

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

P04.06.231

Acta Cryst. (2008). A64, C303

Structural studies of the multidrug-responsible transcriptional repressor protein CgmR

Hiroshi Itou¹, Nobuhisa Watanabe², Min Yao³, Yasuo Shirakihara¹, Isao Tanaka³

¹National Institute of Genetics, Structural Genetics Center, Yata1111, Mishima, Shizuoka, 411-8540, Japan, ²Nagoya University, Chikusa-ku Furo-cho, Nagoya, Aichi, 464-8603, Japan, ³Hokkaido University, N10W8, Sapporo, Hokkaido, 060-0810, Japan, E-mail : hitou@lab.nig.ac.jp

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

P04.06.232

Acta Cryst. (2008). A64, C303

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

Maiko Tanabe¹, Shinichi Kiyonari², Yoshizumi Ishino², Hirokazu Nishida¹

¹Hitachi, Ltd., Central Research Laboratory, Biosystems Research Department, 1-280, Higashi-koigakubo, Kokubunji-shi, Tokyo, 185-8601, Japan, ²Department of Genetic Resources Technology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Fukuoka, Fukuoka 812-8581, Japan, E-mail : maiko.tanabe.za@hitachi.com

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

P04.06.233

Acta Cryst. (2008). A64, C303-304

Structural basis for transcriptional regulation mechanisms by the transcription factor Ets2

Yoshiaki Suwa, Teruya Nakamura, Sachiko Toma, Shinji Ikemizu, Hirofumi Kai, Hiroshi Morioka, Yuriko Yamagata

Kumamoto-Univ., 5-1, Oe-Honmachi, Kumamoto, Kumamoto, 8620973, Japan, E-mail : 053y9010@pharm.stud.kumamoto-u.ac.jp

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

binding, which is proposed in Ets1. Finally, in order to illustrate binding specificity against gene promoter, we measured DNA binding affinity of Ets2ETSD to various DNA sequences using the SPR method. Additionally, we investigated alterations of DNA binding mode by building up model structures based on the Ets2ETSD/DNA structure determined in this work. In conclusion, this research indicates specificity and selectivity of DNA binding by Ets2 in the three-dimensional level and explains some transcriptional regulating mechanisms of Ets2.

Keywords: transcription factor structure, DNA-protein complexes, DNA recognition

P04.06.234

Acta Cryst. (2008). A64, C304

Comparison of crystal structures of NF- κ B p50/RelB/DNA and p52/RelB/DNA complexes

Debin Huang, Amanda Fusco, Anu Moorthy, Gourisankar Ghosh
UCSD, Chem & Biochem, 9500 Gilman Dr, La Jolla, Ca, 92093-0375, USA, E-mail: dehuang@ucsd.edu

NF- κ Bs constitute a family of transcription activators that modulate the expression of a large number of genes that are responsible for key cellular functions including development, proliferation, survival and inflammation. This family consists of five members, p50, p52, RelA, c-Rel and RelB which share a ~300 residues segment of high sequence homology at or near their N-terminus. This homologous segment, known as the Rel Homology Region (RHR) is critical for nearly all the functions, including DNA binding, dimerization, inhibitor binding and nuclear localization. Unlike the other NF- κ B proteins, p50 and p52 lack a transactivation domain but contain inserts within and outside the RHR. These two subunits associate with RelA, c-Rel and RelB to form the predominant NF- κ B dimers responsible for gene activation. RelB does not stringently follow the NF- κ B family rules. It is the only member that is not known to form a homodimer and has restricted ability to heterodimerize. RelB preferentially forms heterodimers with p50 and p52 *in vivo*. We describe here the X-ray crystal structures of NF- κ B p50/RelB heterodimer and p52/RelB heterodimer bound to the same 10-bp kB DNA. Although p50 and p52 have identical DNA contacting amino acids, these two complexes reveal distinctive base-specific contacts. In the p50/RelB complex, the p50 subunit contacts GGG in the 5 bp half-site and RelB subunit contacts GG in the 4 bp half-site. In the p52/RelB complex, p52 subunit contacts CGG with H62 interact overhang cytosine while RelB subunit bound GGG. The specific binding in these two complexes suggests that RelB may allow the recognition of more diverse kB sequences. Our studies thus provide a basis as to why RelB/p50 and RelB/p52 heterodimers display differential biological regulations.

Keywords: DNA-protein complexes, transcription factor, DNA-packing

P04.06.235

Acta Cryst. (2008). A64, C304

Structural basis for regulation of bifunctional roles of the F-plasmid replication initiator RepE

Akira Nakamura¹, Chieko Wada², Kunio Miki^{1,3}

¹Graduate School of Science, Kyoto University, Department of Chemistry, Oiwake-cho, Sakyo-ku, Kyoto, Kyoto, 606-8502, Japan, ²Yoshida Biological Laboratory Inc., 11 Takehanasotoda-cho, Yamashina-ku, Kyoto,

Kyoto, 607-8081, Japan, ³RIKEN SPring-8 Center at Harima Institute, Koto 1-1-1, Sayo, Hyogo, 679-5148, Japan, E-mail: nakamura@kuchem.kyoto-u.ac.jp

RepE is an essential protein for the initiation of the F-plasmid replication, and its initiator activity is fundamentally controlled by interconversion of two molecular association states (i.e., monomer and dimer). The RepE monomers are the replication initiators, binding to iteron DNA sequences of the replication origin. In contrast, the dominant dimeric form of RepE has no initiator activity but functions as an autogenous transcriptional repressor, binding to the promoter/operator region of the *repE* gene, which shares an 8-bp sequence with the iteron. The RepE dimer therefore requires the DnaK molecular chaperone system to be dissociated into monomers and to be activated as initiators. During the past decade, our group determined the crystal structures of RepE in both association forms as RepE-DNA complexes (Komori *et al.*, *EMBO J.* 1999; Nakamura *et al.*, *PNAS* 2007). RepE can be divided into two domains, the N-terminal and C-terminal domains, with a linker connecting them. Both domains include a winged helix-turn-helix motif, and the specific 8-bp of DNA is recognized by the C-terminal domain. Although the N-terminal domain of the monomer also interacts with DNA, that of the dimer does not participate in DNA binding but is involved in RepE dimerization. Surprisingly, the conformations of each domain are similar between the monomer and dimer, while the secondary structure of the domain linker and relative domain orientation differ significantly from each other. Furthermore, there would be interacting areas of DnaK/DnaJ chaperones nearby the domain linker. These structural features suggest that actions of the DnaK system may induce a structural transition to the domain linker and cause a domain rearrangement of RepE, and thereby the dimer must be converted to monomers.

Keywords: DNA replication, conformational change, protein structure and function

P04.06.236

Acta Cryst. (2008). A64, C304-305

Structural study of the C-terminal domain of DNA gyrase

Chan Nei-Li^{1,2}, Tung-Ju Hsieh², Te-Sheng Lin², Hsun-Tang Chang², Shu-Yun Huang², Lynn Farh³

¹National Taiwan University, Institute of Biochemistry and Molecular Biology, Room 912, No.1, Sec. 1, Ren-Ai Rd., Taipei City, Taiwan, 100, Taiwan, ²National Chung Hsing University, 250 Kuo-Kuang Rd., Taichung City, Taiwan, 402, Taiwan, ³National Pingtung University of Education, No.4-18 Ming-Shen Rd., Pingtung, Taiwan 900, Taiwan, E-mail: nlchan@ntu.edu.tw

Most bacteria harbor two essential type IIA DNA topoisomerases, DNA gyrase and topoisomerase IV (TopoIV). While these two enzymes are highly homologous, they exhibit distinct activities. DNA gyrase supports transcription and replication by introducing negative supercoils into DNA, whereas TopoIV preferentially relaxes positive supercoils and serves as the main decatenating enzyme to facilitate chromosome segregation. Based on crystal structures of the C-terminal domains (CTDs) from *Borrelia burgdorferi* gyrase (BbGyrA-CTD) and *Bacillus stearothermophilus* TopoIV (BsTopoIV-CTD), it was proposed originally that the functional divergence of these two enzymes can be attributed to differences in the surface contour of their respective CTDs. Specifically, the DNA-binding surface of gyrase CTD has a steeper curvature and is thus more affective in bending DNA. Surprisingly, later determined crystal structure of the CTD of *Escherichia coli* gyrase (EcGyrA-