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Structural study of the discriminative DNA-recognition mechanism by a bacterial transcription factor

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Introduction

The TetR-family is the protein family of widely spreading bacterial transcriptional repressors. The family members respond to environmental changes, and most of them are predicted to be relating to multidrug export or their detoxification. Upon binding of inducers, they turn on transcriptions by dissociating from their bound operator. Several tertiary structures of TetR-family proteins had been reported, however, only for the *Escherichia coli* TetR and the *Staphyrococcus aureus* QacR, their tertiary structures in three functional forms, free protein, complex with operator, and that with inducer, were obtained. These structures successfully explain the alternative binding of the repressors to their operators or to inducers.

The CgmR from *Corynebacterium glutamicum*, previously called as the CGL2612 protein, is the transcriptional repressor that regulates expression of the immediately upstream gene *cgmA*, which encodes a major facilitator superfamily permiase. The protein pair of CgmR and CgmA confers multidrug-resistance to *C.glutamicum*. The CgmR is structurally homologous to QacR, a multidrug-responsible repressor from *S.aureus*. Despite of their structural similarities, CgmR and QacR have non-homologous primary structures in their regulatory domains, and thus these repressors respond to different drugs as inducers. The mechanisms of inducer binding and controlling its operator binding are still unclear for CgmR. In this study, the crystal structures of CgmR in complex with its inducers and with its operator are respectively obtained. These tertiary structures provided distinct structural explanation of the alternative switch model, which is different from ones for TetR and QacR. The model can provide a feasible explanation for the multidrug responsiveness (H.Itou, a manuscript in preparation).

Results and Discussions

The native diffraction data for two CgmR-inducer complex crystals were collected at the beamlines BL6A and BL17A respectively. For the operator complex crystal, the single anomalous diffraction (SAD) data were collected at the beamline NW12. The tertiary structures of the inducer complexes were determined by molecular replacement method (PDB: 2DH0 and 2YVE), and that of the operator complex was determined by the SAD method (PDB: 2YVH).

In a regulatory system consists of a repressor and its inducer, the repressor must bind either to operator or to inducer at a time. This requirement is satisfied by the existence of two protein conformations, one with a high affinity for operator but a low affinity for inducer (the oval in FIG), and the other with the opposite property (the square in FIG). Thus, if the conformation of free protein is taken into account, two alternative models can exist for the regulatory system. In the distortion model, the conformation of free protein is close to that in the operator complex, and function of the inducer is to alter the conformation to another. On the other hands, in the stabilization model, the conformation of free protein is maintained and stabilized by inducer binding, and the protein carries out induced fitting to its operator to repress. These models use different strategies of regulation. The distortion model is easily designed by a mechanical link between its inducer-binding pocket and DNA-binding domain to maintain high affinity for its counterpart. In contrast, the stabilization model is more thermodynamic and any interaction with its inducer is available, if the inducer binding reduces significant free energy. The both models seem to be possible, and it is supposed that a regulatory system is inclined to choose the distortion model when inducer is required to be specific, and prefers to select the stabilizing model when a broad range of inducers are required. However, all the regulatory systems so far clarified by the structural analyses belong to the distortion model, and QacR is not exceptional. This study proved the existence of the stabilization model for CgmR. The conformation of free CgmR is almost identical to that of inducer complex and quite different from the operator complex. The stabilizing model does not provide a feasible explanation for multidrug responsiveness of CgmR, but also may explain the function of inducers in future structural studies.

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