doi.org/10.36721/PJPS.2020.33.5.REG.1939-1943.1

Molecular docking of the pneumococcal main autolysin (LytA) and deoxycholate ligand

Ghanbar Mahmoodi Chalbatani[1], Samira Karimaei[2], Davoud Afshar[3]*, Solmaz Ohadian Moghadam[4], Habibollah Mahmoodzadeh[1], Masoumeh Amini[2] and Mohammad Shirkhoda[1]

[1]Cancer Research Center, Cancer Institute of Iran, Tehran University of Medical Science, Tehran, Iran
[2]Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Microbiology and Virology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
[4]Uro-Oncology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Abstract: In the *Streptococcus pneumoniae*, the N-acetylmuramoyl-l-alanine amidase known as LytA protein is a main autolysin and in the presence of sodium deoxycholate, it activates and breaks *S. pneumoniae* cell wall. In the present study, the interaction between the LytA protein and deoxycholate as ligand was investigated. The Lyt A protein was retrieved from PDB databank and energetically minimized by Molegro Virtual Docker. The binding sites of LytA protein were detected and molecular docking carried out using MolDock algorithm. Finally, the number of hydrogen and electrostatic bonds were obtained for each predicted pose. A total of 5 binding sites predicted on LytA protein. The number of 5 predicted poses for each binding site also detected and molecular docking showed that all the poses have interactions (by H bonds) with deoxycholate. The interaction of the LytA protein with the deoxycholate ligand reveal five binding sites, which are involved in deoxycholate substrate recognition.

Keywords: LytA protein, sodium deoxycholate, autolysin, molecular docking, *Streptococcus pneumoniae.*

INTRODUCTION

Streptococcus pneumoniae is a Gram-positive bacterium and a common causative agent of bacterial meningitis, pneumonia and sinusitis. Pneumococcal LytA protein is one of the bacterium autolysins with the N-acetylmuramoyl-L-alanine amidase activity (Romero *et al*., 2004). Structurally, the length of protein has three different parts, which are associated with its physiological capacities such as involvement in cell division and daughter cell separation in microorganism. The LytA protein associated autolysis is occurred by degrading the lactyl-amido bond between the glycan strands of the peptidoglycan and stem peptides (Mellroth *et al*., 2012).

It has been shown that all of *S.pneumoniae* strains lyse when they grow in the presence of bile due to stimulation of bacterial amidase enzymes such as LytA protein (Murray, 1979, Obregón *et al*., 2002). The bile salt such as sodium deoxycholate can also use in the bile solubility test, frequently (Wessels *et al*., 2012). Other beta-hemolytic and non-hemolytic streptococci are negative for bile-solubility test, because they do not have dynamic autolytic compounds on their cell walls.

Molecular docking is one of the most commonly used approaches, which predict the best interaction or orientation among two proteins or other molecules including peptides and inorganic ligands at the atomic level (Kini and Evans, 1991, Meng *et al*., 2011, Sobolev *et al*., 1996). In other word, the method enables us to describe the atomic conduct of the target proteins at coupling sites.

The prediction of interaction between the LytA protein and deoxycholate as ligand can be interesting, because data can be evaluated in different studies including drug design. To the best of our knowledge, there aren't documented data that show the preferred orientations between LytA protein and deoxycholate molecule.

In this study, for the first time, the preferred interaction between the LytA protein and deoxycholate ligand was investigated.

MARTIALS AND METHODS

Retrieving structures

The X-ray crystallography structure of LytA protein was downloaded from protein data bank (PDB). The PDB ID of LytA protein was 4X36 with resolution of 2.101 Å; R-Value Free: 0.199 and R-Value Work: 0.183 (Li *et al*., 2015). The water molecules and unwanted interactions were eliminated from protein crystal structure. The deoxycholate (PubChem CID: 9548661) was also used as ligand and imported in Molegro Virtual Docker (MVD) software.

**Corresponding author:* afshar.d@zums.ac.ir

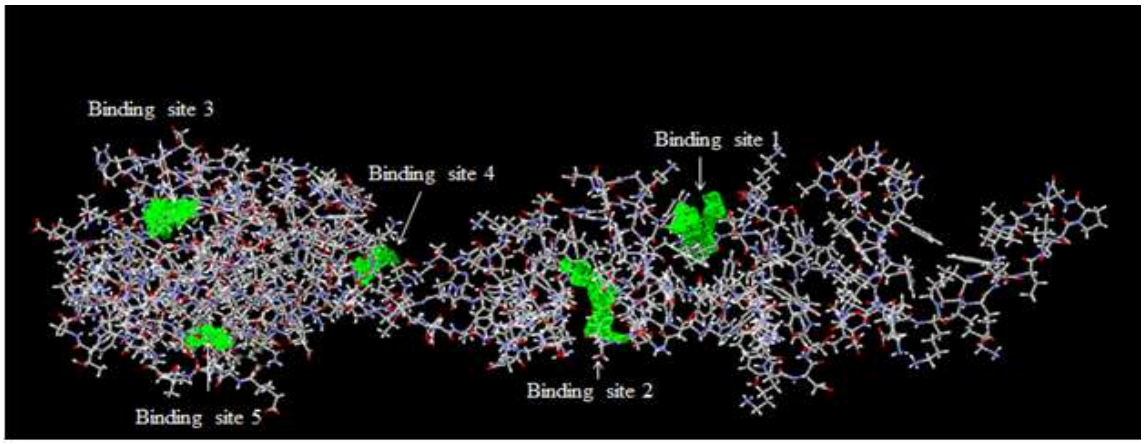

Fig. 1: Predicted cavities on LytA protein. A total of 5 binding sites or cavities were predicted based on software defaults algorithms.

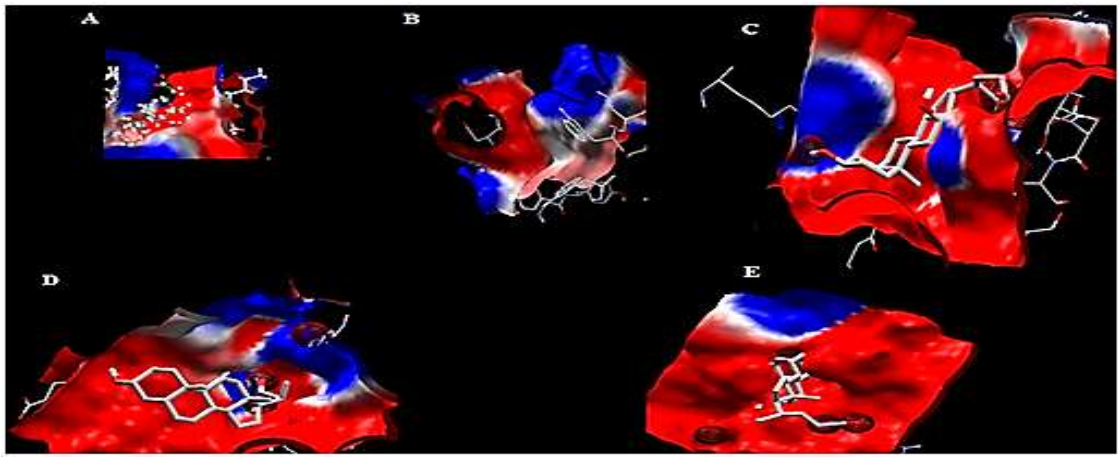

Fig. 2: Electrostatic interactions between LytA protein and deoxycholate ligand on the binding sites A-E.

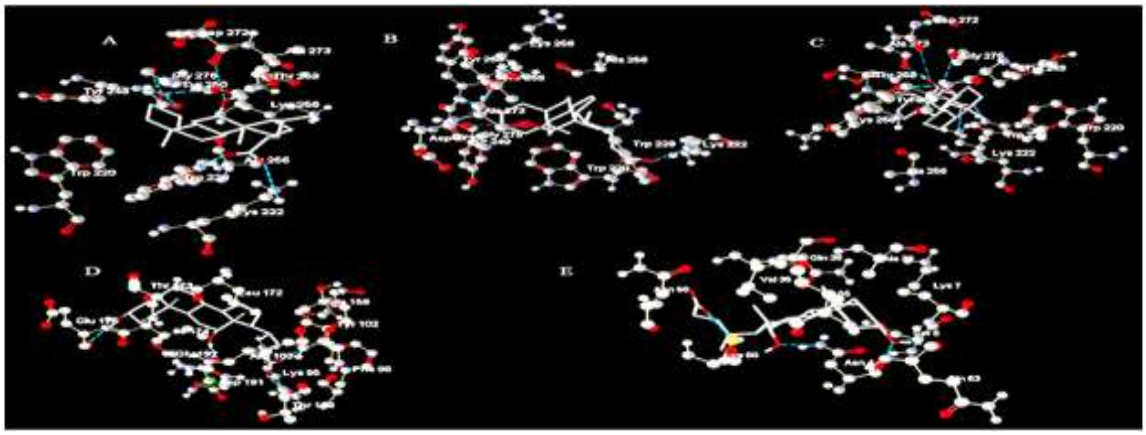

Fig. 3: Hydrogen interactions between LytA protein and deoxycholate ligand on the binding sites A-E

Molecular docking

Binding sites or cavities on the LytA protein were predicted based on software defaults algorithms and each cavity then docked against deoxycholate ligand for 10 runs (Scoring Function (Grid resolution (A): 0.30), Binding site radius: 12, Search algorithm: MolDock SE). In the next step, the file of docking results was imported to software and all of the predicted poses selected for subsequent analysis. Default settings were utilized for every all of the calculations. Number of H bonds and electrostatic energies for each pose were investigated.

Table 1: The number of binding sites and their properties

Number of binding sites	Position (X-Y-Z)	Volume A~3	Surface A~2
1	-8.78117 3.0542 -47.9681	65.024	235.52
2	-15.9568 6.22557 -36.0513	45.056	172.8
3	-63.6462 -3.6596 -23.6714	38.912	157.44
4	-40.0247 -7.66437 -26.324	38.4	143.36
5	-53.371 5.65544 -14.2097	28.672	87.04

Table 2: Scoring results for different poses of the ligand in each binding

Cavity	Pose name	MolDock Score	Rerank Score	HBond
1	[00]222_1	-240.683	-22.4402	-18.3013
	[01]222_1	-237.369	55.1633	-16.6201
	[02]222_1	-233.427	69.8157	-16.8042
	[03]222_1	-230.018	11.2425	-16.8278
	[04]222_1	-222.366	-41.3205	-5.76803
2				
	[00]222_1	-250.901	86.611	-20.2381
	[01] 222_1	-226.438	81.773	-15
	[02] 222_1	-224.695	20.4075	-6.05756
	[03]222_1	-204.654	61.0702	-12.6846
	[04]222_1	-189.319	-32.7649	-7.93047
3				
	[00]222_1	-232.341	-183.313	-11.3569
	[04]222_1	-227.04	-188.297	-8.56735
	[01]222_1	-225.833	-173.426	-15.5748
	[02]222_1	-219.852	-178.06	-13.5559
	[03]222_1	-219.824	-188.228	-6.74489
4				
	[00]222_1	-214.286	-151.289	-13.731
	[01]222_1	-206.677	-132.531	-14.9656
	[03]222_1	-205.489	-113.337	-5.74102
	[02]222_1	-201.026	-84.9622	-9.20169
	[04]222_1	-197.568	15.0048	-9.28098
5				
	[01]222_1	-155.007	39.8957	-2.17015
	[04]222_1	-154.659	60.2159	-3.68612
	[02]222_1	-151.812	509.19	-13.5058
	[03]222_1	-144.881	49.8834	-6.35752
	[00]222_1	-143.508	-44.1516	-3.51337

Table 3: The number of residues involving H bond between the LytA protein and deoxycholate

Binding sites	Residues in LytA protein
1	Asp272, Ala273, Thr259, Lys258, Gly276, Tyr250, Tyr249, Ala256, Trp228, Trp220, Lys222
2	Lys222, Trp228, Trp220, Ala 273, Gly276, Asp272, Tyr249, Thr259, Ala256, Lys258, Tyr 250
3	Asp272, Ala273, Gly276, Thr259, Tyr249, Tyr250, Lys258, Trp228, Trp220, Lys222, Ala256
4	Thr173, Leu172, Ile159, Glu175, Ile174, Tyr102, Gly192, Arg103, Asp191, Lys95, Phe98, Thr100
5	Gln36, Ala39, Asn58, Val35, Ile55, Lys7, Cys60, Asn4, Ser6, Gln63

RESULTS

The molecular docking showed that both LytA protein and deoxycholate ligand have the best binding affinity in the different cavities. A total of 5 binding sites predicted on LytA protein are presented on fig 1. All of the binding sites and their properties are also shown in table 1. The electrostatic and hydrogen interactions between the deoxycholate ligand and the LytA protein are shown in fig. 2 and fig. 3, respectively. The number of predicted poses and their properties including MolDock Score, Rerank Score and H Bonds are shown on table 2. Table 3 also shows the LytA protein residues which have H bonds with deoxycholate.

DISCUSSION

Worldwide, *S. pneumoniae* infections cause great morbidity and mortality among young children and elderly individuals. In this context, it is important to appropriately distinguish *S. pneumoniae* from other mitis group streptococci (Slotved *et al*., 2017). The WHO (World Health Organization) has recommended culture based method in combination with both the optochin susceptibility and bile or deoxycholate solubility tests for identification of *S. pneumoniae* (Satzke *et al*., 2013). Usually, the deoxycholate solubility test considers as an accurate method for differentiating *S. pneumoniae* from other streptococci.

The mechanism of lysis of pneumococcal strains in the presence of deoxycholate is due to the its activating the pneumococcal autolysins (Obregón *et al*., 2002). Autolysins are the bacterial cell wall hydrolytic enzymes that mediate antibiotic-induced cell lysis. LytA protein is the major autolysin of *S. pneumoniae* and has an N-acetylmuramoyl-L-alanine amidase activity. It hydrolyzed the lactyl-amide bond, which links the polypeptides and the glycan strands, resulting in cleaves of the peptidoglycan(Zhang, Zhang *et al*., 2018) . During the logarithmic growth only a small fraction of LytA protein is connected with the extracellular cell wall and this binding indicates its role in bacterium cell division. Bacterial expression of LytA protein and autolytic activities inhibit production of phagocyte-activating cytokines, which lead to phagocytosis inhibition (Mellroth *et al*., 2014).

In the present study, we have taken a structural approach to consider the mode by which the main pneumococcal autolysin LytA protein interacts with the deoxycholate ligand. Our data demonstrate that LytA protein has five binding sites for interaction with the deoxycholate ligand. The carboxyl-terminal domain of LytA protein has a pair of six repeated motifs, which are involved in recognition of the choline residues existing in the cell wall teichoic acids and binds exactly to these residues (GARCiA, GARCiA *et al*., 1988). Thus, this region probably is not suitable for interaction with the deoxycholate ligand. On the other hand, the amino-terminal domain has catalytic function, therefore this region probably interacts with the deoxycholate ligand. In our analysis, two binding sites were predicted (binding sites 3 and 5) in the amino-terminal domain; it seems that these two regions are likely to have interactions with the deoxycholate ligand. Moreover, the capacity to induce bacterium autolysis considers as the role of the N-terminal amidase (Zhang *et al*., 2018).

CONCLUSION

In this study, the interaction of the pneumococcal main autolysin known as LytA protein with the deoxycholate ligand confirmed, bioinformatically. The data presented here reveal five binding sites, which are involved in deoxycholate ligand recognition.

REFERENCES

Garcia E, Garcia JL, Garcia P, Arrarás A, Sánchez-Puelles JM and López R (1988). Molecular evolution of lytic enzymes of Streptococcus pneumoniae and its bacteriophages. *Proc. Natl. Acad. Sci. USA*, **85**(3): 914-918.

Kini RM and Evans HJ (1991). Molecular modeling of proteins: a strategy for energy minimization by molecular mechanics in the AMBER force field. *J. Biomol. Struct. Dyn.*, **9**(3): 475-488.

Li Q, Cheng W, Morlot C, Bai X-H, Jiang Y-L, Wang W, Roper DI, Vernet T, Dong Y-H and Chen Y (2015). Full-length structure of the major autolysin LytA. *Acta Crystallogr. D Biol. Crystallogr.*, **71**(6): 1373-1381.

Mellroth P, Daniels R, Eberhardt A, Rönnlund D, Blom H, Widengren J, Normark S and Henriques-Normark B (2012). LytA, major autolysin of Streptococcus pneumoniae, requires access to nascent peptidoglycan. *J. Biol. Chem.*, **287**(14): 11018-11029.

Mellroth P, Sandalova T, Kikhney A, Vilaplana F, Hesek D, Lee M, Mobashery S, Normark S, Svergun D and Henriques-Normark B (2014). Structural and functional insights into peptidoglycan access for the lytic amidase LytA of *Streptococcus pneumoniae*. *MBio.*, **5**(1): e01120-01113.

Meng X-Y, Zhang H-X, Mezei M and Cui M (2011). Molecular docking: a powerful approach for structure-based drug discovery. *Curr. Comput. Aided. Drug Des.*, **7**(2): 146-157.

Murray PR (1979). Modification of the bile solubility test for rapid identification of *Streptococcus pneumoniae*. *J. Clin. Microbiol.*, **9**(2): 290.

Obregón V, García P, García E, Fenoll A, López R and García JL (2002). Molecular peculiarities of the lytA gene isolated from clinical pneumococcal strains that are bile insoluble. *J. Clin. Microbiol.,* **40**(7): 2545-2554.

Romero P, López R and García E (2004). Characterization of LytA-like N-acetylmuramoyl-L-alanine amidases from two new *Streptococcus mitis* bacteriophages provides insights into the properties of the major pneumococcal autolysin. *J. Bacteriol.*, **186**(24): 8229-8239.

Satzke C, Turner P, Virolainen-Julkunen A, Adrian PV, Antonio M, Hare KM, Henao-Restrepo AM, Leach AJ, Klugman KP and Porter BD (2013). Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: Updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine,* **32**(1): 165-179.

Slotved H-C, Facklam RR and Fuursted K (2017). Assessment of a novel bile solubility test and MALDI-TOF for the differentiation of Streptococcus pneumoniae from other mitis group streptococci. *Sci. Rep.,* 7(1): 7167.

Sobolev V, Wade RC, Vriend G and Edelman M (1996). Molecular docking using surface complementarity. *Proteins,* **25**(1): 120-129.

Wessels E, Schelfaut JJ, Bernards AT and Claas EC (2012). Evaluation of several biochemical and molecular techniques for identification of Streptococcus pneumoniae and *Streptococcus pseudopneumoniae* and their detection in respiratory samples. *J. Clin. Microbiol.,* **50**(4): 1171-1177.

Zhang Z, Zhang X, Zhang L, Wang G, Zeng J, Zhang J, Hu X and Zhou Y (2018). A choline binding polypeptide of LytA inhibits the growth of Streptococcus pneumoniae by binding to choline in the cell wall. *J. Antibiot. (Tokyo)* **71**(12): 1025-1030.