RstB2 Protein, the DNA Binding Protein of CTXΦ Phage from V. cholerae: Current Status and Pending Research

Abstract

The phage-encoded protein RstB is required for CTXϕ phage integration, a lysogenic filamentous phage that contains the genes encoding cholera toxin, the main virulence factor of Vibrio cholerae. This mechanism of integration is catalyzed by the host-encoded tyrosine recombinases, XerC and XerD and some phage-encoded and host-encoded factors. This paper summarizes the results reported so far, of some bioinformatical and biochemical studies of the protein RstB. Moreover, it has also been informed about ongoing bioinformatical studies which may contribute to clearing up the role of this protein in phage integration CTXϕ.

Keywords

RstB2 protein; Vibrio cholerae; CTXϕ phage; DNA-binding protein; Single-stranded DNA; Double-stranded DNA

As above mentioned, the phage-encoded protein RstB is required for CTXϕ integration; hence it would make sense deepen about its role in the integration process, just to try to control this deadly disease, if possible. However, until we know, there are no abundant papers related with the biochemical study of this protein nowadays.

The first report about biochemical characterization demonstrated that RstB is a DNA binding protein [12]; because of its gene position and size homology to other genes encoding single-stranded DNA binding (SSB) proteins in other filamentous phages [13]. Although it preferentially binds ssDNA, it is surprising its capacity to interacting with dsDNA, following similar kinetic reaction in the protein-DNA complex formation [14]. In fact, RstB is the first protein of a filamentous phage to show affinity for both ds and ssDNA in vitro. Also purification methods for both, recombinant RstB-His and the tag-less RstB native proteins were described, using E. Coli as the host. These methods enable obtaining sufficient amounts of protein to carry out further biochemical studies focused at clearing up the role of the protein in the integration process of the phage [12]. Until we know, there are no other reported other expression vectors for this protein.

Recently, we reported that RstB occurs mostly as a monomeric species in solution and it is stable over time, because it retained its ssDNA-binding activity after a year of storage at -20 °C. Moreover, it was found that its C-terminal His-tail promoted oligomerization and decreased its isoelectric point from 8.52 to 6.47. On the other hand, bioinformatics analyses of the amino acid sequence of RstB showed affinity for both dsDNA and ssDNA in vitro. Also purification methods enable obtaining sufficient amounts of protein to carry out further biochemical studies focused at clearing up the role of the protein in the integration process of the phage [12]. Until we know, there are no other reported other expression vectors for this protein.
dimensional structures of the RstB and protein-DNA complex have not been reported yet, to our knowledge.

The three-dimensional structure of the proteins is the key to know how the protein carries out its biological function [15]. Its experimental determination is based on expensive and time-consuming methods, such as X-ray crystallography, Nuclear Magnetic Resonance spectroscopy and Circular Dichroism spectroscopy. However, several computational methods using sequence and/or structural information can be used to propose the three-dimensional structure of both, the RstB protein and the protein-DNA complex. Knowledge of both structures allows us to predict the amino acid residues involved in DNA binding site and binding specificity which is critical for understanding the mechanism of gene regulation and would help to understand the recognition mechanism of protein-DNA complexes [16]. In addition, conformational changes, functional classes and biophysical features of bound proteins and stability of the complex can be predicted. Such information may be used to address experimental studies such as site-directed mutagenesis for functional characterization of this protein.

Due to its binding to both, ssDNA and dsDNA with similar kinetic reaction, we have previously suggested that RstB may be a non-canonical filamentous phage SSB protein of an unusual filamentous phage, such as CTXΦ [14]. Therefore, it would be of interest testing the affinity of this protein by the DNA triplex, which could give more light to outstanding questions related to CTXΦ integration.

On the other hand, it was found that the shape of DNA is the determining factor in the cellular function. [17]. Recently it was reported that a small fraction of genomic DNA can adopt the non-canonical B-DNA structure or unusual DNA structure. The unusual DNA structures like DNA-hairpin, cruciform, Z-DNA, triplex and tetraplex are represented as hotspots of chromosomal breaks, homologous recombination and gross chromosomal rearrangements since they are prone to the structural alterations [18]. These repetitive DNA motifs are abundant in the genomes of various species, which are not random and often co-localize with sites of chromosomal breakage associated with genetic diseases [19].

Therefore, based on the knowledge described above we think that it would be very useful prove if CTXΦ phage have the capacity to adopt non-canonical (i.e. non-B) DNA structures like triplex DNA, which could be addressed by several experimental and bioinformatic methods reported before [20-24].

Thus, considering the above explained, we are carrying out some bioinformatics analyses to predict the three-dimensional structures of both, from the RstB protein and the protein-DNA complex that allows us getting all information possible to pave the way for further studies addressed at clearing up the role of RstB protein in the integration process.

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