MOL2NET, International Conference Series on Multidisciplinary Sciences
CHEMINFOUNC-02: Chemoinformatics Workshop, UNC Chape Hill, USA, 2020

Microalgae pigment with possible anti-caries activity: *In silico* evidences

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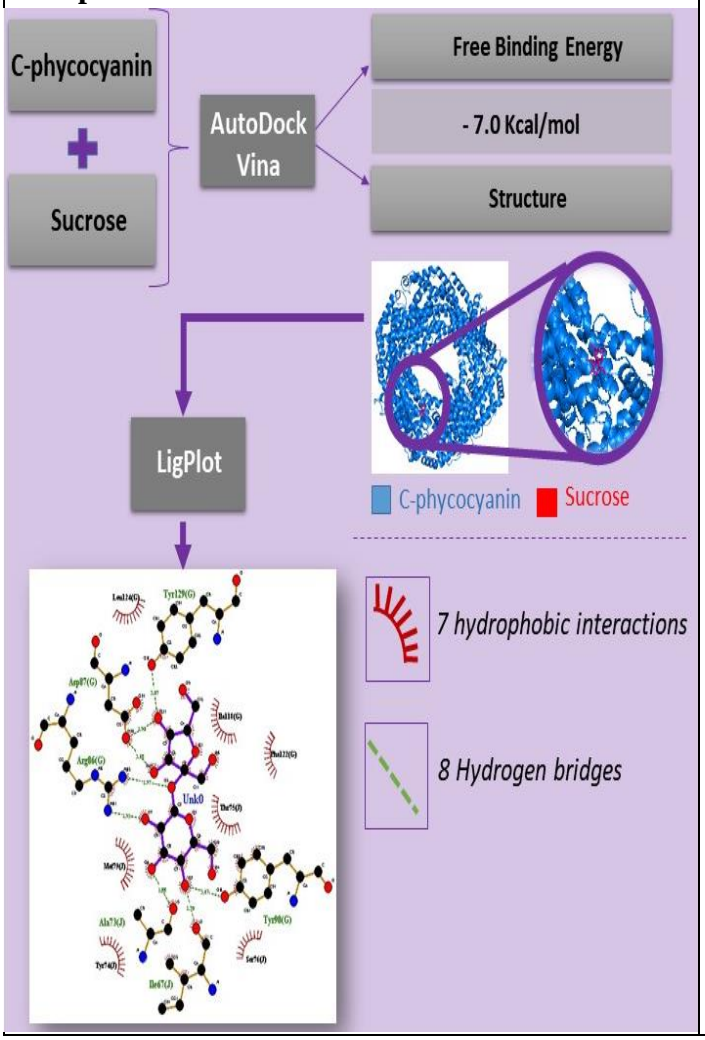
Graphical Abstract



Abstract.

Microalgae such as Spirulina (Arthrospira) platensis are the source of several bioactive compounds. C-phycocyanin (C-PC) is one of these compounds and has been widely used in the food industry. Caries disease is multifactorial and causes destruction of dental tissues. The lesions are sugar dependent, with sucrose being the most cariogenic sugar, which is metabolized by bacteria that cause caries. Thus, our objective was to verify through molecular docking the possible binding of C-PC with sucrose and thus create the possibility of adding C-PC in a mouthwash (which enhances the elimination of sucrose from the dental surface). We used the UCSF Chimera for removing heteroatoms from C-PC, AutoDock Tools for preparing protein (C-PC) and ligand (sucrose), AutoDock Vina for docking simulation, PyMol for viewing and converting files and, finally, the LigPlot to check the types of connections between molecules. The C-PC and sucrose connected with a free bonding energy of -7.0 Kcal/mol. In addition, the molecules established 08 hydrogen bonds and 07 hydrophobic interactions. Our results demonstrate the potential for sucrose sequestration of C-PC and the possibility of adding C-PC in a mouthwash as an aid to dental cleaning and caries preventing.

Introduction

C-phycocyanin (C-PC) is a blue-green heteroprotein which acts as a photosynthetic pigment for cyanobacteria such as Spirulina (Arthrospira) platensis (1). *The S. platensis has GRAS status (Generally Recognized As Safe) issued by Food and Drug Administration (FDA) and can to be legally used as a food supplement or as a bioactive ingredient* (2). *Caries disease is multifactorial and causes destruction of dental tissues and in an advanced stage, the disease can cause the loss of the dental element.* (3). *The lesions are sugar dependent, with sucrose being the most cariogenic sugar, which is easily found in various foods and is metabolized by bacteria that cause caries* (4). *Studies demonstrate the diverse biological activities of C-PC, as well as its ability to interact with different types of molecules* (5). *Thus, the inclusion of C-PC in a mouthwash could prevent caries, as C-PC has the potential to sequester sucrose molecules and thus prevent the metabolization of this sugar by cariogenic microorganisms. Therefore, the objective of this study was to verify, through molecular docking, the interaction of C-PC with sucrose and thus to predict the sucrose sequestration capacity exerted by C-PC.*

Materials and Methods

The C-PC structure (receptor) was obtained from Protein Data Bank PDB (PDB ID: 1GH0) (6) *and sucrose (ligand) was obtained from Pubchem (PubChem ID: 5988)* (7). *Firstly, using the UCSF chimera (available to download at http://www.cgl.ucsf.edu/chimera/download.html) we remove heteroatoms from the C-PC and select only one of its rings to simplify the docking simulation (since the two rings are identical). Then, we prepare receptor and ligand input files using AutoDockTools software for AutoDockVina* (8). *For preparing receptor input PDB file (1GH0), polar hydrogen atoms were added and we add Kollman charges. The ligand (sucrose) was prepared by the addition of Gasteiger charges and set TORSDOF (TORSDOF is the number of torsional degrees of freedom in the ligand, and is independent of the number of rotatable bonds). To perform docking simulations, we configure grid box as: size x = 112 Å; size y = 56 Å; and size z = 100 Å; and center box coordinates are x = 31.081 Å center; y = 12.349 Å center; z = 76.891 Å; considering exhaustiveness as 500. Molecular docking simulations were performed with AutoDock Vina* (8). *Protein was kept as rigid and ligand molecules were kept flexible throughout docking process. Then, Free Energy of Binding (FEB) of docked ligand-receptor was estimated in Kcal/mol. The more negative FEB indicates the greater stability of ligand-receptor complex. Visual analysis of docking results was performed with PyMol (available for download https://pymol.org/2/) and for check the types of connections between molecules we used the LigPlot* (9).

Results and Discussion

The C- formed a complex with sucrose (Fig. 1) and the free binding energy of this complex was -7.0 Kcal/mol. Detailed analysis of the bonding pattern revealed that the molecules established 08 hydrogen bonds and 07 hydrophobic interactions (Tab. 1). Interaction with sucrose may reduce the availability of this sugar for cariogenic microorganisms. In addition to the sequester activity demonstrated by the docking in this article, C-PC has antimicrobial activity (10) *which can represent a great advantage for preventing caries, as it can combat cariogenic microorganisms such as Streptococcus mutans. It is important to highlight that for a possible formulation of mouthwash containing C-PC, toxicity tests could be dispensed with or abbreviated, since the S. platensis (one of the main sources of C-PC) has GRAS status (Generally Recognized As Safe) issued by Food and Drug Administration (FDA) and can to be legally used as a food supplement or as a bioactive ingredient* (2).

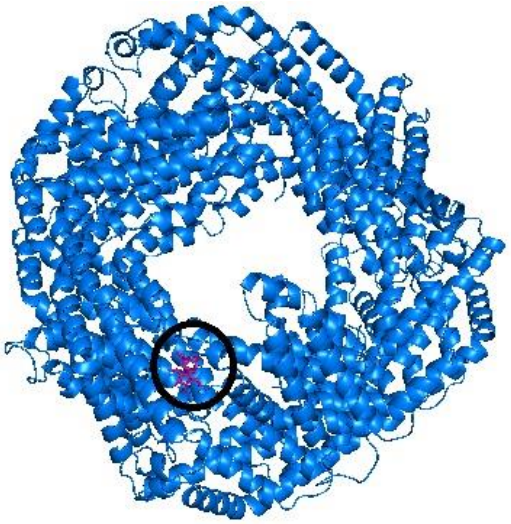


Figure 1: C-phycocyanin (blue) ligada à sacarose (red molecule circled on black).

Table 1: Type of connections between C-PC and Sucrose

Type of connections	Amino acid involved
Hydrogen bond	Arg 86 (two); Asp 87 (two); Tyr 90 and 129; Ile 67; Ala 73
Hydrophobic interactions	Leu 124; Ile 118; Phe 122; Thr 75; Ser 76; Tyr 74; Met 74

Abbreviations: Arg: Arginine; Asp: Aspartate; Tyr: Tyrosine; Ile: Interleucine; Ala: Alanine; Leu: Leucine; Phe: Phenylalanine; Thr: Threonine; Ser: Serine; Met: Metionine.

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Conclusions

The sucrose-sequestering ability of C-PC demonstrated by docking, coupled with its antimicrobial activity make C-PC an attractive molecule for formulating mouthwashes, as it may possibly play a dual role in preventing caries: Reduction of sucrose available on the tooth surface and antimicrobial activity on cariogenic microorganisms. In addition, toxicity tests would be abbreviated or waived, since the main microalgae producing C-PC already has food safety status.

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