

Structural Analysis of a Full-length OmpR Homolog from *Thermotoga Maritima*

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Introduction: Two-component signal transduction pathways are common in bacteria and control a broad spectrum of bacterial functions spanning from chemotaxis to regulated expression of virulence factors. This signaling pathway involves phosphorelay from a sensor histidine kinase to an intracellular response regulator protein (RR). The majority of RRs function as transcription regulators, where phosphorylation-modulated RR-DNA, RR-RNA polymerase, and RR-RR oligimerization interactions provide the basis for regulated gene expression in response to environmental stimuli. The architecture of the response regulator superfamily is typified by an N-terminal phosphoreceiver (regulatory) domain and a C-terminal DNA-binding (effector) domain. The structural basis for activation of RRs by phosphorylation remains a central mechanistic question, and insights into this mechanism may lead to the development of new classes of antibacterial agents. Here, we report the first full-length structure of a member of the largest subfamily of RR transcription regulators, the OmpR/PhoB subfamily, determined by X-ray crystallography using synchrotron radiation.

Methods and Materials: OmpR/PhoB homologs from *Thermotoga maritima* were identified by DNA sequence database searching and cloned and expressed in *E. coli*. Single crystals of one homolog were obtained from 10% PEG 3350, 200 mM KTC, 100 mM MES pH 6.5. Selenomethionyl derivatives were obtained by overexpression in *E. coli* strain B834 and crystallized under similar conditions to those for the native form. X-ray diffraction data were collected at three wavelengths near the selenium K-edge to maximize anomalous and dispersive signals used in phase determination. The resulting data were indexed, integrated, and scaled using the program package Denzo/Scapecapack. Selenium sites were located using the program SOLVE, which also produced phase estimates for all structure factors using data extending to a Bragg spacing of 2.0 Å providing well-defined electron density maps for initial model building. Model refinement proceeded with data collected at the remote wavelength (0.960 Å) extending to 1.5 Å and was carried out using the programs O and CNS (**Figure 1**). The final agreement of model to experimental data is R=22.8% (Rfree=24.5%).

Results: As expected from sequence analysis, the folds of the two subdomains are similar to folds observed in X-ray and NMR studies of isolated effector and regulatory domains from this subfamily of RRs. The N-terminal regulatory domain exhibits a doubly wound α/β sandwich, and the C-terminal effector domain presents a helix-turn-helix motif as well as an unusually large loop, or wing, between HTH helices, consistent with the winged helix subfamily. A 4-stranded antiparallel β -sheet at the N-terminus of the effector domain, that distinguishes the OmpR/PhoB subfamily within the general winged helix fold classification, provides a planar surface that mediates the sole interdomain interactions involving $\alpha 5$ of the regulatory domain (**Figure 2**). This minimal interaction between domains, amounting to an interaction surface of less than 300 Å², is in contrast to interdomain interfaces for the two other full-length RRs from other RR subfamilies for which structural data are available (1,2). In the latter cases the interaction areas amount to more than 1000 Å², similar to solvent-protected interface areas observed for homodimeric proteins (3). Disorder was observed for the interdomain linker, which is further consistent with minimal structural constraints between the two domains, which might otherwise enforce specific interdomain orientations.

Conclusions: Structures of two-domain RRs reported previously show significant interdomain interfaces, which result in an inhibition of function of the effector domain through direct interdomain steric interference. Phosphorylation in these cases presumably leads to activation by causing a repositioning of the two domains with respect to each other. The present structure shows limited enforcement of a specific interdomain orientation, suggesting an alternate mechanism of activation by phosphorylation.

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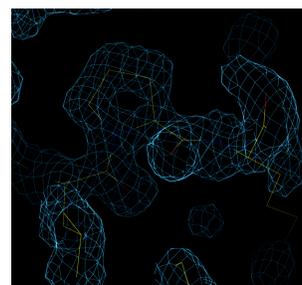


Figure 1. Representative 2fo-fc electron density map near Pro100.



Figure 2. Ribbons diagram showing the N-terminal regulatory domain (top) and C-terminal effector domain (bottom). Sidechains of the few residues involved in interdomain contacts are fully displayed in red (regulatory domain) and green (effector domain)