Motion of the DNA-binding Domain with Respect to the Core of the Diphtheria Toxin Repressor (DtxR) Revealed in the Crystal Structures of Apo- and Holo-DtxR*

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The diphtheria toxin repressor (DtxR) from Corynebacterium diphtheriae is a divalent metal-activated repressor of chromosomal genes that encode proteins responsible for siderophore-mediated iron uptake and also of the gene of certain corynebacteriophages that encodes diphtheria toxin. DtxR consists of two 25.3-kDa three-domain subunits and is a member of a family of related repressor proteins in several Gram-positive bacterial species, some of which are important human pathogens. In this paper, we report on the first high resolution crystal structures of apo-DtxR in two related space groups. In addition, crystal structures of Zn-DtxR were determined in the same two space groups. The resolutions of the structures range from 2.2 to 2.4 Å. The four refined models of the apo- and the holo-repressor exhibit quite similar metal binding centers, which do, however, show higher thermal motion in the apo-structures. All four structures reported differ from each other in one important aspect. The N-terminal DNA-binding domain and the last 20 residues of the dimerization domain of each subunit move significantly with respect to the core of the DtxR dimer, which consists of residues 74–120 from both subunits. These results provide the first indication of a conformational change that may occur upon binding of the holo-repressor to DNA.

Iron is an essential nutrient for almost all living organisms including pathogenic bacteria. The availability of free iron in the mammalian host is extremely limited, since most of the extracellular iron is associated with transferrin and lactoferrin, and most of the intracellular iron is bound to heme-containing proteins (1). The extracellular Fe$^{2+}$ concentration is usually restricted to concentrations below $10^{-18}$ M, which is far too low to satisfy normal bacterial growth requirements. In addition, one important mammalian host response to infection is the release of additional transferrin and ferritin, thereby further reducing the amount of available iron (1). The ability to acquire ferric iron from the mammalian host is therefore an important key element of infection. In order to solve this problem, many bacteria have developed elaborate mechanisms to capture ferric iron, including siderophore synthesis and specific membrane proteins (1, 2). Furthermore, numerous virulence determinants produced by bacterial pathogens, including a variety of toxins, hemolysin, and proteins involved in the iron uptake system, are regulated by iron (3).

In Corynebacterium diphtheriae, the diphtheria toxin repressor (DtxR), $^1$ is a global iron-dependent negative repressor that is activated by ferrous iron. In the presence of the co-repressor Fe$^{2+}$, DtxR binds its target DNA sequences as a homodimer, thereby repressing the genes controlled by the DtxR-regulated promoter (4–9). The most important gene regulated by DtxR is the bacteriophage tox gene that encodes diphtheria toxin (10). It has also been shown that the expression of siderophores in C. diphtheriae is regulated by DtxR (11). Whereas in vivo only Fe$^{2+}$ acts as co-repressor, in vitro several divalent transition metal ions including Fe$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Mn$^{2+}$, and Cd$^{2+}$, as well as Zn$^{2+}$, can also function as activators (8–11).

DtxR also binds to the promoter regions of the iron-regulated irp1, irp2, irp3, irp4, and irp5 promoters/operators (12, 13). Very little is known about the proteins encoded by the genes downstream from DtxR binding regions. The gene product regulated by irp1 has recently been identified as a 38-kDa periplasmic lipoprotein implicated in the iron uptake system (14). In addition, the expression of a heme oxygenase is regulated by iron and DtxR (15). Several DtxR homologs have been discovered in Gram-positive bacteria, including the major human pathogens Mycobacterium tuberculosis (16) and Mycobacterium leprae (17), the soil bacteria Streptomyces pilosus and Streptomyces lividans (18), and Brevibacterium lactofermentum (19). These homologs share an overall amino acid sequence identity of about 60%. DtxR can therefore serve as a model for a related iron-dependent repressors in Gram-positive bacteria in a similar manner as the ferric iron uptake repressor (Fur), which controls more than 30 genes in Escherichia coli and related bacteria, is a model for a different family of iron-dependent repressors in Gram-negative bacteria (20).

In order to understand the activation of DtxR by metals, it is essential to accurately determine the three-dimensional structures of the repressor with and without metal. Crystal structures of wild-type DtxR in complex with different divalent transition metals have been determined at 2.8-Å resolution (21) and of apo-DtxR at 3.0-Å resolution in a different space group (22). Recently, the resolution for Co-DtxR, Mn-DtxR, and Zn-

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The atomic coordinates and structure factors (codes 1bi0–1bi3) have been deposited in the Protein Data Bank, Brookhaven National Laboratory, Upton, NY.

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$ The abbreviations used are: DtxR, diphtheria toxin repressor; r.m.s., root mean square.

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The abbreviations used are: DtxR, diphtheria toxin repressor; r.m.s., root mean square.
DtxR has been extended to the 1.85, 2.2, and 2.4 Å, respectively (23, 24). These structures reveal an N-terminal domain of residues 1–73 that includes the DNA-binding helix-turn-helix motif and a dimerization domain (residues 74–140) that contains two metal binding sites. Binding site 1 in all structures elucidated so far contains a metal ion with high occupancy. The metal at this site is tetrahedrally coordinated by the side chains of His35, Glu83, His86, and an oxygen of a sulfate anion. This site has also been designated the “cation-anion binding site” (23). The metal at site 2 is coordinated by the side chains of Glu105, His114, the carbonyl oxygen of Cys112, and a water molecule. This site had only partial occupancy in the Cd-DtxR and Mn-DtxR crystal structures (23). A similar site was also found to be occupied by Ni2+ in the crystal structure of the Cys112→Asp DtxR variant determined by Ding et al. (25). The third domain, comprising residues 148–226, was found to adopt an Src homology 3-like conformation (23). How ever, this domain is highly flexible in all crystal structures found to adopt an Src homology 3-like conformation (23). However, this domain is highly flexible in all crystal structures determined so far, and its function remains unclear.

In this study, we report the first high resolution crystal structures of apo-DtxR at a resolution of 2.2 Å. Crystal structures were determined in two space groups. In crystal form II the two-fold axis is noncrystallographic, and there is one monomer in the asymmetric unit, whereas in the related crys tal form I the two-fold axis is crystallographic and contains two crystal forms to a resolution of 2.3 Å. The comparison of these four crystal structures provides the first evidence of the conformational changes that might be essential for the regulation of the repressor by its co-repressor. It turns out that the mechanism presumably involves the motion of the DNA-binding domain with respect to the metal binding domain rather than a rigid body motion of the monomers with respect to each other as suggested by Schiering et al. (22) on the basis of their 3.0- and 3.8-Å structures of apo- and Ni-DtxR, respectively.

### EXPERIMENTAL PROCEDURES

**Protein Expression, Purification, and Crystallization**—DtxR was cloned and overexpressed in *E. coli* as described previously (5). The protein was purified using a nickel-nitrilotriacetic acid affinity column followed by anion exchange chromatography (11). DtxR in complex with zinc and sulfate was crystallized by vapor diffusion from 1.8–2.0 mM ammonium sulfate and 10 mM Zn2+ using the hanging drop method as described by Qiu et al. (21). Crystal dimensions were typically 0.5 × 0.3 × 0.3 mm. The metal-free form was crystallized in the presence of 1 mM EDTA in all buffers. Prior to crystallization, the protein was dialyzed twice against a buffer of 10 mM Tris, pH 8, 50 mM NaCl, 10 mM DTT, and 1 mM EDTA to ensure the removal of all residual metal ions. The two crystal modifications were obtained under the same conditions, sometimes with both crystal forms in the same drop.

**Data Collection and Processing**—The data set of form I apo-DtxR was collected from two different crystals at room temperature mounted in capillaries. All other data sets were collected at cryogenic temperatures using crystals frozen in rayon loops (26). Before cryo-cooling, the crystals were transferred into a drop containing 1.2–1.5 mM ammonium sulfate and 15–20% glycerol (24). The data sets for apo-DtxR and for crystal form I of Zn-DtxR were collected on an RAXIS-II imaging plate detector using monochromatic CuKα radiation and focusing mirrors. The data set of Zn-DtxR in form II was collected at beam line 14B at the National Synchrotron Light Source (Brookhaven, NY) at a wavelength of 1.28 Å. In all cases, data were processed using DENZO and SCALEPACK (27). Further key information is summarized in Table S1.

**Structure Solution and Refinement**—The coordinates of Co-DtxR at 1.85 Å resolution (24) were used as a starting point for crystallographic refinement of form I crystals using simulated annealing and conjugate refinement of the Cys102→Asp DtxR variant determined by Ding et al. (25). The third domain, comprising residues 148–226, was found to adopt an Src homology 3-like conformation (23). How ever, this domain is highly flexible in all crystal structures determined so far, and its function remains unclear.

### High Resolution Structure of Apo-DtxR

**TABLE I**

| Data collection parameters | Apo-DtxR form I | Zn-DtxR form I | Apo-DtxR form II | Zn-DtxR form II |
|---------------------------|----------------|----------------|-----------------|----------------|
| T (K)                     | 100            | 100            | 100             | 100            |
| Space group               | P3,21          | P3,21          | P3,21           | P3,21          |
| a = b (Å)                 | 63.2           | 63.5           | 63.0            | 63.4           |
| c (Å)                     | 108.3          | 107.4          | 216.2           | 213.0          |
| No. of observations       | 92,585         | 102,464        | 76,446          | 177,437        |
| Maximum resolution (Å)    | 2.2            | 2.3            | 2.3             | 2.4            |
| Completeness (%)          | 97.2 (92.3)    | 96.2 (99.9)    | 88.9 (69.7)     | 95.8 (91.0)    |
| Rmerge (%)                | 0.048 (0.195)  | 0.083 (0.237)  | 0.064 (0.197)   | 0.077 (0.178)  |

* a Statistics for the last shell in parentheses.

### TABLE II

**Refinement statistics**

|                  | apo-DtxR form I | Zn-DtxR form I | apo-DtxR form II | Zn-DtxR form II |
|------------------|-----------------|----------------|-----------------|----------------|
| No. of reflections used | 12,830 | 10,884 | 18,605 | 19,014 |
| Resolution (Å)   | 8–2.2 | 8–2.3 | 8–2.3 | 8–2.4 |
| Protein atoms    | 1653 | 1654 | 2661 | 2670 |
| No. of solvents  | 174 | 159 | 163 | 169 |
| Rmerge (%)       | 0.209 | 0.191 | 0.257 | 0.253 |
| Rfree (%)        | 0.311 | 0.296 | 0.352 | 0.333 |

### TABLE III

**r.m.s. deviations of the 2.2-Å apo-DtxR from I (this study) and several holo-DtxR structures in crystal form I (Å)**

|                  | apo-DtxR form I | Zn-DtxR form I | apo-DtxR form II | Zn-DtxR form II |
|------------------|-----------------|----------------|-----------------|----------------|
| Domains 1 and 2  | 27              | 27             | 27              | 27             |
| Domain 3         | 51              | 64             | 64              | 64             |
| Site 1 residues  | 15              | 15             | 15              | 15             |
| Site 2 residues  | 19              | 19             | 19              | 19             |

* Average values for both subunits.*

**Holo-repressor structure**

| Reference | R.m.s. deviation with apo-DtxR form I | All C atoms |
|-----------|---------------------------------------|-------------|
| A         | 4–73                                  | 74–140      |
| Zn-DtxR (form I) | 2.3       | 0.37 | 0.34 | 0.21 | 0.49 | This paper |
| Zn-DtxR (form II, subunit A) | 2.4       | 0.43 | 0.51 | 0.25 | 0.47 | This paper |
| Co-DtxR   | 1.85                                    | 0.35 | 0.28 | 0.25 | 0.47 | 24          |
| Mn-DtxR   | 2.2                                     | 0.53 | 0.49 | 0.54 | 0.56 | 23          |
gradient least-squares techniques with the program XPLOR (28). The structure of form II was solved by molecular replacement using the program AMoRe (29) with a dimer comprising residues 4–140 of the Co-DtxR structure (24) as a search model. Using data in the resolution range between 8 and 3.5 Å, the rotation function gave a 5.7 σ peak for the correct solution (second best 3.4 σ). The translation function re-

**FIG. 1.** Stereo views of simulated annealing omit-maps of the anion-cation binding sites. The anion and all water molecules were omitted prior to one round of simulated annealing refinement to avoid any model bias. The $F_o - F_e$ electron densities are contoured at the 3.0 σ level. A, apo-DtxR in crystal form I. The two peaks shown are 5.9 (Wat1), and 3.7 σ (Wat2), respectively. The 5.9 σ peak is the second highest water peak in the difference electron density. B, Zn-DtxR in crystal form I. The peak at the sulfate position has a height of 11.2 σ, and the water peak is 8.3 σ. The final coordinates of the refined model including the sulfate and the water are superimposed onto the density. C, apo-DtxR in crystal form II, monomer A. The water peak shown has a height of 3.9 σ. D, apo-DtxR in form II, monomer B; the water peaks shown are 4.5 (Wat1 and Wat2) and 4.2 σ (Wat3), respectively. All water positions depicted have reasonable distances to hydrogen bond donors and/or acceptors of the protein side chains and/or main chain atoms. This figure was prepared using the program O (35).
High Resolution Structure of Apo-DtxR

RESULTS

Quality of the Models—Crystal form I of both apo- and Zn-DtxR contains one monomer per asymmetric unit. The functional dimer is generated by the crystallographic two-fold axis in space group P3_21. The final models of apo-DtxR and Zn-DtxR consist of the DNA-binding domain 1 (residues 1–73), the dimerization and metal-binding domain 2 (residues 74–140), and residues 148–197 and 201–226 of domain 3. The N-terminal residues 1–3 and two linker regions (residues 141–147 and 198–200) are invisible in the electron density map and were therefore not included in the structure. The model of Zn-DtxR includes one Zn$^{2+}$ ion and a sulfate at the anion cation site and, in addition, a total of 159 well defined solvent molecules. The apo-DtxR structure contains 177 solvent positions, the third domain is partially disordered, while a number of polar surface residues had no electron density for the side chains and were thus refined as alanines. The final models yielded R-factors of 20.9% for apo-DtxR and 19.2% for Zn-DtxR with good geometry (Table II). All residues fall within the allowed regions of the Ramachandran diagram (data not shown).

In crystal form II, the asymmetric unit contains a dimer for both apo- and Zn-DtxR. The dimer is the result of a noncrystalllographic two-fold axis in space group P3_21. The first two domains are well defined in both monomers. Domain 3, however, appears to be severely disordered in one of the monomers where virtually no electron density was visible. The model therefore includes residues 4–140, 148–197, and 201–225 in subunit A of the dimer and only residues 4–140 in subunit B. The Zn-DtxR model includes a Zn$^{2+}$ and a sulfate ion at the cation-anion binding site of both monomers A and B, and 169 well defined waters. The apo-DtxR model contains 163 solvent molecules. The missing third domain in monomer B is presumed responsible for the relatively high crystallographic R-factors of 25.7 (apo-DtxR) and 25.3% (Zn-DtxR) in the P3_21 lattice. The final models possess good geometry for bond lengths and angles with all residues in allowed regions of the

![Fig. 2](image2.png) Stereo view of a superposition of apo-DtxR at 2.2 Å and Zn-DtxR at 2.3 Å at metal binding site 1 after superposition of the Cα atoms in crystal form I. The apo-DtxR structure at 2.2 Å is shown with open bonds; Zn-DtxR is shown in dark gray lines. Figs. 2–4 were prepared using MOLSCRIPT (40) and RASTER3D (41).

![Fig. 3](image3.png) Stereo view of metal binding site 2 after the least-squares superposition of all Cα atoms of apo-DtxR (this study) and Mn-DtxR at 2.2 Å (22). The apo-DtxR structure is shown with open bonds; Mn-DtxR is shown in dark gray.

![Table IV](image4.png) Pairwise comparison of the apo- and Zn-DtxR structures

The upper right hand half of the table gives the result of the A on A superposition of all 137 Cα atoms of domains 1 and 2 of subunit B. The lower left half summarizes the r.m.s. deviations of the B subunits after the superposition of the protein dimers. It is important to note that for this table, as well as for Fig. 4, the Cα atoms of only subunits A from the two DtxR dimers being compared are used for calculating the rotational and translational components of the superposition operation. This superposition operation is subsequently applied to both A and the B subunit of the "second" dimer in the comparison.

|   | apo-DtxR | Zn-DtxR | apo-DtxR | Zn-DtxR |
|---|---------|---------|---------|---------|
| form I | 0.26    | 0.26    | 0.35    | 0.39    |
| form II | 0.66    | 0.66    | 0.88    | 0.88    |
| form II | 0.65    | 0.65    | 0.47    | 0.75    |
metal-activated DNA-binding in vivo assays (9). Elucidation of the role of Cys102 in metal binding might have to await the metal removal. The r.m.s. deviations of all 29 atoms of the metal-coordinating residues His79, Glu83, and His98 range between 0.1 and 0.2 Å when comparing the apo- and holo-DtxR subunits in all four structures with only minor changes in side chain conformations (Fig. 2). The average B-factors for residues His79, Glu83, and His98 are 22 Å² for apo-DtxR and 11 Å² for Zn-DtxR (see Table II). The side chains of the coordinating residues have apparently more degrees of freedom in the apo-structure than in the metal-containing repressor.

The presence of a sulfate anion as the fourth ligand in Zn-DtxR was confirmed by the height (11 σ) of the peak at the anion site in the $F_o - F_c$ electron density and the shape of the density, which strongly suggests the presence of a tetrahedral anion (see Fig. 1B). These peaks have a relative height of 9.9 and 9.1 σ, respectively, in the two subunits of Zn-DtxR form II (data not shown), a clear indication that the anion binding site is occupied in all three independent Zn-DtxR subunits in crystal forms I and II. On the other hand, the anion binding site is empty in all three apo-DtxR subunits determined, and water peaks (of 5.9 σ in apo-DtxR form I and 3.9 and 4.5 σ in the two subunits of form II) appear about 2.6 Å from the center of the sulfate anion observed in the metal-containing structures (Fig. 1, C and D). The absence of the anion in all three apo-DtxR subunits in the two crystal forms suggests that the metal cofactor is necessary for anion binding.

Metal Site 2—In wild type DtxR, metal binding site 2 has so far only been observed to be partially occupied in the 2.8-Å Cd-DtxR (21) and the 2.2-Å Mn-DtxR structures (23). In both of those cases, the metal appears to be coordinated by O$^-$ of Glu105, N$^\nu$ of His106, the carbonyl oxygen of Cys102, and a well ordered water molecule. A comparison of the apo-DtxR and Mn-DtxR structures shows no significant conformational changes of this binding site (Fig. 3) with a r.m.s. deviation for all non-hydrogen atoms of the coordinating residues of 0.2 Å. The average B-factors of these residues are also very similar in the apo- and the manganese-containing structures: 21 Å² for apo-DtxR and 22 Å² for Mn-DtxR.

In both Zn-DtxR structures described in this paper, site 2 does not appear to be occupied by a metal ion. There is no density present except for a water molecule (data not shown). It has been suggested that the lack of metal binding at site 2 is due to the formation of a persulfide or a mixed disulfide at cysteine 102 (23, 24). In all metal-bound DtxR crystal structures investigated so far, including the two Zn-DtxR structures described in this paper, the $F_o - F_c$ difference electron density had its strongest peak at approximately 2 Å from the S⁺⁺ of Cys102, which, based on geometric criteria, can be interpreted as a sulfur atom bound to the S⁺⁺ (23, 24). However, in the present study, this peak is significantly weaker in apo-DtxR crystal form I and not present in crystal form II of apo-DtxR. The latter structure was determined from fresh crystals and crystallized with 10 mM dithiothreitol present at all times to prevent oxidation. Nevertheless, in the apo-structures, the side chain of Cys102 adopts the same conformation as in the metal-bound DtxR structures determined previously. These results may suggest that this might be the biologically relevant conformation for the cysteine side chain in the apo- as well as the holo-repressor. However, there is no steric hindrance that would prevent the cysteine side chain from adopting a conformation that would bring the S⁺⁺ closer to metal site 2. Such a motion would be in agreement with reports that Cys102 plays an important role in the mechanism of activation as evidenced by site-directed mutagenesis studies, which showed that a replacement by all amino acids but Asp results in a loss of metal-activated DNA-binding. Elucidation of the role of Cys102 in metal binding might have to await the

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**Fig. 4.** A, superposition of the 137 C$^\alpha$ atoms of domains 1 and 2 of monomer A in both crystal forms. The transformation matrix was calculated superimposing only monomer A (left side) and consequently applied to the full dimer. Apo-DtxR in form II is depicted in blue; Zn-DtxR in form I is shown in red. The view shown is perpendicular to the two-fold axis and approximately along the putative DNA-binding helices H3 and H3’. Domain 3 is omitted for clarity. B, Cpk representation of apo-DtxR form I showing the two DNA-binding domain in blue, the core domain in yellow, and the last 20 residues of the dimerization domains (residues 121–140) in green.

Ramachandran plot. Further information on the crystallographic refinements is given in Table II. Since the apo- and Zn-DtxR form I crystals diffract to higher resolution and the model is also more complete in these structures, they will mainly be used for the detailed comparison of the metal binding sites of the apo- and holo-repressor described below. The overall structures of apo-DtxR and of DtxR bound to its divalent metal ion co-repressor are very similar; the r.m.s. deviations of C$^\alpha$-atoms range from 0.3 to 0.5 Å (see Table III). However, a careful analysis of the structures reveals significant domain motion of the DtxR subunits, as will be described below after comparing the metal binding sites in apo- and holo-DtxR.

**Metal Site 1**—As mentioned before, metal binding site 1 has been observed to be occupied in all high resolution crystal structures of DtxR determined in the presence of divalent metal ions so far (21, 23, 24). The metal was found to be tetrahedrally coordinated by N$^\nu$ of His79, O$^\gamma$ of Glu83, N$^\delta$ of His98, and a sulfate (or phosphate) ion. In apo-DtxR the metal binding site 1 is clearly not occupied (Fig. 1A). Yet no significant conformational changes appear to occur near site 1 upon
Comparison of Apo- and Zn-DtxR Structures: Domain Motion with Respect to the Core—The crystal structure of DtxR in complex with different divalent cations was originally solved in our laboratory in crystal form I (21), whereas crystal form II was initially reported by Schiering et al. (22). Both forms were crystallized from similar conditions and are closely related. In form I, the protein dimer lies on a crystallographic two-fold axis, and there is one monomer in the asymmetric unit. In form II, the crystallographic two-fold axis is slightly shifted and becomes a noncrystallographic two-fold axis. Consequently, the c axis is doubled, and the space group changes from P3221 (form I) to P32121 (form II). Schiering et al. (22) obtained a structure of apo-DtxR at 3.0 Å resolution in a crystal modification similar to form II ($R = 0.233$, $R_{free} = 0.413$) and suggested on the basis of a comparison with a Ni-DtxR structure at 3.8 Å in form I ($R = 0.193$, $R_{free}$ not reported) that binding of the metal would cause a small change in quaternary structure between the different crystal forms, thereby activating the repressor.

A comparison of the four high resolution structures of apo-DtxR and Zn-DtxR in the same two space groups described in the present paper shows that not differences in quaternary but rather in tertiary structure are most likely to be involved in repressor activation. For this analysis, we superimposed, as a first step, the 137 Cα atoms of the two first domains of monomer A of the DtxR dimer onto each other. Next, we evaluated, overall as well as per residue, how much subunits A and B deviate from each other after this “A on A” superposition. The results clearly show that the B subunits deviate in each case significantly more than the A subunits (Table IV). This suggests that the B subunits, or parts thereof, undergo a motion with respect to subunit A. A more detailed analysis of the most deviating pair of protein dimers, apo-DtxR form II versus Zn-DtxR form I, provides clear evidence for a domain motion within the subunit (see Fig. 4A). The key observation is that residues 74–120 of the B subunits superimpose as well or even better onto each other than any part of subunit A, although none of these atoms was used in calculating the parameters for this “A on A” superposition. Evidently, residues 74–120 of the B subunits do not move with respect to the A subunit. The deviations for B subunit residues 4–74 that form the DNA binding domain and for residues 121–140 at the C terminus of the second domain are much larger than for the B subunit residues 74–120 (Fig. 5). As residues 74–120 of subunits A and B make numerous interactions with each other, we can define an immobile “core” of the DtxR dimer consisting of these residues in both subunits. The rest of each subunit can move with respect to these 92 core residues. All other possible combinations of pairwise superpositions of DtxR dimers exhibit the same result, although the actual differences are smaller. The core deviates by only 0.15–0.21 Å, while the rest of the subunits differ by r.m.s. deviations for the DNA-binding domain up to 0.84 Å (Table V). The DNA-binding domain is observed to be able to rotate with respect to the core and exhibits a change in the angle of the putative DNA recognition helices H3 and H5 by 1.8° (161.5° in Zn-DtxR form I and 159.7° in apo-DtxR form II as calculated using the program EDPDP (39)). In addition, some Cα atoms of the recognition helix H3 are shifted significantly in the four structures determined, up to 1.7 Å of residue 39 when comparing the apo-DtxR form II and Zn-DtxR form I dimers (Figs. 4 and 5). The ability to move the recognition helices with respect to the constant core of the DtxR dimer is likely to be of critical importance to allow the repressor to adopt the optimal conformation for interacting with its cognate DNA.

DISCUSSION

The DtxR dimer can be described as consisting of the following elements: (i) a rigid core formed by residues 74–120 of subunit A plus the same residues in subunit B; (ii) the N-terminal DNA-binding domain, which can rotate with respect to the core; (iii) the C-terminal residues 121–140 of the dimerization domain, which vary more in conformation than the core.
FIG. 6. Pairwise comparison of the deviations of Cα atoms after least-squares superposition of 94 Cα atoms of the core domain (residues 74–120 in each monomer). A, apo-DtxR form I superimposed on Zn-DtxR form I; B, apo-DtxR form I superimposed on Apo-DtxR form II; C, apo-DtxR form I superimposed on Zn-DtxR form II.
and (iv) the hyperflexible C-terminal third domain. Fig. 4B illustrates this domain organization. The core of the apo-DtxR dimer is depicted in yellow, the two DNA-binding domains in each monomer in blue, and the C-terminal residues in green.

Interestingly, our present studies do not reveal any correlation between metal content and structural differences, since the smallest structural changes are observed between apo-DtxR in form I and Zn-DtxR in form I (Fig. 6). Hence, crystal packing effects also play a role regarding the differences observed in the orientation of the DNA-binding domain in the four structures reported. Nevertheless, the motions of the DNA binding domain seen in Figs. 4 and 5 are most intriguing, since they are the type of motions one would expect to occur upon binding the co-repressor. Clearly, the DNA-binding motifs are able to move, presumably, to interact better with DNA. Even small changes in the geometry of the two DNA recognition helices with respect to each other might have a large effect on the affinity. It seems as if the motion observed in the apo- and Zn-DtxR crystals reported in this paper shows a tantalizing glimpse of what may occur in the living cell upon DNA binding. The changes seen in Figs. 5 and 6 are to a first order of approximation a motion of residues 1–73 in subunit B with respect to the rest of this subunit. It is gratifying that this amounts to an experimental determination of the border of the first two domains in DtxR, which coincides quite precisely with the assignment initially given by us in the first report on the DtxR structure (21).

In evaluating these results, it may be useful to note that the modification of Cys102, which is observed time and again in the DtxR structure (21).

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