

because it plays the central role for all kinds of biological events. It is therefore of crucial importance to elucidate the underlying principles that govern the binding selectivity between proteins and DNA. ColE7 is an Escherichia coli toxin, containing the non-specific endonuclease activity with a slight preference for cleaving after thymine. Here we used the nuclease domain of ColE7 (N-ColE7) as a model system to study the interactions between a protein and DNA molecules. Based on computational methods, we constructed nuclease-ColE7 mutants, K490R, D493N, D493Q and K497R, which were predicted to bind DNA with better affinity. The fluorescence steady-state assays showed that D493N, D493Q and K497R indeed had increased DNA-binding affinity. The foot-printing assays further showed that D493N and D493Q digested DNA with a different sequence preference as compared to the wild-type enzyme. Moreover, the N-ColE7 mutant D493Q was co-crystallized with an 18-bp DNA and X-ray diffraction data were collected at NSRRC BL-13B1. The crystal structure of N-ColE7/DNA complex revealed that Q493 formed two hydrogen bonds with DNA. The extra hydrogen bond contributed to the mutant's higher DNA-binding affinity and altered sequence preference in cleavage. These results show that the combination of crystal structural analysis and computational methods is a useful device for generating a re-designed endonuclease with different properties in DNA binding and cleavage.

Keywords: proteins - DNA interaction, endonuclease, computer redesign

P04.06.228

Acta Cryst. (2008). A64, C302

Structural insights into TDP-43 in nucleic acid binding

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TDP-43 is a nucleic acid-binding protein, localized mainly in nucleus in eukaryotes. TDP-43 was firstly characterized as a transcriptional factor bound to the TAR DNA to repress the transcription of HIV-1 gene. Subsequently, TDP-43 was identified as a RNA-binding protein and plays a role as a splicing repressor in the splicing of CFTR (cystic fibrosis transmembrane conductance regulator) gene. Recently, it was further identified as a pathological protein of the pathogenic inclusions in the brains cells of FTL-D and ALS patients. To address the role of TDP-43 in DNA/RNA binding, we characterized its nucleic-acid binding activities by filter binding assays and determined the crystal structure of a truncated TDP-43 in complex with a 10-mer DNA at a resolution of 1.65 Å. We found that TDP-43 binds both single-stranded and double-stranded DNA/RNA, and it prefers to bind TG-rich DNA and UG-rich RNA substrates. The crystal structure of the truncated TDP-43 bound to a ssDNA revealed the structural basis for its preference in TG-rich sequences. Structural comparison of TDP-43 to other RNA-binding proteins containing homologous RRM motifs further showed that the truncated TDP-43 was dimerized in a different way.

Keywords: TDP-43, DNA/RNA binding protein, structure

P04.06.229

Acta Cryst. (2008). A64, C302

Crystal structure of CRN-4: Implications for domain function in apoptotic DNA degradation

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Cell death related nuclease 4 (CRN-4) is one of the apoptotic nucleases involved in DNA degradation in *Caenorhabditis elegans*. CRN-4 contains a N-terminal DEDDh exonuclease domain and a C-terminal domain with unknown function. To reveal the molecular basis of DNA fragmentation in apoptosis, we determined CRN-4's crystal structures in apo-form, Mn²⁺-bound active form and Er³⁺-bound inactive form. Comparison of the Mn²⁺-bound and Er³⁺-bound CRN-4 structures revealed the geometry of the functional nuclease active site in the N-terminal DEDDh domain. The C-terminal domain bound to a structural zinc ion, termed the Zn-domain, and folded into a novel mixed α/β structure, containing basic surface residues ideal for RNA/DNA recognition. Site-directed mutagenesis further confirmed the catalytic residues in the N-terminal DEDDh domain and the DNA-binding residues in the Zn-domain. The structural comparison of CRN-4 to a number of dimeric DEDDh family nucleases, further demonstrated that CRN-4 not only dimerizes but also interacts with DNA in a unique way. Combining all these data, we suggest a structural model where DNA is bound at the Zn-domain and cleaved at the DEDDh nuclease domain in CRN-4 when the cell is triggered for apoptosis.

Keywords: apoptosis, DNA interaction, DNA-binding proteins

P04.06.230

Acta Cryst. (2008). A64, C302-303

Crystallization and structure of human flap endonuclease 1, FEN1, in complex with a DNA product

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In lagging strand DNA synthesis and long-patch repair, primers of Okazaki fragments are displaced to form a single-stranded 5'-flap structure by DNA polymerase. Flap endonuclease-1 (FEN1) is a key enzyme to cleave these flaps specifically at the junction between single- and double-stranded regions, thereby producing the nick for ligation. This reaction is an essential step in replication and repair for maintaining genome stability. However, the mechanism by which FEN1 produces a precise nick is unknown. Here we report crystallization and structure determination of human FEN1 in complex with a 3'-flap nicked DNA product. DNA lengths and FEN1 constructs were systematically screened to find crystals suitable for X-ray works. Furthermore, we designed a DNA-protein complex crystallization screening (DPCS) kit for efficient screening. The kit consists of 338 PEG and MPD solutions collected from commercial crystallization screening kits without similar or overlapping conditions. Solutions containing acidic buffers (pH < 5) or divalent ions (zinc, copper and cadmium) were also excluded since FEN1 tends to denature or aggregate under these conditions. The kit was