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Hypothesis

Function inferences from a molecular structural model of bacterial ParE toxin

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Abstract
Toxin-antitoxin (TA) systems contribute to plasmid stability by a mechanism that relies on the differential stabilities of the toxin and antitoxin proteins and leads to the killing of daughter bacteria that did not receive a plasmid copy at the cell division. ParE is the toxic component of a TA system that constitutes along with RelE an important class of bacterial toxin called RelE/ParE superfamily. For ParE toxin, no crystallographic structure is available so far and rare *in vitro* studies demonstrated that the target of toxin activity is *E. coli* DNA gyrase. Here, a 3D Model for *E. coli* ParE toxin by molecular homology modeling was built using MODELLER, a program for comparative modeling. The Model was energy minimized by CHARMM and validated using PROCHECK and VERIFY3D programs. Resulting Ramachandran plot analysis it was found that the portion residues failing into the most favored and allowed regions was 96.8%. Structural similarity search employing DALI server showed as the best matches RelE and YoeB families. The Model also showed similarities with other microbial ribonucleases but in a small score. A possible homologous deep cleft active site was identified in the Model using CASTp program. Additional studies to investigate the nuclease activity in members of ParE family as well as to confirm the inhibitory replication activity are needed. The predicted Model allows initial inferences about the unexplored 3D structure of the ParE toxin and may be further used in rational design of molecules for structure-function studies.

Key words: ParE toxin; TA systems; RelE/ParE superfamily; Homology modeling.

Background:
In bacteria, one of the most studied programmed cell death (PCD) systems is the toxin-antitoxin (TA) system. TA systems are mechanisms that contribute to plasmid stability through death or growth inhibition of daughter bacteria that did not receive a plasmid copy at the cell division **[1].** Active TA systems have been described for a number of plasmids in a wide range of bacteria **[1],** and are composed of two genes organized in an operon encoding a toxin and the corresponding antitoxin. Additionally, several TA systems are also found on prokaryotic chromosomes, which may have alternative regulatory functions **[2].** ParD and ParE are proteins of a TA system founded on plasmid RK2 with ParE being the toxin and ParD the antitoxin. ParD, is effective in the autoregulation of the *parDE* operon **[3]** and is able to neutralize ParE action by forming a tight inactive complex. ParE and another currently known toxins can be subdivided into five superfamilies called MazF, RelE, Doc, HipA and ζ, each consisting of distinct gene families **[4].** A sixth toxin superfamily (VapC), not yet studied, consists of an N-terminal domain (PilT-N), also found in proteins of the eukaryotic nonsense mediated RNA decay system **[5].** The RelE superfamily includes the families RelE, Yoeb, YafQ, HigB, ParE, Txe and YhaV **[4].** In recent work, three new RelE-homologous, yafNO, higBA and ygiUT, were described in *E. coli* **[6].** The toxins of the RelE superfamily are characterized by a five-stranded β-sheet with four of the strands antiparallel to each other, while the first and last strands are parallel. The β-sheet is flanked on one side by two N-terminal α-helices (α1 and α 2) and on the other side by C-terminal α-helix (α3) that, together with loop connecting β2 and β3, forms a deep cleft that encompasses the RNase active site **[4].** Similar architecture has been found in genuine RNases **[4].** In contrast with the others RelE families, which act on RNA at the translation level, ParE inhibits DNA replication by inactivation of *E.* coli DNA gyrase, as demonstrated by the unique study available of the ParE activity **[7].** Despite of a common origin and significant sequence similarity, these toxins must have had an evolutionary divergence to acquire very distinct functional roles **[5].** In the future, it would be interesting to investigate the possibility of an unexplored nuclease activity in members of the ParE family, similarly to the explored RelE cleaving ribosome-associative activity **[5].** As the TA systems occur in several pathogenic bacteria, such investigations, as well as elucidation of the molecular structure of these toxins, might be useful as a basis for the design of new and alternative drugs **[8].** Several crystallographic structures for members of different bacterial toxins families are available and these are a promise for the rational design of new class of antibiotics. To ParE toxin, no crystallographic structure is available so far. In this study we built a three-dimensional structure model for ParE toxin by molecular homology modeling and the identification of a possible active site was explored in the final Model.

Methodology:

Sequence alignment
Sequence alignment was performed using CLUSTALW program. (http://www.ebi.ac.uk/Tools/clustalw2/index.html). All protein sequences were obtained into NCBI sequence database (http://www.ncbi.nlm.nih.gov).

Molecular homology modeling
The protein sequence of *E. coli* ParE toxin was searched by using RK2 sequence **[9]** (gi 420755) as query in PSI-BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The obtained sequence (gi 194437967) was used to find template proteins through PHYRE sever **[10]** (http://www.sbg.bio.ic.ac.uk/phyre), which is an online tool for searching similar sequences, based on sequence and structure-wise similarity. From the homology searching, templates from RelE and YoeB families were selected. By having greater identity, the protein sequence of *E. coli* RelE toxin was used as template for homology modeling. A 3-D model was building adopting an approach to comparative modeling by satisfaction of spatial restraints using MODELLER9v7 **[11]** (http://salilab.org/modeller). CHARMM **[12]** energy minimization was applied using Swiss-PdbViewer program (http://spdbv.vital-it.ch).

Analysis of the Model
The stereochemical and quality of the final models were assessed by the PROCHECK (http://www.biochem.ucl.ac.uk/~roman/procheck/procheck.html) and VERIFY-3D **[13]** (http://nihserver.mbi.ucla.edu/Verify_3D) programs. Structural similarity search was performed using DALI server **[14]** (http://ekhidna.biocenter.helsinki.fi/dali_server). Structural models were visualized by PyMol (http://pymol.sourceforge.net).

Active sites analysis:
After the final Model was built, the possible active sites of ParE were explored applying CASTp program **[15]** (http://sts.bioengr.uic.edu/castp).

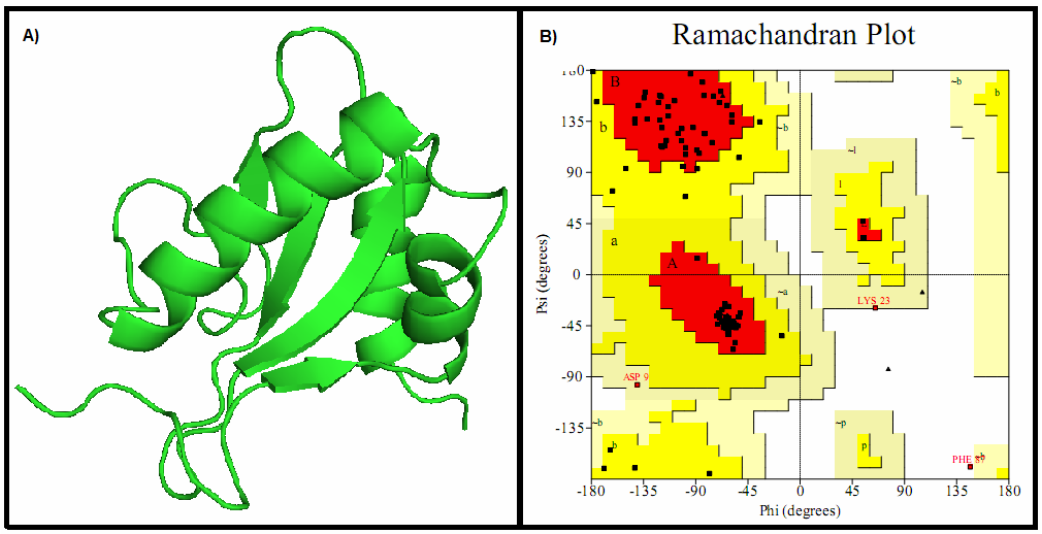

Figure 1: (A) Predicted 3-D structure of *E. coli* ParE toxin. The 3-D model was built from the primary structure of the *E. coli* ParE toxin (105 amino acids) by adopting an approach to comparative modeling by satisfaction of spatial restraints using MODELLER9v7 program and atomic coordinates of the *E. coli* RelE toxin as template. **(B): Validation of the Model using Ramachandram plot**. Ramachandram plot analysis was used to validate the predicted Model. The plot statistics are: 77 (81.9%) residues in most favored regions; 14 (14.9%) residues in additional allowed regions; 2 (2.1%) residues in generously allowed regions; 1 (1.1%) residue in disallowed regions.

Results and Discussion:

Template identification, homology modeling and model quality

The primary structure of the *E. coli* ParE toxin was obtained by subjecting the sequence of the plasmid RK2 that encodes ParE protein (gi 420755), to the PSI-BLAST program. One sequence (gi 194437967) with 105 amino acids was selected. To find homologous sequences with resolved three-dimensional structure, the *E. coli* ParE protein sequence selected was submitted to PHYRE server. This server allows searching similar sequences based on sequence and structure-wise similarity **[10].** As expected, no 3D structure of representatives of the ParE family was matched since there is no three-dimensional structure available to this family. The search identified RelE and YoeB families as the best matches for templates structures. These families belong to the same superfamily that ParE family is included. Three templates were selected, two of the RelE family (PDB id 1WMI and PDB id 3KHA) and one of the Yoeb family (PDB id 2A6S). Each of the sequences templates selected was aligned with the *E. coli* ParE protein sequence. By having major identity, the *E. coli* RelE toxin (PDB id 3KHA) was selected as template. Structural models were built adopting an approach to comparative modeling by satisfaction of spatial restraints **[11]** using MODELLER9v7 program. The quality of the models was assessed by the VERIFY-3D and PROCHECK programs. Initial models did not showed good quality. Thus, the alignment was manually modified in some points to provide better matching in regions of secondary structure of the disrupted template by insertions into target sequence. The modified alignment was used to build a new model which was applied CHARMM energy minimization using Swiss-PdbViewer program. The final model is shown in the **Figure 1A.** Analysis using Verify3D program showed 100% of the positive score values and 90% higher than 0.2. Ramachandram plot calculations showed 96.8% of the residues in favored and allowed regions **(Figure 1B).** The analysis indicates that the model has a good quality.

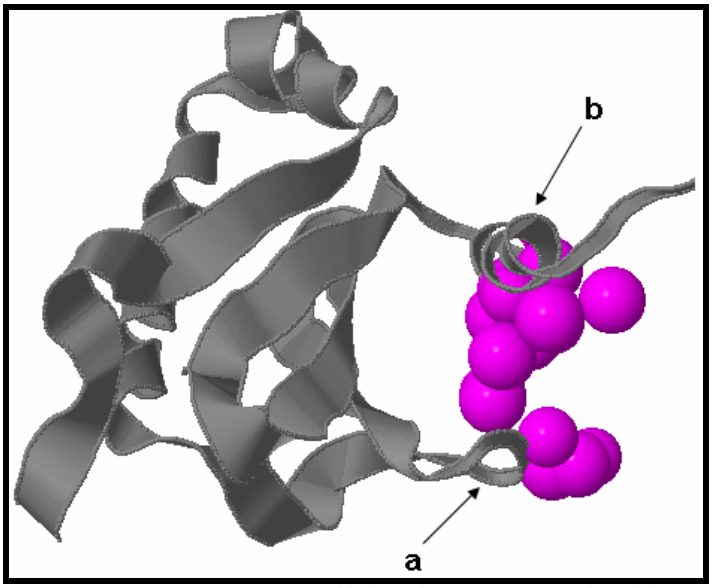

Figure 2: Identified active site of the *E. coli* ParE toxin. A possible deep cleft active site was identified using CASTp program (**a**) Loop connecting α2 and β2; (**b**) C-terminal α-helix (α3).

Structure and active sites analysis

Toxins of the RelE superfamily are characterized by a core of the five-stranded β-sheet, with four of the strands antiparallel each other, while the first and last strands are positioned parallel. The core of β-sheets is flanked on one side by two N-terminal α-helixes and on the other side by one C-terminal α-helix which together with the loop connecting β2 and β3, forms a deep cleft that encompasses the RNase active site **[4].** As the *E. coli* RelE toxin **[16]** was employed as template in molecular modeling, the proposed Model for *E.coli* ParE protein showed a similar molecular structure that which characterizes the RelE superfamily: a core of the four-stranded β-sheets flanked by three α-helixes (two N-terminal and one C-terminal). The atomic coordinates of the Model was submitted to Dali server which is a network service for comparing of a queried protein structure with structures deposited in the Protein Data Bank (PDB) **[14].** In this structural similarity search, RelE toxin from *E. coli* (Z score = 17.9), mutant (R81A/R83A) RelE toxin **[16]** from *E. coli* (Z score = 15.3), RelE toxin from *Mycobacterium tuberculosis* (Z score = 9.8), YoeB toxin [**17]** from *E. coli* (Z score = 9.6) and RelE toxin **[18]** from *Pyrococcus horikoshii* (Z score = 9.2) were identified as the best matches. As reported to other members of the RelE superfamily **[17, 18],** the proposed Model also showed structural similarities, but with small Z score, for other microbial ribonucleases such as colicin-E5 **[19]** from *E. coli* (Z score = 3.7), RNase SA3 **[20]** from *Streptomyces aureofaciens* (Z score = 3.6) and RNase SA **[21]** from *Streptomyces aureofaciens* (Z score = 3.4). The Model predicted for *E. coli* ParE toxin was also submitted to CASTp program to identify accessible surface pockets and/or cavities. The analysis identified several cavities including one formed exactly in region delimited by C-terminal α-helix (α3) and the loop connecting α2 and β2 which is indicate as a possible deep cleft that encompasses the RNase active site in the template protein **(Figure 2).**

Conclusions:

A satisfactory Model for ParE toxin from *E. coli* was obtained by adopting of an approach to comparative modeling with satisfaction of spatial restraints **[11].** This model shows a similar architecture for the general structure of the RelE superfamily. Structural similarity search, using atomic coordinates of the obtained Model as query, showed best matches with RelE and YoeB families, as well as with others microbial ribonucleases, but with small score. A surface topography analysis identified a cavity formed by the region delimited by the C-terminal α-helix (α3) and the loop connecting α2 and β2 sheets, which is indicate as a possible RNase active site for RelE superfamily. However, no reports on nuclease activity for ParE toxin is available at the moment and only one study describes the bacterial DNA gyrase as the intracellular target of this toxin **[7].** Would be interesting additional studies to investigate the possibility of a DNA or RNA-cleaving activity by members of the ParE family. Moreover, new studies are necessary to confirm the ParE inhibitory replication activity as well as to establish a mechanism for its action. The Model presented here allows initial inferences about the structure of the ParE toxin and will allow the rational design of peptide derivatives for structure-function studies, as reported to Ccdb toxin **[22].**

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