CprK Crystal Structures Reveal Mechanism for Transcriptional Control of Halorespiration*

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M. Gordon Joyce‡, Colin Levy§, Kristzina Gábor‡, Stelian M. Pop‡, Benjamin D. Biehl‡, Tzanko I. Doukov‡, Jodi M. Ryter**, Hortense Mazon†‡, Hauke Smidt§, Robert H. H. van den Heuvel‡‡, Stephen W. Ragsdale†, John van der Oost‡, and David Leys††

From the ††Manchester Interdisciplinary Biocentre, P. O. Box 88, Manchester, M60 1QD, United Kingdom, the ‡‡Laboratory of Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands, the †Department of Biochemistry, Beadle Center, University of Nebraska, Lincoln, Nebraska 68588-0664, the §Stanford Synchrotron Radiation Laboratory, Menlo Park, California 94025, the ‡§Department of Chemistry, Nebraska Wesleyan University, Lincoln, Nebraska 68504-2794, and the ‡‡‡Department of Biomolecular Mass Spectrometry, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584-CU, Utrecht, The Netherlands

Halorespiration is a bacterial respiratory process in which haloorganic compounds act as terminal electron acceptors. This process is controlled at transcriptional level by CprK, a member of the ubiquitous CRP-FNR family. Here we present the crystal structures of oxidized CprK in presence of the ligand ortho-chlorophenolacetic acid and of reduced CprK in absence of this ligand. These structures reveal that highly specific binding of chlorinated, rather than the corresponding non-chlorinated, phenolic compounds in the NH₂-terminal β-barrels causes reorientation of these domains with respect to the central α-helix at the dimer interface. Unexpectedly, the COOH-terminal DNA-binding domains dimerize in the non-DNA binding state. We postulate the ligand-induced conformational change allows formation of interdomain contacts that disrupt the DNA domain dimer interface and leads to repositioning of the helix-turn-helix motifs. These structures provide a structural framework for further studies on transcriptional control by CRP-FNR homologs in general and of halorespiration regulation by CprK in particular.

Past and present industrial and agricultural activities have led to the ever increasing presence of haloorganic compounds such as chlorophenols and chlorinated ethenes in the environment (1). Due to both toxicity and recalcitrant nature, increasing amounts of these xenobiotics threaten the integrity of the environment and human health (2). In recent years, it has emerged that several haloorganic compounds are also naturally produced (3) and that several species of strictly anaerobic bacteria are able to conserve energy via the reductive dehalogenation of these compounds by respiratory metabolism (4, 5). In view of their favorable degrading capacities, e.g. high dehalogenation rate and low residual concentration of the contaminant, it has been anticipated that halorespiring microorganisms should be of utmost significance for efficient biological remediation of halogenated hydrocarbons in anoxic environments (6, 7). The versatile, strictly anaerobic Gram-positive bacterium Desulfitibacterium dehalogenans and the closely related Desulfitibacterium hafniense have the capacity of degrading ortho-chlorophenol. Both have been used as model organisms in halorespiration studies, representing one of the most significant groups of halorespiring isolates (8). In these organisms, proteins involved in halorespiration are encoded by the cpr (chlorophenol reductive dehalogenase) operon, of which multiple copies are present within the genome. This potentially allows for reductive dehalogenation of a wide range of haloorganic compounds by the use of a series of paralogous enzymes (9).

The cpr operon is transcriptionally regulated by CprK, a member of the CRP-FNR family of regulators that is ubiquitous in bacteria (10). Recent in vivo and in vitro studies reveal that CprK binds 3-chloro-4-hydroxyphenylacetate (CHPA)2 with micromolar affinity promoting a tight interaction with a specific DNA sequence in the promoter region of the cpr-encoded genes, called the “dehalobox” (11, 12). CHPA-like compounds that lack either the chloride or the hydroxyl group fail to induce DNA binding, even at millimolar concentrations. The cpr gene cluster appears to be subject to a second layer of transcriptional control; under aerobic conditions, oxidation of CprK leads to disulfide bond formation, which prevents DNA binding (11).

Regulators of the CRP-FNR family respond to a broad spectrum of intracellular and exogenous signals (13). To accomplish their roles, these regulatory proteins have intrinsic sensory modules that either bind allosteric effector molecules or chemically modulate prosthetic groups, after which a signal is trans-

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The atomic coordinates and structure factors (code 2H6B and 2H6C) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

1 A Royal Society University Research Fellow and an European Molecular Biology Organization Young Investigator. To whom correspondence should be addressed. Tel.: 44-161-306-51-50; Fax: 44-161-275-55-86; E-mail: david.leys@manchester.ac.uk.

2 The abbreviations used are: CHPA, 3-chloro-4-hydroxyphenylacetate; MES, 4-morpholinethanesulfonic acid; r.m.s.d., root mean square deviation; ESI, electrospray ionization; MS, mass spectrometry; dsDNA, double-stranded DNA; HPA, ortho-hydroxyphenolacetic acid.
mitted to a DNA-binding domain. For example, the paradigmatic *Escherichia coli* cAMP receptor protein (CRP) undergoes a conformational change upon binding cAMP that triggers binding to the promoter region of target genes (14). To date, crystal structures of distinct CRP states provide atomic insights into cAMP-, DNA-, and RNA-polymerase binding (15–17). However, a detailed structure for the ligand-free, non-DNA binding state of CRP is lacking. The structural data available for other CRP-FNR family members are limited to the crystal structures of CooA from *Rhodospirillum rubrum* (18) and the pathogenicity factor PrfA from *Listeria* spp (19), both ligand-free structures. Due to the absence of a family member with both ligand-bound and -free states available, the allosteric mechanism by which effector binding induces a conformational transition to an “active,” DNA binding state is poorly understood, and several models have been put forward to explain how the sensory module can transmit the signal to the distant DNA-binding domain (14, 15, 20, 21).

Here we report the 2.2-Å crystal structure of the oxidized *D. hafniense* CprK in the presence of CHPA and the 2.9-Å crystal structure of the highly related *D. dehalogenans* CprK (both proteins are 232 amino acids long and 89% identical) in the ligand-free, reduced state. Comparison of both structures allows identification of the allosteric changes induced by ligand binding.

**MATERIALS AND METHODS**

**Crystallization and Structure Elucidation of *D. hafniense* CprK**—CprK was prepared as described previously (12), using the pWUR89 expression vector. Crystals of the oxidized CprK-CHPA complex were grown in 100 mM Tris, pH 7.5, 1.8 M ammonium sulfate at 4 °C. Heavy atom derivatization of the crystals was carried out by soaking crystals in mother liquor containing 10 mM potassium tetrachloroplatinate(II) and 10 mM mercury(II) acetate. Data were collected on single crystals at Deutsches Elektron-Synchrotron (DESY), Hamburg, Germany, beamline BW7A. All data were processed and scaled using DENZO and SCALEPACK (22), and crystals belong to the I222 space group ($a = 104.4$ Å, $b = 112.2$ Å, $c = 119.5$ Å). The position of heavy atom sites for isomorphous derivatives was found using difference Patterson functions and verified using (cross) difference Fourier analysis. Coordinates and real and anomalous occupancies were refined for all heavy atom positions and final MIRAS phases calculated using MLPHARE (23). Density modification and non-crystallographic symmetry averaging were carried out using DM (24). Model building was carried out using TURBO-FRODO (25), and maximum like-
CprK Crystal Structures

Maximum likelihood refinement was carried out using REFMAC5 (26). Final coordinates and structure factors have been deposited with the Protein Data Bank (codes 2H6B and RCSB037983, respectively).

Crystallization and Structure Elucidation of D. dehalogenans CprK—CprK was expressed and purified as described previously (11). CprK (7–8 mg/ml) was crystallized in a solution containing 100 mM MES pH 6.5, 115 mM MgCl2, 8% polyethylene glycol-3350, and 10 mM dithiothreitol. Data were collected from a single flash-cooled crystal at beamline 11-1 at Stanford Synchrotron Radiation Laboratory. All data were processed and scaled using Crystal Clear (27), and crystals belong to the P21 space group (\(a = 72.7 \text{ Å}, b = 50.0 \text{ Å}, c = 76.4 \text{ Å}, \beta = 105.5^\circ\)). Molecular replacement using the individual domains of the \(D. \text{ hafniense}\) CprK was carried out using Phaser (28). Model building was carried out using TURBO-FRODO (25) and COOT (29). Maximum likelihood and translation-libration-screw refinement were carried out using REFMAC5 (26, 30). Final coordinates and structure factors have been deposited with the Protein Data Bank (codes 2H6C and RCSB037984, respectively).

Trp Fluorescence Quenching—Fluorescence experiments were carried out using a Cary Eclipse fluorescence spectrophotometer with constant temperature at 25 °C maintained using a Cary single cell peltier regulator. Samples were measured using a Hellma precision quartz cuvette with a 10-mm light path. Excitation was carried out at 295 nm with a 5 nm wavepath and emission fluorescence measured at 370 nm with a 10 nm wavepath. CHPA was added to a 1-mL solution of 10 \(\mu\text{M}\) wild-type or mutant CprK in 50 mM potassium phosphate buffer, pH 7.2. Quenching observed was corrected for the inner filter effect by titration of CHPA versus tryptophan. To determine the binding constant for ortho-hydroxyphenolacetic acid (HPA), titrations versus CHPA were carried out in the presence of 1–10 mM HPA.

Native Macromolecular Mass Spectrometry—4 \(\mu\text{M}\) CprK dimer was used for mass spectrometry studies in the presence of 10 mM dithiothreitol. For DNA binding measurements, 4 \(\mu\text{M}\) CprK was incubated with 400 \(\mu\text{M}\) CHPA and 5 \(\mu\text{M}\) dsDNA for 30 min at room temperature. Native macromolecular mass spectrometry measurements were performed in positive ion mode using an electrospray ionization time-of-flight instrument (LC-T; Micromass, Manchester, UK) equipped with a Z-spray nano-electrospray ionization source. To produce intact ions \textit{in vacuo} from large complexes in solution the ions were cooled by increasing the pressure in the first vacuum stages of the mass spectrometer (31, 32). Source pressure conditions and electrospray voltages were optimized for transmission of the macromolecular protein complexes (31). The needle and sample cone voltage were 1,400 and 140 V, respectively. The pressure in the interface region was adjusted to 8 millibars. The spectra were mass calibrated by using a solution of 10 mg/ml cesium iodide in isopropanol:water 50:50. Deconvoluted spectra were generated by using the transform tool of the MassLynx software.

RESULTS AND DISCUSSION

Crystal Structure of Oxidized, Ligand-bound \(D. \text{ hafniense}\) CprK—The crystal structure of oxidized \(D. \text{ hafniense}\) CprK in complex with CHPA was determined to 2.2 Å

![Oligomeric structure of oxidized CprK](image)

**FIGURE 2. Oligomeric structure of oxidized CprK.** A, overlap of both monomers within the oxidized CprK dimer observed in the asymmetric unit. One monomer is colored gray, while the other is colored as described for Fig. 1. B, schematic representation of the putative tetrameric CprK molecule obtained by applying 2-fold crystal symmetry; one CprK dimer is colored gray, while the other is colored as described for Fig. 1.

![D. hafniense CprK CHPA-binding site](image)

**FIGURE 3. D. hafniense CprK CHPA-binding site.** Stereo representation of the binding site for CHPA with key residues depicted in sticks in addition to the Cα trace for residues 63–94 (part of the β-barrel) and 129–135 (part of the C helix). Color coding is the same as described for Fig. 1. Hydrogen bonds between binding site residues and CHPA are indicated by black dashed lines. The SigmaA weighted \(F_p - F_o\) omit map is superimposed on the bound CHPA molecules contoured at 3\(\sigma\) (in blue) and 6\(\sigma\) (in magenta).
using MIRAS and non-crystallographic symmetry averaging techniques (Fig. 1A). The asymmetric unit contains a single CprK dimer with each monomer folded in two distinct domains connected by a long α-helix. The r.m.s.d. in the Cα position between both monomers in the asymmetric unit is 0.455 Å for the DNA-binding domain and 0.256 Å for the sensory module. A search of the protein structure data base reveals that CprK is most similar to CRP (Protein Data Bank code 2CGP; Fig. 1C) (sensory module: Z score of 13.9 and r.m.s.d. of 3.0 Å for 124 Ca atoms; DNA-binding domain: Z score of 9.3 and r.m.s.d. of 1.9 Å for 68 Ca atoms). The relative position of the sensory modules within the CprK dimer is similar to CRP, the dimer interface predominantly made by the Cα-helices. However, in contrast to CRP and other crystal structures of related regulators, CprK is a-domain swapped dimer, with the COOH-terminal DNA-binding domain of one monomer interacting with the NH2-terminal effector-binding domain of the other. While a non-crystallographic 2-fold axis relates both sensory modules, the relative position of both DNA-binding domains is drastically different, resulting in a distinctly asymmetric CprK dimer (Fig. 2A). Within the dimer, two intersubunit disulfide bonds are present between Cys-11 from the sensory module and Cys-200 located in the DNA-binding helix F. For both DNA-binding domains, contacts with β-strands 3 and 4 of the sensory module are mainly made by residues of the COOH-terminal helix G (not present in CRP), leading to a surface area buried of, respectively, ~70 Å² and ~220 Å² for the individual contacts. In contrast, in the CRP-DNA complex (Fig. 1C), contacts between β-strands 4 and 5 and the DNA-binding domain occur via residues provided by helices D and E. Thus, whereas in CRP both DNA-binding helices are positioned roughly perpendicular to the central α-helical pair along the same face of the molecule, the CprK DNA-binding helices are near parallel to the Cα-helices. This conformation is not compatible with tight binding to the dehalobox DNA. Reorientation of both domains to a position similar to that observed in CRP-DNA complexes requires breaking the disulfide bond between Cys-11 and Cys-200 and reorientation of the NH2-terminal polypeptide stretch preceding Pro-16. In CRP, residues from helices D and E are involved in interdomain contacts; surprisingly, the corresponding residues for both CprK DNA domains are in close contact with the same residues from a symmetry related dimer (Fig. 2B). The interface formed between two CprK dimers involves the close association of four DNA-binding domains: a 870-Å² surface that is mainly hydrophobic, containing four salt bridges in addition to several hydrogen bonds. The interface has a surface complementarity 0.68 (33) suggesting it is a physiologically relevant interaction in the non-DNA binding state.

**CHPA Binding by CprK**—Clear electron density is observed for a tightly bound CHPA molecule in the sensory modules of both monomers in a position similar to the binding site of cAMP in CRP (Fig. 3). CprK makes six direct hydrogen bonds to CHPA and two salt bridge contacts, one between Lys-86 and the acetate group of the effector and another between the CHPA phenol hydroxyl group (assuming this is deprotonated) and the conserved residue Lys-133. The phenol hydroxyl group forms hydrogen bonding contacts with Tyr-76 (2.3 Å), Lys-133 (2.7 Å), and the backbone nitrogen of Gly-85 (3.0 Å) of one monomer. The chloride atom is positioned in van der Waals contact with residues of the...
between several hydrophobic residues in the Asn-92 (2.8 Å), while the aromatic moiety is sandwiched hydrogen bonded to Lys-86 (2.7 Å), Thr-90 (2.6 Å), and terminal group (3.6 Å). The CHPA acetic acid group is main chain atoms of Gly-85 (3.7 Å) and the Lys-133 amino-
Å) in addition to a buried water molecule (3.9 Å), several residues Tyr-130 (3.7 Å), Leu-131 (3.8 Å), and Val-134 (3.9 Å) pocket for the chloride atom is made up of hydrophobic charges of the different ion series are indicated. The green circle and blue square indicate monomeric CprK and dehalobox DNA, respectively. The insets represent the deconvoluted mass spectra of dimeric CprK (I) and the complex between CprK and dsDNA (II). B, Trp fluorescence quenching by CHPA titration. Tryptophan fluorescence quenching obtained by titration of C200S D. hafniense CprK and oxidized wild-type (WT) D. hafniense CprK with CHPA, in the presence or absence of HPA. Data were fitted to a single exponential quadratic function.

central α-helix of the opposite monomer. The binding pocket for the chloride atom is made up of hydrophobic residues Tyr-130 (3.7 Å), Leu-131 (3.8 Å), and Val-134 (3.9 Å) in addition to a buried water molecule (3.9 Å), several main chain atoms of Gly-85 (3.7 Å) and the Lys-133 amino-terminal group (3.6 Å). The CHPA acetic acid group is hydrogen bonded to Lys-86 (2.7 Å), Thr-90 (2.6 Å), and Asn-92 (2.8 Å), while the aromatic moiety is sandwiched between several hydrophobic residues in the β-barrel.

Crystal Structure of Reduced, Ligand-free D. dehalogenans CprK—The crystal structure of the reduced D. dehalogenans CprK was solved to 2.9 Å by molecular replacement using the individual D. hafniense CprK domains as search models. The asymmetric unit contains a single CprK dimer with the sensory modules and central α-helix coiled coil in highly similar orientations as observed for oxidized CprK (Fig. 1, A and B). The r.m.s.d. in Ca position between both monomers in the asymmetric unit is 0.251 Å for the DNA-binding domain and 0.319 Å for the sensory module. Close comparison of both the ligand-free and ligand-bound sensory modules reveals no significant change in the relative position of helices B and C, which provide the majority of the CprK dimer interface (r.m.s.d. 0.3 Å for 46 Ca atoms). In contrast, the positions of both NH2-terminal β-barrels have shifted with respect to the B and C helices in the ligand-free structure (Fig. 4). The motion relating the position of the NH2-terminal β-barrel in both crystal structures constitutes a rigid body hinge movement around residue Ser 108 immediately preceding helix B. This leads to a maximum shift in position of ~3.8 Å for β-strands 4 and 5. Due to the relatively small shift in position and the flexibility of some side chains, many of the direct contacts between the β-barrel and the central C helix are conserved between both conformations. The CHPA-binding site is empty (water molecules cannot be reliably observed at this resolution) and is part of a tunnel extending across the molecule and providing solvent access. While the residues involved in binding CHPA are completely conserved between both D. hafniense and D. dehalogenans CprK (except for the conservative replacement of Lys/Arg 86), they occupy different conformations in both crystal structures (Fig. 5). A notable exception being residue Lys 85 which does not show a ligation dependent conformational shift. The 3.7 Å shift in the Ca position of Gly 85 is one of the largest differences observed when comparing both structures.

In contrast to the relatively minor changes observed in the sensory modules, the short linker regions connecting the C α-helix with the DNA-binding domain are projecting outward from the central coiled coil, positioning the DNA-binding domains at a distance from the sensory modules rather than the close contacts observed in the oxidized CprK structure. Both DNA-binding domains form a close association that buries hydrophobic residues from helices D and E and is identical to the dimerization observed in the oxidized CprK dimers (Fig. 2B), providing further evidence for the physiological relevance of this interaction.

Native ESI-MS Reveals a Single CprK Dimer That Binds dsDNA in Presence of CHPA—The ESI-MS spectrum of D. hafniense CprK under reducing conditions (Fig. 6A) revealed that CprK is mainly dimeric with a measured mass of 52,739.4 ± 3.7 Da (calculated mass 52,736 Da). Fig. 6 shows the ESI-MS spectrum of CprK incubated with a 30-bp DNA frag-
ment containing the dehalobox recognition site, and CHPA under reducing conditions. The spectrum clearly shows that dimeric CprK interacts specifically with one molecule of dsDNA (measured mass 71,165.0 ± 2.7) with very low amounts of free dsDNA and CprK present. The observed mass increase of the analyzed complex as compared with the calculated mass (71,143 Da) is likely due to water or buffer molecules still present in the protein-DNA complex. In the absence of CHPA, the level of CprK-DNA complex was very low. These results show that active CprK is a dimer that binds dehalobox DNA in equimolar amounts when CHPA is present.

Trp Fluorescence Quenching Indicates High Specificity for CHPA—Trp fluorescence quenching experiments demonstrate up to 55% quenching upon titration of oxidized D. hafniense CprK with CHPA, leading to an apparent K_D of 4.1 ± 0.4 μM for CHPA. This binding becomes slightly tighter (to an apparent K_D of 0.83 ± 0.11 μM) when using a C200S mutant (12) that is unable to form the disulfide bond (Fig. 6B and Table 1). A single Trp is situated at the bottom of the β-barrel, which is not in close contact with the CHPA-binding site. It is therefore most likely that binding of CHPA triggers a conformational change that results in the fluorescence quenching. Titration with HPA, which only differs from CHPA in lacking the chloride substituent, does not result in significant quenching even at millimolar concentrations. Binding of HPA to CprK can indirectly be observed through inhibition of CHPA-induced Trp fluorescence quenching, leading to an observed K_D for HPA of 6.2 ± 0.4 mM. Thus, despite the relatively small difference in structure, CprK has a remarkable preference for the chlorinated compound, in terms of both affinity (>1000-fold higher for CHPA over HPA) and functionality (HPA binding does not induce any measurable increase in DNA binding). Thus, the
product of CHPA respiration does not induce transcription of the cprBA dehalogenase or significantly interfere with the CHPA-induced allosteric transition.

**Allosteric Changes following Binding of ortho-Chlorophenolic Compounds**—Although binding of HPA, unlike CHPA, does not elicit a measurable allosteric transition, one can postulate that the local structure of the binding pocket in the HPA-CprK complex is similar to that in the CHPA-CprK complex, given the very high similarity between both compounds. However, as HPA does not induce any DNA binding, the overall structure of the HPA-CprK complex likely resembles that of ligand-free CprK. To model this putative CprK-HPA complex, the β-barrel of the ligand-bound structure was repositioned to occupy the conformation observed in the ligand-free structure. The resulting “hybrid” CprK structure thus contains local active site changes induced by ligand binding, but the overall structure retains the unbound state (Fig. 7B). Then, overlaying this hybrid CprK structure on the rigid body motion of the NH₂-terminal β-barrel (Figs. 3 and 7C).

While the pKₐ for the phenol group of HPA is approximately ~10, this value is lowered to ~8.4 in CHPA due to the chloride substituent at the ortho-position (Spectrum Laboratories: Chemical Fact Sheet-Chemical Abstract Number 95578). We postulate that CprK can distinguish between CHPA and HPA not only on the basis of the additional steric bulk provided by the chloride atom but also by a "pKₐ interrogation" mechanism (35), which is based on the ability of ligands to ionize to the phenolate form and consequently interact with Lys-133, driving the conformational change that promotes DNA binding. Among the several CprK homologues in the genome of *D. hafniense*, Lys-133 is strictly conserved, which supports the pKₐ interrogation concept. The other side chain interaction with the phenol hydroxyl group, Tyr-76 is not conserved, and the Y76F variant of *D. hafniense* CprK still undergoes allosteric changes upon binding of CHPA (albeit at 10-fold higher CHPA concentrations than the wild type, Table 2), indicating this residue is not essential for activity. Most residues involved in binding the ortho-chlorophenol moiety are conserved or highly similar in CprK paralogs, while residues involved in binding the acidic group (e.g. Lys/Arg-86, Thr-90, Asn-92) are specific to CprK, indicating that these CprK paralogs will likely interact with other compounds that have been found to serve as terminal electron acceptors such as 2,4-dichlorophenol and/or 2,4,6-trichlorophenol (36).

**CprK Crystal Structures**

| Soak time/Concentration                  | CprK *D. hafniense* (2H6B) | Potassium tetrachloroplatinate soak | Mercury acetate soak | CprK *D. dehalogenans* (2H6C) |
|-----------------------------------------|-----------------------------|------------------------------------|---------------------|-------------------------------|
| Space group                             |                             | 10 min/10 mM                      | 10 min/10 mM        |                               |
| Cell dimensions                         |                             | 1 222                              | 1 222               |                               |
| a (Å)                                   |                             | 104.4                              | 104.9               | 105.2                         |
| b (Å)                                   |                             | 112.1                              | 113.0               | 112.3                         |
| c (Å)                                   |                             | 119.4                              | 119.6               | 117.8                         |
| X-ray source                            |                             | DESY BW7A                          | DESY BW7A           | DESY BW7A                     |
| Resolution (Å)                          |                             | 50-2.2 (2.25-2.2)                  | 50-2.5 (2.55-2.5)   | 50-3.1 (3.2-3.1)              |
| No. of observations                     |                             |                                    |                     |                               |
| Total                                   |                             | 766,373                            | 313,230             | 423,430                       |
| Unique                                  |                             | 35,512                             | 24,333              | 12,468                        |
| Completeness (%)                        |                             | 99.2 (100)                         | 97.6 (98.6)         | 95.8 (99.8)                   |
| r/rl                                    |                             | 16.6 (2.5)                         | 17.08 (2.906)       | 8.97 (2.31)                   |
| Rcryst/Rfree                            |                             | 0.102 (0.573)                      | 0.078 (0.427)       | 0.147 (0.415)                 |
| Model                                   |                             | Residues 3–250 for chain A, 9–243 for chain B, residues 233–250 are part of the COOH-terminal His-tag, 2 CHPA, 2 sulfate ions, 230 water molecules | Residues 19–226 for chain A, 19–226 for chain B |
| Rcryst/Rfree (Å)                        |                             | 18.4 (41.5)/22.9 (53.6)            | 23.2 (34.0)/30.7 (44.0) |
| Average β-factor                        |                             | 39.9                               | 70.1                |
| Ramachandran plot: core/allowed/generously allowed | | 89.5/7.0/7.0 | 83.6/15.9/0.3 |
| r.m.s.d. bond lengths (Å)               |                             | 0.024                               | 0.019               | 2.075                         |
| r.m.s.d. bond angles (°)                |                             | 1.989                               |                     |                               |

* DESY, Deutsches Elektronen-Synchrotron; SSRL, Stanford Synchrotron Radiation Laboratory.
CONCLUSIONS

Crystal structures of CprK with and without CHPA bound reveal the binding mode for halogenated orthophenolic compounds and suggest a mechanism whereby both the phenol hydroxyl group and the chloride ortho-substituent are required for tight binding and integrally involved in promoting the conformational change that leads to DNA binding. We postulate that binding of CHPA and concomitant phenol deprotonation leads to a rigid body hinge motion of the β-barrel that docks the chloride atom in the binding pocket provided by the central coiled coil and leads to formation of a tight phenolate-Lys-133 interaction. While in absence of a crystal structure for the reduced, CHPA-bound CprK, it is difficult to determine the exact mechanism whereby the observed rigid body motions in the β-barrel are communicated to the DNA-binding domains, we postulate the sensory module reorganization repositions β-strands 3 and 4 with respect to the central coiled coil and allows for a direct contact between the DNA-binding domain and the sensory module to be formed. This disrupts the DNA-binding domain dimer interface and positions the helix-turn-helix motifs in the required conformation for productive binding to the dehalobox DNA. The relative motion of the β-barrel to the B-C helix is reminiscent of similar motions postulated to occur in functionally unrelated proteins containing related sensory modules, such as cyclic nucleotide-gated channels and cAMP- and cGMP-dependent protein kinases (34, 37–40). Formation of a disulfide bond under aerobic conditions between Cys-11 and Cys-200 does not impact CHPA binding but disrupts formation of the correct interdomain contacts and hence provides a possible means for redox regulation of halorespiration.

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