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In silico molecular docking study of milk-derived peptide against biofilm forming *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

In this *in-silico* study lactoferrin was docked to the active site of four receptors of *Staphylococcus aureus* and two receptors of *Escherichia coli*. The rise of antimicrobial resistance highlights the significance of bioactive compounds as crucial therapeutic agents. The current study investigated on the binding energy of lactoferrin to these receptors by stabilising their structures. The receptors taken were with PDB IDs 3FRA, 3FYV, 3VUS, 2RKZ, 3GEU and 6F86. All the binding energy was negative which indicated that lactoferrin has activity against these receptors. The results revealed that lactoferrin bound to the fibronectin binding protein of S. aureus with least binding energy as -2.70 Kcal/mol and highest binding energy with oxidoreductase of *S. aureus* as -8.36Kcal/mol. These evidences showed that by proper synthesis and advances in designing of the lactoferrin structure to improve its stability it could be used as potential therapeutic agent against these organisms.

Keywords: Biofilm, Lactoferrin, Milk-derived peptide, *In-silico* docking.

INTRODUCTION

Recently, the adaptation and natural selection of bacteria, combined with the excessive use of antimicrobials have led to an increase in resistance [1]. The term biofilm denotes a collection of bacterial cells either from one cell or from various species adhering to surfaces of living or non-living and enveloped within an extracellular polymeric substance (EPS) [2]. The formation of biofilm involves several sequential steps, commencing with attachment of organism, followed by the secretion of EPS and signalling components responsible for intercellular communication. It terminates in the dispersion of cells into a planktonic state [3]. Biofilm architecture offers notable advantages by providing increased resilience against various stressors such as antimicrobials, predation, and toxic compounds [4]. These biofilms typically develop in high-density environments, where bacteria engage in communication and the regulation of their physiological processes through a phenomenon known as quorum sensing (QS). Quorum sensing facilitates intercellular interactions, allowing organisms to organize their social behaviours and exhibit various patterns of behaviour. The bacteria employ chemical signals called autoinducers (AIs) for communication, which are produced by bacterial organisms specifically for chemical signalling purposes [5]. The quorum sensing system can be categorized into two groups such as QS in Gram-negative bacteria, which follows the LuxI/LuxR type, and QS in Gram-positive bacteria, which involves oligopeptide/two-component-type sensor histidine kinases [6]. Among Gram-negative bacteria, the most widely recognized type of AI used is acylated homoserine lactone (AHL), while Gram-positive bacteria utilize peptides, specifically autoinducer peptides (AIP) [7]. Beyond these QS systems, there is another type of QS that exists in both Gram-negative and Gram-positive bacteria. This is known as Autoinducer-2 (AI-2), which serves as a non-species-specific AI, essentially acting as a "universal language" to enable communication both between different species and within the same species.

Goat milk exerts various effects on human health, taking into account its composition of total solids, fats, proteins, lactose, minerals, and vitamins [8]. The lipids found in goat milk enhance digestibility due to their small fat globule size and high content of fatty acids. Conjugated linoleic acids are more in goat milk which play pivotal part in stimulating immunity, promoting development, and preventing diseases. Among the significant effects of proteins present in goat milk is their ability to alleviate cow milk hypersensitivity, a common and potentially life-threatening food allergy, particularly in infants [9]. So, goat milk is used as replacement for individuals with allergies or sensitivities to cow's milk [10]. Furthermore, the ratio of β-casein to αs1-casein in goat milk proteins closely resembles that of human milk, resulting in better digestibility compared to other kinds of milk due to the higher susceptibility of β-casein protein to protease enzymes. In contrast, goat milk, which is rich in oligosaccharides, plays a crucial role in protecting the intestinal flora against pathogens and contributes to the development of the

brain and nervous system. Colostrum from dairy goats serves as a source of lactoferrin, providing several advantages, including its antimicrobial properties.

Lactoferrin, a cationic glycoprotein that binds to iron and belongs to the transferrin family in mammals, was first discovered several decades ago. This glycoprotein is extensively present in various organic fluids and is produced by cells responsible for immunity [11]. Lactoferrin serves multiple functions, including metal transport. However, it is also a crucial component of the nonspecific immune system due to its antimicrobial properties, effective against bacteria, fungi, and several viruses. Initially, its robust antimicrobial abilities were allocated to its capacity to seize vital iron, but it is now recognized that lactoferrin and lactoferrin-derived peptides directly interact to exhibit bactericidal effects [12]. Furthermore, lactoferrin plays a role in protecting against cancer development and metastasis. From a nutritional perspective, lactoferrin is noteworthy as a dietary source of amino acids and for its role in enhancing iron bioavailability [13]. In addition to its role as a dietary supplement, lactoferrin along with its derivatives are active against numerous clinical diseases which are resistant to conventional antibiotics [14]. Lactoferricin therapy is effective against urinary tract infections and has been applied as an oral treatment for irritable bowel syndrome [15].

There are several factors responsible for biofilm formation in bacterial organisms which includes fibronectin binding protein, dihydrofolate reductase, cell adhesion receptor, oxidoreductase, gyrase, glycoside hydrolase etc. These are essential for the attachment aswell as survival of the biofilm-forming bacteria. Once these structures get destroyed it will lead to disruption of the biofilm leading to suppression of bacterial growth [16].

Accordingly, the present paper focuses on *in-silico* molecular docking studies of lactoferrin, an antimicrobial peptide from goat milk against the receptors of biofilm forming bacteria, *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)*.

MATERIALS AND METHODS

In the present paper, in-silico molecular docking was used as a tool to predict the binding affinity of lactoferrin to receptors of *S. aureus* and *E. coli*.

Protein receptors / Macromolecules

Four receptors from *S. aureus* and two receptors from *E. coli* were selected from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (Figure 1). The crystal structure of fibronectin binding protein of *S. aureus* (PDB ID: 2RKZ), Dihydrofolate reductase of *S. aureus* (PDB ID: 3FYV), Cell adhesion receptor of *S. aureus* (PDB ID: 3GEU), oxidoreductase of *S. aureus* (PDB ID: 3FRA), Gyrase B (PDB ID: 6F86) and PgaB (PDB ID: 3VUS) of *E. coli* were downloaded. Receptors were viewed in Discovery studio 3.5 Client.

Ligand structure

Lactoferrin structure was obtained from PubChem, National Center for Biotechnology Information (NCBI). The structure was saved in SDF format and was cleaned in Marvin view software both in 2-dimensional and 3-dimensional view (Figure 2).

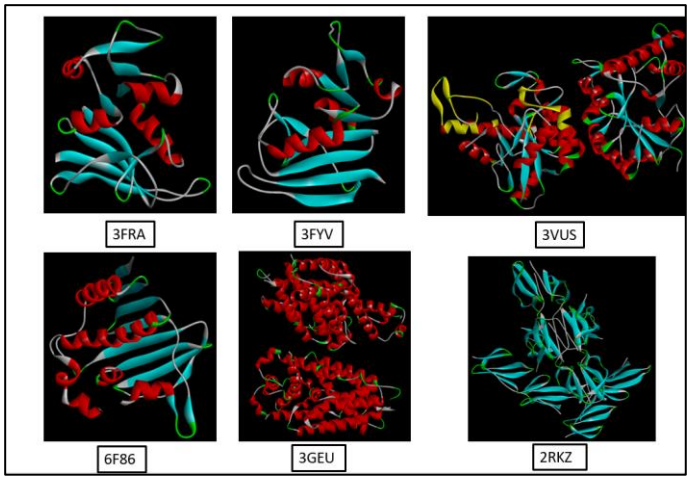

Figure 1: Structure of receptors from RCSB PDB

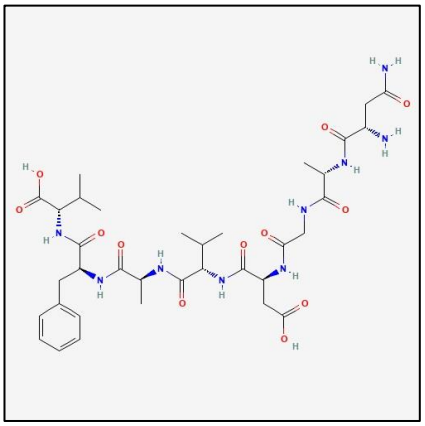

Figure 2: Structure of lactoferrin

Molecular docking

Lactoferrin was docked against these receptors using Autodock tools 1.5.6. The grid box coordinates for the receptor were adjusted to cover the binding cavity area for each receptor depicted in table 1.

Table 1: Grid map of each receptor used for docking

Receptor	Grid points			Central Grid points			Grid point spacing (A˚)
	X	Y	Z	X	Y	Z	
3FRA	40	40	40	24.7	11.7	38.8	0.531
3FYV	40	40	40	24.441	12.503	36.034	0.419
3VUS	40	40	60	15.063	12.863	35.683	0.619
6F86	40	40	40	67.364	31.987	54.425	0.48
3GEU	40	40	60	21.932	34.221	11.893	1
2RKZ	40	40	40	-3.01	9.518	-20.17	0.81

The docking tool utilized a Lamarckian genetic algorithm to generate ligand conformers and examined the docked position of the lowest binding free energy conformer with the receptors. For this analysis, we employed Discovery Studio 3.5 client to ensure the accurate binding of the ligand to the appropriate receptor binding pocket post-docking (Figure 3).

RESULTS AND DISCUSSION

The values of binding energy of lactoferrin against all receptors is tabularized in table 2. The value with more negativity indicates better binding between ligand and the target receptor. All values were negative with least binding energy value of -2.70 kcal/mol for fibronectin binding protein of *S. aureus* and highest binding energy value of -8.36 kcal/mol for oxidoreductase of *S. aureus*. The binding conformers is depicted in Figure 1.

The binding energy of lactoferrin with a value above -5 kcal/mol shows good binding energy with the receptors and can be more effective in the treatment of infections caused by biofilm forming bacteria. In this study the highest binding energy was -8.36 kcal/mol for oxidoreductase of *S. aureus*, which indicated that more affinity was shown towards this receptor. Thus, the results exhibited that biofilm inhibition of lactoferrin was more through oxidoreductase inhibition of *S. aureus*. The binding energy value with -2 kcal/mol would be having least affinity towards the lactoferrin which depicted a least effectiveness towards inhibition of fibronectin binding protein of *S. aureus*.

Table 2: Binding energy values of lactoferrin with receptors

Ligand	Receptors	Binding energy (Kcal/mol)
LACTOFERRIN	3FRA	-8.36
	3FYV	-7.97
	3VUS	-6.24
	6F86	-6.32
	3GEU	-4.17
	2RKZ	-2.70

In our in-silico study it was found that lactoferrin had a good capacity to act against biofilm forming bacteria and with specific modifications it could be used as a treatment for those diseases which are caused by these organisms.

Ongoing research is actively exploring the mechanisms responsible for the anti-biofilm effectiveness exhibited by lactoferrin and its derivatives [17]. The primary action of lactoferrin responsible for its antimicrobial activity appeared to involve binding and isolating environmental iron. These peptides essentially deprived the biofilm of this crucial nutrient, thereby restricting the biofilm's ability to thrive. However, certain studies indicated a more intricate interaction between lactoferrin and the biofilm, particularly considering that bacterial siderophores can extract iron from lactoferrin [18]. Laboratory assessments using iron-saturated lactoferrin didn't completely diminished the anti-biofilm potential of the original molecule which suggested the presence of additional mechanisms also governed its activity. Furthermore, lactoferrin exhibited an iron saturation mechanism that reduced its bactericidal effectiveness on planktonic bacteria compared to biofilm-grown bacteria [19].

These results showed another mechanism of action through binding and inhibition of receptors responsible for biofilm formation in bacterial organisms, thereby inhibiting the pathway involved in the synthesis of biofilm. Also, adherence to a surface is important for the biofilm growth which was demonstrated on *Pseudomonas aeruginosa* (*P. aeruginosa*) through inhibition of bacterial adhesion by lectin and increasing the bacterial movement [20]. The peptide also inhibited the attachment of enterotoxigenic *E. coli* on digestive tract of mice which was infected with the organism [21]. Amphipathic nature of the peptide also allowed penetration to the biofilm membrane thereby degrading the essential factors responsible for virulence and destroying the nutrition uptake affecting the metabolisms essential for its formation [22].

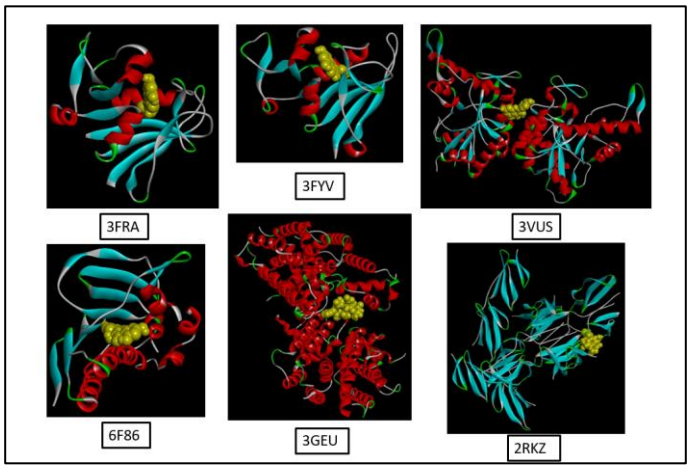

Figure 3: Discovery studio 3.5 images of receptor-ligand interaction. Ligand is represented as yellow balls

CONCLUSION

The increase of antibiotic resistance in bacterial species persists due to the ongoing inappropriate utilization of antibiotics. In an effort to find and create novel treatments, we performed virtual screenings of peptide and receptors. Molecular docking investigations provided valuable insights into how peptides bind to different receptors in *S. aureus* and *E. coli*, and these interactions were compared based on their binding energy values. This research has pointed lactoferrin as a promising candidate for drug development against organisms that form biofilms. As lactoferrin is versatile in nature it is attributable to numerous derivatives of peptide by modifying the N- and C-termini which can further increases its activity against organisms. This highlighted the likelihood of alternative modes of action for lactoferrin and its derivatives, potentially better suited for targeting biofilm growth. The N- and C- terminal truncation can lead to stabilisation of alpha helical structure. To verify the stability of lactoferrin, further *in-vitro* and *in-vivo* analysis is necessary. Validation on activity studies like broth microdilution on suitable medium and biofilm assessment on *in-vivo* surfaces also.

Conflict of interest

The authors declare that they have no conflict of interest.

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