Journal of Materials and Electronic Devices 3 (2023) 28-31

Interactions of AFB2 Aflatoxin and Cinnamycin bacteriocin by Molecular Docking

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Aflatoxins, produced by fungi, can be found in various environments and cause severe harm to humans and animals. The most notable type is AFB2, which has a cyclopentane ring and is present in human and animal milk. AFB2 is produced by molds like *A. flavus* and *A. parasiticus* among the 18 identified types. Aflatoxin B2 has been linked to liver damage and classified as carcinogenic, teratogenic, and immunosuppressive. On the other hand, Class IB bacteriocins are negatively and uncharged, with antimicrobial properties that inhibit specific enzymes. This paper investigated the interaction between AFB2 and Cinnamycin using the molecular insertion method. This study determined that the interaction between AFB2 and cinnamycin resulted in a binding energy (Gibbs free energy, ΔG) of -5.01 kcal/mol, indicating that the reaction is exothermic and occurs voluntarily.

Keywords: *Aflatoxins, Bacteriocins, Molecular docking, Cinnamycin*

Submission Date: 30 June 2023
Acceptance Date: 19 August 2023

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1. Introduction

Mold fungi can create harmful mycotoxins in crops, especially when exposed to certain environmental conditions like humidity and temperature. The most toxic mycotoxins are called aflatoxins, which have low molecular weight and are synthesized by some Aspergillus species [1, 2]. Aflatoxins (AFs) are the most potent natural chemical carcinogen, consisting of a coumarin and double furan ring. They have been known to cause hepatocellular carcinoma, chronic hepatitis, Reye's syndrome, and aflatoxicosis [3]. Corn, millet, rice, peanuts, hazelnuts, figs, ginger, coconut, and milk are particularly susceptible to *Aspergillus flavus, Aspergillus parasiticus*, and *Aspergillus nomius* contamination [4, 5]. These fungi can produce aflatoxins, which can harm the liver. Improper agricultural practices and environmental factors can contribute to the spread of aflatoxin contamination [6].

One of the dangerous fungal toxins that can lead to cancer and other diseases is AFB2 (Figure 1) [7, 8]. This toxin is produced as a secondery metabolite by various types of Aspergillus fungi, including *A. flavus*, *A. arachidicola*, *A. nomius*, *A. minisclerotigenes*, and *A. parasiticus*, and is a dihydro derivative of AFB1 [3]. AFB2 is commonly present in sunflower seed, cottonseed, peanuts, groundnut, pistachio, maize, rice, sorghum, barley, and wheat, making it a frequent contaminant in food [7, 8, 9, 10]. Studies have shown that AFB2 can cause sister chromatid exchange, DNA damage, and cell transformation [11].

To minimize the risk of exposure to aflatoxin, there are certain practices that can be followed before and after harvesting. By implementing Good Manufacturing Practices (GAPs) like proper harvesting techniques and storage conditions with low moisture content and temperature, mold growth and aflatoxin production in grains can be prevented. However, to completely eliminate the risk of contamination, post-harvest intervention is necessary [12]. Aflatoxins are highly resistant to cooking, frying, baking, and roasting as their decomposition temperature ranges between 237 and 306 °C [13]. Fortunately, there are physical, chemical, and biological methods available to break down aflatoxins in food [14]. Biological techniques, such as microbial and enzymatic conversion, can effectively transform aflatoxins into non-toxic or less toxic metabolites [15].

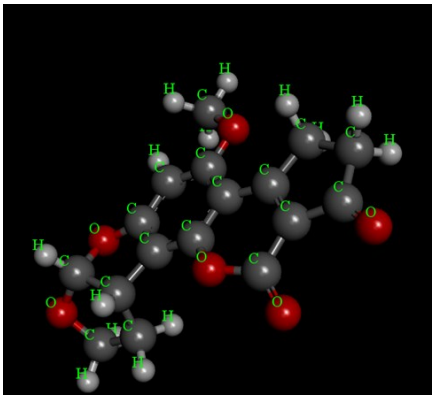


Fig. 1. Molecular structure of AFB2.

Cinnamycin, a Class IB bacteriocin, is synthesized by *Streptomyces griseoverticillatus* (synonym: *Streptomyces cinnamoneus*), a Gram-positive bacteria species. Class I bacteriocins are small peptides of 19–38 amino acids in length with lanthionine or β-methyllanthionine residues, also called lantibiotics, and contain antibiotics [16]. Cinnamycin, which have a compact globular electrically neutral peptide structure, have a one Lan and two MeLan residues in addition to an unusual lysinoalanine (Lal) formed from lysine 19 and serine 6 [17, 18, 19]. Cinnamycin's precursor peptide, CinA (Figure 2), consists of 19 amino acids (CRQXCSFGPFXFVCXGNXK) and is converted to cinnamycin by post-translational modifications [20].

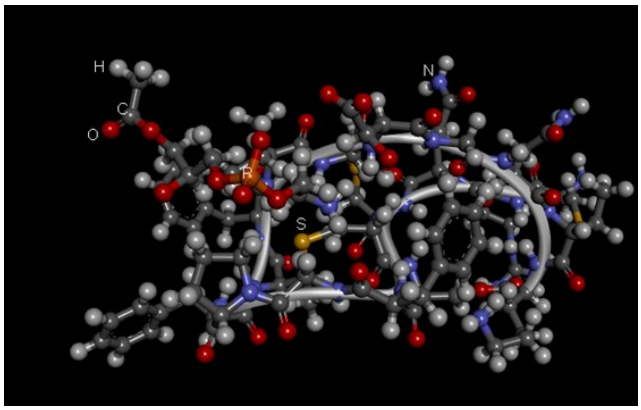


Fig. 2. Molecular structure of Cinnamycin's precursor peptide, CinA.

In this study, protein-ligand interactions of AFB2, which poses a threat to human and animal health, and Cinnamycin, a Class IB bacteriocin, were studied by molecular docking method.

2. Experimental

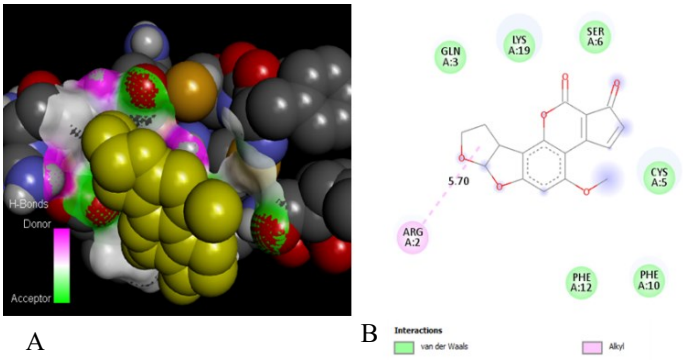
The Cinnamycin protein structure, identified by PDB ID 2DDE, was obtained from The Protein Data Bank. Chain A of the protein was selected and prepared in PDB file format using AutoDockTools (ver.1.5.6). H_2O molecules were removed and the protein files were saved in pdbqt format. Aflatoxin B2 ligand (PubChem CID: 2724360) was obtained from the National Library of Medicine. The ligand's torsions were examined and the files were saved in pdbqt format using AutodockTools. We performed molecular docking using Autodock 4.1 [21] following standard Autodock steps [22]. We analyzed the most suitable binding modes obtained from the molecular docking process using Biovia Discovery Studio Visualizer 2021 program.

3. Results and Discussion

With the significant development of studies at the molecular level and the integration of the obtained molecular structures into the computer environment, the interaction of many molecules to be carried out in the laboratory environment is primarily investigated by computer simulation. In this way, information about parameters such as possible interactions of molecules with each other, binding energies, physicochemical properties and biological activities can be obtained [23,24].

In this study, the binding energy (Gibbs free energy, ΔG) resulting from the ligand-protein (AFB2-cinnamycin) interaction was calculated as -5.01 kcal/mol. A negative result indicates that the reaction is exothermic and occurs voluntarily. The figure 3A,B shows the interaction between

AFB2 and cinnamycin. In Figure 3A, the interaction surface formed between the ligand (AFB2) represented in light yellow and the receptor cinnamycin is shown. Here, the ligand appears to be completely embedded in the receptor. On Cinnamycin, dark gray represents carbon atom, light gray represents hydrogen atom, red represents oxygen atom, blue represents nitrogen, yellow represents sulfur.



A B

Fig. 3. A-3D visualizations of the interaction of the ligand with the amino acids of the Cinnamycin binding site with discovery studio. B-2D ligand-protein interaction.

The presence of alkyl and pi-alkyl bonds can enhance the hydrophobic interaction of a ligand within a receptor's binding pocket, as supported by research [25]. In the case of AFB2, it forms six van der Waals interactions with specific residues (Gly3, Cys5, Ser6, Phe10, Phe12, Lys19) as shown in Figure 3-B. These van der Waals forces are important in protein-ligand complexes, and scientific studies have demonstrated their significance in determining the binding affinity of the ligand to the protein [26, 27].

The values of intermolecular energy, electrostatic energy, total internal energy, and ΔGbind for the docked positions of AFB2-cinnamycin are listed in Table 1.

Table 1. Molecular docking analysis of AFB2 and cinnamycin

Protein	Cinnamycin
Ligand	$C_{17}H_{14}O_6$
Binding Energy/ΔG (kcal/mol)	-5.01
Intermol energy (kcal/mol)	-5.3
Electrostatic energy (kcal/mol)	-0.13
Total internal energy (kcal/mol)	-0.13

According to the results, AFB2 interacts with the active site of Cinnamycin and showed high binding affinity.

4. Conclusions

Aflatoxins, which have the potential to be found in many types of food and feed, cause both serious health problems and great economic losses in domestic and foreign trade. In vitro research on the detoxification of aflatoxins is both costly and time-consuming. Ligand-protein interaction studies are carried out rapidly with molecular docking-based studies. This study showed how AFB2 and cinnamycin, a type of bacteriocin, interact with each other. The findings of this research could be useful in further studies on how to break down aflatoxin.

Acknowledgement

We thank Assoc. Dr. Ali Kemal Garip for his help with the molecular docking.

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