

Abstract No. toth260

Crystal Structure of the Bacteriophage T7 Single-Stranded DNA Binding Protein Bound to ssDNA

E. A. Toth and T. Ellenberger (Harvard Medical School)

Beamline(s): X25, X12B

Introduction: Unprotected single-stranded DNA (ssDNA) exists transiently during cellular processes such as DNA replication, recombination, and repair and is therefore vulnerable to chemically reactive agents in the cell. In order to circumvent this vulnerability, cells have evolved a class of proteins, the ssDNA binding proteins, that not only protect the naked DNA from chemical reactants, but also regulate and/or assist the above processes.¹ The gene 2.5 protein (gp2.5) from bacteriophage T7 is a ssDNA binding protein that interacts with both the helicase/primase (gp4) and the polymerase (gp5) at the replication fork. Gene 2.5 assists in both leading and lagging strand synthesis by stimulating the primer synthesis activity of gp4 and the polymerase activity of gp5 through physical interactions with both proteins.² In addition, gp2.5 exhibits a strand annealing activity that is unique among the ssDNA binding proteins.^{3,4} This strand annealing activity contributes to genetic recombination during phage T7 growth.⁵ In order to gain some understanding of how gp2.5 functions in the context of a replisome, and to gain insight into its strand annealing activity, we have undertaken the crystal structure determination of gp2.5 in complex with ssDNA.

Methods and Materials: Native x-ray diffraction data for the complex of gp2.5 with poly(dT) were collected to 2.6Å at NSLS Beamline X25. Data for a mercury-substituted crystal were collected to 2.9Å at NSLS Beamline X12B. Structure solution via molecular replacement using the apo-gp2.5 structure⁶ as a search model unambiguously placed a single monomer in the asymmetric unit and established the space group as P4₁2₁2.

Results and Conclusions: The initial molecular replacement phases failed, even after considerable model rebuilding and refinement, to reveal the location of the bound ssDNA. However, subsequent SIRAS phasing using the mercury substituted protein revealed a stretch of unaccounted for density in the proposed DNA-binding cleft⁶ that was significantly enhanced after solvent flattening and histogram matching. Modeling of the bound ssDNA is in progress.

Acknowledgments: This work was supported by grants from the NIH. We thank the staffs of Beamlines X25 and X12B for their assistance with data collection.

References:

1. J.W. Chase and K.R. Williams, "Single-stranded DNA binding proteins required for DNA replication," Annu. Rev. Biochem., **55**,103-136, 1986.
2. H. Nakai and C.C. Richardson, "The effect of the T7 and Escherichia coli DNA-binding proteins at the replication fork of bacteriophage T7," J. Biol. Chem., **263**, 9818-9830, 1988.
3. Y.T. Kim et al., "Purification and characterization of the bacteriophage T7 gene 2.5 protein. A single-stranded DNA-binding protein," J. Biol. Chem., **267**, 15022-15031, 1992.
4. D. Kong and C.C. Richardson, "Single-stranded DNA binding protein and DNA helicase of bacteriophage T7 mediate homologous DNA strand exchange," EMBO J., **15**, 2010-2019, 1996.
5. D. Kong, J.D. Griffith, and C.C. Richardson, "Gene 4 helicase of bacteriophage T7 mediates strand transfer through pyrimidine dimers, mismatches, and nonhomologous regions," Proc. Nat. Acad. Sci. USA, **94**, 2987-2992, 1997.
6. T. Hollis et al., "Structure of the gene 2.5 protein, a single-stranded DNA binding protein encoded by bacteriophage T7," Proc. Nat. Acad. Sci. USA, **98**, 9557-9562, 2001.