Elzbieta Zabrocka
TrfA structural motifs required for DNA-TrfA complex assembly
Supervisor: Prof. dr hab. Igor Konieczny

Abstract

Many aspects of DNA replication process remains unknown although has been extensively investigated. Detailed understanding of molecular basics of DNA replication initiation in bacteria is crucial for development of new therapeutic strategies. The subject of this study was TrfA protein, the replication initiator of broad-host-range plasmid RK2. TrfA protein triggers DNA replication by binding repeated sequences (iterons) in the origin, then specifically interacts with ssDNA in the DUE. Structures of TrfA in complex with dsDNA or ssDNA has not been described so far.

The purpose of this study was the analysis of structural motifs required for DNA-TrfA complex assembly. First, replication activity of TrfA variants with different ability to dsDNA binding were tested. Results of SPR analysis and *in vivo* binding assay confirmed influence of point mutations within TrfA for dsDNA binding and protein variants replication activity. Next, it was examined whether separate TrfA domains can interact with dsDNA. Obtained results demonstrated that TrfA binds with dsDNA within WH1WH2 domains as well as in N-terminal region. By means of crosslinking, hydrolysis and mass spectrometry analysis TrfA residues interacting with dsDNA have been identified. In this study, it has been shown that TrfA binds with dsDNA using LMCGSDSTRVK (339-349 aa) motif located in WH2 domain and AMPNDTARSALFTTR (148-163 aa) located in the N-terminal region of the protein. In the second part of the project, the influence of point mutations in TrfA protein binding with ssDNA was tested. It was shown that main influence on TrfA ssDNA interaction is seen by mutation located within N-terminal region of the protein. TrfA binds with ssDNA using AMPNDTAR (148-156 aa) and also MFDYFSSHR (318-327 aa) located at WH2. Identified regions were verified by analysis of proteins with substitutions within regions described as interacting with dsDNA (TrfA R347E) or ssDNA (TrfA R327E). It has been shown that protein with point mutation within region binding dsDNA (TrfA R347R) did not interact with dsDNA, but binds ssDNA. Protein with mutation in region interacting with ssDNA (TrfA R327E) was not binding ssDNA, but interact with dsDNA. Protein with mutation in N-terminal region (TrfA P151S) was not interacting with dsDNA or with ssDNA. Based on results of genetic and biochemical experiments homology modeling followed by molecular dynamic was performed to determine

structure of N-terminal of TrfA as well as model of TrfA-dsDNA complex. Based of those experiments N-terminal of the TrfA protein was describes and Wined Helix domain.