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

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Introduction

The CGL2612 homodimeric protein from *Corynebacterium glutamicum* is a multidrug-binding transcriptional regulator represses transcription of the *cgl2611* gene encoding multidrug exporter protein. This protein belongs to the TetR-family proteins, and it has been known that binding of structurally dissimilar compounds to the CGL2612 protein induces dissociation of the protein from its operator site located just upstream of the *cgl2611* gene [1]. In this study, crystal structures of the CGL2612 protein in complex with its operator DNA or two different drugs were determined respectively to discuss how the multidrug resistance related transcription factor dissociated from its operator DNA responding to binding of diverse drugs.

Methods and Results

Crystallization and Data Collection

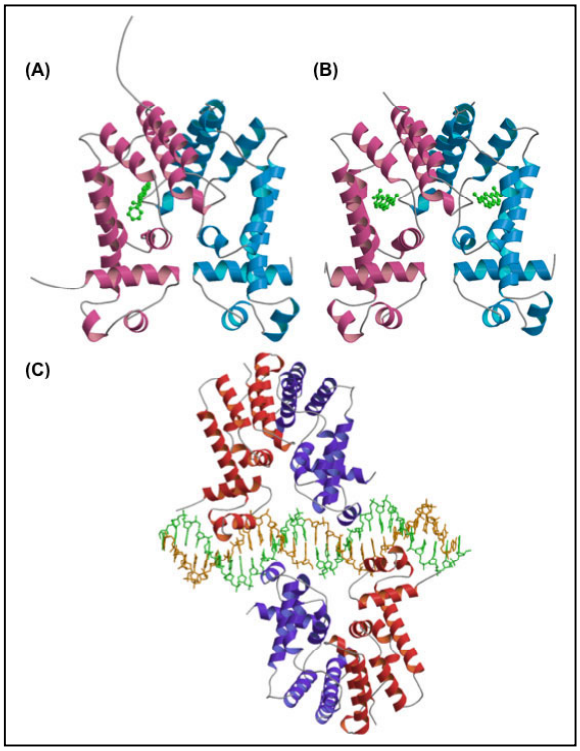
The recombinant CGL2612 protein was purified using Ni-chelating affinity chromatography and size exclusion chromatography. The purified protein was mixed with drugs ethidium bromide (ET) or methylene blue (MB), and co-crystallized respectively. The CGL2612 protein binds to its operator DNA composed of inverted repeat sequence [1]. The protein – DNA complex crystal was obtained by co-crystallization with the DNA fragments harboring a sequence resembling its half. All crystallization experiments were carried out using vapor diffusion methods at 293 K.

The native data of two drug complex crystals were collected at a wavelength 1.000 Å under cryogenic condition (100 K) on beamlines BL6A (ET complex) and BL17A (MB complex), respectively. For the protein – DNA complex crystal, Se-Met substituted crystal was prepared, and the single anomalous diffraction (SAD) data were collected at a wavelength 0.97909 Å under cryogenic condition on the beamline NW12. All collected data were processed using the program *HKL2000*.

Structure Analysis

Structures of the CGL2612 protein bound to drugs were determined by molecular replacement method using the program *AMoRe*. Tertiary structure of the ligand-free CGL2612 (PDB: 1V7B) [1] was used as the search model. Model fitting and structure refinement were carried out automatically using the program *Lafire* with the *CNS*. Final models had an R-factor of 20.7 % and a free R-factor of 23.5 % for data between 20 and 1.95 Å resolution (ET-complex, Figure (A). PDB: 2DH0), and R-factor of 20.7 % and a free R-factor of 23.5 % for data between 10 and 1.4 Å resolution (MB-complex, Figure (B). PDB: 2YVE), respectively.

The structure of the DNA-complex was determined using the SAD method. Twelve of total 16 selenium sites in the asymmetric unit were found using the program *SHELXD* in *SHELX97* package. Initial phases were calculated by the program *SOLVE* using these selenium sites, and the best electron density map was obtained after the phase improvement by non-crystallographic symmetry averaging using the program *DM*. Model was manually built using the graphic program *O,* and structure refinement was performed using the program *Lafire* with the *Refmac5*. The final model had an R-factor of 22.0 % and a free R-factor of 25.9 % for data between 10 and 2.5 Å resolution (Figure (C). PDB: 2YVH).



Ribbon representations of the ET-complex (A) and the MB-complex (B). Bound ligands were shown in green ball-and-stick models. Figure (C) represents the CGL2612 protein – DNA complex structure.

References

[1] H. Itou et al., J. Biol. Chem. 280, 38711 (2005).

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