Crystal Structure of the MexR Repressor of the mexRAB-oprM Multidrug Efflux Operon of Pseudomonas aeruginosa*

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MexR is a member of the MarR family of bacterial transcriptional regulators and is the repressor for the MexAB-OprM operon, which encodes a tripartite multidrug efflux system in Pseudomonas aeruginosa. Mutations in MexR result in increased resistance to multiple antibiotics due to overexpression of this efflux system. We have determined the crystal structure of MexR to 2.1-Å resolution in the absence of effector. The four copies of the MexR dimer in the asymmetric unit are observed in multiple conformations. Analysis of these conformational states in the context of a model of the MexR-DNA complex proposed in this study suggests that an effector-induced conformational change may inhibit DNA binding by reducing the spacing of the DNA binding domains. The inhibited conformation is exhibited by one of the four MexR dimers, which contains an ordered C-terminal tail from a neighboring monomer inserted between its DNA binding domains and which we propose may resemble the MexR-effector complex. Our results indicate that MexR may differ from the other described member of this family, MarR, in the nature of its effector, mode of DNA binding, and mechanism of regulation.

Pseudomonas aeruginosa is an opportunistic human pathogen of increasing clinical concern due to its intrinsic resistance to multiple antibiotics and to the appearance of strains showing high level multidrug resistance (1, 2). The intrinsic resistance of P. aeruginosa results from a synergy of low outer membrane permeability and chromosomally encoded tripartite efflux systems (2–5). To date, four such efflux systems have been identified in P. aeruginosa: MexAB-OprM (6, 7), MexCD-OprJ (8), MexEF-OprN (9), and MexXY-OprM (10). These efflux systems consist of an inner membrane drug-proton antiporter of the resistance-nodulation-cell division (RND) family (MexB, MexD, MexF, and MexY), an outer membrane channel-forming component (OprM, OprJ, and OprN), and a periplasmic membrane fusion protein (MexA, MexC, MexE, and MexX), which couples the inner and outer membranes for direct extrusion of antibiotics across both membranes (5). The MexAB-OprM system is the first of the four efflux systems that have been described in P. aeruginosa and shows the broadest substrate range (11, 12). The MexAB-oprM operon is negatively regulated by the product of the mexR gene (11, 13), which is located upstream of mexA and transcribed in the opposite direction. Mutations in mexR lead to hyperexpression of the mexAB-oprM operon, resulting in increased resistance to multiple antimicrobials, including fluoroquinolones, β-lactams, tetracycline, macrolides, chloramphenicol, novobiocin, trimethoprim, and sulfonamides (13–16). Signaling molecules utilized in quorom sensing have also been implied as substrates for the efflux pumps in P. aeruginosa. Overexpression of the MexAB-OprM efflux system has been found to decrease intracellular accumulation of the P. aeruginosa autoinducer 3-O-dodecanoylhomoserine lactone (PAI-I) resulting in reduced expression of autoinducer regulated virulence factors (17, 18).

MexR is a member of the MarR family of bacterial transcriptional regulators (19). The marR gene is a component and negative regulator of the marAB locus in Escherichia coli (20). Members of the MarR family regulate the expression of resistance to multiple antibiotics, organic solvents, detergents and oxidative stress agents, and of pathogenic factors (21, 22). By regulating the expression of the transcriptional activator MarA, MarR is able to regulate several MarA-regulated genes involved in antimicrobial resistance. The recently determined crystal structure of MarR in complex with the effector salicylate (23), revealed a dimeric structure with a winged helix DNA binding motif (24) in each MarR monomer. Two molecules of the effector salicylate, which inhibits DNA binding by MarR (21, 25), were bound to each subunit and located on either side of the proposed recognition helix in the DNA binding domain (denoted SAL-A and SAL-B). To understand the specific regulation of MexAB-OprM expression in Pseudomonas and to gain further insights into the molecular regulation of the DNA binding activity of other members of the MarR family, we have determined the crystal structure of MexR. We show that salicylate is not an effector for MexR and have also modeled the MexR-DNA complex, using the MexR operator sequence identified by previous footprinting studies (26). Based on our structural analysis of MexR, our model of the MexR-DNA complex and a comparison with the crystal structure of the MarR-salicylate complex (23), we propose an allosteric mechanism of regulation of the DNA binding activity of MexR that likely differs from that of MarR.
Table I

Data collection, MAD phasing, and structure refinement statistics

|                         | \(\lambda_1\) (peak) | \(\lambda_2\) (remote) | High resolution |
|-------------------------|-----------------------|-------------------------|-----------------|
| Wavelength (\(\lambda\))| 0.979                 | 0.932                   | 1               |
| Cell axes a, b, c (\(\AA\)) | 67.9, 72.2, 241.3 | 67.9, 72.2, 241.4 | 67.9, 72.7, 240.5 |
| Resolution (\(\AA^{n}\)) | 25.0–2.6 (2.69–2.6) | 25.0–2.5 (2.59–2.5) | 25–2 (2.18–2.1) |
| No. of observed reflections | 192,756              | 164,169                 | 352,292         |
| No. of unique reflections | 69,848               | 78,058                  | 63,205          |
| \(R(I)^{a}\)           | 22.5 (4.7)           | 21.3 (4.7)              | 30.7 (3.1)      |
| \(R_{sym}(\%)^{b,c}\)  | 3.3 (8.6)            | 2.5 (11.3)              | 4.5 (34.0)      |
| Overall completeness (\(\%)^{n}\) | 99.1 (97.0) | 99.0 (95.9)          | 90.3 (60.5)     |
| Figure of merit,\(^{d}\) | 0.60/0.47/0.48       |                         |                 |

No. of non-H atoms, protein/water 8924/214

\(R_{crys}/R_{free}^{d}\) 24.2/29.4

r.m.s. deviations, bond lengths (\(\AA\)/\(\circ\)) 0.0060/1.14

Average B-factor (\(\AA^2\)) 38.1/44.3/36.0

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\(^{a}\) Values in parentheses correspond to the highest resolution shell.

\(^{b}\) \(R_{sym} = \frac{\sum |I_i - <I>|}{\sum |I_i|}, \text{ where } I_i \text{ is the intensity for reflection } j, \text{ and } I \text{ is the mean intensity.}

\(^{c}\) Figure of merit is defined in CNS (29).

\(^{d}\) \(R_{crys} = |F_o| - |F_c| / |F_c|, \text{ calculated with the working set. } R_{free} \text{ is similarly calculated but with 5\% of the data excluded from the calculation of } R_{crys}.

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**FIG. 1. Overall structure of the MexR dimer.** a, MexR dimer in ribbon representation. The secondary structure elements are labeled and colored individually in the monomer on the left. The winged helix domain (colored blue in the monomer on the left) consists of \(\alpha2\) (H1)–\(\beta1\) (S1)–\(\alpha3\) (H2)–\(\alpha4\) (H3, recognition helix)–\(\beta2\) (S2)–W1 (wing)–\(\beta3\) (S3), where the terminology used by Gajiwala and Burley (24) is given in brackets. The N and C termini are labeled N and C, respectively. b, MexR dimer shown in similar orientation as in a and with a GRASP (43) molecular-surface representation of one subunit highlighting the hydrophobic dimerization interface. Low, medium, and high hydrophobic potentials are colored gray, yellow, and green, respectively. The second subunit is shown in ribbon representation (magenta).

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**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—A wild type expression vector was constructed by PCR amplification of the mexR open reading frame (encoding residues 1–147 with no tags or additional residues) from the previously described vector pKLE1 (26). The PCR product was cloned into the pET41a vector (Novagen) and used for the production of native and selenomethionine (SeMet)-labeled MexR protein from *Escherichia coli* strain BL21 Ade3. An overnight seed culture (grown in LB with 50 \(\mu\)g/ml of kanamycin) was used to inoculate M9 medium (with 1:100 dilution) supplemented with 2 mM MgSO\(_4\), 0.1 mM CaCl\(_2\), and 4 g/liter glycerol. For the production of native protein, the medium was also supplemented with 2.5 g/liter of casamino acids. Cultures were grown at 37 °C to an optical density at 600 nm (OD\(_{600}\)) of -0.5, at which point isopropyl b-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM for the production of native protein. For the production SeMet-labeled protein, 100 mg each of Thr, Lys, Phe, and 50 mg each of Leu, Ile, and SeMet were added per liter of culture at an OD\(_{600}\) of -0.5 with the subsequent addition of 0.5 mM IPTG after 15 min. Cells were shifted to room temperature and MexR expression was allowed to proceed overnight.

All purification steps were carried out at 4 °C, and the same procedure was used for both native and SeMet-labeled proteins. Cells were pelleted by centrifugation at 5000 \(\times\) g for 30 min and frozen at -80 °C. Frozen cell pellets were resuspended in a harvest buffer consisting of 5 mM sodium phosphate buffer (pH 7), 1 mM EDTA, 10% (v/v) glycerol, and 14 mM \(\beta\)-mercaptoethanol. The cells were sonicated on ice (3 \(\times\) 10 min, 30% pulse, 50% power), and insoluble material was removed by centrifugation at 25,000 \(\times\) g for 30 min. The supernatant was applied onto a SP-Sepharose (Amersham Biosciences) column (2.5 \(\times\) 10 cm) equilibrated with harvest buffer. The protein was eluted with a linear gradient of 0 to 0.2 mM NaCl. Unbound MexR protein in the initial flow-through was recovered and purified with a second pass over the SP-Sepharose column. Peak fractions as assessed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) were pooled, diluted 5-fold with harvest buffer, and applied onto a heparin-agrose (Sigma) column (2.5 \(\times\) 5 cm) equilibrated with harvest buffer. MexR protein was eluted with a linear gradient of 0 to 0.5 mM NaCl. Peak fractions were pooled, concentrated to ~0.5 ml, and applied onto a Sephacryl-100 (Amersham Biosciences) gel filtration column (2.5 \(\times\) 10 cm) equilibrated with harvest buffer. MexR was eluted with a linear gradient of 0 to 0.5 mM NaCl. Peak fractions were pooled, concentrated to 80–128 mg/ml as measured by the Bradford assay. The protein was assessed to be >90% pure by SDS-PAGE. Complete substitution of methionines in SeMet-labeled protein was confirmed by electrospray mass spectrometry.

**Crystallization**—Crystals of MexR were grown by the hanging drop vapor diffusion method using protein solutions diluted to 8.8 mg/ml with water and with the addition of triis(carboxymethyl)phosphine hydrochloride to a final concentration 10 mM. For native MexR, 1 \(\mu\)l of protein solution was mixed with an equal volume of reservoir solution consisting of 20 mM CaCl\(_2\), 65 mM NaCl, 6 mM MES (pH 6.4), 4 mM sodium acetate (pH 5), and 10 mM dithiothreitol (DTT). For SeMet-labeled

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\(^{1}\) The abbreviations used are: SeMet, selenomethionine; MES, morpholinooethanesulfonic acid; DTT, dithiothreitol; MAD, multiwavelength anomalous diffraction; NCS, non-crystallographic symmetry; r.m.s., root mean square.
MexR crystals were harvested in buffer containing 10 mM CaCl₂, 6 mM SeMet-labeled crystals. Reservoir solution consisting of 46 mM CaCl₂, 105 mM NaCl, 6 mM MES (pH 6), 4 mM sodium acetate (pH 5), and 10 mM DTT. Crystals of both native and SeMet-labeled MexR grew after equilibration over 1 ml of reservoir solution for 12–24 h at 20 °C reaching dimensions of up to ~0.15 × 0.15 × 0.3 mm for native crystals and 0.2 × 0.2 × 0.5 mm for SeMet-labeled crystals.

X-ray Data Collection, Structure Determination, and Refinement—MexR crystals were harvested in buffer containing 10 mM CaCl₂, 6 mM MES (pH 6), 4 mM sodium acetate (pH 5), and 10 mM DTT. Crystals were cryo-protected by gradual transfer in ten increments to 33% (w/v) polyethylene glycol 400, 20 mM CaCl₂, 128 mM NaCl, 6 mM MES (pH 6), 4 mM sodium acetate (pH 5), and 10 mM DTT and were flash-frozen in a nitrogen cold stream prior to data collection. The increase in salt concentration with the addition of polyethylene glycol 400 was necessary to prevent shrinkage of the unit cell to a pseudo-tetragonal cell with pseudo-merohedral twinning. Due to their superior diffraction over native crystals, data from SeMet-labeled crystals were used for both structure determination and refinement. MAD data were collected at beam line X8-C at the National Synchrotron Light Source. Data were processed using the HKL package (27) and programs from the CCP4 software suite (28). Based on systematic absences, the space group was determined to be P2₁2₁2₁ with unit cell dimensions of a = 67.9 Å, b = 72.7 Å, and c = 240.5 Å. Data collection statistics are listed in Table I. Structure superpositions were made using LSQKAB (28) and ALIGN (32). Interhelical angles and distances were measured using the program INTERHLX (33). Unless otherwise stated, all figures were created with Molscript (34) and Raster3D (35).

Molecular Modeling of the MexR-DNA Complex—The MexR crystal structure and the MexR operator sequences identified by previous footprinting studies (26) provide geometric constraints to guide the modeling of the MexR-DNA complex. Both the MexR dimer and the operator DNA sequence contain a 2-fold rotation axis relating the two MexR monomers and the inverted repeats, respectively. These axes are expected to coincide in the MexR-DNA complex. Each inverted repeat is five base pairs in length (5'–GTTGA) and is spaced five base pairs apart. Assuming that the MexR operator DNA adopts a canonical search routine in CNS (29). Five additional sites were located by log-likelihood gradient maps after refinement and phasing with the initial sites. Following solvent flattening with RESOLVE (30), an initial electron density map was sufficiently interpretable to allow Co tracing (in XtalView; Ref. 31) of one of the four MexR dimers in the asymmetric unit. This initial trace was manually positioned into the density regions for the remaining dimers, which were then used to derive non-crystallographic symmetry (NCS) operators. A significantly improved electron density map was obtained after NCS averaging and phase extension to 2.15 Å with DM (28), from which a nearly complete model of the asymmetric unit was built (Fig. 1). Due to the asymmetry of the MexR dimer, 4-fold multidomain averaging was done with the two monomers in each dimer treated as separate domains. The model was completed with iterative rounds of manual fitting and refinement in CNS (without NCS restraints). MAD phasing and model refinement statistics are given in Table I. Structure superpositions were made using LSQKAB (28) and ALIGN (32). Interhelical angles and distances were measured using the program INTERHLX (33). Unless otherwise stated, all figures were created with Molscript (34) and Raster3D (35).
linear B-DNA conformation with 10.5 bp per turn, the major grooves of the inverted repeats would be positioned on the same side of the DNA, to which MexR is expected to bind. Taking these geometric constraints into consideration, MexR was manually docked onto an ideal model of the operator DNA with the MexR dimer oriented such that the DNA binding domains faced the major grooves of the inverted repeats.

RESULTS AND DISCUSSION

Overall Structure—The crystal structure of MexR was determined using MAD data collected from a selenomethionine-substituted crystal. The final refined model shows good stereochemistry with 94.0% of residues in the most favorable region of the Ramachandran plot (36) and one residue in the disallowed region (Gln90 in chain D), which is located in a region of poor electron density. The overall structure (Fig. 1a) is predominantly α-helical and appears to resemble that of MarR (23). The MexR dimer is triangular in shape and contains two winged helix DNA binding domains (residues 36–97 of each monomer), each connected via two long helices (a1 and a5) to a

![Proposed mechanism of regulation of MexR.](image-url)

- **a**, molecular modeling of MexR-DNA complex. The MexR dimer CD (ribbon representation) depicts the “open” or DNA-bound conformation. The DNA molecule is shown in stick representation, with the inverted repeats colored green and highlighted by thick rendering. Residues on MexR which correspond to the MarR residues observed to be in contact with salicylate (23) are shown in stick rendering.
- **b**, insertion of the C-terminal tail (residues 140–147 shown with magenta carbons) from chain C in between the DNA binding domains results in a “closed” conformation depicted by dimer AB (ribbon representation), in which the reduced spacing between the DNA binding domains is incompatible with the spacing of the inverted repeats (green with thick rendering) of the operator. SigmaA-weighted 2F_0 − F_2 electron density (29) is contoured at 1σ to 2.1 Å around the C-terminal tail. The electron density object was created in O (44).
- **c**, close-up view of the interactions between the C-terminal tail of monomer C (magenta) with side chains on monomer A, close-up view of the interactions between the C-terminal tail of monomer C (magenta) with the side chains on monomer B. Water molecules are shown as red spheres in c and d.
- **d**, close-up view of the interactions between the C-terminal tail of monomer C (magenta) with the side chains on monomer B. Water molecules are shown as red spheres in c and d.
- **e**, electrophoretic mobility shift assays of DNA binding by SeMet MexR with and without treatment with Hin c II restriction endonuclease. All samples contained 247 ng of a 28-bp DNA oligonucleotide with the sequence 5′ATTTTACGTGAAGCCTCATTTCTGTTTT (the Hin cII site is in bold with the cleavage site indicated by ‖) corresponding to the MexR binding site II identified by previous footprinting studies (26). The DNA in lanes 3 and 4 were digested with Hin cII for 1 h and 40 min at 37 °C. Prior to loading onto the gel, 12.8 µg of SeMet-substituted MexR protein were added to samples in lanes 2 and 4.
dimerization domain made up of the N- and C-terminal regions (residues 5–17 and 120–139) from the two monomers (Fig. 1, a and b). Hydrophobic residues from α1, α5, and α6 are buried at the dimerization interface. The total buried surface area ranges from 4360 to 4930 Å² for the four MexR dimers in the asymmetric unit. The electrostatic interactions observed between the DNA binding domains of MarR (Asp67 of one monomer with Arg73 of the other monomer and the reciprocal pair; Ref. 23) are not present in MexR.

Conformational Flexibility of the MexR Dimer—The molecular packing in the MexR crystal has provided eight independent observations of the MexR monomer structure. A significant degree of flexibility is seen in the MexR structure as evidenced by the large deviations between the eight copies of the MexR monomer in the asymmetric unit, with overall r.m.s. (root mean square) deviations of 0.70 Å relative to monomer A for 512 pairs of main chain atoms (Fig. 2a). The regions encompassing helices α1, α5, and α6 (residues 4–32 and 99–139) show main chain r.m.s. deviations of 0.40–1.29 Å relative to monomer A, with the largest differences seen between monomers A and B. Flexibility at loop regions (residues 33–34, 97–98, and 121–123) allows helix α1 to deviate by 17.0° and 4.9 Å, α5 by 6.8° and 1.7 Å, and α6 by 12.1° and 8.2 Å in helix orientation and midpoint position, respectively. In contrast to MarR, clear main chain density is observed for α5, including Ser113, which corresponds to the poorly ordered region of Gly116 in MarR (a proposed flexible hinge region; Ref. 23).

In addition to the conformational differences among individual monomers, we also observe significant variations in the relative disposition of the two monomers at the dimerization interface in each of the four independent dimers in the asymmetric unit. These positional variations in the MexR dimer give rise to major differences in the disposition of the two DNA binding domains relative to each other (Fig. 2b). The interhedral interactions at the dimerization interface primarily consist of van der Waals contacts between hydrophobic side chains, which unlike hydrogen bonds and salt bridges, do not require specific geometric arrangements for the interacting atoms and therefore could accommodate significant reorganization of the dimerization helices relative to each other. With the exception of the W1 region, the winged-helix domain moves as a relatively rigid and well ordered entity. There is little deviation between the eight copies in the asymmetric unit (r.m.s. deviations of 0.31–0.57 Å relative to main chain atoms in residues 33–84 and 93–98). Superposition of the four observed MexR dimers using main chain atoms from one DNA binding domain per dimer (residues 33–84 and 93–98) shows a maximum helix midpoint displacement of 7.7 Å (between dimers AB and CD) for the recognition helix α4 in the other DNA binding domain.

Model of the MexR-DNA Complex—Of the four MexR dimers in the asymmetric unit, dimer CD shows the largest spacing between the DNA binding domains with a Ca-Ca distance of 29.2 Å between Arg73 and Arg73 (from helix α4), which is close to the 34 Å spacing between major grooves in linear B-DNA. Manual docking of dimer CD onto the operator DNA produced a model with a reasonable match of the spacing between the recognition helices (α4 and α4') with the spacing between the major grooves in the inverted repeats (Fig. 3a). Given the observed conformational adaptability of the dimerization interface, it is likely that in solution the spacing between the recognition helices may be further increased to provide an even closer match with the major groove spacing on the operator DNA. In our current model, the wings (W1) are well positioned to make minor groove or phosphate backbone contacts, presumably allowing for increased affinity in the interaction. Loss of one of the two inverted repeats on a 28-bp oligonucleotide corresponding to the MexR binding site II (26) by HincII digestion prevents binding by MexR (Fig. 3b) and is consistent with a single MexR dimer binding to both inverted repeats simultaneously. The predicted interactions of the recognition helices and wings with the major and minor grooves of the operator DNA for MexR would be similar to those observed in the CAP-DNA complex (37, 38).

Proposed Allosteric Mechanism of Regulation of MexR—An analysis of the interactions of the MexR C-terminal tail region with a neighboring MexR dimer in the crystal structure suggests that different conformations of the MexR dimer observed in the crystal structure may represent each of the distinct DNA- and effector-bound conformations. The C-terminal tail immediately follows α6 and consists of residues 140–147. While the C-terminal tail is disordered in most of the MexR monomers, interpretable electron density is visible for the C-terminal tail of monomer C (average B-factor of 38.7 Å² for residues 140–147), which is inserted in between the DNA binding domains of dimer AB (Fig. 3c). Interestingly, dimer AB exhibits the shortest spacing between its DNA binding domains.
(Ca-Ca distance of 22.6 Å between Arg73 and Arg73'). In contrast, dimer CD exhibits the largest spacing (Ca-Ca distance of 29.2 Å between Arg73 and Arg73) between its DNA binding domains and shows no density for the C-terminal tail in the region between its DNA binding domains (Fig. 2). While dimer CD can be manually docked onto a linear model of the MexR operator DNA, the spacing of the recognition helices in dimer AB is incompatible with DNA binding as predicted by our model (Fig. 3, a and c), so that the conformations shown by dimers CD and AB can be interpreted as open (able to bind DNA) and closed (unable to bind DNA) states, respectively. The observation of an ordered C-terminal tail in between the DNA binding domains in the closed conformation (but not in the open conformation) suggests that binding of the C-terminal tail or a ligand resembling this peptide can inhibit DNA binding by inducing the closed conformation. In the absence of an effector or an effector-like ligand, repulsion between positively charged side chains (Arg21, His41, Arg63, and Arg70) lining the crevice between the DNA binding domains likely help to maintain the MexR dimer in the DNA binding conformation (Fig. 3, d and e).

An examination of the crystal packing of MexR predicts that interdimer interactions between the MexR C-terminal tail and DNA binding domains lead to the formation of MexR filaments, which is inconsistent with the high solubility of this protein. The interaction of the DNA binding domains with the C-terminal tail therefore likely occurs only under crystallization conditions, and the affinity is likely to be weak in solution, so that the C-terminal tail itself is unlikely to be the in vivo effector. However in the context of the MexR conformations observed in the crystal structure and the proposed model of the MexR-DNA complex, the C-terminal tail has identified a potential effector binding site on MexR and a conformation of the MexR dimer.
that is expected to be unable to bind DNA is apparently linked to interaction with the C-terminal tail.

Contrast with MarR—An overall structure-based alignment of MexR (monomer D) with the MarR monomer gives an r.m.s. deviation of 1.8 Å for 116 Ca positions. This relatively large overall r.m.s. deviation mostly results from conformational differences in the dimerization helices (α1, α5, and α6), since a superposition of the DNA binding domains gives an r.m.s. deviation of 1.2 Å (for 256 main chain atoms). The multiple conformations observed in the crystal structure of MexR contrast with the situation in MarR, in which the dimer 2-fold rotation axis coincides with a crystallographic 2-fold axis, so that the MarR asymmetric unit contains only one monomer (23). The conformational flexibility seen in MexR is therefore not observed in MarR and may be accounted for in part by the salt bridge interactions, which tether the DNA binding domains of MarR and which are not conserved in MexR. The single dimer conformation observed for MarR differs drastically from the observed MexR dimer conformations. A superposition of the MexR and MarR (CD) dimers using the main chain atoms from one DNA binding domain for each dimer shows that the second recognition helix (α4') of MarR is displaced 18.8 Å relative to that of MexR (Fig. 4a). Given the unusual “zigzag” arrangement of the recognition helices in MarR and the expected positions of the inverted repeats being on opposite faces of the MarR operator DNA (42), the mode of DNA binding by MarR is unclear and does not appear to resemble our proposed model of DNA binding for MexR.

The salicylate binding sites in the MarR-salicylate complex were located on either side of the recognition helix (Fig. 4b; Ref. 23). The SAL-A site is located in a crevice between the recognition helix (α4) and the β-sheet (from which W1 loops out), while the SAL-B site is located on the opposite side of α4. In the absence of an apo MarR structure, the effect of salicylate binding to MarR on the dimer conformation (if any) is unclear; however, the observed positions of the SAL-A and SAL-B sites are distant from and seem unlikely to affect the conformation of the dimerization domain. Of the two salicylate binding sites observed in the MarR crystal structure, only for SAL-A are the structural characteristics similar for the corresponding region in MexR, while for SAL-B the binding of salicylate as observed in MarR would not be possible in MexR. However, sodium salicylate at a concentration of 5 mM is unable to dissociate the MexR-DNA complex as assessed by an electrophoretic mobility shift assay (Fig. 4c), which directly indicates differences in effector specificity between MexR and MarR.

The results of this study indicate that the effector binding and mode of regulation between MexR and MarR are very different despite clear similarities in sequence and structure. This raises the possibility that members of the MarR family of transcriptional regulators may be diverse in the types of effector molecules recognized and in the ways their DNA binding domains interact with the DNA binding domains of the MarR dimer suggest that the effector for MarR may be a peptide signaling molecule or the C terminus of a protein ligand. Further work is needed to identify the in vivo effector(s) for MexR, which will also shed light on the physiological function of the MexAB-OprM multidrug efflux system regulated by MexR.

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