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Structural study of the replication initiator protein RepE of the F-plasmid

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Introduction

RepE, the replication initiator protein of the F-plasmid in *Escherichia coli*, is a bifunctional protein and plays an essential role for the copy number control of the F-plasmid. RepE intrinsically has two molecular association states, i.e., the monomer and dimer, to regulate its initiator activity. The RepE monomer functions as a DNA replication initiator, whereas the dominant dimer acts as an autogenous transcriptional repressor, exhibiting no initiator activity. The DnaK molecular chaperone system of *E. coli* has been known to be necessary for the F-plasmid replication through the dissociation of the RepE dimer into active monomers. To date, although the crystal structure of the monomeric form of RepE, a mutant RepE54 in complex with DNA, has been reported [1], no structural information of the dimer is available. In this study, the crystal structure of the wild-type RepE dimer in complex with the *repE* operator DNA was determined and compared with that of the monomer to elucidate how RepE is activated as a DNA replication initiator.

Methods

The full-length RepE protein with N-terminal His-tag was overexpressed in *E. coli* and purified by Ni affinity column chromatography under high salt conditions [2]. The RepE dimer was crystallized in complex with the *repE* operator DNA using the sitting-drop vapor-diffusion technique. The crystals belong to the space group $P2_1$ with unit-cell parameters of a = 60.7, b = 99.3, c = 95.0 Å, and β = 108.6°. The structure of the RepE dimer-DNA complex was determined by the molecular replacement method using the monomeric structure of RepE (PDB ID code, 1REP [1]) as a search model. Manual model building and structure refinement were performed using the programs O and CNS, respectively. Coordinates and structure factors have been deposited in the Protein Data Bank (PDB ID code, 2Z9O [3]).

Results and Discussion

Prior to X-ray exposure, the application of dehydration and crystal annealing were indispensable to improve the resolution limit of X-ray diffraction from the crystals [2]. The crystal structure of the dimeric form of RepE bound to the *repE* operator DNA was determined at 3.14 Å resolution [3]. The protomers of the RepE dimer can be divided into the two domains, N- and C-terminal domains, like the RepE monomer. Although the monomer interacts with DNA through the both domains, there is no contact between DNA and the N-terminal domain of the dimer, which is responsible for RepE dimerization. Comparing the structures of the two forms, the conformations of the N- and C-terminal domains are essentially the same, but the secondary structure of a domain linker connecting the two domains and the relative domain orientation are significantly changed. Based on a biochemical study of a homologous protein and on amino acid sequence analysis, interaction sites of DnaK and DnaJ proteins of the DnaK chaperone system are predicted within the domain linker region of RepE. These findings suggest that the chaperone-binding would induce a local structural alteration of the RepE domain linker and then cause dynamic domain rearrangement and dissociation of the RepE dimer, resulting in activation of RepE as a replication initiator.

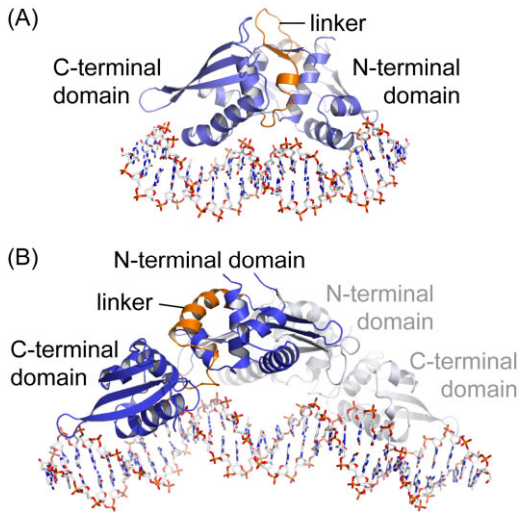

Figure. RepE-DNA structures. Crystal structures of the monomer (A) and the dimer (B) of RepE in complex with DNA are shown. The monomer and one protomer of the dimer are colored blue, and the domain linkers are colored orange.

References

[1] H. Komori et al., EMBO J. 18, 4597 (1999).
[2] A. Nakamura, C. Wada, and K. Miki, Acta Crystallogr. F63, 346 (2007).
[3] A. Nakamura, C. Wada, and K. Miki, Proc. Natl. Acad. Sci. USA 104, 18484 (2007).

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