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Modeling of the Anthrax Protective Antigen Binding to the VWA/I Domain of Integrins

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Modeling of the Anthrax Protective Antigen Binding to the VWA/I Domain of Integrins[#]

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Abstract

Motivation. Anthrax toxin receptor (ATR) is a cell surface receptor that has been recently recognized as having a homology to Tumor endothelial marker 8 and human capillary morphogenesis protein 2. Both these proteins have been found to have an extracellular von Willebrand factor type A domain (VWA), also called integrin inserted (I) domain. VWA/I domain of the anthrax toxin receptor is located between AAs 44 and 216 of the extracellular domain of the receptor. Protective antigen (PA) is the binding moiety of the anthrax toxin and has been proved to precisely bind to the VWA/I domain of the toxin receptor. VWA domain/ I domain of ATR is highly related to VWA/I domain of the integrins and matrilins. Integrins and matrilins are adhesion molecules with non-covalently associated alpha and beta subunits that mediate cell–cell, cell–extracellular matrix, and cell–pathogen interactions. In order to understand the molecular interaction of PA to extracellular VWA/I domain of integrins/ATR, *in silico* docking of PA was done to the VWA / I domain of the α -2 integrin. This VWA/I domain is the most extensively studied domain and also is a homolog of VWA/I domain of anthrax toxin receptor. Understanding the molecular interactions between VWA/I domain and protective antigen of the anthrax toxin is crucial for designing novel therapeutics targeted to block the binding of the toxin to its cell surface receptor.

Method. BiGGER soft docking algorithm was used for predicting the interactions between anthrax toxin PA and VWA/I domain of the integrins. Results were visualized using Chemera 2.0 interface. VEGA open GL1.4.3 software was used for importing the PDB files for anthrax protective antigen (PDB ID: 1ACC) and VWA/I (PDB ID: 1AOX) domain of α -2 integrin.

Results. Global scoring functions for the first 10 docking solutions were in the range of 10.43 to 6.5. In the present study the top ranking solution has been discussed. Results show that PA domain 4 AAs from I603 to T716 were involved in binding to the VWA/I AAs V193–T199 and Q215–R243. Also, domain 2 amino acids of PA from L271 to L450 were also observed to be involved in the interaction with VWA domain of the integrin. MIDAS site AAs D151, D153, S155, T221 and D254 were in close vicinity of the target: probe interface. Lysine at 679 position of PA was observed to be at the key position in interaction with the VWA domain. PA residues from K679 to 684 were found to be in making the close contact with the target (<2.2 Å distance). Chemical character of the amino acids at the target: probe recognition site comprised mainly of hydrophobic non-polar amino acids. The amino acids of domain 2 of protective antigen were also observed in contact with the VWA/I domain of the integrin. Domain 2 amino acids are mainly involved in the transportation of the toxin across the membrane.

Conclusions. In the present study the binding of PA to the VWA/I integrin domain mainly involved domain 4

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and domain 2 of the protective antigen in a predominantly hydrophobic, nonpolar environment. AAs K679–K684 of the PA have been speculated to play a significant role in binding to the VWA/I domain of the receptor. The present study contributes towards making a framework for future rational design of anti-receptors/ anti-toxins.

Keywords. Anthrax toxin receptor; protective antigen; von Willebrand factor A domain; integrins; BiGGER; soft docking; tumor endothelial marker 8.

Abbreviations and notations

| | |
|--|-------------------------------------|
| AA, amino acid | PA, protective antigen |
| ATR, anthrax toxin receptor | PDB, protein databank |
| MIDAS, metal Ion dependent adhesion site | VWA, von Willebrand factor A domain |

1 INTRODUCTION

Anthrax is caused by the spore forming organism *Bacillus anthracis*. Recently this disease attracted considerable attention as a major bioterrorist threat, because of the deliberate mailing of *B.anthraxis* spores via the U.S. postal system that resulted in deaths of five people [1]. *Bacillus anthracis* secretes a tripartite toxin (AT) that forms a complex comprising protective antigen (PA; 83kDa; 735 amino acids), lethal factor (LF; 90kDa; 776 amino acids) and edema factor (EF; 89kDa; 767 amino acids). A combination of PA and LF is termed the lethal toxin (LeTx), whereas a combination of PA with EF is termed as edema toxin. Both lethal toxin and edema toxin conform to the A/B toxin model, where PA acts as the B-receptor binding subunit for both LF and EF A-subunits. PA binds to a recently identified anthrax toxin receptor (ATR) and is then cleaved by a furin-like protease into PA63 and PA20 [2,3]. This processing facilitates the heptamerization of PA63 and the subsequent binding of EF or LF [4–7]. These toxic complexes are internalized by receptor-mediated endocytosis. EF and LF are further translocated into the cytoplasm to exert their toxic effects. Thus it is obvious that PA is the key protein of the toxin, promoting both the binding of the toxins and the translocation of their enzymatic moieties facilitating the action of the LF and EF. The three dimensional structure of the monomeric PA has been solved by X-ray diffraction at 2.1 Å resolution and consists of 735 amino acids with four folding domains. Domain 4 of PA (–C terminal end) encompasses the last 139 carboxyl-terminal amino acids (596 to 735) [8]. Also, the anthrax toxin receptor (ATR) has been recently recognized and is a 368 amino acid protein with a short cytoplasmic tail and recently has been shown to have a homology to tumor endothelial marker 8(TEM8) and human capillary morphogenesis protein 2 [9,10]. Extracellular domain of ATR/TEM8/Human Capillary morphogenesis protein 2 contains a von Willebrand factor A-type domain (VWA) also known as I domain. This domain is located between residues 44 and 216 of ATR. The VWA/I domains are conserved and are present in a large number of extracellular proteins including integrins, matrilins, collagens, and complement components [11,12]. VWA/I domain of ATR has been reported to be the binding site for PA [9,10]. In addition, a MIDAS (metal ion dependent adhesion site) motif (defined by the amino acid sequence DXSXS...T.... D where X is any amino acid) located within the VWA domain is suggested to have an important role in ligand binding in case of integrins and is also the part of VWA/I domain of the ATR. MIDAS Motif

functions to coordinate Mg^{2+} or Mn^{2+} , which is usually necessary for ligand binding [13].

So far a detailed model of anthrax toxin receptor is not available for the study of its molecular interaction with the ligand. Also, the detailed interaction of PA to the VWA/I domain of the ATR/Integrins is not very well elucidated and reported in the literature. Therefore, in order to understand the molecular and chemical interactions between the PA and the VWA/I domain of the ATR/Integrins and other extracellular proteins with VWA/I domain, the present work was undertaken and *in silico* protein docking of anthrax protective antigen was done to extensively understand α -2 integrin VWA/I domain using BiGGER soft docking algorithm. This work will help to understand the interaction of PA with the ATR at molecular level and open the gateway for the further studies on designing the anti-receptor/anti-toxins that would downplay the effect of the anthrax toxin.

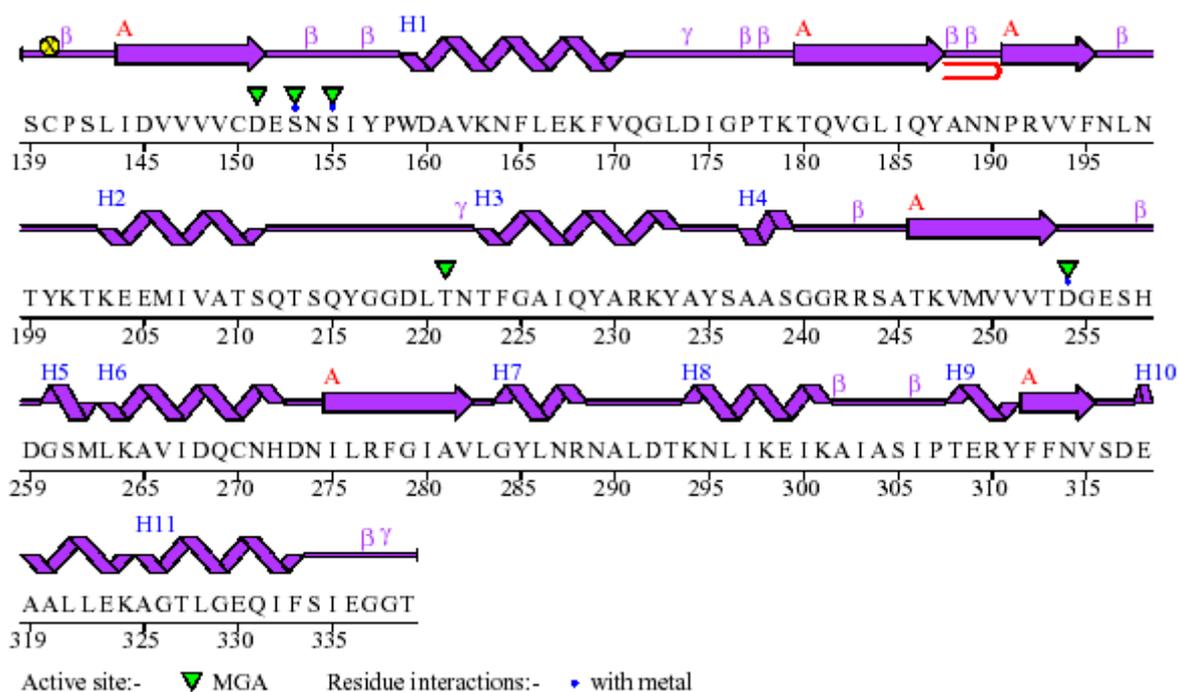


Figure 1a. PDB summary of the secondary structure of the file 1AOX, (set as target). The green inverted triangles represent the positions of the MIDAS amino acids A151, S153, S155, T221 and D254 (Reproduced from PDB).

2 MATERIALS AND METHODS

2.1 Test Files

Integrin VWA/I domain (PDB ID: 1AOX, species: *Homo sapiens*) and Protective antigen (PDB ID: 1ACC, species: *Bacillus anthracis*) files were imported from protein databank [14] to VEGA Open GL1.4.3 software and converted to appropriate format (.pdb) for BiGGER docking software.

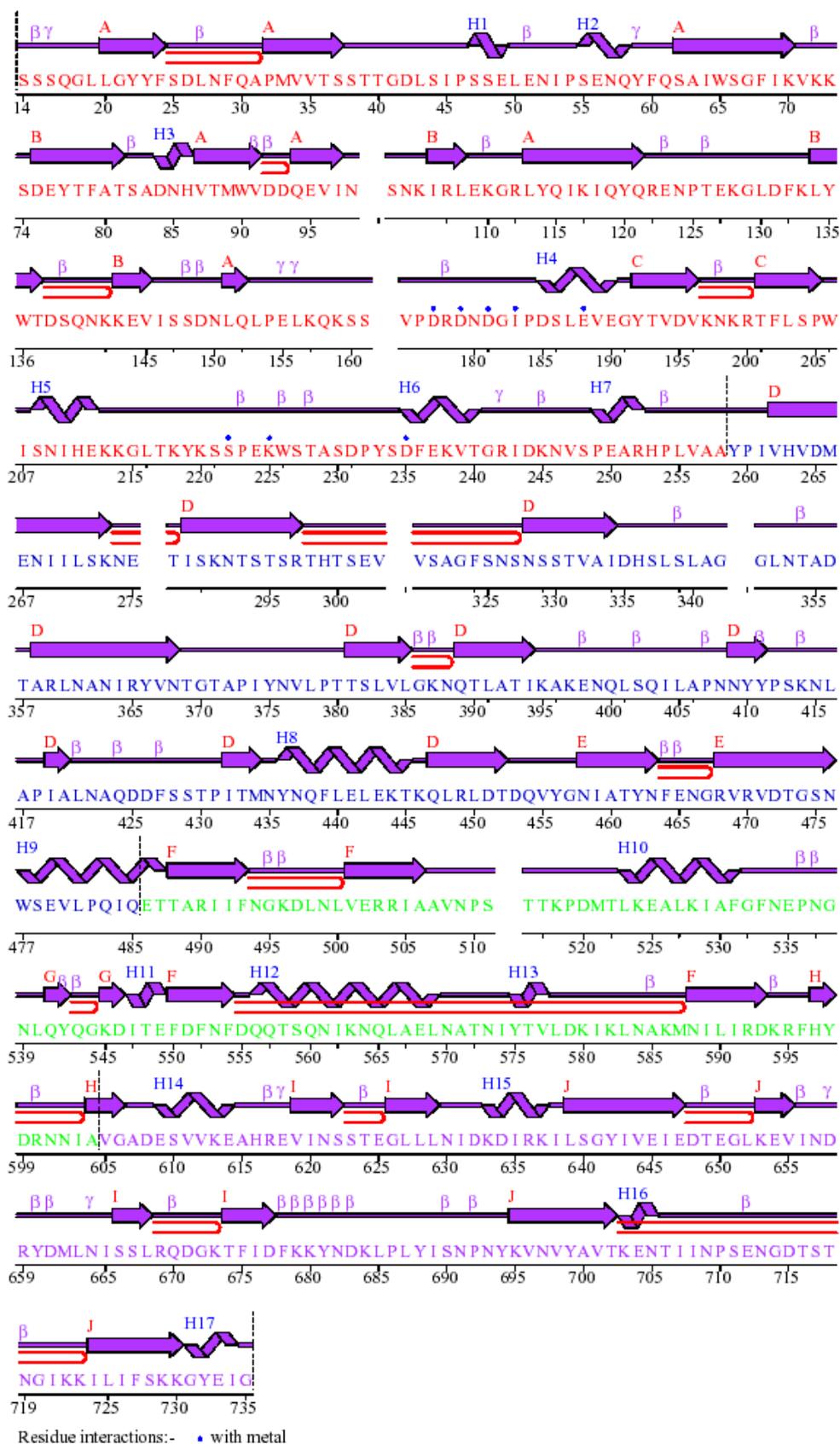


Figure 1b. Secondary structure of the file 1ACC, (set as probe): red, blue, green and purple colored amino acids depict the four domains of the PA (Reproduced from PDB).

VWA domain used for the study is the synthetic gene with mutations at T137R, Q138S, P139S and T338G. The structure of this domain has been solved by X-ray diffraction at 2.1 Å resolution [15]. SCOP (Structural Classification of Proteins) classifies I domain as alpha and beta proteins (a/b, comprising mainly of parallel β sheets). It has two polymer identical chains A and B (each has 201 residues). Active site residues of the 1AOX are Asp151, Ser153, Ser155, Thr221 and Asp254. Two Mg²⁺ are bound to S153 and S155 of the chain and are suggested to play a crucial role in the ligand binding. Figure 1a summarizes the various amino acid positions and secondary structure of the chain (A) of VWA/I domain (set as target) in the present study.

PA is a monomeric protein comprised of 735 AAs and has four folded domains organized mainly into antiparallel beta-sheets: N-terminal domain (domain 1) containing two calcium ions and the cleavage site for activating proteases; a heptamerisation domain (domain 2) containing a large flexible loop implicated in membrane insertion; a small domain of unknown function (domain 3); and a carboxy terminal end (domain 4). SCOP classification categorizes PA as a membrane and cell surface proteins mainly comprised of β sheets. Figure 1b summarizes the PDB secondary structure and sequence of PA as taken for the present study.

2.2 Docking Of The PA To The VWA/I Domain Of The Integrins

BiGGER soft protein-protein docking (<http://www.cqfb.fct.unl.pt/bioin/chemera/>) algorithm was used to dock the PA to VWA domain/I domain of the integrins [16]. In this algorithm the overall evaluation of docked solutions is given by the Interaction Global Score. This is the estimated probability (in percentage) that a docking solution with that Neural Network response is an accurate model of the complex [17–20]. The algorithm consists of two stages: 1) Searching and filtering and 2) Scoring. First stage is a step-by-step search through all the possible docking configurations. The first step consists of generating the Target Grid. This is a three dimensional matrix representing the shape of the target molecule, distinguishing surface and core regions. Core regions are adjusted to model side chain flexibility. For every orientation of the Probe molecule a Probe Grid is generated and moved relative to the TARGET grid (similar to Target Grid) in 1Å steps, which correspond to the grid cell size. Every relative position of two grids is a candidate solution. Once the Maximum Solutions are reached (default: 5000), every new solution kept will remove one solution from the final set. The solution removed is always among those with the least surface contact score. Stringent filtering criteria retain only a small fraction of the possibilities. The first stage generates a set of Docked Configurations that are evaluated in the second stage, using a Neural Network with the following inputs:

1. Surface Contact Score as measured during the Searching and Filtering stage.
2. The output of the Side Chain Contact Filter.

3. Electrostatics Interaction Score, based on the Coulomb model.

4. Hydrophobic score, based on an estimate of the solvation energy variation caused by the formation of the complex.

5. Side Chain Contacts Score, as used in the Side Chain Contact Filter.

The obtained theoretical model of interaction between PA and VWA/I domain was submitted to Protein Data Bank and has the PDB ID 1PQB.

Table 1. Interaction global score of the 10 best solutions produced by BiGGER for the 1AOX and 1ACC docking: Interaction global score for the top 10 ranking solutions were in the range of 10.43 to 5.14. The top ranking solution with 10.43 score was selected for the analysis of the interactions.

| Solution x of 5000 | Global Score |
|--------------------|--------------|
| 1 | 10.43 |
| 2 | 9.85 |
| 3 | 8.61 |
| 4 | 8.06 |
| 5 | 7.48 |
| 6 | 6.96 |
| 7 | 6.59 |
| 8 | 5.49 |
| 9 | 5.41 |
| 10 | 5.14 |

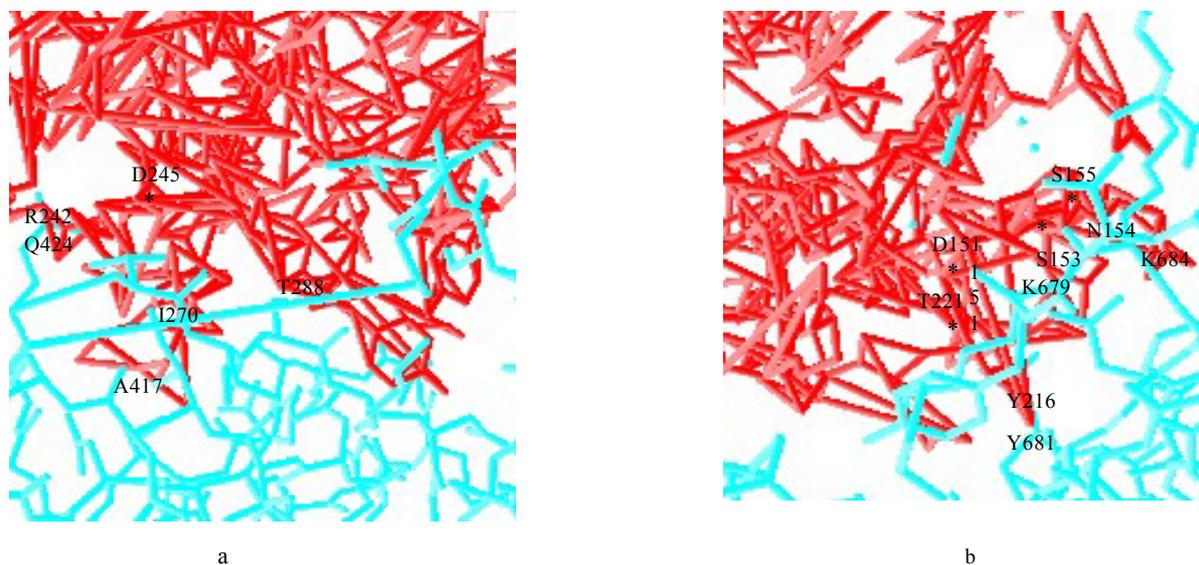


Figure 2. Interaction of PA (blue) and VWA/I domain of the integrin (red): alpha carbon structure of the complex is shown. Interactions are shown rotating the domains along the X-axis by 122°, Y-axis by 54° and Z-axis by 19°. (a) Shows the interaction of domain 2 residues of PA to the VWA/I domain residues. MIDAS amino acid D245 is in the vicinity. (b): Shows the interaction of domain 4 amino acids K679, Y681 and K684 to VWA/I amino acids T221, Y216. MIDAS amino acids are shown in the vicinity. These regions are speculated to have a potential role in interaction.

3 RESULTS AND DISCUSSION

3.1 Interaction Global Score Of The Docking Solutions

The present docking produced 5,000 solutions with 300 overlaps set as default. Total steps taken were 6384 where each angular step was at 15Å and total docking time was 1day, 19 hours and 8 minutes on a Pentium III 1.3 Ghz computer. Interaction global score of the top 10 ranking solutions were in the range of 10.43 to 5.41 (Table1). In this study the top ranking solution (Global score 10.43) has been analyzed to predict the interactions. Results were visualized at Chemera 2.0 interface and the presence of PA domain 4 amino acids from I603 to T716 were revealed to be in close proximity of VWA/I domain amino acids from V193 to R243. The MIDAS site AAs D151, S153, S155, T221 and D254 were also visualized in the close proximity of PA and VWA/I interface. At the same time the presence of domain 2 amino acids of PA from L271 to L450 were also observed in the interaction with VWA/I at a site distal to the target: probe interaction site (Figure 2).

3.2 Interatomic Distances of MIDAS From PA And VWA/ I Amino Acids

Interatomic distances of MIDAS residues were calculated from VWA and PA amino acids. K679 from PA appeared to be in close proximity of the metal chelating amino acids of the VWA/I domain (Table 2). K679 of PA was at 6.40 Å from S153 and S155, 12.72 Å from D151, 13.95 Å from D254 and 14.55 from T221. Of the VWA/I amino acids G255 was closest at 1.32 Å from D254 of MIDAS, and Q215 was at 6.41Å of D151.

Table 2. Interatomic distances between the MIDAS and closest AAs in the target and probe molecules: distance (D in angstroms) was calculated between the amino acids of metal ion dependent adhesion site of VWA/I domain and the amino acids closest to them from target (VWA/I) and probe (PA) molecules. Lysine (K) at position 679 of domain 4 of PA was in close vicinity of the MIDAS site.

| MIDAS | TARGET (VWA/I) | | PROBE (PA) | |
|-------|----------------|-------|------------|-------|
| AA | AA | D (Å) | AA | D (Å) |
| D151 | Q215 | 6.41 | K679 | 12.72 |
| S153 | N154 | 2.46 | K679 | 6.40 |
| S155 | I156 | 3.49 | K679 | 6.40 |
| T221 | D219 | 2.15 | K679 | 14.55 |
| D245 | G255 | 1.32 | K679 | 13.95 |

3.3 Site of Contact between the VWA/I and Protective Antigen

PA amino acid residues from I603 to T716 were observed to be in close proximity of the VWA/I domain residues V215–R243. Table 3 depicts residues from chain 1AOX and 1ACC that were calculated to have a close contact (<2.2Å distance between the residues) with each other at the interface. Thus it is clear that both domain 2 and domain 4 amino acids were involved in binding with the VWA domain of the $\alpha 2\beta 1$ integrin.

Table 3. Close contact residues (<2.2 Å) of VWA/I domain and the PA located within 2.2Å distance as observed by the BiGGER soft docking. Both domain 2 and 4 residues were involved in making a close contact with the VWA residues

| Chain | Res | Seq | Chain | Res | Seq | Distance |
|-------|-----|-----|-------|-----|-----|----------|
| | Val | 194 | | Leu | 340 | 0.61 |
| 1AOX | Arg | 243 | 1ACC | Gln | 424 | 1.08 |
| | Tyr | 216 | | Lys | 618 | 1.41 |
| | Gln | 215 | | Lys | 684 | 1.66 |

3.4 Chemical Character of the Target: Probe Recognition Site

Chemical character of the target: probe interface was calculated by estimating the percentage of different types of amino acids involved in the interaction. Figure 3 represents the chemical character of the target: probe interface. Thus, it is obvious that interface mainly was predominantly of hydrophobic non-polar amino acids. This is in agreement with the reports in the literature regarding the protein interaction sites where a hydrophobic environment is observed in most of the protein-protein interactions. Hence it is suggested that a change in the chemical atmosphere of the recognition site may have an impact on the binding characteristics of the anthrax toxin to its receptor.

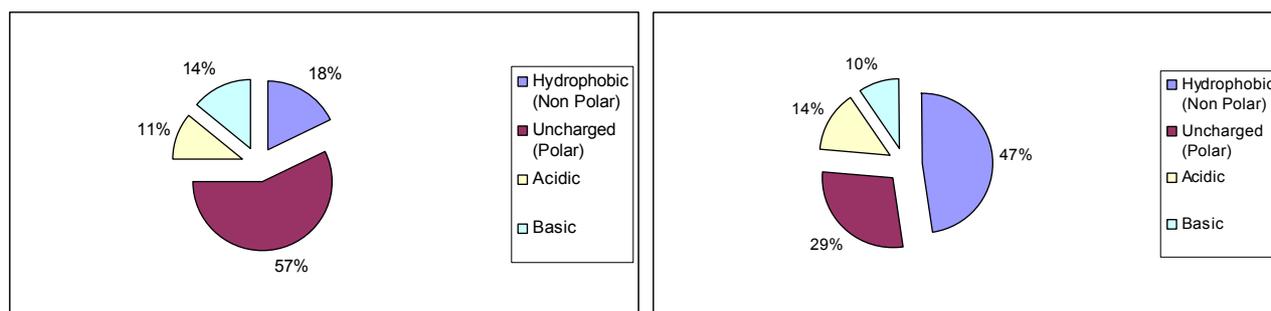


Figure 3. Chemical Character of the Probe (PA, a) and Target (VWA/I, b) at the interface was mainly of hydrophobic non-polar amino acids: Chemical character of the amino acids at the interface in case of PA (a) comprised of 47.61% hydrophobic AAs, 28.51% uncharged polar AAs, 14.28% acidic and 9.52% basic amino acids. (b) In case of the VWA it was mainly comprised of 57.14% hydrophobic AAs, 17.85% uncharged polar AAs, 10.71% acidic AAs and 14.28% basic amino acids.

3.5 Discussion

Pathogenesis of anthrax and methods of treating the disease have recently been the topics of great interest due to concerns over the use of *B. anthracis* as a biological weapon and the recent mail anthrax attacks in the US. One of the reasons that this bacterium is so difficult to treat is that symptoms appear after *Bacillus anthracis* has already multiplied inside its human host and started to produce large amounts of the tripartite toxin [21–24]. Thus, although antibiotics may kill or suppress growth of the bacteria, it is the toxin that eventually kills the human patient. Clearly a two-pronged counter attack is required: killing of *Bacillus anthracis* with antibiotics and neutralization of the toxin. Understanding and manipulating the interaction between the anthrax toxin and its receptor is one of the approaches towards neutralization of the toxin.

As PA is the receptor binding moiety of the anthrax toxin, in the present study the binding of PA to the VWA domain of the integrins was performed and analyzed. The VWA domain of the ATR has been reported to be a homolog of VWA domain found in integrins and matrilins. PA of the anthrax toxin is reported precisely to bind to the extracellular VWA/I domain of the receptor [25]. PA is divided into four domains. Domain one (AAs from 1 to 258) has a role in binding the lethal factor and edema factor of the toxin, domain two (AAs from 259 to 487) is involved in the transport of the PA across the membrane, domain three (AAs 488 to 735) is a short domain with unknown functions whereas the domain four from 596 to 735 AAs is recognized as the receptor binding domain [26]. In the present study the PA residues from I603 to T716 of domain four were observed to be in contact with the target (VWA/I domain of the integrins). Residues between K679 and K684 had a close contact ($<2.2 \text{ \AA}$) with VWA residues between T193–I243. Leppla SH et al [27] in their Alanine scanning mutations done in the small loop of the anthrax PA have reported that residues between Y682–Y688 have a critical role in binding of PA to the ATR. Thus our current study is in good agreement with the experimental studies reported in the literature. Importantly, the residues that had a mutation in the VWA/I domain taken in the present study (T137R, Q138S, P139S and T338G) did not have any significant role to play in the interactions.

However, we also observed domain II residues Ile270, Ser290, Ser339, and Leu 340 of PA to be in close contact with the VWA AAs Asn 198, Asn 196, Val193, and Phe 195 respectively. The involvement of domain 2 AAs in the binding suggests a role of these residues in the binding of the PA to the receptor.

This binding was observed at the distal end of the active site. Our finding is in agreement with the mutagenesis studies reported by Bhatnagar *et al.* [28]. They showed that deletions in domain 2 AAs D425 and F427 reduced anthrax toxicity both in vivo and in vitro. Thus although the role of PA domain 2 amino acids in anthrax toxin receptor binding is not very well elucidated in the literature, the present study along with the mutagenesis studies of Bhatnagar *et al.* does suggest a role of Domain 2 in the interaction with the receptor, which needs further investigation. VWA/I residues from V193–R243 are reported to interact with PA in the current study, which has a close agreement with the study done by Tuckwell *et al.* [29], where the residues between T199–Y216 of VWA were identified as functionally important regions of the $\alpha 2\beta$ integrin.

In the present study Lys at position 679 of PA was found to be in close vicinity of the MIDAS amino acids of the target and it is speculated that the K679 may have an important role in the toxin binding to the receptor. Also, the MIDAS amino acids were observed to be in vicinity of the interface confirming their role in the ligand–receptor complex. The present study is in agreement with the reports available in the literature based on the experimental studies, where the domain 4 residues of the PA has been reported to be involved in binding to the ATR, which has been found to be important for bringing about the effect of anthrax toxin in the cells [30–36]. The chemical

character of the interface was found to be mostly hydrophobic nonpolar, which is the key feature of the protein–protein recognition sites [37].

Presence of VWA/I domain in the extracellular domain ATR adds complexity to the pathogenesis of anthrax infection, as VWA domains are present in Eukaryotes, Eubacteria and Archaea. Frequently VWA domain containing proteins have been found to function in multiprotein complexes [38–40]. In order to understand the pathogenicity of anthrax toxin, its interaction with proteins containing VWA domain needs further investigation.

4 CONCLUSIONS

Identification of ATR has opened new avenues for the development of antitoxins. Antitoxins are small molecules or proteins that block toxin action and that may be used in conjunction with antibiotics to prevent disease. There is currently much interest in developing anti–toxins for anthrax, as the anthrax toxin plays a major role in virulence. There is a need for new treatments for anthrax that improves the efficacy. Since PA is the binding moiety of the toxin, blocking PA function is a viable antitoxin approach. Recently it has been recognized that the extracellular domain of the anthrax toxin receptor has a VWA domain homologous to that of the integrins and PA of the anthrax toxin is reported to bind to it. In the present work the best understood VWA domains from integrins is considered in order to understand the binding of anthrax toxin PA to the VWA/I domain of the ATR. Keeping this in view it was feasible to recognize the points of contact between the PA and the VWA domain of the integrins. Also, binding of PA to the VWA/I domain of the integrin opens a new insight into the pathogenicity of the anthrax toxin that needs further investigation. The present study analyzing the binding of PA to VWA/I domain of the integrins may form a framework for the future rational design of anthrax anti–toxins, targeted to the anthrax toxin receptor.

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