Structure
Article

Coiled-Coil Helix Rotation Selects Repressing or Activating State of Transcriptional Regulator DhaR

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SUMMARY

Escherichia coli dihydroxyacetone (Dha) kinase consists of two subunits, DhaK and DhaL. Transcription of *dha* operon is regulated by the DhaR transcription factor and its action is under control of the kinase subunits. DhaR is activated by interaction with DhaL while it is repressed by DhaK. We have determined the structures of DhaK and DhaL bound to the tandem GAF-like and PAS domains of the DhaR, providing an architectural model for how GAF/PAS tandem domains work together in binding protein partners. The structures reveal a mechanism of opposite transcriptional regulation by the DhaK and DhaL subunits. The kinase subunits interface with DhaR through surfaces that partially overlap with their active sites, allowing sensing of ATP- versus ADP-loaded DhaL subunit and also precluding a ternary complex between DhaK-DhaL and DhaR. The rotation of helices within the DhaR coiled-coil linker upon DhaL binding provides the mechanism for transmitting the binding signal from the GAF/PAS domains to the C-terminal DNA-binding domain.

INTRODUCTION

Bacterial enhancer binding proteins (bEBP) constitute a large group of transcription regulators, with common architecture consisting of the N-terminal regulatory region, central AAA+ ATPase domain and C-terminal DNA-binding helix-turn-helix motif (Schumacher et al., 2006; Tucker and Sallai, 2007; Bush and Dixon, 2012; Joly et al., 2012). The regulatory region contains diverse input domains, sensing a wide range of environmental clues (Studholme and Dixon, 2003). Often, regulation is accomplished through binding of a low-molecular-weight ligand to sensory input domains, such as GAF (named after cGMP-specific phosphodiesterases, Adenylyl cyclases and FhlA) and PAS (after Period circadian protein, Aryl hydrocarbon receptor nuclear translocator protein and Single-minded protein), or through phosphorylation of a response regulation domain. Other groups of bEBPs respond to environmental clues via interactions with proteins; these bEBPs have been less studied (Buck et al., 2000) and remain poorly understood to date.

One interesting bEBP, which regulates transcription through protein-protein interactions, is the *Escherichia coli* DhaR. It controls the expression of the phosphoenolpyruvate-dependent dihydroxyacetone (Dha) kinase, an enzyme that produces dihydroxyacetone phosphate. Dha kinase consists of three subunits DhaK, DhaL, and DhaM, which are encoded by the genes *dhaK*, *dhaL*, and *dhaM* of the *dha* operon. DhaM is a phosphotransferase component of the phosphoenolpyruvate: sugar phosphotransferase system (PTS). This subunit is phosphorylated by the small phospho-carrier protein HPr of the PTS (Erni et al., 2006) and transfers the phosphate to the ADP moiety tightly bound to DhaL (Gutknecht et al., 2001; Bächler et al., 2005a; Oberholzer et al., 2005). Therefore, ADP functions as a phosphate transferring coenzyme. The DhaL-ATP complex subsequently associates with the DhaK subunit containing the Dha substrate covalently bound to His218 through a hemiaminal bond (Siebold et al., 2003), whereupon the phosphate is transferred from ATP to Dha yielding Dha-P (Gutknecht et al., 2001; Bächler et al., 2005a; Oberholzer et al., 2005).

The transcription regulator DhaR functions as an autorepressor of the *dhaR* gene and a Dha-dependent activator of the *dha* operon (Bächler et al., 2005b). The DhaK kinase subunit functions as a corepressor while DhaL is a coactivator of DhaR. They belong to so called "trigger enzymes" that have dual roles: in catalysis and in transcriptional regulation (Commichau and Stülke, 2008). The activation is triggered by phosphorylation of Dha by the kinase that restores the DhaL-ADP complex, which through direct interactions with the DhaR N-terminal sensor region activates the transcription of the *dha* operon (Bächler et al., 2005b).

The majority of bEBPs activate RNA polymerase by conformational changes and/or oligomerization induced by environmental signals, resulting in an active form of AAA+ ATPase, which interacts with the σ^{54} factor (Bush and Dixon, 2012;

Table 1. X-Ray Data Collection and Refinement Statistics

Data Set	DhaR-DhaK	DhaR-DhaK (T79L)	DhaR-DhaL
Space group	$P6_1$	$P6_1$	$P1$
a, b, c (Å)	232.1, 232.1, 79.9	231.4, 231.4, 79.8	89.8, 91.5, 93.8
α, β, γ (°)	–	–	84.2, 72.4, 90.0
Wavelength (Å)	0.9793	0.9793	0.9793
Resolution (Å)	50–3.25 (3.37–3.25)	50–2.83 (2.93–2.83)	50–2.32 (2.40–2.32)
Observed *hkl*	300,714	340,124	461,931
Unique *hkl*	39,071	56,455	119,216
Redundancy	7.7 (5.7)	6.0 (3.7)	3.9 (3.4)
Completeness (%)	99.8 (99.1)	97.0 (87.7)	97.5 (92.1)
R_{sym}[a]	0.134 (0.525)	0.091 (0.552)	0.090 (0.588)
I/(σI)	13.1 (3.1)	13.4 (2.0)	13.3 (2.0)
Wilson B (Å^2)	47.1	64.2	48.3
R_{work} (no. *hkl*)[b]	0.191 (37,079)	0.201 (53,562)	0.200 (113,145)
R_{free} (no. *hkl*)	0.228 (1,958)	0.241 (2,860)	0.247 (6,008)
B factors (no. atoms)			
Protein	68.4 (9,900)	59.6 (9,900)	46.4 (15,394)
Solvent	–	31.6 (30)	35.8 (445)
Ligands	75.6 (12)	63.6 (12)	32.8 (116)
Ramachandran			
Allowed (%)	98.8	98.6	99.3
Generous (%)	1.0	1.2	0.7
Disallowed (%)	0.2	0.2	0
Rmsd			
Bonds (Å)	0.015	0.018	0.018
Angles (°)	1.63	1.94	1.74
PDB code	4LRX	4LRY	4LRZ

Data for the highest resolution shell are given in parentheses.
[a] $R_{sym} = (\sum|I_{obs}-I_{avg}|)/\sum I_{avg}$.
[b] $R_{work} = (\sum|F_{obs}-F_{calc}|)/\sum F_{obs}$.

Joly et al., 2012). DhaR belongs to a subgroup of bEBPs that control transcription through the σ^{70} factor (Bächler et al., 2005b). The mechanism of regulation by this group is relatively poorly understood, but their ATPase domain does not have a σ^{54} binding sequence motif (Bächler et al., 2005b), a critical element for RNA polymerase activation. Thus downstream signal propagation by DhaR is likely very different from σ^{54}-dependent systems and may not require signal transmission from the sensor region to the ATPase domain. Some of these bEBPs function via direct interactions between their sensory region and the C-terminal domain of RNA polymerase (Pittard et al., 2005); DhaR may use a similar mechanism.

The architecture of bEBP reveals a frequent presence of multiple small domains in the regulatory region, the role of which is often not clear in sensing and signaling (Studholme and Dixon, 2003). The dihydroxyacetone kinase system provides a new mechanism of sensing, in which transcription regulation by DhaL and DhaK involves physical interaction of these subunits with the sensory region of DhaR comprising a tandem PAS and GAF-like domain. Such domains are frequently present in sensory regions of bEBPs. PAS and GAF domains have a related fold and belong to the profilin-like superfold in the SCOP classification (Murzin et al., 1995; Lewis et al., 2013). They are one of the largest and most widespread folds in all kingdoms of life, have versatile functions, and are often involved in signal transduction pathways and protein regulatory systems. Most of the structurally characterized PAS and GAF domains bind low-molecular-weight ligands or serve as homodimerization modules (Ho et al., 2000; Möglich et al., 2009; Henry and Crosson, 2011). Dha kinase regulatory system uniquely involves interactions of these domains with other proteins.

In this report, we provide extensive mechanistic studies of this system through crystal structures of the *E. coli* DhaR N-terminal regulatory region complexed with the DhaK or DhaL subunits of the kinase. Our studies have uncovered the molecular basis of recognition between a transcriptional regulator, its corepressor and co-activator, and provide the first example of how interactions of two different kinase subunits with the sensory domain of the transcription regulator trigger different downstream responses.

RESULTS

Overall Structures of the DhaR(N)-DhaK and DhaR(N)-DhaL Complexes

The pertinent details of data processing and refinement are shown in Table 1.

DhaR(N)-DhaK Complex

The crystals of the DhaR(N)-DhaK complex diffract to only 3.25 Å resolution, possibly due to their high solvent content. Diffraction has been moderately improved (to 2.83 Å) by using bigger crystals of DhaR(N)-DhaK(T79L). Other than the mutation itself, no structural difference (root-mean-square deviation [rmsd] of 0.34 Å for all 1,300 Cα atoms) could be detected between the wild-type and mutant crystals of this complex. We therefore used the DhaR(N)-DhaK(T79L) model for the description below. Each asymmetric unit contains one DhaK dimer and one DhaR(N) dimer, in which each DhaK monomer contacts only one DhaR(N) (Figure 1A). However, the molecules pack in the crystal in such a way that the second DhaK subunit in the DhaK dimer interacts with one DhaR subunit belonging to another DhaR dimer, thus forming helical chains extending throughout the crystal (Figure S1A available online). This interaction pattern can be represented as …$DhaK_A'$-$DhaK_B'$-$DhaR(N)_A$-$DhaR(N)_B$-$DhaK_A$-$DhaK_B$…, where subscript denotes dimer components and superscript (′) indicates a symmetry-related heterotetramer (Figure 1A). The pseudo 2-fold symmetry that relates two monomers of DhaR(N) extends to the $DhaK_B'$-$DhaR(N)_A$-$DhaR(N)_B$-$DhaK_A$ segment. Thus the interface contacts between $DhaR(N)_B$-$DhaK_A$ (within one asymmetric unit) are nearly identical to those of $DhaR(N)_A$-$DhaK_B'$ (between two asymmetric units; Figure 1A). The interface is large and encompasses an area of ~1,150 Å^2 on each molecule as calculated by PISA (Krissinel and Henrick, 2007). These values are nearly identical for the $DhaR(N)_B$-$DhaK_A$ and $DhaR(N)_A$-$DhaK_B'$ interfaces. In comparison, the DhaR dimerization interface has an area of ~2,350 Å^2, the $DhaK_A$-$DhaK_B$ dimerization interface is ~1,960 Å^2 and the next largest contact between symmetry related molecules is only 350 Å^2. Moreover, the DhaR surface