Full Length Research Paper

Computational Prediction and Analysis of Interaction of Silver Nitrate with Peptidoglycan-Associated lipoprotein (Pal)

Mohammad Mehrasa[1], Sayed Rasoul Zaker[2], Behrouz Larki[3], Mohammad Mehmandoust[4], Hojjat Baghshahi[5], Mohammad Hadi Sekhavati[6], Shahin Gavanji[7]*

[1]Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran
[2]Department of Biology Faculty of Science University of Isfahan, Isfahan, Iran
[3]Department of Plant protection, Khorasgan (Isfahan) Branch, Islamic Azad University, Isfahan, Iran
[4]Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
[5]Department of Animal Sciences, College of Agriculture, Isfahan University of Technology (IUT), Isfahan, Iran
[6]Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran
[7]Young Researchers Club, Khorasgan Branch, Islamic Azad University, Khorasgan, Isfahan, Iran
*Corresponding Author: shahin.gavanji@yahoo.com

Abstract. Silver nitrate is an inorganic compound with chemical formula $AgNO_3$. Silver or silver ions have long been used in many areas due to their strong antimicrobial activity against pathogenic microbes such as bacteria, yeast, fungi and algae. The protein Pal (peptidoglycan-associated lipoprotein) is anchored in the outer membrane (OM) of Gram-negative bacteria and interacts with Tol proteins. We used Molegro virtual docker (MVD). The results obtained from docking showed us that the best pose which is derived from MolDock score for Chitinase was -23.4702 with reranking score equal to -33.6883. Bioinformatic studies show that silver nitrate has interaction with protein Pal.

1. INTRODUCTION

Silver nitrate is an inorganic compound with chemical formula AgNO3. Silver salts have antiseptic properties. Until the development and common adoption of antibiotics, dilute solutions of AgNO3 is used to be dropped into newborn babies' eyes at birth to prevent contraction of gonorrhea from the mother. Eye infections and blindness of newborns was reduced by this method, but incorrect dosage could cause blindness in extreme cases. This protection was used by Credé in 1881 for the first time (Credé, 1881). The antimicrobial properties of silver were first detected thousands of years ago when silver containers have been used to store water for preservation. In recent years, research has focused mostly on generated silver ions or colloidal silver electrolytically (Chambers et al., 1962). Therefore studies must both minimize the external factors affecting the concentration and to measure the changes in concentration that take place throughout the experiment. The researchers tested the effect of pH on the kinetics, finding that a higher pH increased the bactericidal action (Chambers et al., 1962; Gavanji

et al., 2011; Gavanji et al., 2012). Wuhrmann and Zobrist added that at a higher temperature, inactivation occurs faster (Wuhrmann and Zobrist 1958). Elemental silver and silver salts have been used as antimicrobial agents for a long time (Gavanji et al., 2012). Silver or silver ions have long been used in many areas due to their strong antimicrobial activity against pathogenic microbes such as bacteria, yeast, fungi and algae (Gavanji et al., 2013).

It may be used for controlling different plant pathogens in a relatively safer way compared to synthetic fungicides (Park et al., 2006). Until now, limited studies have provided pieces of evidence of the applicability of silver for controlling plant diseases (Park et al., 2006). Silver ions are very reactive, which are known to cause the inhibition of microbial respiration and metabolism as well as physical damage (Gavanji et al., 2013; Bragg and Rannie, 1974; Thurman and Gerba, 1989). Ionic silver has some disadvantages such as its high reactivity which made it unstable and thus easily oxidized or reduced into a metal depending on the surrounding environment. In addition, ionic silver causes discoloration by itself or allows other materials to

cause undesirable coloration and it does not continuously exert antimicrobial activity. Also, silver in the form of a metal or oxide, which is stable in the environment, is applied in a relatively increased amount due to its low antimicrobial activity (Park et al., 2006). In recent years, the use of silver as a biocide in the form of micro crystals or nanoparticles has grown significantly, as these preparations are useful against many resistant populations and 'biofilms' aggregates of microorganisms that grow on the surfaces of bodies of water and inside water pipes (Silver, 2003; Panyala et al., 2008; Gaidau et al., 2009).

Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism. Furthermore, it has been suggested that silver ions penetrate into bacterial DNA once entering the cell, which prevents further proliferation of the pathogen (Woo et al., 2009) The protein Pal (peptidoglycan-associated lipoprotein) is anchored in the outer membrane (OM) of Gram-negative bacteria and interacts with Tol proteins. Tol–Pal proteins form two complexes: the first is composed of three inner membrane Tol proteins (TolA, TolQ and TolR); the second consists of the TolB and Pal proteins linked to the cell's OM. These complexes interact with one another forming a multiprotein membrane-spanning system. It has recently been demonstrated that Pal is essential for bacterial survival and pathogenesis, although its role in virulence has not been clearly defined (Godlewska et al., 2009). The aim of present study was to study bioinformatic interaction of silver nitrate and Peptidoglycan-associated lipoprotein

2. MATERIALS AND METHODS

2.1. Preparing 3 dimensional structures of silver nitrate and Peptidoglycan-associated lipoprotein

In the first step, amino acid sequences of Peptidoglycan-associated lipoprotein of the *E. Coli* (Pal Protein- excC) with accession number of P0A912 were taken from NCBI website (www.ncbi.nlm.nih.gov/) (Figure 1). Then the Peptidoglycan-associated lipoprotein with the number of 1OAP was obtained from Protein Data Bank website (www.rcsb.com) (Figure 2). In the next step, Silver Nitrate with AgNo3 molecular formula (number 22878) was provided from ChemSpider website (www.chemispider.com) (Figure 2).

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MQLNKVLKGLMIALPVMAIAACSSNKNASNDGSEGMLGAGTGMDANGGNGNMSSEEQARLQMQQLQQNNIVYFDLDKYD
IRSDFAQMLDAHANFLRSNPSYKVTVEGHADERGTPEYNISLGERRANAVKMYLQGKGVSADQISIVSYGKEKPAVLGH
DEAAYSKNRRAVLVY
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Fig. 1: Amino acid sequence of structure of Peptidoglycan-associated lipoprotein
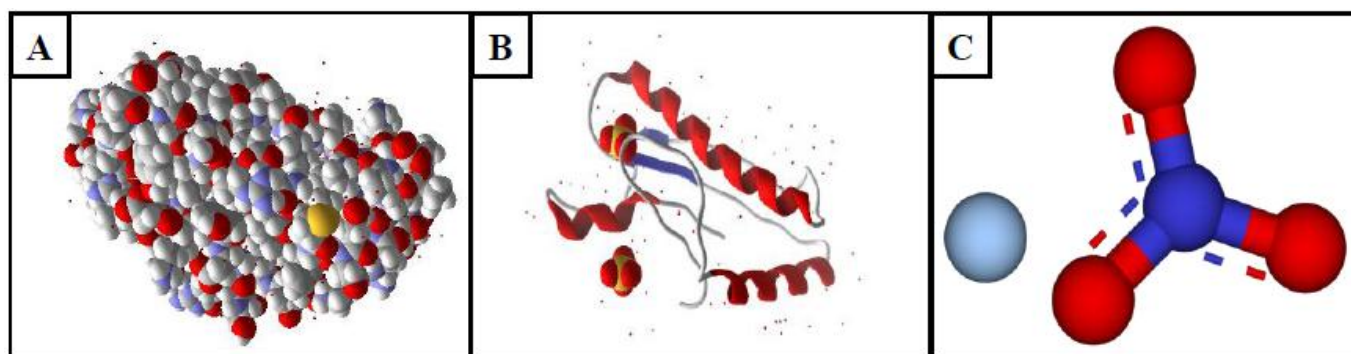

Fig. 2: A and B: Structure of Pal Protein, C: Structure of silver nitrate

2.2. Molecular docking study

Molegro virtual docker (MVD) 2011.4.3.0 is used for computer simulated docking study. Before initiation the docking operation, protein and ligand structures were prepared using MVD. For this purpose, charges assigned to the model of protein and ligands structures and flexible torsions in ligands were detected by this software (Gavanji et al., 2013) (Figure 3).
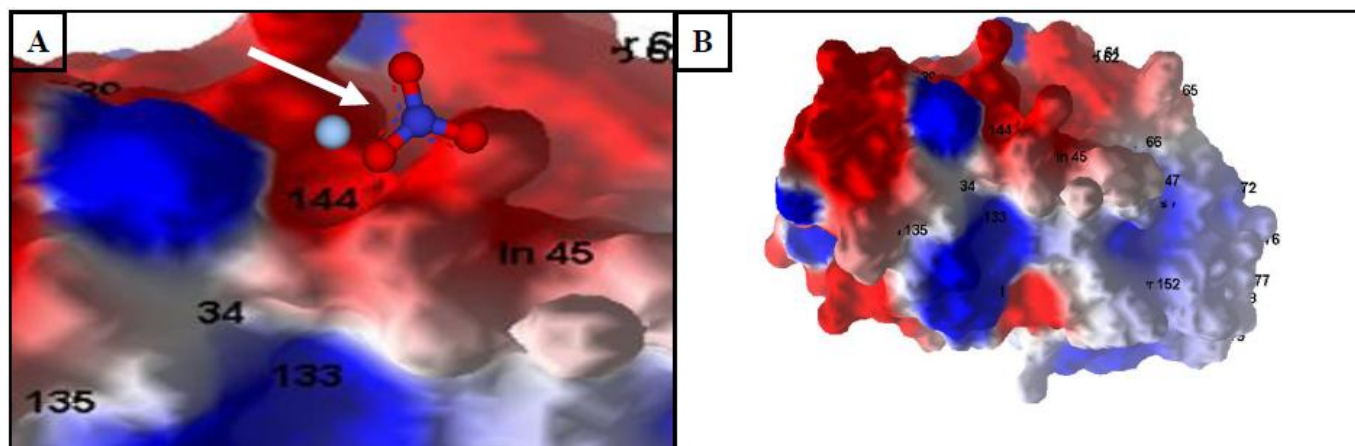
Fig. 3: The hydrophobic regions and negative charge. A and B: Pal protein

3.2. Finding ligand binding sites

3DLigand Site server (http://www.sbg.bio.ic.ac.uk) was used for prediction of potentially binding sites of Peptidoglycan-associated lipoprotein. In the server output GLU, LE, TYR, ASP, LEU, ASP , LYS, PHE, HIS, ALA, ASP, LEU, GLY, ARG, GLU, TYR, SER, LYS, ASN, ARG and ARG were predicted as present in binding site(Figure 5). The position and the percentage of each amino acide is shown in table 1. Also as an alternative approach, MVD is used for finding cavities of model. For this purpose, probe size was 1.2, max number of ray checks was 16, minimum number of ray hits was 12 and Grid Resolution was 0.8. Five cavities were found by MVD (Figure 4).
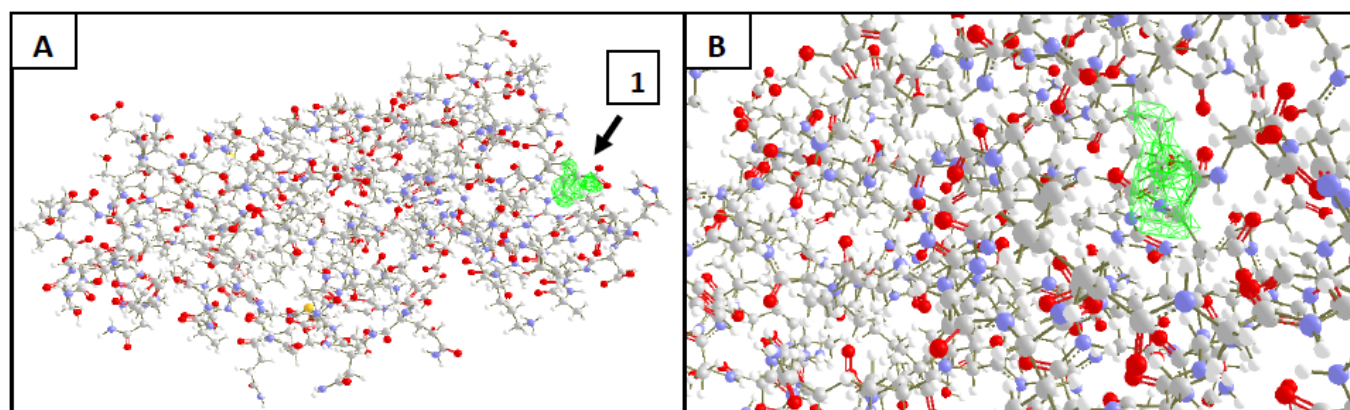

Fig. 4: Cavities of Periplasmic Domain predicted model. MVD used for cavity detection. Detecting parameters: probe size 1.2, max number of ray checks was 16, minimum number of ray hits 12 and Grid Resolution 0.8

MolDock score with a grid resolution of 0.30 Â was used as scoring function for docking (Figure 5). Internal electrostatic interaction and hydrogen bond between ligand and protein were permitted. MolDock SE was used as the docking algorithm and ten runs for ligands were carried out. After docking, energy minimization and optimization of hydrogen bonds were performed. The energy threshold was 100.00 and similar poses were ignored. Docking results are evaluated based on MolDock and reranking score. Rerank score is estimated for interaction. For the defined docking radius in Pal protein, the best pose which is derived from MolDock score for Pal protein was -23.4702 with Reranking score equal to -33.6883 (Table 2).

Cell envelopes of Gram-negative bacteria consist of three layers: a lipid–protein inner (cytoplasmic) membrane (IM), an outer membrane (OM) composed mainly of lipopolysaccharides and proteins, and a thin rigid layer of peptidoglycan (polymeric chains of N-acetylmuramic acid and N-acetylglucosamine linked by short peptides) located in the periplasmic space. Peptidoglycan has the ability to interact with many proteins of the cell envelope, conditioning their stability. One of these is the peptidoglycan-associated lipoprotein (Pal), which constitutes part of the Tol–Pal protein system. In the majority of Gram-negative bacteria, the Tol–Pal complex is composed of five core proteins: TolQ, TolR, TolA, TolB and Pal. The Tol–Pal system forms a membrane-spanning multiprotein complex that exhibits structural and functional similarities to the MotAB proteins (flagellar motor) and the TonB system (TonB, ExbB and ExbD) (Godlewska et al., 2009).
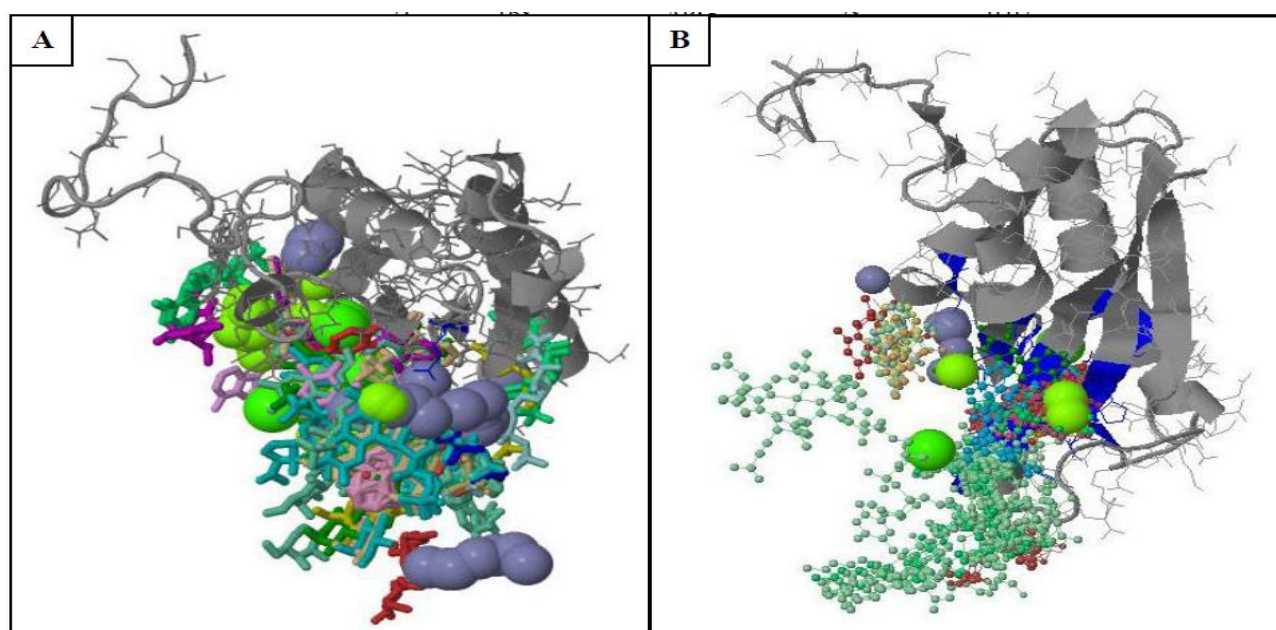
Fig. 5: Ligand bound to amino acids located in A and B position in Pal protein.

Table 1: Ligand binding sites by computing interactions using 3D Ligand Site server for Peptidoglycan-associated lipoprotein

No	Residue	Amino acid	contact	av distance
1	106	GLU	40	0.12
2	70	ILE	24	0.10
3	72	TYR	40	0.21
4	74	ASP	24	0.00
5	75	LEU	24	0.00
6	76	ASP	26	0.00
7	77	LYS	27	0.01
8	84	PHE	33	0.00
9	108	HIS	25	0.00
10	109	ALA	24	0.00
11	110	ASP	24	0.00
12	121	LEU	26	0.03
13	122	GLY	23	0.027
14	125	ARG	26	0.02
15	160	GLU	23	0.21
16	163	TYR	27	0.04
17	164	SER	25	0.00
18	165	LYS	23	0.01
19	166	ASN	26	0.00
20	167	ARG	26	0.02
21	168	ARG	28	0.02

4. CONCLUSION

Based on obtained results using Bioinformatic softwares, it is clear that silver nitrate can make an interaction with PAL. So in order for environment not to be damaged, it suggests that silver nitrate, due to its heavy interaction with proteins, should be applied extremely carefully in a lower doses.

Table 2: Binding energy level of five top poses of silver nitrate to Pal protein

| No | Ligand name | Pal protein | |
		MolDock Score	Rerank Score
1	silver nitrat	-23.4702	-33.6883
2	silver nitrat	-20.0614	-30.0089
3	silver nitrat	-20.1549	-30.1117
4	silver nitrat	-13.6203	-23.6203
5	silver nitrat	-22.8051	-22.0817

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Mohammad Mehrasa graduated in Biotechnology at MSc at the Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran.

Dr. Sayed Rasoul Zaker, Department of Biology Faculty of Science University of Isfahan

Behrouz Larki is MSc student at Isfahan (Khorasgan) Branch, Islamic Azad University. Behrouz Larki 's research has focused on Biology, Pharmacy, Linguistic and Translation.

Mohammad Mehmandoust, Department of basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, IRAN

Hojjat Baghshahi graduated in Animal Science, Department of Animal Sciences, College of Agriculture, Isfahan University of Technology (IUT), Isfahan, IRAN

Dr Mohammad Hadi Sekhavati, Assistant Professor, Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran.

Shahin Gavanji graduated in Biotechnology at MSc at the Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran. He has over 10 international medals in invention. Shahin Gavanji's research has focused on Pharmacy and Pharmacology, Nano Biotechnology, Bioinformatics, Biotechnology - Medical Biotechnology. He is editor in chief of International Journal of Scientific Research in Inventions and New Ideas.