successful to produce crystals in our screening. Initial diffraction tests were performed using a home-source X-ray generator (Rigaku FR-E) equipped with a Rigaku R-AXIS detector at 100 K. The best crystal was found to diffract up to 2.75 Å resolution. The structure was determined by molecular replacement using our previous structure of human FEN1 in the FEN1-PCNA complex crystal (PDB code 1UL1) as a search model. The structure shows that the enzyme holds both the upstream and downstream duplexes and induces a sharp kink of the DNA by embedding the kinked template strand in a basic cleft.

Keywords: DNA replication, DNA-protein complexes, endonucleases

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Structural studies of the multidrug-responsible transcriptional repressor protein CgmR

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The CgmR protein from Corynebacterium glutamicum is a multidrug-responsible transcriptional repressor belonging to the TetR-family. The crystal structure analyses of CgmR in its three different functional conformations, a DNA-binding form, drug-binding forms, and a drug-free form revealed this protein is inherently in a DNA-releasing conformation. Repressor proteins are molecular switches and dissociate from their bound operator in inducer-binding dependent manner. There are two possible two-state mechanistic models explaining functional cycle of repressors. These models are composed of two functional protein conformations, DNA-binding form and DNA-releasing form. In the first model (Inducing model), the default conformation of its inducer-free condition is the DNA-binding form, and binding of inducers provokes its structural change to dissociate from operator. In an alternative model, the default conformation is the DNA-releasing form, and binding of inducers stabilizes it to prevent changing to the DNA-binding form (Stabilizing model). Functional appearances of these models as transcriptional repressors are identical, however their mechanistic aspects as molecular switches are different each other. Repressors adopting the former inducing model have been known (e.g. TetR), while another model has not been confirmed yet. Our structural study revealed that CgmR adopts the latter stabilizing model, and this is the first example experimentally demonstrating the unconfirmed model. From structural viewpoints, this model adopted by CgmR has innate ability to respond against divergent ligand molecules. The model is consistent with molecular function of the CgmR protein, and also explains functional propensity of other TetR-family proteins as multidrug responsible repressors.

Keywords: crystal structure analysis, multidrug-binding protein, transcriptional regulation

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The C-terminal extension in archaeal and eukaryotic DNA ligases modulates the DNA binding activity

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ATP-dependent DNA ligase encoded by the hyperthermophilic archaeon Pyrococcus furiosus (PfuLig) seals single-strand breaks (nicks) in DNA duplex substrates. The thermostability of this enzyme is exploited in the ligase chain reaction and the ligase detection reaction to distinguish single base mutations associated with genetic diseases. Here we report the identification of an essential residue responsible for the improvement of the ligation activity. PfuLig comprises the N-terminal DNA-binding domain, the middle adenylation domain, and the C-terminal OB-fold domain. The architecture of each domain resembles those of human DNA ligase I (hLig I). The closed form (PfuLig) compared to the substrate-bound open form (hLig I), the helical extension conserved at the C termini in archaeal and eukaryotic DNA ligases appears to play crucial role in stabilizing a closed form without DNA substrate, therefore this helical moiety might affect the substrate-DNA-binding activity. The deletion of extensional helix caused the increased DNA binding activity. But the ligation activity of this mutant became lower than wild type. After the several mutational experiments on the C-terminal helical moiety, we demonstrated that Asp540, one of the selected amino acid residues, is accounted for the improved DNA binding and ligation activity over the optimum temperature.

Keywords: protein engineering and biotechnology, DNA-protein interactions, mutational analysis

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Structural basis for transcriptional regulation mechanisms by the transcription factor Ets2

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The Ets transcription factor family has been known to regulate gene transcription related to cellular differentiation, proliferation, senescence, apoptosis and oncogenic transformation. All family members have highly conserved DNA binding domain, termed the Ets domain (ETSD). To date, three-dimensional structures of some Ets transcription family members complexed with DNA containing their target sequences have been determined. However, the specificity and selectivity of DNA recognition and subsequent transcription by each family member are poorly understood. In this study, we focused on Ets2. Firstly, in order to elucidate DNA recognition mechanism of Ets2, we determined the three-dimensional structure of the ETSD from Ets2 complexed with the DNA containing the Ets2 target sequence. Secondly, we evaluated the kinetic parameters to DNA binding by Ets2ETSD and Ets2ΔN307 including the inhibitory domains flanking at both N- and C-termini of ETSD by the surface plasmon resonance (SPR) method and clarified transcriptional regulation by auto-inhibition mechanism to DNA

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