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Research Article

The Interaction of AFB1 Aflatoxin and Lactococcin A; Molecular Docking

Arzu Özgen[1*], Nihan Ünlü[1], Canan Aksu Canbay[2]

[1] Istanbul Gelisim University, Vocational School of Health Sciences, Department of Medical Services and Techniques, Istanbul, Türkiye, aozgen@gelisim.edu.tr, nunlu@gelisim.edu.tr
[2] Firat University, Faculty of Science, Department of Physics, Elazig, Türkiye, caksu@firat.edu.tr
[*]Corresponding Author

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ABSTRACT

Aflatoxins (AF), which cause diseases in humans and animals, are mycotoxins produced by certain types of fungi. Bacteriocins are natural antimicrobial substances synthesized by bacteria. These substances that are in protein structure, generally have short chain and small molecular weight. According to the classification made by Klaenhammer, especially considering Gram (+) bacteria, bacteriocins are divided into 4 different classes. These are Class I (Class IA, Class IB), Class II (Class IIA, Class IIB, Class IIC, Class IID), Class III and Class IV. Enterocin A, Sakacin A, Lactococcin A can be given as examples of Class II bacteriocins. In this study, we examined the interaction of AFB1 aflatoxin (ligand) and Lactococcin A (protein) bacteriocin, which is in Class II, using Molecular Docking. The results showed that Lactococcin A molecule have the potential to be used for aflatoxin degradation.

1. Introduction

Mycotoxins, produced by fungi that can live in all ecosystems, including soil, are evaluated in the group of toxic secondary metabolites. Suitable ambient temperature and humidity promote fungal growth and toxin production. Mycotoxins are divided into six classes: aflatoxins, fumonisins, ochratoxins (OTA), trichothecenes, zeranol, and ergot alkaloids [1]. In particular, *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* species are aflatoxin-producing species. Mycotoxins contaminate various food and agricultural products and significantly threaten human and animal health [2]. Long-term exposure to aflatoxin may cause DNA damage, cancer, and developmental abnormalities in embryos [3]. According to the International Agency for Research on Cancer (IARC), many mycotoxins that have been officially proven to be carcinogenic to humans are classified as Group 1 (Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2)), and Group 2B (OTA, FB1 and FB2, AFM1) [4] and ranked as AFB1>AFG1>AFB2>AFG2 according to the level of toxicity [5].

AFB1 is one of the most potent naturally occurring carcinogens and possesses hepatotoxic, immunotoxin, and teratogenic properties [6-7]. AFB1 is the most common type of aflatoxin and accounts for 75% of all aflatoxins in food and feed contamination [8]. Cereals, millet, rice, sorghum, hazelnuts, and legumes contaminated with AFB1 pose a risk of liver cancer due to AFB1 exposure, especially in children. Children, especially infants and young children, are at a higher risk of exposure to this toxin due to their immature metabolic pathways, higher intake-to-body weight ratio, more rapid metabolic rates, and lesser detoxification capability. Studies have shown that mycotoxins can have more harmful effects on children's health than on adults [9,10]. In a study, AFB1 was detected in at least 47% of popcorn samples, and it was reported that there was a risk of liver

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cancer due to the consumption of AF-contaminated popcorn [11].

Today, physical, chemical and biological methods are used to prevent the formation of aflatoxins in food and feed or to reduce toxin production. In addition to being inefficient, physical and chemical methods have disadvantages such as being costly and causing loss of nutritional value [12]. At the same time, very few of the physical and chemical techniques meet the Food and Agriculture Organization (FAO) criteria [13]. Plant extracts, microorganisms and enzymes are used in biological procedures, which is the most preferred method [14], and many studies in this context suggest the use of probiotic strains in particular [15].

Bacteriocins are antimicrobial peptides (AMP) encoded by a gene in the bacterial genome and thus synthesized via ribosomes. Bacteriocins have a low molecular weight. Bacteriocins have the potential to be used in various fields of application, including the food industry, livestock industry, medicine and agriculture. Many gram-positive and gram-negative bacteria produce bacteriocins [16]. Especially bacteriocins produced by lactic acid bacteria (LAB) are important in food industry applications [17].

Bacteriocins, which do not pose a danger to humans, do not change the nutritional value of foods, are effective even at low concentrations and do not lose their activity under storage conditions [18]. Bacteriocins are examined in 4 classes according to their molecular size, chemical structures, mechanisms of action and heat stability properties. Class IV bacteriocins have not been adequately characterized [19]. Class II bacteriocins are small peptide sequences that do not contain lanthionine and are divided into four subclasses: IIa, IIb, IIc, and IId [20]. Class II bacteriocins are heat-stable, and often synthesized as pre-bacteriocins containing an N-terminal signal sequence that is cleaved during secretion [20-22].

Numerous studies show that LAB can neutralize aflatoxins [23]. In addition to being an antagonist against fungi, LAB also acts as an antidote against mycotoxins, and the exact mechanisms of the process remain unclear. However, research suggests that the complex on the cell surface of LAB, which comprises teichoic and lipoteichoic acids, S-layer proteins, and exopolysaccharides, plays a crucial role in the adsorption of mycotoxins [24].

Lactococcus lactis subsp. *lactis*, a lactic acid bacterium (LAB) is widely used in fermented milk products [25]. Lactococcin A, belong to the class IId group synthesized by Lactococcus lactis subsp. lactis. The pore-forming class IId bacteriocin lactococcin A, [26] targets The mannose phosphotransferase system (man-PTS) [27].

In this study, the interactions of AFB1 and Lactococcin A were studied using the molecular docking method. Our study is essential in laying the groundwork for other in-vitro and in-vivo studies. The existence of the interaction between AFB1-Lactococcin A may enable the detoxification of this dangerous aflatoxin species. Therefore, it will contribute to the protection of human and animal health and prevent economic losses.

2. Materials and Methods

2.1.Bioinformatic analysis of Lactococcin A

The protein sequence of the Lactococcin A was obtained from the NCBI Gene Database (https://www.ncbi.nlm.nih.gov/protein/5LFI_A) (access date: 01.02.2024). The chemical properties of Lactococcin A protein were examined using the Expasy online server (http://web.expasy.org/protparam/) address (access date: 04.02.2024), sourced from (Swiss Institute of Bioinformatics, Zurich, Switzerland). The Lactococcin A protein predicted subcellular location was displayed using the online server DeepLoc-2.0 ((https://services.healthtech.dtu.dk/services/DeepLoc-2.0/) (access date 07.02.2024). Conserved motifs of Lactococcin A were obtained from NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (access date: 09.02.2024).

2.2. Molecular docking

Lactococcin A (PDB ID: 5LFI) molecule structure was obtained from https://www.rcsb.org/. The pdb file of the 5LFI molecule was performed using chain A and transferred to AutoDockTools (ADT ver.1.5.6). H_2O molecules were removed and the pdbqt files of the proteins were constructed. The Aflatoxin B1 (PubChem CID: 186907) ligand chemical structure was obtained from https://www.ncbi.nlm.nih.gov/. Torsions of the ligand were processed and the pdbqt files of the AFB1 were constructed by program.

The molecular docking study was performed using Autodock 4.1 [28]. Each docking was performed according to standard process [29]. Their analyzes and visuals were acquired with the Biovia Discovery Studio Visualizer 2021 program.

3. Results and Discussion

Globally, approximately 25% of the food produced is contaminated by mycotoxins, and this causes significant economic loss [30]. Aflatoxins are one of the most common and most dangerous food pollutants [31]. The cancer-causing and immunosuppressive potential of Aflatoxin B1 (AFB1), which is considered highly harmful, has been reported in many animal species, including poultry [32], trout [33], cattle [34], and rats [35, 36]. Toxicity in humans has been evaluated as different outbreaks of acute poisoning, particularly in developing countries [37]. Today, studies are showing that aflatoxin degradation can be possible using bacteriocins, but they are limited in number. It has been reported that AFB1 can be highly effectively detoxified by bacteriocins [38]. Previous studies were carried out under laboratory conditions. With the advancing computer technology and biotechnology, the structures of molecules are understood in more detail, and the interactions of molecules with each other are shown by computer simulation. In this way, the interaction of many molecules can be determined, and time and material can be used effectively.

Molecular docking can predict binding sites. It can also provide a reference for characterizing the thermodynamic and dynamic changes of intermolecular interactions by simulating the spontaneous binding of ligands to receptors. Therefore, molecular docking verifies experimental results at the molecular level and guides the actual experiment [39]. In food safety research, molecular-level studies of drug residues, biotoxins, and foodborne pathogens are gaining momentum, and molecular docking can be widely used for these studies [40].

The protein properties of the bacteriocin Lactococcin A are given in Table 1. Additionally, the conserved domain is shown in Figure 1. This region is called the LciA-like superfamily domain.

Table 1. The protein properties of the bacteriocin Lactococcin A

Species	*Lactococcus lactis* subsp. *lactis*
Protein description	Lactococcin A
Conserved domain LciA-like: pfam08951	36→96
Number of aa	117
Mw (kDa)	13,249.16
pI	9.52
SL	Cytoplasm

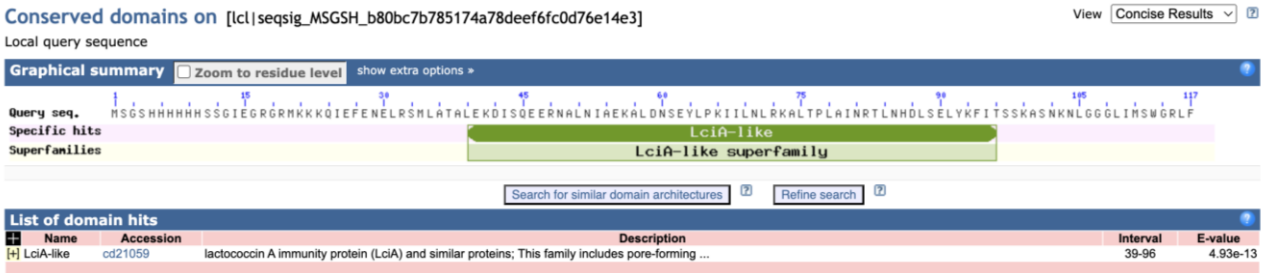

Figure 1. The conserved domain of the bacteriocin Lactococcin A

The existence of interaction between these two molecules, Lactococcin A as the receptor and interaction between Lactococcin A and AFB1. A conventional hydrogen bond between -O atom in AFB1 and Lys-66 residue (Lenght: 2.09 Å) was found by moleculer docking. A carbon hydrogen bond exists between -C atom in AFB1 and Glu-62 (Lenght: 3.56 Å) residue. A π-sigma interaction between AFB1 and Ile-53, a π-anion interaction between AFB1 and Glu-62, and π-AFB1 as the ligand, was investigated by using molecular docking method. Figure 2 shows the cation interaction between AFB1 and Lys-66 residue were found by moleculer docking. It has been observed that there is an interaction between AFB1 and amino acids (36→96) in the protected region called the LciA-like superfamily. These are amino acids 53, 57, 62, 66, 67, 69, 70, 73, respectively.

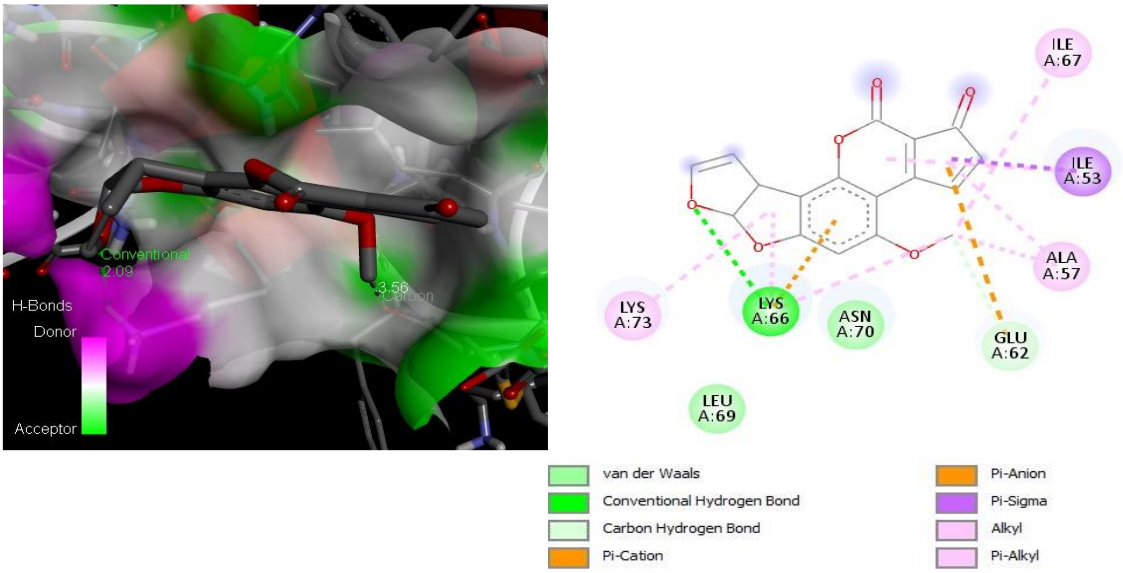

Figure 2. 3D and 2D visualizations of interaction of the ligand with the amino acids of the Lactococcin A binding site with discovery studio

The binding energy values (ΔGbind) of the docked positions, intermolecular energy, electrostatic energy and total internal energy between Lactococcin A and AFB1 are listed in Table 2. According to the results, AFB1 interacts with the active site of Lactococcin A and shows high binding affinity.

The binding surface is between the ligand (AFB1) shown in light yellow and the receptor (Lactococcin A) (Figure 3). On Lactococcin A, dark gray represents carbon atom, light gray represents hydrogen atom, and red represents oxygen atom, blue nitrogen, yellow sulfur.

Table 2. Molecular docking analysis of Lactococcin A and the AFB1

Protein	Lactococcin A
Ligand	$C_{17}H_{12}O_6$
Binding Energy/ΔG (kcal/mol)	-4.89
Intermol energy (kcal/mol)	-5.19
Electrostatic energy (kcal/mol)	-0.33
Total internal energy (kcal/mol)	-0.1

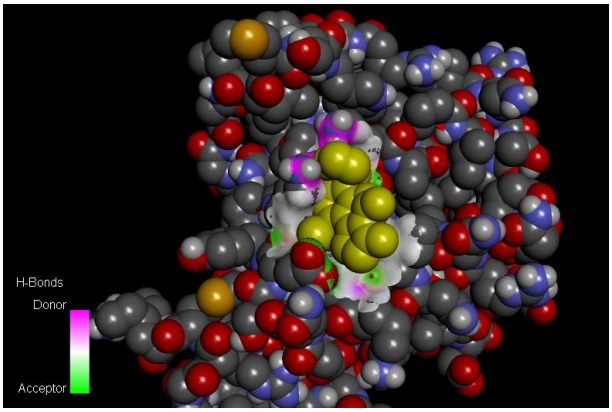

Figure 3. The binding sites in the pocket of protein (5LFI)

4. Conclusion

As a result, it is of great importance that the harvested agricultural products are purified from factors that threaten human and animal health, such as aflatoxins, considering the increasing human population, decreasing agricultural lands and the problems experienced in global food access. Biological methods are safer than other physical and chemical methods for the removal of aflatoxins from food, which cause cancer and other health problems. Mycotoxins are a severe problem in the food chain, and aflatoxin detoxification studies using lactic acid bacteria and their metabolites (bacteriocins) will accelerate. According to the results obtained in this study, it has been shown that Lactococcin A molecule have the potential to be used for aflatoxin degradation.

Article Information Form

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This study does not require ethics committee permission or any special permission.

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