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Full Length Research Paper

Structural and functional analysis of seeligeriolysin O by homology modeling

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Seeligeriolysin O (LSO) is a cholesterol-dependant cytolysin of *Listeria seeligeri*. These toxins are produced by various species of Gram-positive bacteria, including members of the genera *Streptococcus*, *Clostridium*, and *Listeria.* Apart from the cytolytic, LSO has been reported to perform cytokine-inducing activity as well. The present study deals with the prediction of three dimensional model, as well as structural and functional analysis of Seeligeriolysin O. MODELLER9 v8 was used for building the homology model. These predicted 3-dimensional models were evaluated with ProSa and PROCHECK software, and the best 3-dimensional models were selected. Multiple alignment was performed with CLUSTALX. Based on the similarity of predicted three dimensional structure of seeligeriolysin O with perfringolysin O, the seeligeriolysin might have similar structure and function with the later. The predicted three dimensional model of seeligeriolysin O had extended rod shaped structure, having ample beta sheets arranged in four domains. The C-terminal region of seeligeriolysin O might have function similar to perfringolysin O. It has been predicted that seeligeriolysin O insertion occurs more readily in an environment having loosely packed lipid.

Key words: Bacterial toxins, tryptophan, Perfringolysin, *Listeria seeligeri,* cholesterol-dependant cytolysins and domain 4, target protein, template protein.

INTRODUCTION

Seeligeriolysin O (LSO) is a cholesterol-dependant cytolysin of *Listeria seeligeri*. The cholesterol-dependant cytolysins (CDCs) are a family of structurally related cytolytic toxins. These toxins are produced by various species of Gram-positive bacteria, including members of the genera *Streptococcus*, *Clostridium*, and *Listeria* (Billington et al., 2000). The mechanism of action of CDCs is multi-step complicated process in which the toxin monomers bind to the cholesterol-containing membranes, and then form a stable association with it (Oberley and Duncan, 1971; Ohno-Iwashita et al., 1988). Then, sidewise diffusion takes place on the bi-layer, and along with this diffusion, cytolysin forms oligomers (Harris et al., 1991; Hotze et al., 2001). After the diffusion and cytolysin oligo-merization, step-wise conformational changes forms pores in the membrane (Heuck et al., 2000; Hotze et al., 2002). The CDCs were first called hemolysins because of having the beta-hemolytic proteins. The CDCs has the potential to lyse different types of nucleated cells *in vitro*. This ability of lysis has been used by many researchers to permealize variegated eukaryotic cell types with CDCs (Bhakdi et al., 1993). The CDCs monomers are highly water soluble but when these monomers bind to the cholesterol-containing membranes, they then self-associate to form aqueous pore in the bi-layer. These oligomers, which are formed by CDCs monomers, vary in size and may be composed of up to

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fifty individual monomers (Bhakdi et al., 1985; Morgan et al., 1995).

Membrane cholesterol is necessary for the activity of CDCs, and for more than two decades, cholesterol was considered as the receptor for the CDCs (Alouf, 2000). This has been supported by evidences, including one that CDCs are not active on membranes having no cholesterol. Secondly, reduction of membrane cholesterol reduces the extent of membrane binding to different eukaryotic cell types (Waheed et al., 2001). The function of cholesterol as a CDCs receptor has been questioned up to some extent in the yester decade by studies suggesting that the function of cholesterol comes after the membrane recognition step of CDC mechanism (Billington et al., 2002; Jacobs et al., 1998). Apart from the cytolytic, LSO has been reported to perform cytokine-inducing activity as well (Ito et al., 2003). These proteins have a highly conserved undecappetide sequence (ECTGLAWEWWR), which is mainly responsible for the cytolytic activity (Michel et al., 1990; Ruiz et al., 1998; Sekino-Suzuki et al., 1996; Shimada et al., 1999). LSO is a unique bacterial protein that stimulates macrophage cytokine production (Ito et al., 2005). The present study deals with the prediction of 3D model of LSO, based on comparative homology modeling. This study further deals with the structure and functional features of the protein.

MATERIALS AND METHODS

Primary sequence analysis

The sequence of LSO was retrieved from SwissProt Data resource (Boeckmann et al., 2003). Multiple sequence alignment of LSO sequence, with other similar sequences, was done with CLUSTAL X (Larkin et al., 2007).

Homology model building

Comparative modeling techniques were employed to gain an insight into the structural characteristics of the LSO. The methodology used to derive the model has been divided into four steps: template selection, sequence alignment, model generation and evaluation.

Template identification

After performing similarity searches against PDB database using PSI-BLASTp of NCBI-BLAST (Altschul et al., 1997, 2005) package with default parameters, the crystal structure coordinates of perfringolysin O (pdb ID: 1PFO) (Rossjohn et al., 1997) of *Clostridium perfringenes* was selected as the template because of its highest homology with the target sequence which shows 45% identity with the target sequence. The 3D structure coordinates of the template was obtained from Brookhaven protein Databank (PDB) (Berman et al., 2000).

Target-template alignment

The sequences were aligned using the align2D command of MODDELLER.

Model generation and evaluation (NCBI)

MODELLER 9v8 (Eswar et al., 2008) was used to build the 3D homology models of LSO. Reliabilities of the predicted homology models were assessed by PROCHECK (Laskowski et al., 1993) and ProSA (Sippl, 1993). The best model was selected on the basis of PROCHECK and ProSA results. In order to examine any alteration in the Cα backbone of the model, it was superposed onto the template, with the help of SUPPERPOSE command of MODELLER. Superposition allowed us to calculate the root mean square deviation (RMASD) values for positional differences between equivalent atoms of the model and template. All protein structures and models were visualized and analyzed with the help of DS Visualizer® (v. 2, Accelrys Software Inc).

RESULTS AND DISCUSSION

Homology model building

Target and template sequences were aligned with the align2D command of MODDELLER. Starting from the alignment (Figure 1), ten homology models were created for target sequence. All the homology models were evaluated, and the best model was selected on the basis of results of PROCHECK, ProSA, and by superposition as described below. According to Procheck results, all the models had zero percent residues in disallowed region. All models had higher values for core region, as compared to the template. The models were selected on the basis of core + allowed region. The models that have the highest percentage of residues in this region would be the best models. According to these results, models having the least value for the generously allowed region would have the highest value for core + allowed region. Model number 1, 6, 7 and 9 were selected according to these results because, these models have the highest value for core + allowed region, which is equal to the template. All the remaining models had less value for the core + allowed region, as compared to the template. Although the core region of model number 9 and 7 are higher, as compared to the other two selected models, but the number of bad contacts are greater in these models. All of these four models have equal values for core + allowed regions, therefore they can be considered as equal according to this parameter. Now, the selection will be made on the bases of another parameter that is, number of bad contacts, which shows that model number 6 could be the best model because it has the least number of bad contacts as compared to the other three models. As far as the number of bad contacts are concerned, model number 2 has only 8 bad contacts but we did not select it because its core + allowed region was lesser than the selected four models. Similarly, its maximum deviation value was also higher than the selected four models. All the selected models were evaluated further with ProSA.

From the energy diagram (Figure 2), it is clear that model 6 has the lowest energy value, as compared to the rest of the selected models. Model 6 was selected as the

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1PFO ----------------DIT----------DKNQ---S----------------------IDSGISSLSY
LSO  MKIFGLVIMSLLFVSLPITQQPEARDVPAYDRSEVTISPAETPESPPATPKTPVEKKHAEEINKYIWGLNY

1PFO NRNEVLASNGDKIESFVPKEGKKAGNKFIVVERQKRSLTTSPVDISIIDSVNDRTYPGALQLADKALVENR
LSO  DKNSILVYQGEAVTNVPPKKGYKDGSEYIVVEKKKKGINQNNADISVINAISSLTYPGALVKANRELVENQ

1PFO PTILMVKRKPININIDLPGL-KGENSIKVDDPTYGKVSGAIDELVSKWNEKYSST-HTLPARTQYSESMVY
LSO  PNVLPVKRDSLTLSVDLPGMTKKDNKIFVKNPTKSNVNNAVNTLVERWNDKYSKAYPNINAKIDYSDEMAY

1PFO SKSQISSALNVNAKVLENSLGVDFNAVANNEKKVMILAYKQIFYTVSADLPKNPSDLFDDSVTFNDLKQKG
LSO  SESQLIAKFGTAFKAVNNSLNVNFEAISDGKVQEEVISFKQIYYNINVNEPTSPSKFFGGSVTKEQLDALG

1PFO VSNEAPPLMVSNVAYGRTIYVKLETTSSSKDVQAAFKALIKNTDIKNSQQYKDIYENSSFTAVVLGGDAQE
LSO  VNAENPPAYISSVAYGRQVYVKLSSSSHSNKVKTAFEAAMSGKSVKGDVELTNIIKNSSFKAVIYGGSAKE

1PFO HNKVVTKDFDEIRKVIKDNATFSTKNPAYPISYTSVFLKDNSVAAVHNKTDYIETTSTEYSKGKINLDHSG
LSO  EVEIIDGNLGELRDILKKGSTYDRENPGVPISYTTNFLKDNDLAVVKNNSEYIETTSKSYTDGKINIDHSG

1PFO AYVAQFEVAWDEVSYDKEGNEVLTHKTWDGNYQDKTAHYSTVIPLEANARNIRIKARECTGLAWEWWRDVI
LSO  GYVAQFNISWDEVSYDENGNEIKVHKKWGENYKSKLAHFTSSIYLPGNARNINIYARECTGLFWEWWRTVI

1PFO SEYDVPLTNNINVSIWGTTLYPG-SSITYN---
LSO  DDRNLPLVKNRNVSIWGTTLYPRHSNNVDNPIQ
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Figure 1. Pairwise alignment of the target seeligeriolysin O (LSO) and template perfringolysin O (1PFO) sequences obtained with align2D command of MODELLER9 v8.

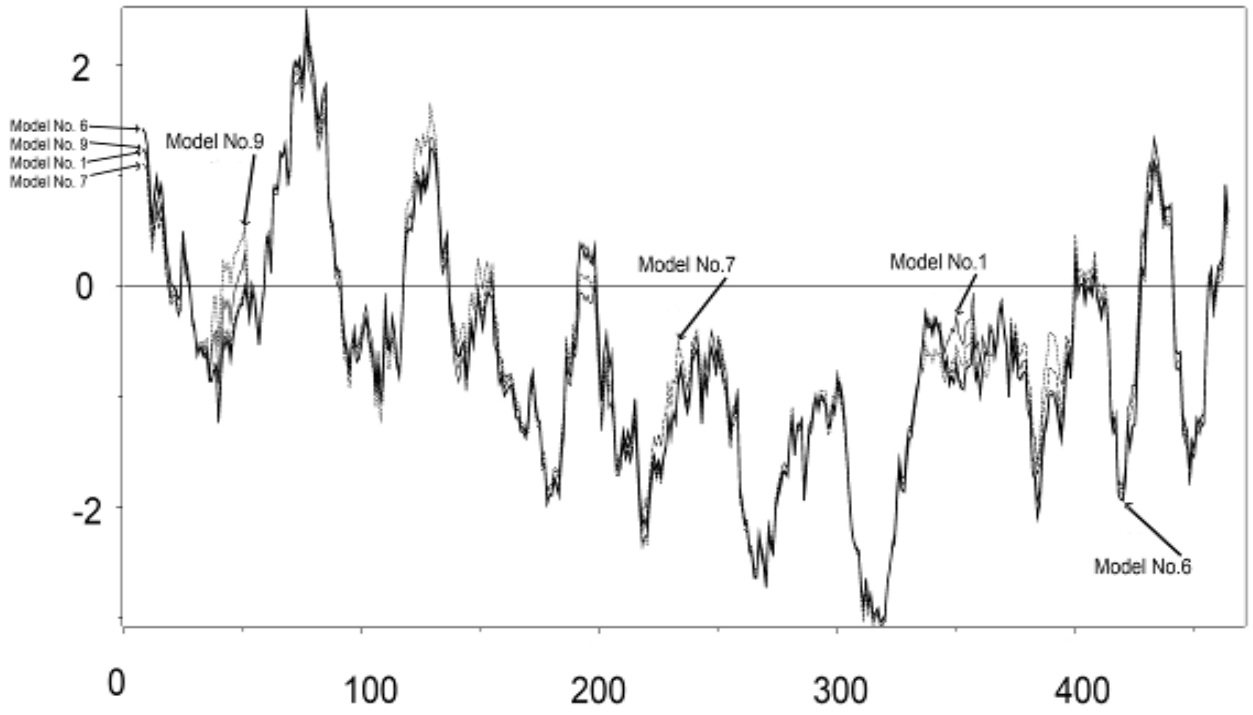

Figure 2. Comparison of ProSa energy plots (winsize: 15) of four selected models i.e. models No. 1, 6, 7 and 9. The energy plots of models have been represented with different lines as explained. Model number 1: Solid thinner gray line, model number 6: Solid thicker dark line, model number 7: Gray dashed line, model number 9: gray dotted line.

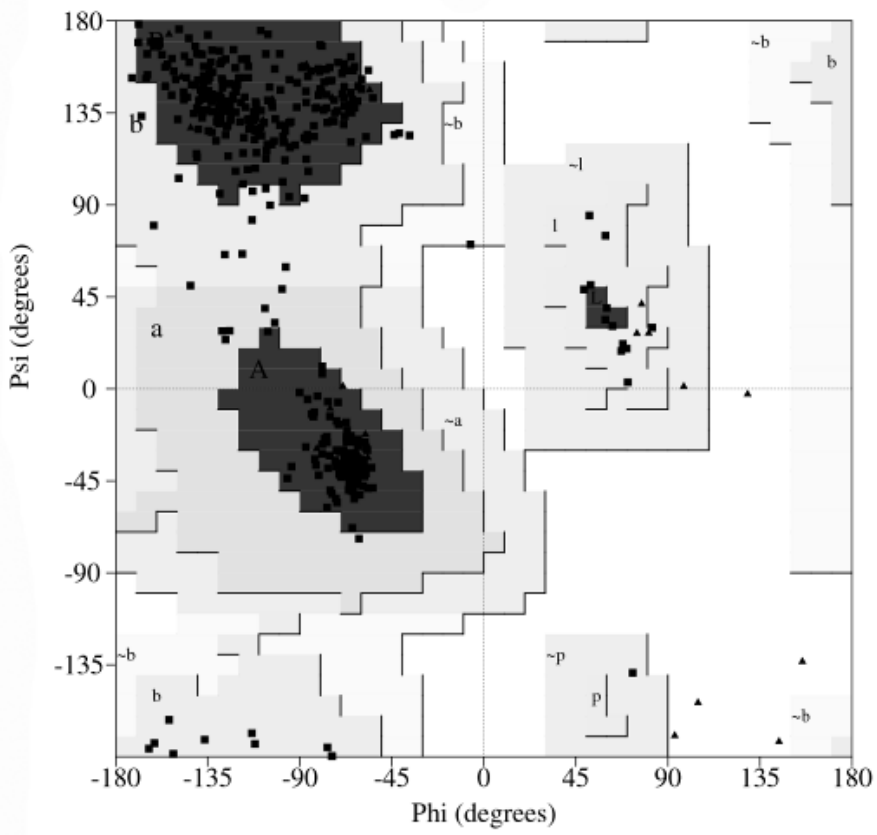

Figure 3. Ramachandran plot of the predicted model (model number 6) of seeligeriolysin O (LSO).

best model as it has the lowest overall energy value. Ramachandran plot (Figure 3) showed that model number 6 had 89.4% residues in the most favoured region, 10.4% residues in additional allowed region, 0.2% residues in generously allowed region, and 0.0% residues in disallowed region. Models having more than 80% residues in the most favoured region are considered as quite satisfactory (Andreini et al., 2004). For comparison, an analogous analysis was performed also on the experimental templates (Andreini et al., 2004). Ramachandran plot for the template 1PFO (Figure 4) showed that there, values are either identical or very close to that of model's values. The comparison of the model's values with the templates' values shows that the model has been made accurately and can be considered as a good quality model. Another important thing is that this does not have any residues in the disallowed region. For further evaluation the model was superposed over all the three templates separately. The model superimposed (Figure 5) well over the template with RMSD of 0.18, which showed that the model is fairly accurate (Arnold et al., 2006). As our sequence showed only 45% identity with the template, errors were expected (Chothia and Lesk, 1986) in the model; however, the RMSD value showed that it is fairly accurate and is identical to a greater extent to the template. No observable deviation was noticed in the model.

3D structure of the model

Cholesterol dependent cytolysins (Alouf, 2000) are a family of bacterial toxins that are produced by Gram-positive bacteria. These protein toxins have high degree of similarity at their amino acid sequence level (40 to 70%) (Geoffroy et al., 1990; Rossjohn et al., 1997). The X-ray crystallographic structures of 1PFO and intermedilysin (ILY) of *Streptococcus intermedius*, which are members of CDC family, showed that the three-dimensional structures are also similar (Polekhina et al., 2005; Rossjoh et al., 1997). Functional similarity has also been observed in the CDC family (Hotze et al., 2001; Heuck et al., 2000). As LSO is a member of the CDC family, so it might also have similar structure, function and mechanism of action.

Tryptophan rich C-terminus conserved region

The three dimensional structure of 1PFO has been

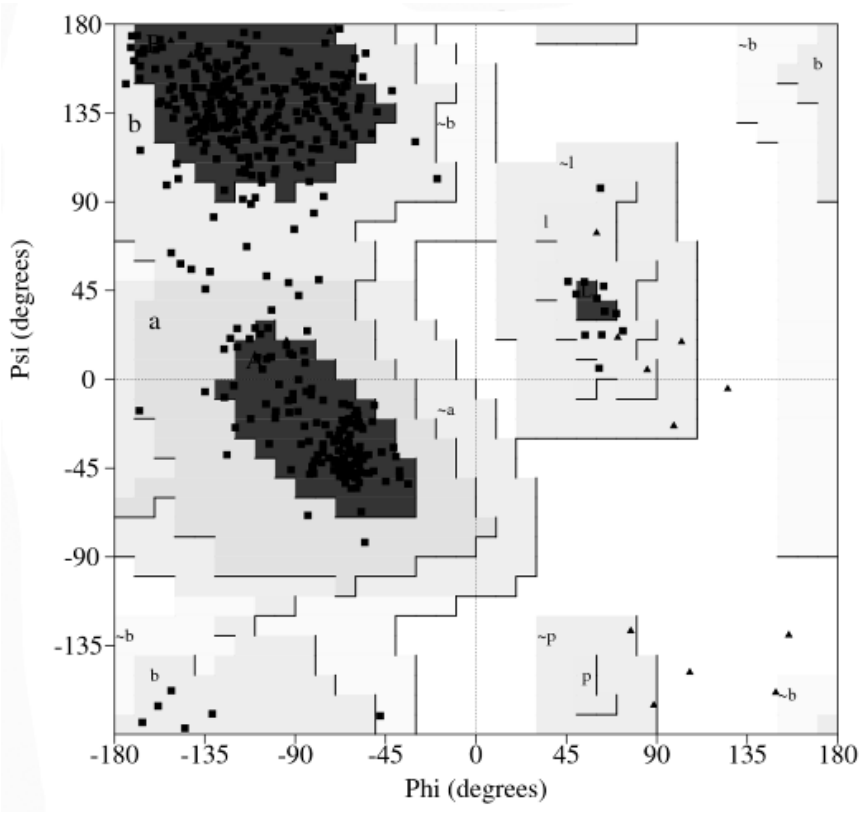

Figure 4. Ramachandran plot of the crystal coordinates of the template that is, perfringolysin O (1PFO).

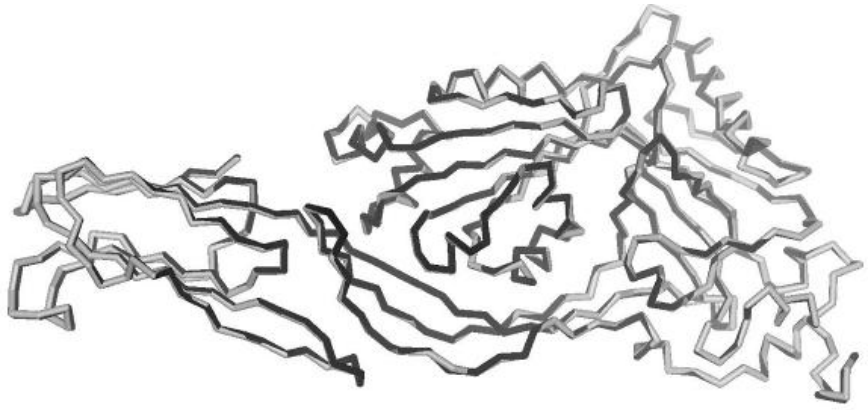

Figure 5. Supperposition of the Cα backbone of the predicted model of seeligeriolysin O (LSO) and the crystal coordinates of perfringolysin O (1PFO).

determined by x-ray diffraction (Rossjohn et al., 1997). X-ray diffraction analysis demonstrated that 1PFO (Figure 6) had extended rod shaped structure, having ample beta sheets. It had four domains in discontinuous fashion. Domain 4 (residues 363 to 472) also called the C-terminal domain, consisted of amino acid chain in continuous manner acting independently. Domain 4 had six tryptophan residues out of a total seven tryptophan residues of the molecule. Furthermore, three out of the six tryptophan residues of the domain 4 were present in the sequence of ECTGLAWEWWR (residues 430 to 440). This sequence is the longest sequence which remained conserved in the thiol-activated cytolysin (Shimizu et al., 1991; Tweten, 1988). In our 3D predicted

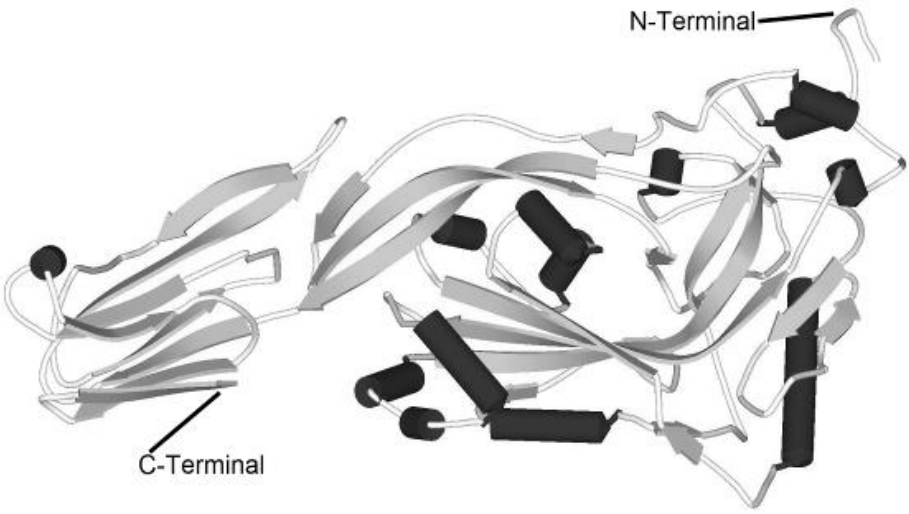

Figure 6. Schematic representation of the crystal coordinates of perfringolysin O (1PFO). Alpha chains have been shown in dark black solid cylinders and the beta sheets have been shown in gray flat arrows.

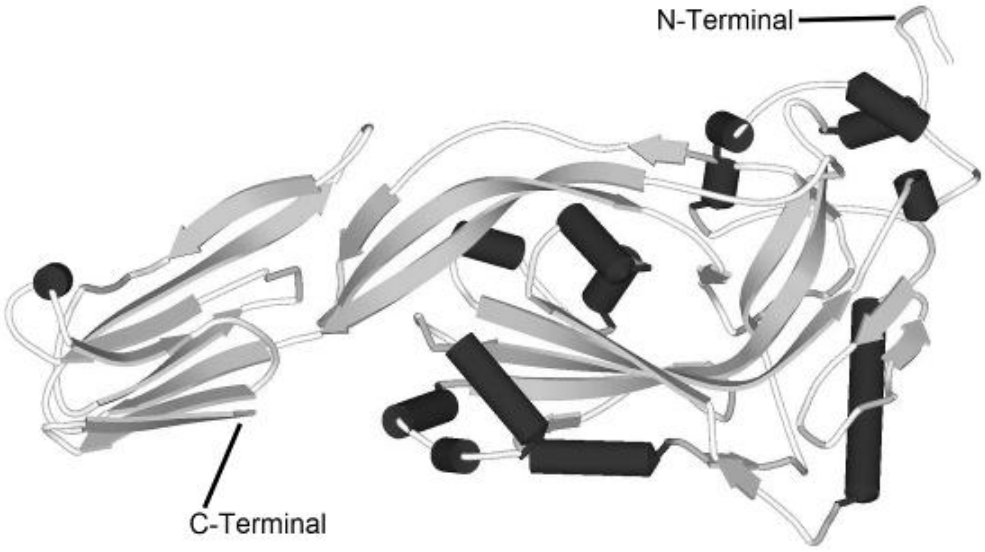

Figure 7. Schematic representation of the predicted model of seeligeriolysin O (LSO). Alpha chains have been shown in dark black solid cylinders and the beta sheets have been shown in gray flat arrows.

protein are 381, 499, 435, 437, and 458 which are exactly similar to the positions of tryptophan residues in the template protein; moreover, like the template protein where three of the six tryptophan of the C-terminal domain are present within the amino acid residues (430 to 440). Similar to the template protein, three out of the six tryptophan residues of the C-terminal region in the target protein are present in the same region. So it can be inferred from the conservation of tryptophan residues within the C-terminal domain of the target protein that the function of the C-terminal region might be similar to 1PFO.

Membrane recognition and cell binding

Domain 4, located at one end of the 1PFO monomer, performs the role of membrane recognition and initial binding in 1PFO (Heuck et al., 2000) that a monoclonal antibody block the cell binding by binding to the toxin

near the C-terminal region. However, the exact epitope remained unidentified (de los Toyos et al., 1996). Membrane binding activity of 1PFO has been significantly decreased upon chemical modification of the cysteine sulfhydryl of the 1PFO (Iwamoto et al., 1987). From this study, it is inferred that LSO domain 4 might also be involved in the membrane recognition and initial binding to the cholesterol containing membrane. It has been demonstrated that PFO insertion occurs more readily in an environment having loosely packed lipid. This approach of PFO does not show that this protein has more affinity for environment having disordered lipid domains than for lipid raft domains. It appears that PFO may contact with the disordered domain first and then move to ordered domains prior to insertion. In addition to this, in membranes having disordered and ordered domains, it is more favorable that ordered domains will be having more cholesterol than disordered domains (Niu and Litman, 2002; Leventis and Silvius, 2001). As PFO is our template protein, so it is possible that our target protein LSO may show same mechanism of insertion in membranes having lipid domains.

Conclusion

The predicted homology model of seeligeriolysin O has extended rod shaped structure having ample beta sheets arranged in four domains. Structure and function of seeligeriolysin O might be similar to that of perfringolysin O, as peringolysin O was our template.

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