

Rational Drug Designing Strategies & Inhibitor Optimization: Anthrax Lethal Toxin Factor

Pawan Kumar Jayaswal^{1*†}, Ganesh Chandra Sahoo¹, Pradeep Das¹

¹Rajendra Memorial Research Institute of Medical Sciences
Agam Kuan, Patna, India - 80007

*Department of Biotechnology
Shri Rawat Pura Sarkar Institute of Technology and Science
Datia, Madhya Pradesh, India

†National Research Centre on Plant Biotechnology, IARI
Pusa, New Delhi - 110012
Email: er.pawanjayaswal@gmail.com

*Corresponding author

Received: July 13, 2012

Accepted: December 28, 2012

Published: January 8, 2013

Abstract: Anthrax toxin protein protective antigen, edema factor and lethal factor are secreted by *Bacillus anthracis* bacteria causes several adverse effects on human as well as on ruminant animals and considered as serious biological weapons. Lethal toxin protein (combination of lethal factor and protective antigen) is highly lethal to the host and responsible for the disruption of signalling pathways, cell destruction, and circulatory shock. 1YQY is one of the crystal structures of lethal toxin protein. It has two domains - Anthrax_M_tox & ATLF where the hydroxymate as well as Zn cofactor are attached. Known inhibitor of the protein 1YQY was identified and downloaded from pubchem. Interaction of the inhibitors with the protein was examined through in silico docking approach with AutoDock 3.0.5 and Hex. Some of the inhibitors apparently interact with several-conserved residue in the cofactor-binding site. The docking work suggests virtual derivatives of the predicted inhibitor that can improve hydrogen bond interaction between inhibitor and protein. From structural and docking analyses, it is hypothesized that 1YQY protein interacts with azelastine molecule shows the lowest docking energy in AutoDock software.

Keywords: Anthrax toxin protein, 1YQY, Docking, Hex, AutoDock, Energy minimization.

Introduction

Infectious disease anthrax caused by Gram positive, anaerobic bacterium *Bacillus anthracis*. Anthrax toxin protein secreted by *Bacillus anthracis* bacteria made up of three different monomeric proteins: edema factor (EF), lethal factor (LF) and protective antigen (PA) [1]. Among three proteins EF is larger in size (89 kDa) while other two PA and LF are comparatively small in size (85 kDa and 83 kDa respectively) [2, 3]. Lethal and Edema toxin protein organized in two different domains A and B. PA which is domain B, act as a receptor, domain A either EF or LF. Combination of PA and LF (Zn metalloprotease) [4] forms the lethal toxin (LT) protein, PA and EF combination produce edema toxin responsible for edema [5-7] individually neither LF nor EF are toxic alone. Protective antigen forms a heptameric structure at the surface of host cell by cleavage of N-terminal domain by furin like protease [8, 9]. After translocation of PA, metalloprotease [10] LF cleaves members of mitogen activated protein kinase (MAPK) family MEK1, MEK2, MKK3, MKK4, MKK6 and MKK7 but not MEK5 [11] this process interrupt the normal function of host cell [12]. MAPK family categorized into ERK, p38, JNK/SAPK and ERK5 [13-18] sub category, activation of these protein responsible for different types of responses in cell like gene expression, apoptosis and cell proliferation. In contrast, high level lethal toxin protein causes lysis of macrophages and

reduce the immune response and also disrupt the vascular barriers [19, 20]. Crystal structure of LF enzyme consists of four domains: domain I is the N terminal binded with PA; domain II, III, IV closely associated with each other and also hold the N terminal tail of MAPKK2 [21]. Protein data bank accession 1YQY plays an important role in catalytic activity. Due to lack of bioavailability and selectivity, it is necessary to develop a lead molecule against LF. In humans mostly skin, lungs and gastrointestinal tract are often in which inhalation of anthrax is most lethal [22].

Materials and methods

Crystal structure and its protein sequence of 1YQY lethal toxin protein were downloaded from Protein Data Bank. It was visualized in open-source software PyMOL 1.1 (Fig. 1) and hydrogen moieties were added. *In silico* lethal protein 1YQY protein interaction studied with different methodology are discussed below.

Template selection and validation

Retrieval of protein sequence of lethal toxin protein of *Bacillus anthracis* bacteria is done through protein data bank. Nine crystal structure of lethal toxin proteins complexed with different ligands are available in Protein Data Bank (<http://www.rcsb.org/pdb/home>) [23] PDB ID of these protein strains are 1J7N, 1JKY, 1PWP, 1PWQ, 1PWU, 1PWV, 1PWW, 1YQY and 1ZXV. Lethal toxin protein 1YQY associated with two different domains, domain II and domain IV (residues 297-809) attached with two ligand molecule (2r) 2 {[(4 Fluoro 3 Methylphenyl)sulfonyl]amino} N Hydroxy 2 Tetrahydro 2h Pyran 4 Y lacetamide and zinc molecule. PFAM is used for the study of functional domain of 1YQY lethal toxin protein. Physicochemical properties of protein are studied by protparam [24]. Downloaded protein sequence was validated by SAVES server (Procheck, What_check, Verify_3D, Errat, Prove) [25].

Energy minimization

Lethal toxin protein 1YQY of bacillus strain downloaded from protein data bank and further refined by molecular dynamic programme YASARA (Yet Another Scientific Artificial Reality Application, <http://www.yasara.com>) [26]. YASARA is based on NOVA (Nucleotide Optimization in VAcuo) which looks like common molecular force field. Monte Carlo simulation has been used for the optimization of NOVA force field. In this study the initial and final energy of 1YQY protein is calculated.

Ligand binding site prediction

Identification of ligand binding sites has own importance in *in silico* drug designing. Q-Site finder is used for the detection of different ligand binding sites in lethal toxin protein of *Bacillus anthracis* uses non- bonded interaction energy and van der Waals probe to locate the favourable binding sites. Nonbonded interaction energy of a probe is calculated by liggrid programme. Complete protein sequence is enclosed in 3D grid and the grid resolution of interaction of protein and methyl probe is 0.9 Å. Most favourable binding sites are decided on the basis of total interaction energy. In comparison of Pocket-Finder, Q-Site finder used 5.0 Å value for the site volume estimation and protein residue identification [27]. Nissink et al. [28] tested the Q-Site algorithm and found the success rate was 90% in top three predicted sites.

Virtual screening

Fourteen different potent inhibitors of 1YQY lethal factor protein molecules are downloaded from PubChem database of NCBI (<http://pubchem.ncbi.nlm.nih.gov/>). Downloaded .sdf structure of ligand molecules are further converted into .pdb with PyMOL software [29] shown in Table 1, and performed the docking study with two different software AutoDock 3.0.5 [30] and Hex 6.1 [31].

Protein-ligand interaction study

Molecular *in silico* docking study of 1YQY lethal toxin protein and 15 corresponding ligand molecule have done with two different software AutoDock 3.0.5 and Hex. AutoDock is graphical user interface program maintained by The Scripps Research Institute and Olson Laboratory used to dock with selected inhibitors listed in Table 1. AutoDock requires the protein and ligand molecule in PDB format or in .mol2. During file preparation nonpolar hydrogen atoms were removed from the 1YQY protein molecule and partial charges were added. Protein file are further converted in PDBQS format with partial charges and salvation parameters, similarly ligand molecule transformed into PDBQ file and torsion angles are defined. The default setting of three thumbwheel widgets 40×40×40 were used to center the protein molecule for the interaction with ligand in *x*, *y* and *z* directions. Lamarckian genetic algorithm (LGA) [32] used by AutoDock to perform the docking. Docking conformation is based on the lowest energy. Hex version 6.1 [31] software is used for the docking study. Default parameter like grid dimension (0.6), receptor range (180), ligand range (180), twist range (360) distance range (40), scan step (0.8) is used for the interaction study, assuming ligand is rigid. Docking study of Hex is based on spherical polar Fourier (SPF) algorithm and in this study shape only approach is used. Lethal toxin protein 1YQY downloaded from protein data bank and energy of the structure is minimised by YASARA software.

Results

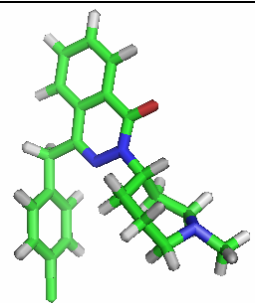
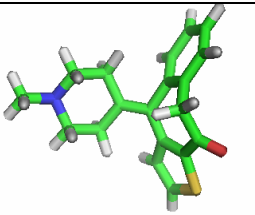
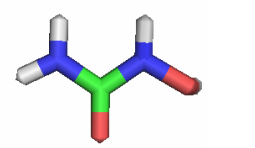
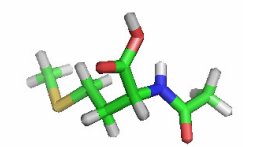
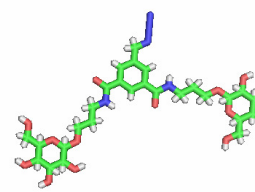
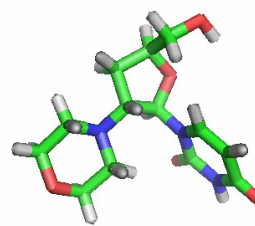
Template selection and validation

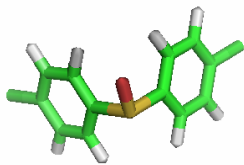
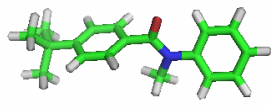
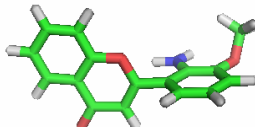
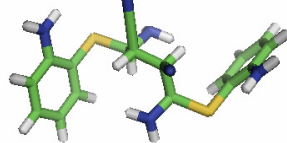
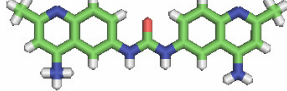
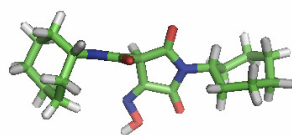
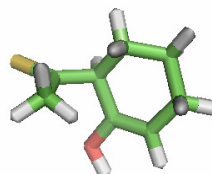
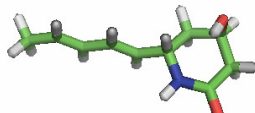
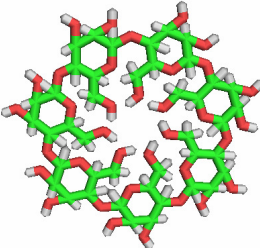
Protein sequences of lethal toxin protein 1YQY (Fig. 1A) downloaded from protein data bank. Middle domain and terminal domain of 1YQY protein having residues 264-550 and 551-775 respectively studied with PFAM. According to the physiochemical study (Prot-Param) 1YQY chain A of lethal toxin protein having 523 amino acid, leucine amino acid is having maximum number of residues 55 (10.8%) of total composition. Total number of negatively charged residues (Asp + Glu): 80 and positively charged residues (Arg + Lys): 74. The estimated half-life of lethal toxin protein is >20 hours (yeast, *in vivo*), >10 hours (*Escherichia coli*, *in vivo*) predicted from prot-param. SAVES server used for the validation of crystal structure of A chain of 1YQY protein (Fig. 1B). Stereochemical quality was checked by procheck programme and ramachandran plot was computed which show 88.1% residues in most favored regions, 54% in additional allowed region while one residue (0.2%) in disallowed region (Fig. 1C). ERRAT programme is used for the analysis of non bonded interaction study between different atoms types and overall quality factor of the model is 95.257 (Fig. 1D).

Energy minimization

Obtained model of lethal toxin protein of *Bacillus anthracis* strain refined by YASARA which is based on NOVA force field. Initial energy and Zscore of the 1YQY protein was 143144188.4 kJ/mol, 2.11 respectively while the end energy of the model is -322902.5 kJ/mol, and Zscore is 0.49 shown in Fig. 2.

Table 1. List of inhibitors against anthrax lethal factor protein

S.No	Inhibitor name	Chemical formula	Chemical structure	Molecular weight (g/mol)	IUPAC Name
1.	Azelastine	C ₂₂ H ₂₄ ClN ₃ O		381.89846	4-[(4-chlorophenyl)methyl]-2-(1-methylazepan-4-yl)phthalazin-1-one
2.	Ketotifen	C ₁₉ H ₁₉ NOS		309.42526	10-(1-methylpiperidin-4-ylidene)-5H-benzo[1,2]cyclohepta[3,4-b]thiophen-4-one
3	Hydroxyurea	CH ₄ N ₂ O ₂		76.05466	hydroxyurea
4	Methionamine	C ₇ H ₁₃ NO ₃ S		191.24802	2-acetamido-4-methylsulfanylbutanoic acid
5	AC1NRAY5	C ₂₇ H ₄₁ N ₅ O ₁₄		659.63954	5-(azidomethyl)-N,N'-bis[3-[(2S,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxypropyl]benzene-1,3-dicarboxamide
6	2'-Morpholino-ddU	C ₁₃ H ₁₉ N ₃ O ₅		297.30706	1-[(2R,3R,5S)-5-(hydroxymethyl)-3-morpholin-4-yl-oxolan-2-yl]pyrimidine-2,4-dione

7	4-Chlorophenyl sulfoxide	$C_{12}H_8Cl_2OS$		271.16232	1-chloro-4-(4-chlorophenyl)sulfinylbenzene
8	Neprilysin	$C_{18}H_{21}NO$		267.36544	4-tert-butyl-N-methyl-N-phenylbenzamide
9	PD 98,059	$C_{16}H_{13}NO_3$		267.27932	2-(2-amino-3-methoxyphenyl)chromen-4-one
10	K00211	$C_{18}H_{20}N_6S_2$		384.5216	2,3-bis[amino-(2-aminophenyl)sulfanyl-methyl]butanedinitrile
11	NSC12155	$C_{21}H_{21}ClN_6O$		408.88404	[6-[(4-amino-2-methyl-quinolin-6-yl)carbamoylamino]-2-methyl-quinolin-4-yl]azanium chloride
12	NSC623899	$C_{19}H_{29}N_3O_4$		363.45126	(4Z)-N,1-dicycloheptyl-4-hydroxyimino-2,5-dioxo-pyrrolidine-3-carboxamide
13	2-Thioacetylcyclohexen-1-ol	$C_8H_{12}OS$		156.24528	1-(2-hydroxy-1-cyclohex-2-enyl)ethanethione
14	CID 11217604	$C_{10}H_{15}NO_2$		181.2316	(4R,6S)-4-hydroxy-6-[(1E,3E)-penta-1,3-dienyl]piperidin-2-one
15	beta-CYCLODEXTRIN	$C_{42}H_{70}O_{35}$		1134.9842	-

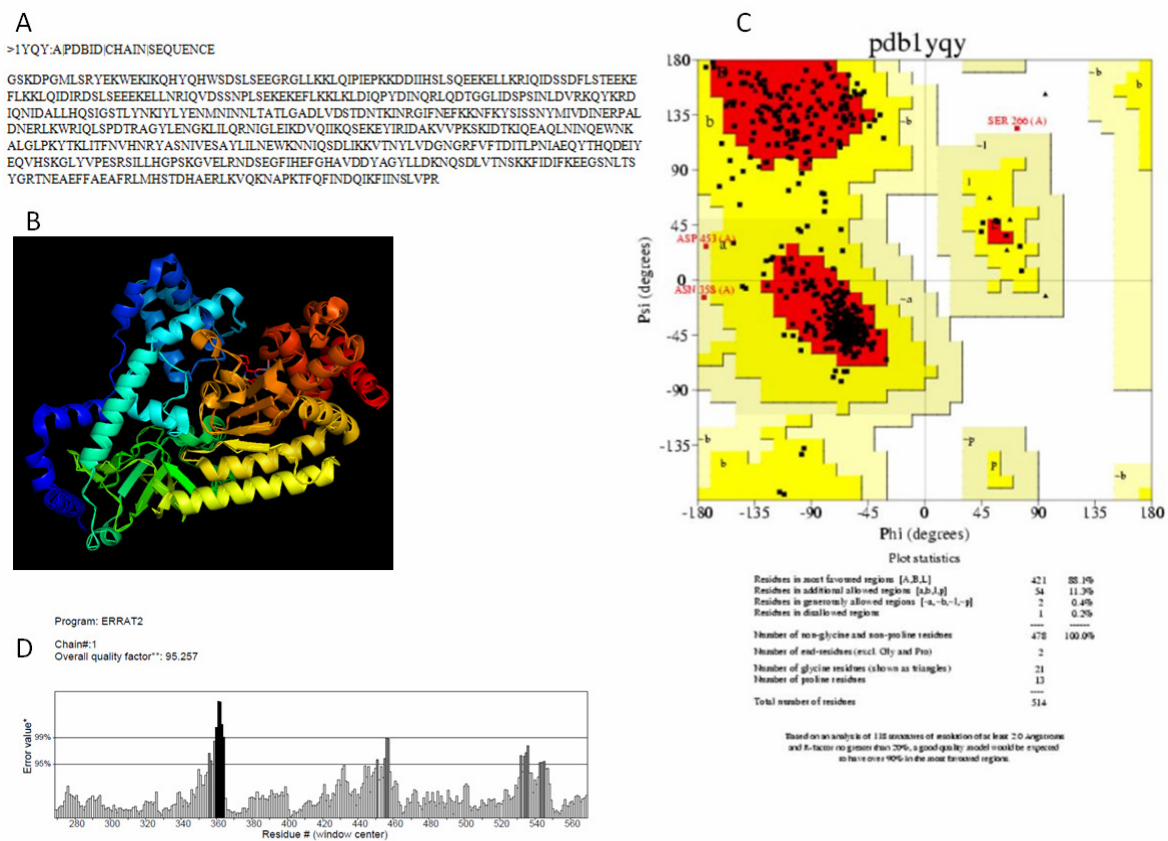


Fig. 1 (A) Fasta format sequence of 1YQY protein downloaded from protein data bank; (B) 1YQY A lethal toxin of protein associated with hydroxamate (2r) 2 [(4 Fluoro 3 Methylphenyl)sulfonyl]amino} N Hydroxy 2 Tetrahydro 2h Pyran 4 Ylacetamide and LF-active site; (C) Ramachandran plot of A chain of 1YQY protein, 88.1% amino acid residues are in favored region; (D) Statistical validation of crystal structure of the 1YQY protein using ERRAT programme.

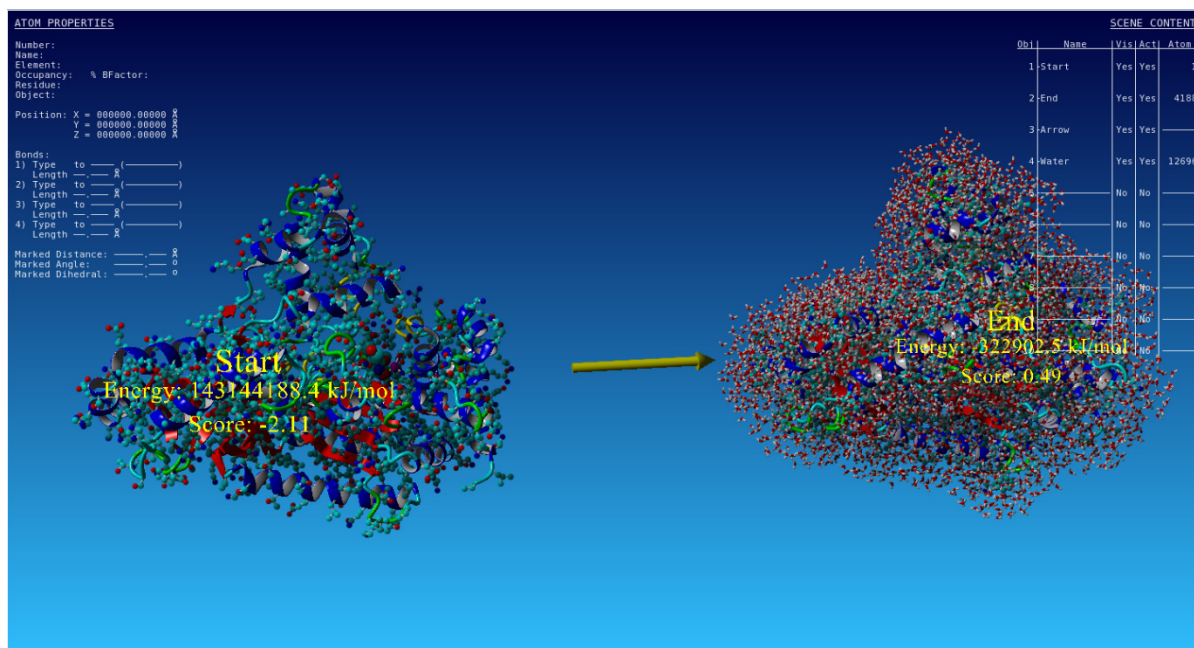


Fig. 2 Energy minimization of 1YQY protein by YASARA software based on NOVA force field

Ligand binding site of protein

Ten potential ligand binding sites in 1YQY protein have been found by using Q-Site finder programme by clustering favourable regions for van der Waals probes i.e. (-CH₃) on the protein surface shown in Fig. 3. Graph shows (Fig. 4) that the first ligand binding site is high in volume i.e. 776 Å³ and decreases respectively in up to nine other sites. Similarly 179 residues involved in the formation of binding sites while other top two sites are having less residues. 47 number of residue reported in site 9 which is very less in all ten predicted sites. Over all protein volume of 1YQY protein is 50515 cubic Å.

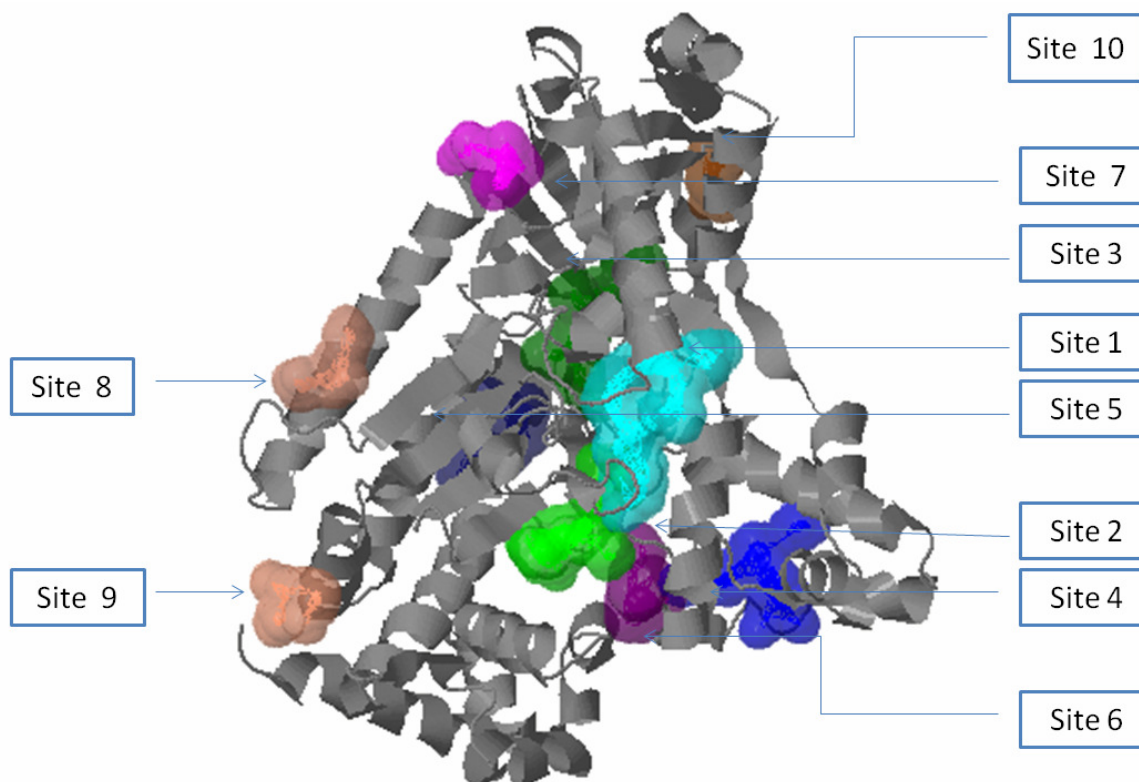


Fig. 3 Q-Site finder program showing the ligand binding site prediction of 1YQY lethal toxin protein. The protein is shown in cartoon model in grey colour with ten different colour-coded binding sites.

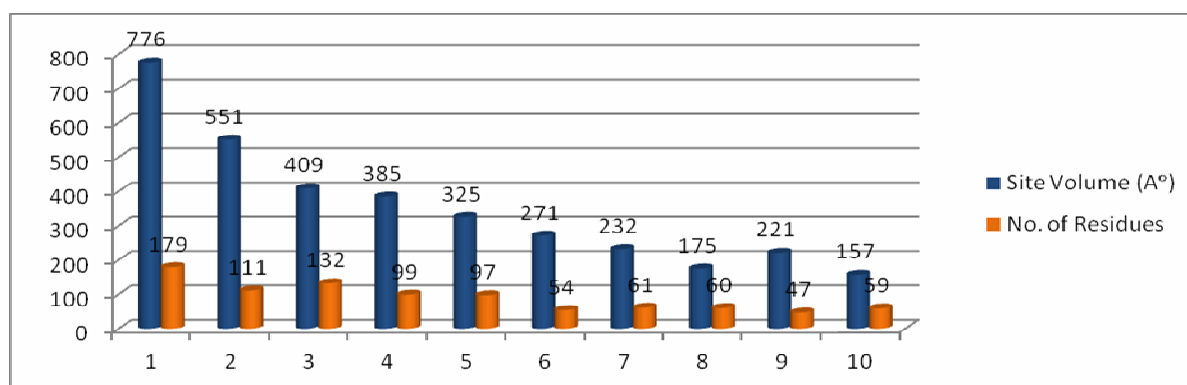


Fig. 4 Graph shows the relation between site volume (dark blue colour) and number of residues (orange colour) in predicted ligand binding sites of 1YQY protein. X-axis shows the number of sites while y-axis represents volume and number.

AutoDock result

Ligand-protein interaction between anthrax lethal toxin protein 1YQY and anti anthrax compound has been reported in this research work. For this purpose, docking study has been carried out by AutoDock 3.0.5 and Hex. Different ligand binding sites in the 1YQY protein were detected. Out of fifteen ligand compounds, eleven shows the interaction with 1YQY protein. Azelastine shows the lowest energy i.e. -18.48 kcal/mol and one hydrogen bond formed with active site residue HIS686: NE2 shown in Fig. 5 which is followed by methionine (-18.24 kcal/mol), ketotifen (-17.16 kcal/mol) and PD 98,059 (-16.26 kcal/mol) respectively shown in Table 2. Azelastine also shows the lowest inter molecular energy level (-18.18 kcal/mol) in comparison to other interacted ligand molecule. In this study 2-Thioacetylcyclohexen-1-ol shows the highest docking energy (-3.77 kcal/mol) followed by hydroxyurea (-4.0 kcal/mol). Azelastine is likely to be the best drug target molecule against anthrax.



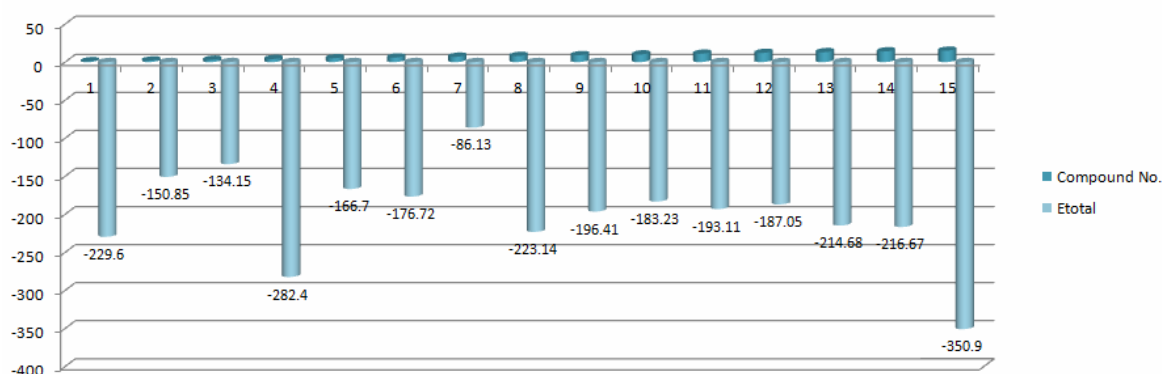
Fig. 5 Azelastine inhibitor - 1 hydrogen bond formed between active site residue (HIS686: NE2)

Table 2. Distribution of AutoDock result

Inhibitor	Docked energy	Free energy	KI	Inter molecular energy	Internal energy
Azelastine	-18.48	-17.25	2.3e-013	-18.18	-0.3
NSC623899	-6.76	-5.73	6.25e-005	-6.98	0.22
2'-Morpholino-ddU	-6.1	-5.17	0.000164	-6.1	0.0
β -Cyclodextrin	-15.67	-14.98	1.03e-011	-16.23	-0.03
Ketotifen	-17.16	-17.16	2.65e-013	-17.16	0.0
NSC12155	-13.24	-11.83	2.13e-009	-13.39	0.15
2-Thioacetylcyclohexen-1-ol	-3.77	-3.88	0.0	-7.93	4.16
K00211	-11.88	-9.91	5.45e-008	-11.78	-0.1
Hydroxyurea	-4.0	-3.68	0.0	-3.99	-0.1
Methionamine	-18.24	-13.0	2.985e-010	-17.98	-0.26
PD 98,059	-16.26	-14.94	1.12e-011	-16.19	-0.07

Hex docking results

Graphical representation shows the receptor ligand interaction results between 1YQY lethal toxin protein and fifteen different drug molecules library (Fig. 6). Stable structure of 1YQY protein is achieved by applying NOVA force field. Hex 6.1 version is used for the docking analysis. β -cyclodextrin shows the lowest Etotal in the Hex docking study (-350.9) shown in Fig. 7. AC1NRAY5 compound shows the second lowest Etotal value -282.4. Azelastine which shows the lowest energy in AutoDock study is having -229.6 Etotal. Hydroxyurea and 2-Thioacetylcyclohexen-1-ol shows the highest Etotal values i.e. -86.13, -134.15 respectively.



- | | | |
|-----------------------|-----------------|--------------------------------|
| 1. Azelastine | 2. Methionamine | 3. 2-Thioacetylcyclohexen-1-ol |
| 4. AC1NRAY5 | 5. CID_11217604 | 6. 4-Chlorophenyl sulfoxide |
| 7. Hydroxyurea | 8. K00211 | 9. Ketotifin |
| 10. 2'-Morpholino-ddU | 11. Neprilysin | 12. PD 98,059 |
| 13. NSC623899 | 14. NSC12155 | 15. β -cyclodextrin |

Fig. 6 Docking result of fifteen different ligand molecules in 1YQY protein. X-axis shows the number of compound while y-axis shows the Ettotal score of Hex.

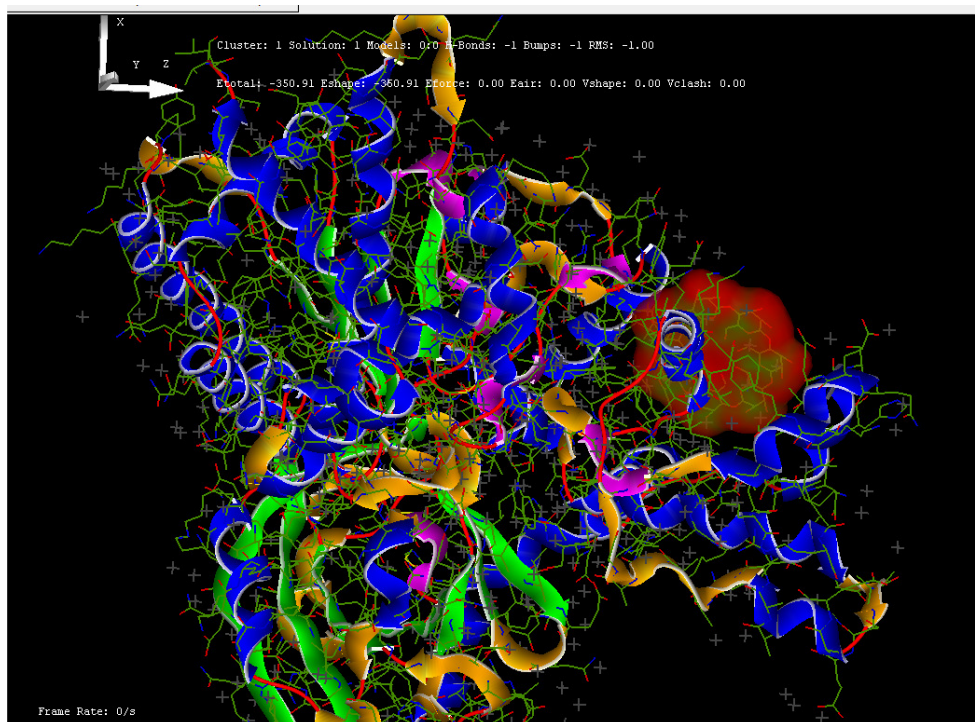


Fig. 7 β -cyclodextrin, Ettotal -350.9

Discussion

Rational structure approaches are increasingly being used in pharmaceutical industry because of the potential decrease of cost and time of drug discovery. Over recent years, the computational or Mathematical modeling of biological system has become increasingly valuable and can provide useful information to understand their behaviors. Coupled with the increase in the number of techniques and software tools available to simulate and analyze them. As the methodology is concerned, the main limitation is the computation of free energy of binding a ligand to the enzyme or a receptor. And in this work Azelastine is found as the best inhibitor among these three, because it has low Docked Energy.

Acknowledgements

P. K. J. is grateful to Biobrainz, Lucknow, India for the provided technical support for this work.

Reference

1. Pezard C., P. Berche, M. Mock (1991). Contribution of Individual Toxin Components to Virulence of *Bacillus anthracis*, *Infection and Immunity*, 59, 3472-3477.
2. Fish D. C., B. G. Mahlandt, J. P. Dobbs, R. E. Lincoln (1968). Purification and Properties of *In vitro*-produced Anthrax Toxin Components, *Journal of Bacteriology*, 95, 907-918.
3. Stanley J. L., H. Smith (1961). Purification of Factor I and Recognition of the Third Factor of Anthrax Toxin, *J. Gen. Microbiology*, 26, 49-66.
4. Chopra A., S. Boone, X. Liang, N. Duesbery (2003). Anthrax Lethal Factor Proteolysis and Inactivation of MAPK Kinase, *The Journal of Biological Chemistry*, 278, 9402-9406.
5. Leppla S. H. (1984). *Bacillus anthracis* Calmodulin Dependent Adenylate Cyclase: Chemical and Enzymatic Properties and Interactions with Eucaryotic Cells, *Adv. Cyclic Nucleotide Protein Phosphorylation Res.*, 17, 189-198.
6. Smith H., H. B. Stoner (1967). Anthrax Toxic Complex, *Fed. Proc.*, 26, 1554-1557.
7. Corinne P., B. Patrick, M. Michele (1991). Contribution of Individual Toxin Components to Virulence of *Bacillus anthracis*, *Infection and Immunity*, 59, 3472-3477.
8. Petosa C., R. J. Collier, K. R. Klimpel, S. H. Leppla, R. C. Liddington (1997). Crystal Structure of the Anthrax Toxin Protective Antigen, *Nature*, 385, 833-838.
9. Benson E. L., P. D. Huynh, A. Finkelstein, R. J. Collier (1998). Identification of Residues Lining the Anthrax Protective Antigen Channel, *Biochemistry*, 37, 3941-3948.
10. Klimpel K. R., N. Arora, S. H. Leppla (1994). Anthrax Toxin Lethal Factor Contains a Zinc Metalloprotease Consensus Sequence which is Required for Lethal Toxin Activity, *Mol. Microbiol.*, 13, 1093-1100.
11. Vitale G., L. Bernardi, G. Napolitani, M. Mock, C. Montecucco (2000). Susceptibility of Mitogen-activated Protein Kinase Kinase Family Members to Proteolysis by Anthrax Lethal Factor, *Biochem. J.*, 352, 739-745.
12. Duesbery N. S., C. P. Webb, S. H. Leppla, V. M. Gordon, K. R. Klimpel, T. D. Copeland, N. G. Ahn, M. K. Oskarsson, K. Fukasawa, K. D. Paull, G. F. Vande Woude (1998). Proteolytic Inactivation of MAP-kinase-kinase by Anthrax Lethal Factor, *Science*, 280, 734-737.
13. Nishida E., Y. Gotoh (1993). The MAP Kinase Cascade is Essential for Diverse Signal Transduction Pathways, *Trends Biochem. Sci.*, 18, 128-131.
14. Marshall C. J. (1995). Specificity of Receptor Tyrosine Kinase Signaling: Transient versus Sustained Extracellular Signal-regulated Kinase Activation, *Cell*, 80, 179-185.
15. Kyriakis J. M., J. Avruch (2001). Mammalian Mitogen-activated Protein Kinase Signal Transduction Pathways activated by Stress and Inflammation, *Physiol. Rev.*, 81, 807-869.

16. Treisman R. (1996). Regulation of Transcription by MAP Kinase Cascades, *Curr. Opin. Cell Biol.*, 8, 205-215.
17. Robinson M. J., M. H. Cobb (1997). Mitogen-activated Protein Kinase Pathways, *Curr. Opin. Cell Biol.*, 9, 180-186.
18. Schaeffer H. J., M. J. Weber (1999). Mitogen-activated Protein Kinases: Specific Messages from Ubiquitous Messengers, *Mol. Cell Biol.*, 19(4), 2435-2444.
19. Warfel J. M., A. D. Steele, F. D'Agnillo (2005). Anthrax Lethal Toxin Induces Endothelial Barrier Dysfunction, *American Journal of Pathology*, 166, 1871-1881.
20. Friedlander A. M. (1986). Macrophages are Sensitive to Anthrax Lethal Toxin through an Acid-dependent Process, *J. Biol. Chem.*, 261, 7123-7126.
21. Pannifer A., T. Wong, R. Schwarzenbacher, M. Renatus, C. Petosa, J. Bienkowska, D. Lacy, R. Collier, S. Park, S. Leppla, P. Hanna, R. Liddington (2001). Crystal Structure of the Anthrax Lethal Factor, *Nature*, 414, 229-233.
22. Borio L., D. Frank, V. Mani, C. Chiriboga, M. Pollanen, M. Ripple, S. Ali, C. DiAngelo, J. Lee, J. Arden, J. Titus, D. Fowler, T. O'Toole, H. Masur, J. Bartlett, T. Inglesby (2001). Death due to Bioterrorism-related Inhalational Anthrax: Report of 2 Patients, *JAMA*, 286, 2554-2559.
23. <http://www.rcsb.org/pdb/home/home.do>
24. Gasteiger E., C. Hoogland, A. Gattiker, S. Duvaud, M. R. Wilkins, R. D. Appel, A. Bairoch (2005). Protein Identification and Analysis Tools on the ExPASy Server, In: John M. Walker (Ed.): *The Proteomics Protocols Handbook*, Humana Press, 571-607.
25. Laskowski R. A., M. W. MacArthur, D. S. Moss, J. M. Thornton (1993). PROCHECK: A Program to Check the Stereochemical Quality of Protein Structures, *J. Appl. Cryst.*, 26, 283-291.
26. Elmar K., K. Guenther, V. Gert (2002). Increasing the Precision of Comparative Models with YASARA NOVA - A Self-parameterizing Force Field, *Proteins: Structure, Function, and Genetics*, 47, 393-402.
27. Laurie A. T. R., R. M. Jackson (2005). Q-SiteFinder: An Energy-based Method for the Prediction of Protein-ligand Binding Sites, *Bioinformatics*, 21, 1908-1916.
28. Nissink J. W., C. Murray, M. Hartshorn, M. L. Verdonk, J. C. Cole, R. Taylor (2002). A New Test Set for Validating Predictions of Protein-ligand Interaction, *Proteins*, 49, 457-471.
29. <http://pubchem.ncbi.nlm.nih.gov>
30. The PyMOL Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC.
31. Morris G. M., D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson (1998). Automated Docking using a Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function, *J. Computational Chemistry*, 19, 1639-1662.
32. Ritchie D. W. (2003). Evaluation of Protein Docking Predictions using Hex 3.1 in CAPRI Rounds 1-2, *Proteins: Structure, Function and Genetics*, Wiley-Liss Inc., 52(1), 98-106.

Pawan Kumar Jayaswal, Res. Assoc.Email: er.pawanjayaswal@gmail.com

Pawan Kumar Jayaswal is at present working as a research associate at National Research Centre on Plant Biotechnology, New Delhi, India. His Bachelor of Engineering degree in Biotechnology from RGPV University, Bhopal, India in 2007 and Master of Technology degree in Bioinformatics from SASTRA University, Tamilnadu, India in 2009. His research interests include next generation sequence data analysis, database development and *in silico* drug designing.

Ganesh Chandra Sahoo, Ph.D.E-mail: ganeshiitkcp@gmail.com, ganeshcs@icmr.org.in

He is Ph.D. in science from Jadavpur University, India in 2008. He has expertise in bioinformatics, virology and nanotechnology. Presently involved in research carried out at BioMedical Informatics Center of Rajendra Memorial Research Institute of Medical Science (RMRIMS), Patna, India. He is Editor and members of reviewing committee of various international journals related to bioinformatics, drug discovery, HIV, nanotechnology, next generation sequencing technology, databases. Guided more than fifty master students for their projects related to sequence analysis, microarray data analysis, protein structure analysis and virtual screening of different chemical compounds. PI and Co-PI of various projects related to biomedical informatics, nanotechnology and virology. Visiting faculty to various national universities in India. Attended various international workshops and conferences such as APBC and IUMS. Organized national level workshops on bioinformatics involving next generation sequence analysis by using NextGene program and SOLiD BioScope Software (ABI). Published more than 40 research articles in various international journals and conferences.

Pradeep Das, Ph.D.E-mail: drpradeep.das@gmail.com

He is Ph.D. from Central Drug Research Institute, India in parasitic immunology during 1981. Director of RMRIMS, Patna and NIPER, Patna. Various international projects are running under his leadership in RMRIMS and NIPER. Received various prestigious national and international awards. He has expertise in molecular parasitology, parasitic immunology, drug resistance mechanism; pathogenesis, cell signalling and oxidative stress. Published more than seventy papers in national and international journals during last five years.