the location of the side chain of Glu222 in the PurR-hypoxanthine-purF operator complex (9), we anticipated that upon guanine binding by PurR, this side chain would swing into the corepressor-binding pocket, eject a tightly bound water molecule (Wat-1), and make a direct hydrogen bond with the exocyclic N-2 atom. However, in the PurR-guanine-purF operator complex, the position of the side chain of Glu222 has changed little, and the water molecule is still present, although slightly shifted in position (Fig. 1). Rather, now the water molecule is positioned perfectly to hydrogen bond to the exocyclic N-2 of the guanine ring (2.91 Å, H₂O–N̈O-2). This interaction is specific and discriminatory as both hydrogen bond donors of the water molecule are taken: one by the carbonyl oxygen atom of Phe221 (3.10 Å, H₂O–O) and the other by O-41 of Glu222 (3.06 Å, H₂O–O-1) (Fig. 1). This leaves only the two acceptor lone pairs of this water molecule, one of which interacts with the guanine N-2 atom and presumably the other with a hydrogen bond donor from the bulk solvent. Such a hydrogen bonding network would disfavor the binding of xanthine, which has an exocyclic acceptor atom (O-2) at this position. Consistent with this hypothesis is the in vitro binding of xanthine to PurR, which is ~200-fold weaker than guanine binding (data not shown). Remarkably, this water molecule is found not only in the PurR-guanine-purF operator and the PurR-hypoxanthine-purF operator complexes, but also in the corepressor-free form of PurR, where the identical water–protein hydrogen bonds are made (13). This demonstrates that this tightly bound water
molecule acts as an extension of the protein.

The importance of water-mediated contacts in ligand binding specificity is now well documented. Notable examples include members of the periplasmic binding protein family such as the arabinose-binding protein (21, 22), lysine/arginine/ornithine-binding protein (23), and oligopeptide-binding protein (OppA) (24); enzymes, e.g. α-chymotrypsin (25); and DNA-binding proteins as exemplified in the TrpR-tryptophan-trp operator complex (26, 27). In the arabinose-binding protein, two hydrogen-bonded water molecules, located in the binding pocket, aid in L-arabinose binding while creating an unfavorable interaction with the C-5 methyl group of D-fucose, thus explaining the low affinity of the latter. The near equal affinity of the arabinose-binding protein for D-galactose is explained by the replacement of one of these water molecules with the D-galactose -CH2OH group (21, 22). A similar situation is observed in the lysine/arginine/ornithine-binding protein, where water molecules assist in the binding of lysine, ornithine, and arginine. However, one of the water molecules must be displaced to permit binding of the larger arginine ligand (23). In contrast, OppA provides an example of the use of solvent in decreasing ligand binding specificity through the accommodation of multiple substrates with different peptide side chains in voluminous hydrated cavities from which water molecules are readily gained or lost (24).

In the structure of α-chymotrypsin complexed with a turkey ovomucoid inhibitor, it was demonstrated that a water molecule, also present in the apo structure, mediated an ionic interaction that was critical for inhibitor binding specificity (25). Thus, as in the case of PurR, this water molecule can be considered as a constitutive element of the protein. Finally, the TrpR-tryptophan trp operator complex reveals quite dramatically the use of water molecules to nearly completely specify protein-DNA binding specificity (26, 27). Thus, through its small molecular size, which allows it to pack around ligands of different size and shape, and its ability to provide hydrogen bond donors as well as acceptors, water provides a key element in the formation of a wide variety of protein-ligand complexes.

In addition to the direct water-mediated discrimination against xanthine, an examination of the electrostatic potential of the PurR corepressor-binding pocket and its proximity reveals a second possible source of unfavorable xanthine binding as well as the chemical basis of the more favorable binding of guanine over hypoxanthine. PurR displays a negative electrostatic potential surface near the exocyclic 2-position of the guanine ring (Fig. 3). Glu222, Glu222, and Glu70 and Glu70 from the other subunit contribute to the negative potential of the binding pocket of PurR. This surface is complemented by N-2 of guanine, which imparts a significant positive potential to this base. Hypoxanthine, which lacks any exocyclic group at the ring 2-position, is nearly neutral, but is still weakly positive. In contrast to the physiological corepressors, xanthine possesses a negative electrostatic potential at its exocyclic O-2 position, which likely contributes further to its very low affinity (Fig. 4).
the exocyclic N-2 atom of the guanine base of GDP and ARF-1 (28), where the N-2 atom is enclosed in a positive pocket in the protein. This suggests that such electrostatic complementarity may play a significant role in purine-protein binding and recognition. In addition to the patch of negative electrostatic potential near the exocyclic 2-atom of guanine, there is another pocket of negative potential within the corepressor-binding domain. Corepressor binding activates DNA binding allosterically by repositioning the hinge regions of each PurR monomer such that they may interact, undergo a coil-to-helix transition (37, 38), and bind the DNA minor groove.

In summary, the structure of the PurR-guanine-purF operon complex has established that binding of either hypoxanthine or guanine induces the same activated conformation of PurR, which can then bind the pur regulon operators with high affinity. The structure also reveals the likely basis for the higher affinity of PurR for its guanine corepressor versus hypoxanthine and the stereochemical mechanism of xanthine discrimination: 1) water-mediated readout of the exocyclic 2-position of the purine and 2) better protein-corepressor electrostatic complementarity, notably about the exocyclic 2-position of the purine ring.

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FIG. 4. Electrostatic potentials of guanine, hypoxanthine, and xanthine. The atomic charges and electrostatic potentials are mapped on the van der Waals surfaces of guanine (A), hypoxanthine (B), and xanthine (C) and were calculated with a 3–21G* basis set using Spartan software (Wavefunction Inc., Irvine, CA).
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