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BACTERIAL OFFENSE AND DEFENSE STRATEGIES USING NUCLEASE TOXINS

H. Fu[1] Y. Cheng[1] W. Ku[1] M. Sui[1] K. Hsia[1] L. Tsai[1] K. Chak[2] H.S. Yuan[1]
[1]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, 11529 TAIPEI 11529 TAIWAN [2]Institute of Biochemistry, National Yang-Ming University, Taipei, Taiwan

Some of the *Escherichia coli* cells produce plasmid-encoded toxic colicins that are secreted to kill closely related bacteria cells thereby they have better survival advantage during times of stress. The colicin E7 (ColE7) is one of the nuclease-type colicins, which contains a non-specific nuclease domain capable of hydrolyzing DNA in target cells. The nuclease activity of ColE7 is inhibited by the co-expressed immunity E7 protein (Im7), which binds to the nuclease domain of ColE7 in the host cell. The nuclease domain of ColE7 (nuclease-ColE7) contains a HNH motif, which has been identified in many endonucleases. The crystal structure of the phosphate-bound nuclease-ColE7/Im7 complex determined at 2.0 Å resolution with different metal ions showed that a phosphate ion was bound directly to the zinc ion in the HNH motif, suggesting that the zinc ion not only stabilizes the folding of the enzyme, but is also likely involved in DNA hydrolysis. Several residues located at positions close to the zinc-binding site were mutated to alanine and these mutants showed decreased or no nuclease activities. A gel retardation assay further demonstrated that the nuclease-ColE7 hydrolyzed DNA in the presence of zinc ions, but only bound to DNA in the absence of zinc ions. These results demonstrate that the zinc ion in the HNH motif of nuclease-ColE7 is not required for DNA binding, but is essential for DNA hydrolysis, suggesting that the zinc ion not only stabilizes the folding of the enzyme, but is also likely involved in DNA hydrolysis.

Keywords: COLICIN, DNASE, HNH MOTIF

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STRUCTURE OF A BACTERIAL QUORUM-SENSING TRANSCRIPTION FACTOR COMPLEXED WITH AUTOINDUCER-TYPE PHEROMONE AND DNA

A. Joachimiak[1] R. Zhang[1] T. Pappas[2] J. Brace[1] P. Miller[3] J. Bashkin[3] S. Winans[2]
[1]Argonne National Laboratory Biosciences Division 9700 S. Cass Ave ARGONNE IL 60439 USA [2]Cornell University [3]Monsanto Company

Many proteobacteria release N-acylhomoserine lactones as pheromones to measure their population density and to initiate gene expression at high cell density, resulting in diverse responses, including bioluminesence, biofilm formation, production of antimicrobials, DNA exchange, pathogenesis, and symbiosis (Whitehead et al., 2001). Many of these regulatory systems require a pheromone-dependent transcription factor similar to LuxR of *Vibrio fisheri*, whose N-terminal domain binds the pheromone and whose C-terminal domain binds DNA. Here we present the structure of the LuxR-type protein TraR protein of *Agrobacterium tumefaciens* complexed with the pheromone N-3-oxooctanoyl-L-homoserine lactone (OOHL) and a DNA at a resolution of 1.6 Å. The amino terminal domain of TraR (169 amino acids) is an αβα sandwich that binds OOHL, while the carboxyl terminal domain (60 amino acids) contains a helix-turn-helix motif DNA binding domain. The TraR dimer displays a two-fold rotational symmetry in each domain. However, these two axes of symmetry intersect at a 90° angle resulting in a pronounced overall asymmetry. The pheromone lies fully engulfed within the protein with virtually no contact to bulk solvent, and is stabilized by numerous hydrophobic interactions and by three direct hydrogen bonds and one water-mediated hydrogen bond.
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Keywords: QUORUM SENSING, TRAR/DNA COMPLEX, N-ACYLHOMOSERINE LACTONE BINDING

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CRYSTAL STRUCTURES OF THE TDM-2 ANTIBODY FAB FRAGMENT IN COMPLEX WITH *CIS-SYN* CYCLOBUTANE THYMIDINE NUCLEOTIDES.

T. Kimura[1] R. Mizutani[1] Y. Satow[1] Y. Komatsu[2] E. Ohtsuka[2] O. Nikaido[3]
[1]Graduate School of Pharmaceutical Sciences, Faculty of Pharmaceutical Sci., University of Tokyo Laboratory of Protein Structural Biology Hongo 7-3-1, Bunkyo TOKYO 113-0033 JAPAN [2]Graduate School of Pharmaceutical Sciences, Hokkaido University [3]Faculty of Pharmaceutical Sciences, Kanazawa University

Murine antibody TDM-2 specifically recognizes ultraviolet-induced *cis-syn* cyclobutane thymidine dimer (CTD). In order to elucidate the structure of CTD as well as recognition mechanisms by the antibody, we determined the crystal structures of the TDM-2 Fab fragment in complex with CTD dinucleotide (d(T[c,s]T)) and tetranucleotide (d(GT[c,s]TG). In these complexes, the side-chains of Tyr 32L, His 34L, Arg 50L, Ser 95H and Pro 100H, and the Asn 31L carbonyl O atom interact with the T[c,s]T bases. The 5- and 3-side moieties of the bases are respectively in the high-anti and anti conformations relative to each deoxyribose ring. The torsion angle γ about the C5-C4 bond is in the anti range, and is ascribable to the conformational restraints caused by the cyclobutane ring. Trp 91L, Tyr 94L, Phe 33H, Ser 50H and Arg 52H are situated near the sugar-phosphate backbone. In the tetranucleotide complex, the phosphate groups in the 5- and 3-sides of the T[c,s]T dinucleotide form hydrogen bonds with the Tyr 32L side-chain and the Phe 33H main-chain, respectively. Fab residues form no hydrogen bond with 5- and 3-terminal guanine bases. This indicates that the antigen specificity is attained for the central cyclobutane thymidylate moiety and its adjacent phosphate groups.

Keywords: ANTIBODY DNA PHOTOPRODUCT CYCLOBUTANE PRYMIDINE DIMER

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CRYSTAL STRUCTURE OF INTEIN HOMING ENDONUCLEASE II ENCODED IN THE ARCHAEAL DNA POLYMERASE GENE

H. Matsumura[1] H. Takahashi[1] H. Hashimoto[2] M. Nishioka[3] S. Fujiwara[4] M. Takagi[5] T. Imanaka[6] T. Inoue[1] Y. Kai[1]
[1]Graduate School of Engineering, Osaka University Dept. of Materials Chemistry 2-1 Yamada-Oka SUITA OSAKA 565-0871 JAPAN [2]Science of Biological Supramolecular Systems, Grad. Sch. Integrated Science, Yokohama City University [3]Dept. Chemical Science and Engineering, Grad. Sch. Engineering Science, Osaka University [4]Dept. Biotechnology, Grad. Sch. Engineering, Osaka University [5]Dept. Chemical Materials Science, Sch. Materials Science, Japan Advanced Insititute of Science and Technology [6]Dept. Synthetic Chemistry and Biological Chemistry, Grad. Sch. Engineering, Kyoto University

Intein homing endonuclease is a bifunctional protein catalyzing both protein splicing and site-specific DNA double-strand cleavage. The endonuclease exhibits the site-specific recognition of the intein-less DNA, and its recognition sequences are asymmetrical and long, with size of 12-40 bp. PI-Tko II, intein endonuclease II from the hyperthermophilic archaeon Thermococcus kodakaraensis strain KOD1 was cloned and expressed at high levels as the native and selenomethionine-substituted (SeMet) protein. Both native and SeMet recombinant proteins were purified and crystallized by the hanging-drop vapor diffusion method at 293 K. X-ray diffraction data for native and SeMet crystals were collected at the synchrotron radiation source of Photon Factory and SPring-8, respectively. The diffraction pattern of the native and SeMet crystals extend to 2.8 Å and 2.5 Å resolution and both crystals belong to an orthogonal space group *C*2221 with the lattice constants of a = 104.0, b = 150.6, and c = 145.5 Å. SeMet crystal was used to collect a complete MAD data set. The SeMet crystal structure of PI-Tko II has been determined at 2.5 Å using MAD method. The present R-factor and free R-factor are 25.4% and 28.5% to 2.5 Å resolution, respectively. The PI-Tko II molecule is composed of four domains. Two of them correspond to splicing and endonuclease domains, which are responsible for protein splicing and DNA-recognition/cleavage activities, respectively. Since the positively charged residues are distributed on the surfaces of the third and fourth domains, the domains may participate in the recognition of the intein-less DNA.

Keywords: INTEIN ENDONUCLEASE ARCHAEA