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Homology Modeling and physiochemical analysis of Toxic Protein Verotoxin and its Model Validation

Anil K. Singh*

Environmental Toxicology Group, CSIR-Indian Institute of Toxicology Research Lucknow, U.P., 226010, India

*Corresponding Author E-mail: phd.anil@yahoo.com

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ABSTRACT

Verotoxin is a toxin produced by some strain of Escherichia coli bacteria. Verotoxin also known as Shiga-like toxin. Two types are of Verotoxin are known as SLT1 and SLT2. The Verotoxin or Shiga-like toxin associated with hemolytic-uremic syndrome in human. This toxin is a multi-subunit protein made up one molecule of A subunit responsible for the toxic action, while other five molecules of the B unit responsible for binding to a specific cell's receptor. Present study is being performed towards understand and structure prediction of this toxic protein. Verotoxin structures of both subunits are being predicted through homology modeling. Three dimensional structures are being predicted and validate through various online modeling tools and server.

Keyword: *Protein modeling, Structure prediction, Toxic protein, in silico, validation.*

INTRODUCTION

Shiga toxin or Verotoxin is a highly toxic protein, produced by some strain of *E.coli*[1-3]. The *E. coli* version of the toxin was named "verotoxin" because of its ability to kill *Vero* cells (monkey kidney cells) in culture[46]. Shiga-toxin-producing *Escherichia coli* (STEC), O157: H7 has the strongest association worldwide with HUS[7]. This toxin has a multi subunit protein made up one molecule of A subunit which has Mw about 32000k DA and responsible for the toxic action as it's a toxic protein[8-10]. While other B subunit of this protein contains 5 molecules Mw about 7700 kDA. A subunit responsible for toxic action, B subunit responsible for binding to cell membrane or receptors[11-12]. When verotoxin enters into cell, A subunit of verotoxin interacts with the ribosomes to inactive them[13]. The A subunit of Shiga toxin is an N-glycoside, which bring modification in RNA component of the ribosome to inactivate it and bring a halt to protein synthesis leading to the death of the cell[14-15]. More detail about structure and its interactivity can be predicted with structure prediction. In this present study verotoxin is being performed through homology modeling and structure also being validated[16-18]. Homology structure of verotoxin provides the information about its structure, twisting and other protein related properties.

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Homology modeling of this toxin provides some visual information about its structure and physiochemical properties also can be predicted with the help of appropriate tools[19-20]. After modeling, structure is being validated and discussed in present study.

MATERIAL AND METHOD

Sequence Retrieval

The amino acid sequence for verotoxin was obtained from NCBI server with accession number **ADF78102.1** for subunit A[21]. And sequence for subunit B was obtained with same server with accession number **ADB77951.1**[22].

>Sequence of A subunit of Verotoxin

MKCILLKWILCLLLGFSSVSYSQEFTIDFSTQQSYVSSLNSIRTAISTPLEHISQGATSVSVINHTPPGSYISVGI
RGLDVYQERFDHLRLIIERNNLYVAGFVNTTTNTFYRFSDFAHISLPGVTTISMTTDSSYTTLQRVAALERSG
MQISRHSLVSSYLALMEFSGNTMTRDASRAVLRFVTVTAEALRFRQIQREFRLALSETAPVYTMTPEDVDL
TLNWGRISNVLPEYRGEAGVRVGRISFNNISAILGTVAVILNCHHQGARSVRAVNEESQPECQITGDRPVIKI
NNTLWESNTAAAFLNRKSQSLYTTGE

>Sequence of B subunit of Verotoxin

MKKMFIAVLFALVSVNAMAADCAKGKIEFSKYNEDNTFTVKVSGREYWTNRWNLQPLLQSAQLTGMTV
TIISNTCSSGSGFAQVKFN

Homology modeling prediction:

Modeling for both A and B chain was performed using SWISS-MODEL Workspace with automated mode[23]. After entering of amino acid sequence model for target protein was built for each subunit. Automatic mode provides the modeled PDB file, with other model's informative details.

Physiochemical analysis

The physiochemical properties of Verotoxin were analyzed using ProtParam tools.ProtParam is online tool and easily accessible through ExPasy web URL address[24-25.]

3D structure visualization

Three dimensional structure of each subunit of Verotoxin was predicted using SWISS-MODEL Workspace, and modeled PDB file of each protein subjected to visualization. Structure analysis and visualization was performed using PyMOL software[26-27]. This software provides the structural information of modeled proteins in different form.

Validation of model:

After model built the validity of the predicted 3D model, the PROCHECK server was used [28]. This tool calculates the phi (Φ) and psi (Ψ) angles thus generate a Ramachandran plot for the model[29-30].

RESULT AND DISCUSSION

The physiochemical properties of both subunit of verotoxin were analyzed using ProtParam tools and after analyzed, results were obtained and shown in Table 1. The 3d structure of verotoxin is modeled by SWISS-Workspace in automated mode. Homology model was validated by PROCHECK server. PROCHECK summery listed in Figure 1 and 2. Model validation result shown in Table 2, while plot shown in Figure 3 and 4. The 3d structure of verotoxin was predicted by PyMol in color form. 3d structure of subunit A reveals in Figure 5, while 3d structure of subunit B of verotoxin revealed in Figure 6.

Table 1: Reveals the different physiochemical properties of Verotoxin, Predicted by Prot Param tools.

Physiochemical analysis	Subunit AVerotoxin	Subunit B of Verotoxin
No of amino acids	319	87
Molecular weight	35570	9650.1
Theoretical pI	8.35	9.34
Instability index	38.96	33.42
Aliphatic index	93.20	77.36
GRAVY	-0.042	0.028

```
+----------<<<  P  R  O  C  H  E  C  K     S  U  M  M  A  R  Y  >>>----------+
  |                                                                           |
  | input_atom_only.pdb   2.5                                  296 residues |
  |                                                                           |
*| Ramachandran plot:   80.4% core   14.1% allow    3.3% gener    2.2% disall |
  |                                                                           |
*| All Ramachandrans:   23 labelled residues (out of 294)                    |
+| Chi1-chi2 plots:      1 labelled residues (out of 165)                    |
  | Main-chain params:    6 better     0 inside      0 worse                  |
  | Side-chain params:    5 better     0 inside      0 worse                  |
  |                                                                           |
+| Residue properties: Max.deviation:     5.0             Bad contacts:    1 |
+|                     Bond len/angle:    4.2    Morris et al class:  1  1  2 |
+|     1 cis-peptides                                                        |
  | G-factors           Dihedrals:  -0.28  Covalent:   0.37    Overall:  -0.02 |
  |                                                                           |
  | M/c bond lengths:100.0% within limits   0.0% highlighted                  |
  | M/c bond angles:  97.6% within limits   2.4% highlighted                  |
*| Planar groups:    82.0% within limits  18.0% highlighted       3 off graph |
  |                                                                           |
 +---------------------------------------------------------------------------+
   + May be worth investigating further.  * Worth investigating further.
```

Fig. 1: Summery of model validation of A subunit of Verotoxin.

```
+----------<<<  P  R  O  C  H  E  C  K     S  U  M  M  A  R  Y  >>>----------+
  |                                                                           |
  | input_atom_only.pdb   2.5                                   68 residues |
  |                                                                           |
*| Ramachandran plot:   88.3% core   10.0% allow    0.0% gener    1.7% disall |
  |                                                                           |
+| All Ramachandrans:    1 labelled residues (out of  66)                    |
  | Chi1-chi2 plots:      0 labelled residues (out of  40)                    |
  | Main-chain params:    6 better     0 inside      0 worse                  |
  | Side-chain params:    5 better     0 inside      0 worse                  |
  |                                                                           |
+| Residue properties: Max.deviation:     4.0             Bad contacts:    0 |
+|                     Bond len/angle:    4.0    Morris et al class:  1  1  2 |
  |                                                                           |
  | G-factors           Dihedrals:  -0.10  Covalent:   0.41    Overall:   0.10 |
  |                                                                           |
  | M/c bond lengths:100.0% within limits   0.0% highlighted                  |
  | M/c bond angles:  97.8% within limits   2.2% highlighted                  |
+| Planar groups:    92.0% within limits   8.0% highlighted                   |
  |                                                                           |
 +---------------------------------------------------------------------------+
   + May be worth investigating further.  * Worth investigating further.
```

Fig. 2: Summery of model validation of B subunit of Verotoxin.

Table 2: Contains the model validation details, validated by PROCHECK server.

Protein/ subunit	Subunit A of verotoxin	Subunit B of verotoxin
Number of residues in favoured region	80.4%	88.3%
Number of residues in allowed region	14.1%	10.0%
Number of residues in outlier region	3.3%	0.0%
Residues in disallowed regions	2.2%	1.7%
Total	100%	100%

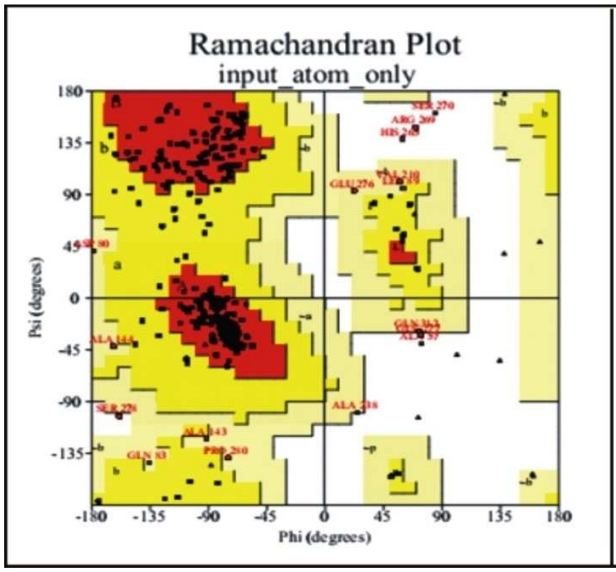

Fig. 3: Ramachandran plot of A subunit.

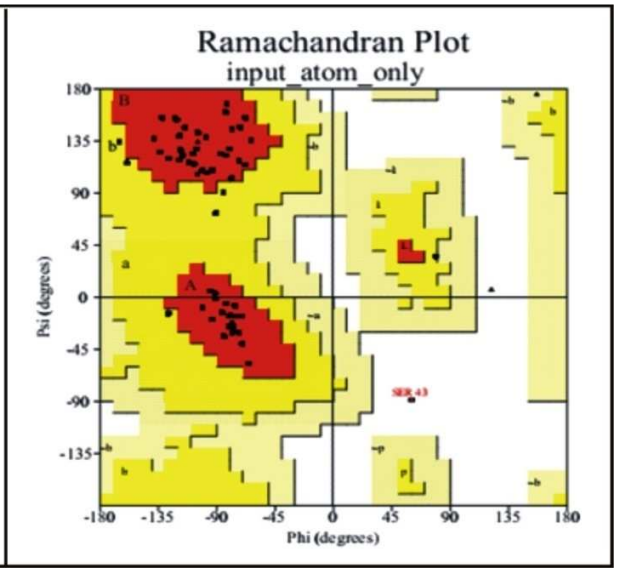

Fig. 4 Ramachandran plot of B subunit.

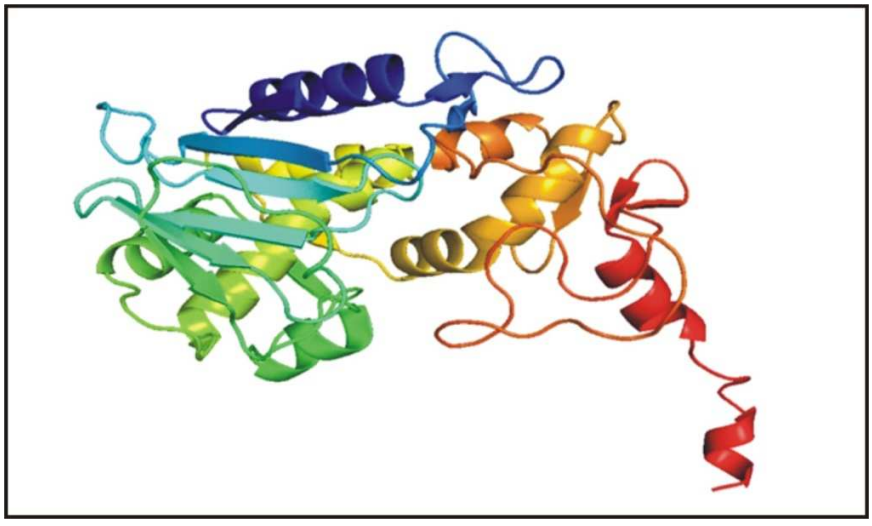

Fig. 5: 3D structure of Verotoxin (A subunit).

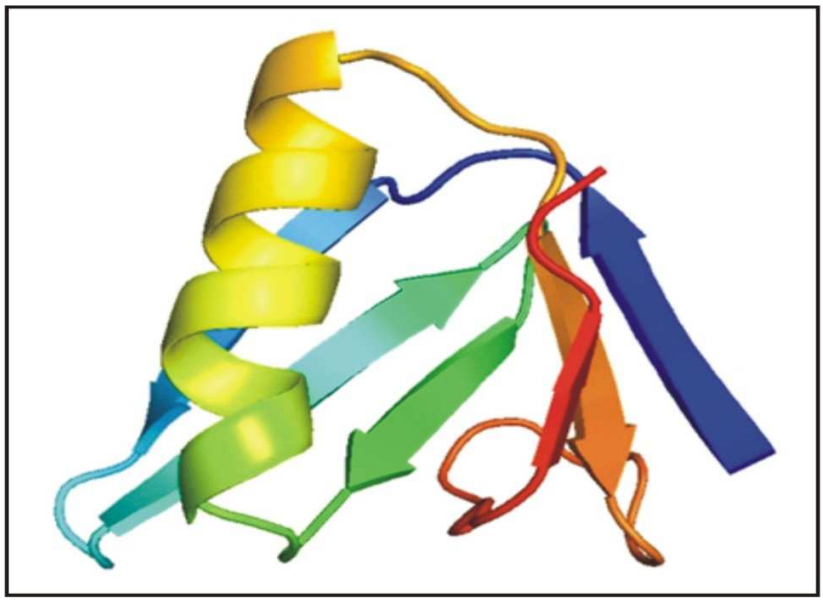

Fig. 6: 3D structure of Verotoxin (B subunit).

CONCLUSION

Present study was performed as Homology modeling, analysis and validation of the verotoxin, that's reveals the various physiochemical properties of verotoxin as toxic protein. As we find, verotoxin has two subunit while modeling of each subunit performed and reveals the 3d structure which provides the information about its structure virtually in different form. Structure predicted and images of this toxic protein predicted in color cartoon form. Structure of Verotoxin can be further analyzed for protein-protein docking or other protein-protein interaction activity.

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