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Biogenic synthesis and antibiofilm efficacy of iron nanoparticles via computer simulation

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Abstract

The search for new drugs can be accelerated by in silico methods, i.e., fully computational methods known for their speed and low cost, allowing the analysis of a large amount of data, e.g., thousands of possible antimicrobials, in a few weeks. Molecular docking and first-principles calculations are great allies in this quest. They enable the assessment of protein-ligand interactions and can predict interactions between NPs and macromolecules to provide more information about the interactions and dynamics of NPs in biological systems. In this context, this work aims to use in silico methods to detect the formation of biogenic metallic nanoparticles from functional microalgal biomolecules of the genus Chlorella, which have chelation of metal ions as a fundamental property, and to verify the possible antibacterial biofilm efficacy using computational tools such as molecular docking. In a first analysis, it was found that the iron salt $FeSO_4$ was the most suitable to bind the microalgal enzyme and produce its phytochelatin protein. Following this result, an analysis of the electronic structure of the phytochelatin complex with the iron salt was carried out, proving its structural modification at the nanometric level, after which an analysis of its therapeutic effect on antibiofilm activity was performed. S. aureus, a bacterium known for its multiresistant to antibiotics, these results demonstrate, through alternative in silico methods, the physiological role of phytochelatin from microalgae in the detoxification and bioremediation of metallic contaminants.

Keywords: Microalgae, Nanoparticle, Phytochelatin, in silico.

1. Introduction

Many methods adopted in the field of nanoparticle synthesis are chemical and physical. Currently, the biological method of nanoparticle synthesis is a growing technique in the field of nanotechnology, in which it fits into the green synthetic processes, which do not use reagents or processes that are harmful to the environment. Among biological materials, algae are the most used as bionanofactories, due to their biomass that is used in the production of metallic nanoparticles. Its biomass is a low-cost, environmentally friendly, and efficient macroscopic structured material, and it has a distinct advantage due to its high metal absorption capacity (Jacob *et al.*, 2021).

Microalgae are photosynthetic beings, valuable natural sources of products used in consumer goods and

biotechnological applications. The broad spectrum of high-value microalgae-derived products currently includes fatty acids, pigments, and enzymes, as well as microalgal biomass, which is considered a healthy supply of food and raw material for the pharmaceutical industry. Microalgal biomass can catalyze the production of gold, palladium, rhodium, and silver nanoparticles (among others) when in the presence of an appropriate metal ion source. They have a wide tolerance range for temperatures, salinities, pH, and different light intensities (Jacob *et al.*, 2021).

The search for new drugs can be accelerated through *in silico* methodologies, that is, fully computational, known for their speed and low cost, allowing the analysis of a large amount of data, such as thousands of possible antimicrobials, in a few weeks. Molecular *Docking* and first-principles calculations are great allies in this search, allowing the evaluation of the interaction between protein and ligand, capable of predicting interactions between NPs and macromolecules, in obtaining more information about the interactions and dynamics of NPs within biological systems.

In this context, this work aims to prove, by *in silico* methodologies, the formation of biogenic metallic nanoparticles, from functional microalgal biomolecules of the Chlorella genus that have the chelation of metallic ions as a fundamental property and to verify the possible antibacterial biofilm effectiveness through of computational tools such as molecular docking.

2. Methodology

For the development of this work in silico methodologies were used (theoretical study - computer simulation).

2.1 in silico study

In this study, computer simulations were performed to analyze the interaction between iron sulfate and microalgal proteins. This analysis was performed in two steps: (1) Molecular interactions by molecular docking and (2) calculation via first-principles methodology.

2.1.1 Molecular *Docking*

The 3D structures of the proteins used in the simulation were acquired from the PDB (Protein Data Bank) database (Berman *et al.*, 2000), the places used for docking were generated from the Deepsite® software through a neural network predicts which are the best binding sites for the protein of interest. The 3D structures of the ligands such as iron sulfate (FeSO4) were created using the Chemcraft® software and their geometry was optimized using the Avogadro® software (Snyder and Kucukkal, 2021). The computer code applied in the simulations was the AutoDock Vina through the AutoDockTools 4 software (Eberhardt *et al.*, 2021).

Through this software, the interactions between iron sulfates and iron chloride with the microalgae protein were first evaluated, then the antibiofilm activity of the different structural steps of the nanobiogenic was evaluated, starting with separate molecules to compare the activities before and after then when they are together (figure 1). The analysis of the 3D results of the simulations was performed using the software

Pymol™ 1.7.x and the regions of docking interactions were verified using the LigPlus program (Laskowski and Swindells, 2011).

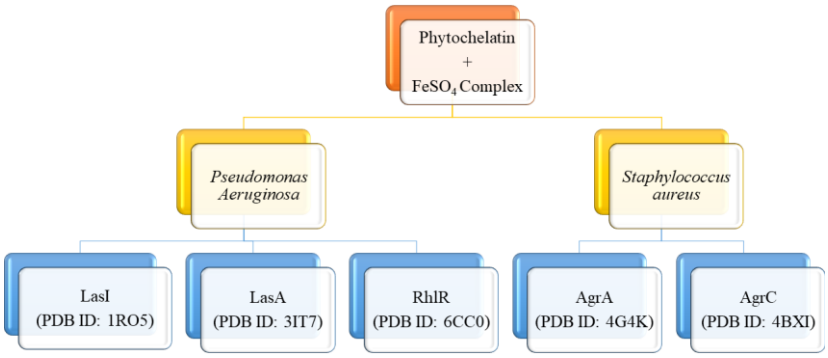

Figure 1. Molecular *docking* of biofilm inducers.

2.1.2 First-Principles Calculations

First-principles calculations are precise calculations that are based on Schrödinger's Quantum Theory. In this type of methodology, it is possible to obtain several properties of the systems under study simply with the knowledge of the atomic positions of the constituent atoms. Because it is a quantum analysis, in which all the electrons and nuclei of the atoms in the system are taken into account, the number of atoms that can be studied is limited to a few hundred. Thus, the study of computer simulation involving proteins, as is the case of this work, in which the number of atoms is very large, needs to be approached using another type of model. However, if the characteristics that are sought to be understood about a particular system depend on information about the electronic part, it is possible to carry out a mixed calculation, that is, an initial analysis, using a simple model, in such a way that keep in the system only the atoms that have greater interaction with each other and then a more complete analysis, with a more elaborate model.

In this work, with the information obtained from the molecular docking calculation, the interaction of sulfate with the proteins that have greater affinity was studied through the calculation of first principles based on the Density Functional Theory (Hohenberg and Kohn, 1964). In a very generic and superficial way, this theory makes it possible to determine the properties of a physical or chemical system based on the knowledge of its electron density. The study of interacting systems was carried out using the SIESTA code (Spanish Initiative for Electronic Simulations with Thousands of Atoms) (Soler *et al.*, 2002), which performs self-consistent calculations by solving the spin-polarized Kohn-Sham equations using orbital basis sets. atomic numbers. In all simulations, we use a zeta base plus a defined polarized function (DZP) to describe the pseudo-orbitals. To represent the electronic charge in real space, 300 Ry mesh cutoff was used. For the exchange and correlation potential, we used a generalized gradient approximation (GGA) (Perdew, Burke and Ernzerhof, 1996). All atomic structures were relaxed until the residual forces were less than 0.05 eV/Å for all atoms. To calculate binding energy, we use basic overlap error correction (BSSE). The electron-ion interactions were described by the pseudopotential method (Troullier and Martins, 1991) while the exchange potential was treated within the local density approximation (Ceperley and Alder, 1980).

3. Results and Discussion

3.1 Molecular *Docking*

AutoDock Vina is a set of computer simulation software that allows the modeling of structures, more specifically the analysis of the geometric and energetic 3D coupling between a protein and a ligand via molecular docking. Protein binding docking predicts whether the binding substance will potentially bind to the receptor (protein) *in vitro* or *in vivo*. The results of the best complexation configurations of the different sulfates with the phytochelatin synthase enzyme are presented in Table 1.

Table 1. Affinity and RMSD values for the best tuning settings for each system studied.

Ligand	Protein	Affinity (kcal/mol)	RMSD (Å)	*Docking* Favorable?*
$FeSO_4$	Phytochelatin Synthase	-3.2	0.094	**Yes**
FeS		-1.1	1.101	**Yes**
$FeCl_3$		-2.1	0.086	**Yes**

**** Based on RMSD < 2.0 A and RMSD different of 0 A***

According to the results found in Table 1, it was possible to observe the initial interaction of metal ions with the enzyme responsible for defending against metal toxicity in the medium where the microalgae are found. Phytochelatin synthase, the enzyme responsible for the production of the protein called phytochelatin-2, is responsible for binding to metal ions, in this first interaction, demonstrates the strong bond that the enzyme has with metal ions. These findings corroborate studies by Dennis et al., 2019.

When we opened this interaction to a more visual scope, we realized that the amino acid responsible for the interaction was Tyrosine 36 of the B chain, as shown in figures 2, which in other studies, such as those by (Chia *et al.*, 2013), searched for new sites of interaction with a new model of phytochelatin synthase. Both our structure and the authors were created from a model of a cyanobacterium, which has a high similarity to the structure of a microalgae phytochelatin synthase. The study shows that one of the amino acid residues present in the enzyme, Tyrosine 36, reveals that the binding site corresponds to the site of GSH production, as described by (Chia, 2021), which will later transform into a phytochelatin-2.

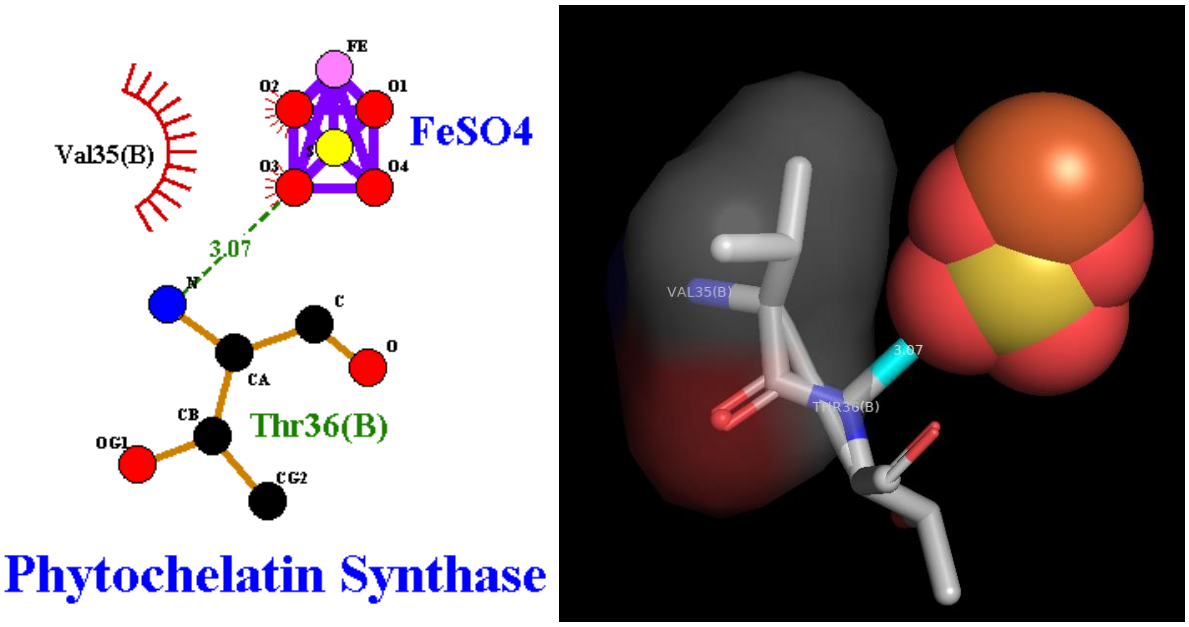

Figure 2. schematics representation of Phytochelatin Synthase bonding with $FeSO_4$.

The presence of biofilm-forming bacteria in different healthcare environments has made the battle against opportunistic infections increasingly difficult, increasingly encouraging the use of stronger antimicrobials, but further increasing the chances of these pathogens to adapt and breed. resistance to new antibiotics (Costa *et al.*, 2019).

The search for new alternatives to combat biofilm-forming bacteria has become a necessity, and the use of nanoparticles as a therapeutic strategy has demonstrated its effectiveness in direct action on biofilm formation, through quorum-sensing (qs) inhibitors. These are present in both gram-positive and gram-negative bacteria, regulating several virulence mechanisms, such as the formation of biofilms, called autoinducers (Mukherjee and Bassler, 2019). Table 2 shows the interactions of the biogenic metallic nanoparticle and its components under the proteins related to the quorum sensing mechanism, involved in the formation of biofilms in *P. aeruginosa* and *S. aureus*.

Table 1. Affinity and RMSD values for the best tuning settings for each system studied.

Bacterium	Receptor	Ligand	Affinity (kcal/mol)	*Docking* Favorable?*
P. aeruginosa	LasI	Phytochelatin	-5.2	**Yes**
		Complex2	—	**No**
		$FeSO_4$	-0.9	**Yes**
	LasA	Phytochelatin	—	**No**
		Complex2	—	**No**
		$FeSO_4$	-3.0	**Yes**
	QscR	Phytochelatin	-3.8	**Yes**
		Complex2	—	**No**
		$FeSO_4$	-3.3	**Yes**
S. *aureus*	AgrA	Phytochelatin	—	**No**

		Complex2	-3.9	**Yes**
		$FeSO_4$	-2.8	**Yes**
	AgrC	Phytochelatin	—	**Yes**
		Complex2	-4.7	**Yes**
		$FeSO_4$	-2.9	**Yes**

Com base em RMSD < 2.0 A e RMSD diferente de 0 A

For Pseudomonas aeruginosa proteins LasI (PDB-ID: 1RO5), LasA (PDB-ID: 3IT7) and QscR (6CC0) were used. It was possible to observe the interaction of phytochelatin and FeSO4 in the aminoacids threonine 145 for phytochelatin and phenylalanine 105 of the LasI protein (Figures 3, 4, and 5), showing a possible molecular interaction where it can interfere in the biological actions of this protein, but when together it was possible to observe an increase in the interaction distances between them and the protein. As it is a new structure, the literature still does not have an explanation to give to this phenomenon, but it was possible to notice that they have a connection that is not as strong as the individual components.

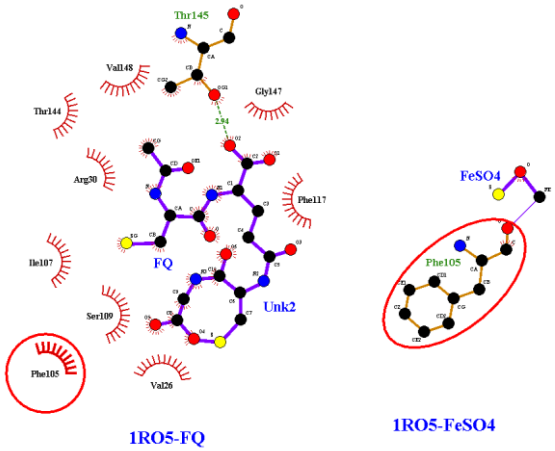

Figure 3. 2-D schematic representation of Phytochelatin and $FeSO_4$ bonding with LasI protein.

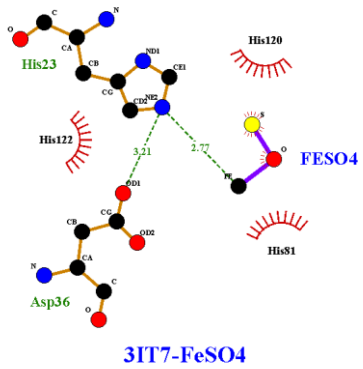

Figure 3. 2-D schematic representation of $FeSO_4$ bonding with LasA protein.

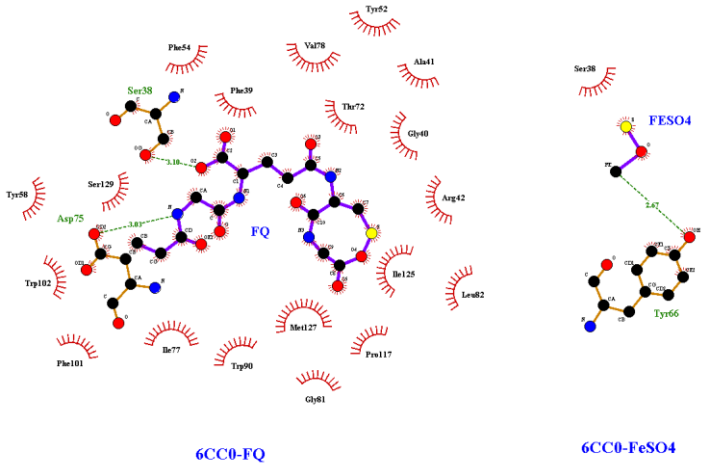

Figure 4. 2-D schematic representation of $FeSO_4$ and Phytochelatin bonding with QscR protein.

For S. aureus, AgrA (PDB-ID: 4G4K) and AgrC (PDB-ID: 4BXI) proteins were used, in the interaction with AgrA protein (Figure 5) only the complex did not show a favorable interaction, but when separated it was It is possible to notice an interaction between the amino acids Tyrosine 229 and Histidine 227 with iron sulfate and the amino acid Threonine 165 with phytochelatin-2.

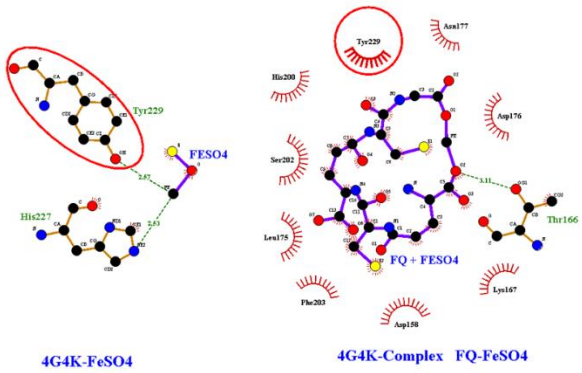

Figure 5. 2-D schematic representation of $FeSO_4$ and Phytochelatin + $FeSO_4$ complex bonding with AgrA protein.

In the interaction with the AgrC protein, it was possible to notice the total interaction of the biogenic iron nanoparticle components with the biofilm-producing proteins (Figure 6), showing a possible antibiofilm activity, where such interaction can modify the biological effect of this protein against this structure. These results corroborate the results found in the literature, where biogenic magnetic iron nanoparticles were highly biocompatible and showed antimicrobial activity against bacteria and fungi (Sandhya and Kalaiselvam, 2020).

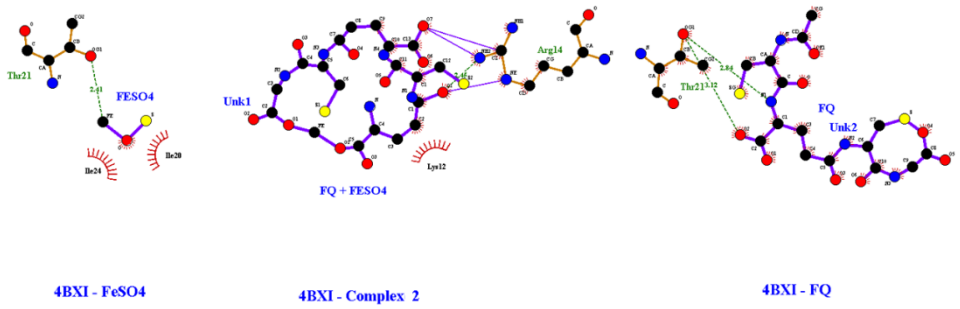

Figure 6. 2-D schematic representation of $FeSO_4$, Phytochelatin and Phytochelatin+$FeSO_4$ complex bonding with QscR protein.

3.2 First-Principles Calculations

The electronic and structural properties of phytochelatin-2 pentapeptide (FQ) and phytochelatin-2 with iron were evaluated and are represented in figure 12 (A) and (B), respectively. The geometry calculated for the phytochelatin-2 protein and the corresponding electronic levels can be seen in Figure 12 (A). The energy difference observed between the HOMO – LUMO states of phytochelatin is 2.9 eV (system A) and with iron (system B) it is 0.69 eV.

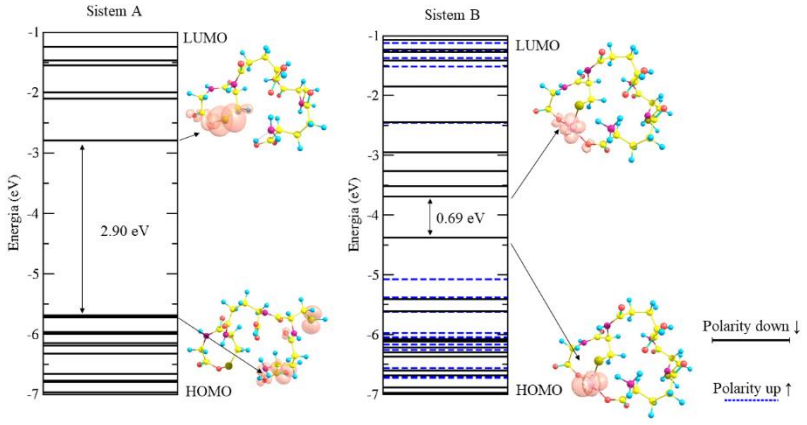

Figure 7. Structural configuration and energy levels for: (A) phytochelatin without iron and (B) iron phytochelatin complex.

It was possible to observe that the interaction of phytochelatin-2 with iron changes the phytochelatin ground state at a deeper electronic level, from the outermost layers (LUMO) to the innermost layers (HOMO) of the electronic orbitals, presenting interaction energy of -4.58 eV. The changes that occur in the electronic structural properties configure a chemical bond compared to the isolated structure, demonstrating a completely new structure. Its polarity remained the same as that of iron, this is due to the intrinsic characteristic of iron, where it keeps the electrons unpaired, maintaining the character of the metal. Through

this 3D plot (7) it is possible to visualize and confirm that the site of interaction of iron ions to phytochelatin-2 is through the cysteine region as reported (Sharma *et al.*, 2015), where depending on the species that produces this protein, the number of cysteine repeats can be high and can carry more iron ions in its structure.

In view of the results shown so far, this work praises the use of computational tools and in silico simulations to demonstrate the interaction of microalgal protein, phytochelatin, exploring its high capacity for synthesis/production of materials with nanometer size, naturally and spontaneously. These corroborate with studies in the literature that demonstrate the great ability to chelate metals, as well as the reduction of metal ions as a detoxification process by microalgae. In addition, the results also demonstrate possible antimicrobial mechanisms to be explored *in vitro* and *in vivo*.

4. Conclusion

The purpose of this work was to attempt for the first time to elucidate, by alternative methods in silico, the physiological role of phytochelatin from microalgae in the detoxification and bioremediation of metallic pollutants. By confirming the physicochemical interactions as well as the structural electronic properties of phytochelatins and the iron ion, we have theoretically demonstrated the biogenic process of nanoparticle formation that occurs naturally and physiologically by microorganisms, particularly microalgae, in the chelation and compartmentalization of this metal in the environment.

We have also theoretically verified the role of iron and phytochelatin in isolated form as well as in its complexed form (nanoparticles) in therapeutic interventions, such as their activity on proteins forming bacterial biofilms. The effective anti-biofilm effect of the nanoparticle was directed against the biofilm-forming proteins of the bacterium S. aureus, a pathogen with a high incidence that poses a threat to human health due to its multi-resistance to antimicrobial agents, which led the World Organization (WHO) to decide in 2018 to search for new therapeutic alternatives (Shrivastava, Shrivastava and Ramasamy, 2018; Pinto *et al.*, 2019). In general, we conclude that this work shows that through theoretical methods relevant results are obtained in biomedical research, in the determination of nanostructured substances produced in a natural (biogenic), economically viable way (they are in the environment) in relation to drugs synthesized in industry and sustainable.

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