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C-Phycocyan in the Prevention and Treatment of COVID-19: A Possibility Based on *In silico* Evidences

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Abstract

Coronavirus disease (COVID-19) is responsible for the current pandemic that worries the world. C-Phycocyanin (C-PC) appears as an attractive alternative for the prevention and treatment of COVID-19 due to its diverse biological activities. We perform protein-protein (P-P) docking with the PRISM webserver for evaluate the interaction of C-PC (in its complete form or just its F chain) with ACE2 (human cell receptor) and with viral proteins that participate in the onset and development of the infection. The F chain interacted with the ACE2 receptor (*in vivo*, C-PC may prevent the virus fusing with human cells) and with several viral proteins fundamental to infection and replication (*in vivo*, C-PC may block viral replication). Our results encourage the development of new studies for the use of C-PC as an anti-SARS-CoV-2 drug.

Keywords: Docking; Drug-discovery; Biological activities; Pandemic

Introduction

The current pandemic caused by coronavirus disease 19 or simply COVID-19 started in December 2019 in the city of Wuhan, China when the first cases of an infection caused by a new coronavirus, a retrovirus called SARS-CoV-2 (Severe Acute Respiratory Syndrome coronavirus 2; formally called HCoV-19) were identified [1]. As it is a new pathogen, many questions remain unclear about SARS-CoV-2, contributing to the seriousness of the current scenario [2].

According to the World Health Organization, the number of cases has grown steadily, with a total of (3,349,786) cases and (238,628) deaths reported worldwide on May 3 [3]. The disease results in the overload of hospitals and health systems with a high number of deaths due to serious acute respiratory problems caused by the virus. In addition to this serious situation, there are still no approved vaccines or treatments against COVID-19, which makes the study and development of effective drugs against the disease extremely necessary [4].

Phycocyanin C (C-PC) is a bluish-green hetero protein (has a portion of protein associated with chromophores) that acts as a photosynthetic pigment in cyanobacteria, such as Spirulina (Arthrospira) platensis. This molecule showed several biological activities, including: Antioxidant; anticancer; anti-inflammatory and antiviral being the antiviral ability an important indication of the possible anti-SARS-CoV-2 character [5-8]. The S Platens is one of the main sources of C-PC has GRAS status (generally recognized as safe) issued by the Food and Drug Administration (FDA) which allows the Spirulina is legally marketed as a food supplement or as a bioactive ingredient in functional foods and drinks [9].

Knowledge of the molecular mechanisms involved in the pathology of COVID-19 has evolved and two main groups of viral proteins are important for the disease: Structural proteins (such as capsid proteins) and non-structural proteins (involved in transcription and translation) [10]. In addition the importance of carboxy peptidase related to the Angiotensin-Converting Enzyme (ACE2) of human cells as a functional SARS-CoV-2 receptor has been evident [11].

The *silico* Molecular Docking (MD) approach was originally developed to help understand the mechanisms of molecular recognition between small and large molecules, but the uses and applications of DM in drug discovery have changed greatly in recent years [12]. The MD has proven to be a valid support for drug discovery programs, which can reduce the time and costs associated with conventional screening. In addition, the MD predicts the likely mode of attachment, providing a molecular basis for its optimization in terms of affinity [13]. Thus, our goal was to use molecular docking (protein-protein for C-PC *versus* host and viral proteins) to determine the possible preventive and therapeutic role of C-PC against COVID-19.

Case Representation

Target selection

We chose as targets for C-PC some of the main proteins involved in the disease. Subsequently, we organized these targets into two blocks presented in Tables 1 and 2 (Figures 1a, 1b, 2a, and 2b).

PDB ID	Protein name	Summary of Importance
1R42	Native human ACE2	Regulation of cardiac function and functional coronavirus receptor by the viral protein called Spike.
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		coronavirus receptor by the viral protein called Spike.

Table 1: Target protein in the host cell (human).

PDB ID	General Classification	Short name	Summary of Importance
6M03	Non-Structural Proteins (NSPs)	Main Protease	All NSPs form a giant replication complex that participates in numerous functions during viral infection, such as RNA genome replication, subgenomic RNA processing and packaging of newly formed virions.
6W9C		Papain-like protease	
6M71		RNA polymerase RNA-dependent	
6W4H		NSP16-NSP10 complex	
6LVN	Structural proteins	Spike protein (S): HR2 domain of the S2 subunit	Mediate the membrane fusion process (between the virus and the host cell by the ACE2 receptor) .
6M3M		Nucleocapsid protein (N-terminal domain of RNA binding)	Binds to RNA sense (+) and results in a complex of ribonucleoproteins, essential for virus replication.
5X29		Envelope Protein (E) (ion channel pentameric protein)	In animal models, deletion of protein E reduced pathogenicity and mortality.

Table 2: Viral proteins as targets.

Protein-protein docking

Protein structures were obtained from the Protein Data Bank (PDB) according to the PDB ID shown in the Tables 1 and 2 [14]. The docking was performed with the PRISM web server considering the C-PC (PDB ID: 1GH0), host cell protein (Table 1) and proteins of the virus (Figures 3a and 3b) (Table 2). Each target (host and virus) was subjected to docking simulation with: 1-complete C-PC structure and 2-only the C-PC F chain (the F chain demonstrated biological activity in a previous study) totaling 16 docking simulations (Figures 4a and 4b) [15]. The PRISM algorithm provides a large-scale forecast that combines structural similarity and explains evolutionary conservation at model interfaces. The method consists of two components: structural comparisons of the target protein rigid body with known protein-protein model interfaces and flexible refinement using a coupling energy function. The energy function is based on the Fiberdock energy score and the more negative the better, showing greater stability of the protein-protein complex [16,17]. Thus for the fitting simulation process provided on the server for each pair of proteins (one pair at a time, for example, 1GH0 x 1R42). The possible connection modes were generated by the server (number of possible connection modes depending on the pair analyzed) and only the most negative connection energy (evidence of greater stability) was shown in the results (Figures 4a and 4b) [18,19].

Docking images

The protein-protein complexes were visualized and their colors were edited to facilitate interpretation through UCSF Chimera (Figures 5a and 5b).

Results

Protein-protein docking

The binding energy attributed to the C-PC linked to different protein targets is shown in Tables 3 and 4. Only the HR2 domain of the S2 subunit (Spike protein) was fitted with the complete structure of the C-PC, the other proteins formed complex only with the F chain (Figures 6a, 6b, 6c and 6d). The most negative binding energy (most stable protein complex) was obtained for the C-PC complex with Protein Spike (S): Domain HR2 of the S2 subunit. C-PC and protein E did not form a complex (Figure 7a and 7b) [20,21].

Target	C-PC	
	Full	F chain
ACE2	-	-29.27

Table 3: Protein-protein docking: Binding energy to target in the host cell (based on the FiberDock energy score). **Note:** Abbreviations: ACE2: Carboxypeptidase related to the enzyme that converts angiotensin; C-PC: C-Phycocyanin.

Target	C-PC	
	Full	F Chain
Main Protease	-	-26.58
Papain-like protease	-	-46.18
RNA polymerase RNA-dependent	-	-37.05
Complexo NSP16-NSP10	-	-37.62
Spike protein (S): HR2 domain of the S2 subunit	-25.59	-48.79
Nucleocapsid protein (N-terminal domain of RNA binding)	-	-36.81
Envelope Protein (E) (ion channel pentameric protein)	-	-

Table 4: Protein-protein docking: Binding energy for viral proteins (based on FiberDock energy score).

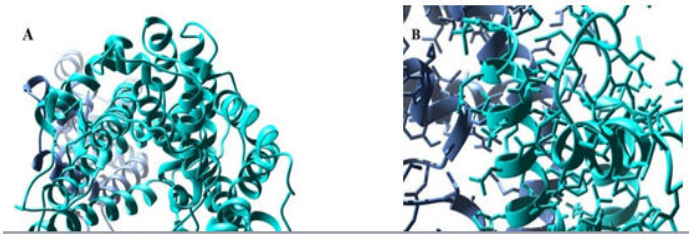


Figure 1: Protein-protein docking: a) C-Phycocyanin F chain (C-PC) (cornflower blue) and ACE2 (cyan); b) (Approximation): F and ACE2 chain amino acids in contact.

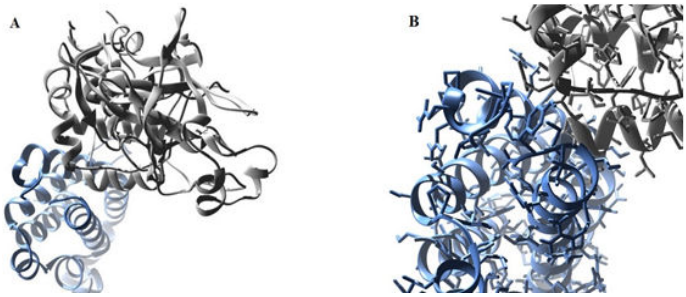


Figure 2: Protein-protein docking: a) C-Phycocyanin F chain (C-PC) (cornflower blue) and main protease (gray) b) Approximation: F chain amino acids and main Protease in contact.

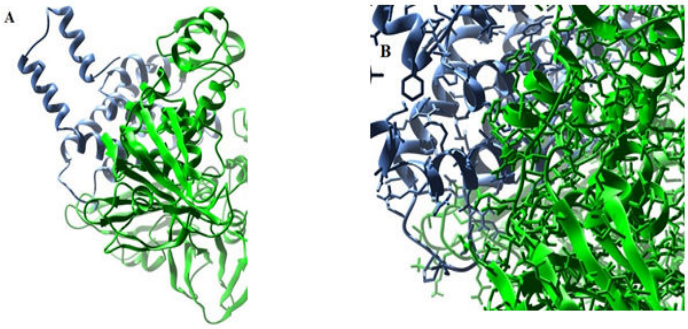


Figure 3: Protein-protein docking: a) C-Phycocyanin F chain (C-PC) (cornflower blue) and papain-like protease (light green) b) Approximation: F-chain amino acids and papain-like protease.

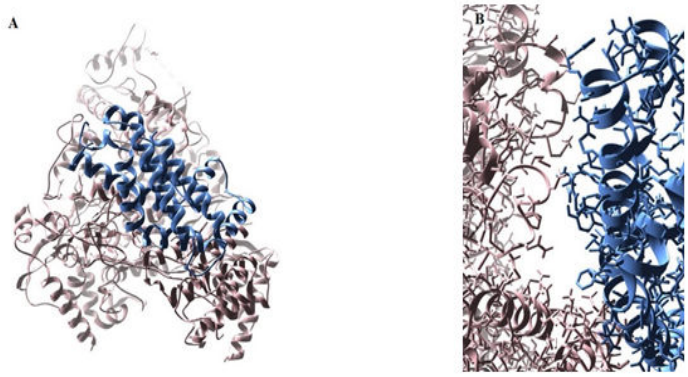


Figure 4: Protein-protein docking: a) C-Phycocyanin F chain (C-PC) (cornflower blue) and RNA-dependent RNA polymerase (light pink) b) Approximation: F-chain amino acids and RNA- RNA polymerase dependent in contact.

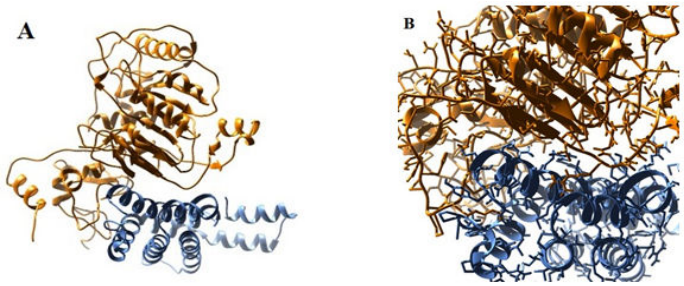
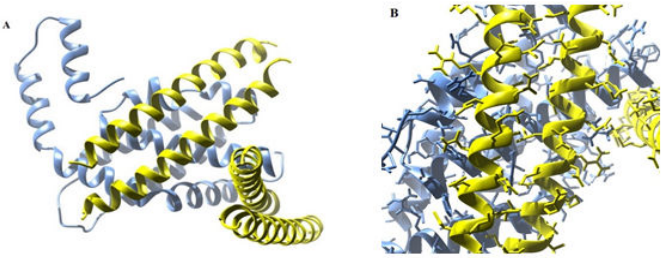


Figure 5: Protein-protein docking: a) C-Phycocyanin F chain (C-PC) (cornflower blue) and NSP16-NSP10 complex (orange) b) Approximation: F chain amino acids and NSP16-NSP10 complex in contact.



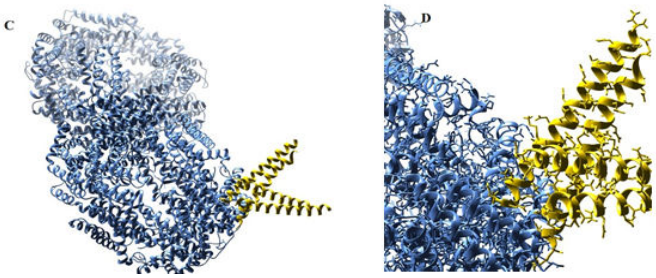


Figure 6: Protein-protein docking: a) Complete structure of C-Phycocyanin (C-PC) (cornflower blue) and Spike (S) (HR2 domain of the S2 subunit) (yellow) b). Approximation: C amino acids -PC and Spike Protein (S) (HR2 domain of S2 subunit) in contact c) C-PC F chain (cornflower blue) and Spike protein (S) (HR2 domain of S2 subunit) (yellow) d) Approximation: F chain amino acids and Spike (S) protein (HR2 domain of the S2 subunit) in contact.

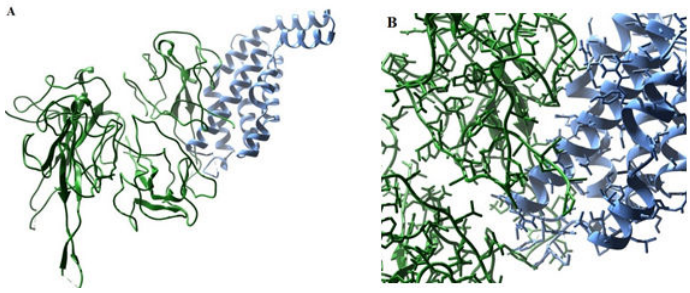


Figure 7: Protein-protein docking: a) C-Phycocyanin F chain (C-PC) (cornflower blue) and Nucleocapsid protein (N-terminal RNA binding domain) (green) b) Approximation: Amino acids from F chain and Nucleocapsid Protein (N-terminal RNA binding domain) in contact.

Discussion

The biological and pharmacological properties of S. Platens is including its antiviral capacity, have been attributed mainly to calcium-spirulane and C-PC which demonstrates the feasibility of investigating the anti-SARS-CoV-2 potential of C-PC [22]. Our article showed the possibility of using C-PC for the prevention and treatment of COVID-19. Our evidence was based on molecular docking to simulate the interaction between C-PC and a host cell target or viral targets.

The development of computing and the rapid growth in the availability of structural, chemical and biological data in databases have led to an increase in the use of *in silico* tools to investigate new drugs and our article is part of this new trend in studies [23–26].

Two proteins play a key role in the onset of SARS-CoV-2 infection: ACE2 (present in human cells) and Spike (viral capsid protein). Spike and ACE2 team up to start the virus-cell fusion process. The ability of C-PC to interact with ACE2 and Spike may indicate a role for C-PC in disease prevention: (1) the C-PC-ACE2 complex would occupy the binding site for Spike and/or (2) the C-PC-Spike complex would avoid the connection between Spike and ACE2 (in both cases, the onset of infection would be avoided).

Although proteins E (envelope) and S (Spike) are structural proteins and are present in the viral capsid, they showed a difference in the pattern of interaction with C-PC. While E protein did not form a complex with C-PC, the HR2 domain of S protein was the only one (among all protein targets) that formed a complex with the two forms of C-PC. In addition, the coupling between the Spike HR2 domain and the C-PC F chain showed the most negative binding energy among all protein targets, which indicates greater stability of the protein complex. Thus, Spike could be one of the main targets of the C-PC.

The interaction between C-PC and the Nucleocapsid protein may be another important therapeutic mechanism. The complex with C-PC can compromise the functions of this viral protein which can destabilize the replication of the virus and prevent certain cellular changes in the host (because the Nucleocapsid protein regulates viral RNA replication and transcription and changes in host metabolism and cell cycle) [27,28].

All protein targets, except Spike's HR2 domain, complexed only with the F-chain of C-PC. This pattern was expected, because in a previous study this chain was coupled to drug efflux proteins (*in silico* analysis) demonstrating the importance of this portion of the C-PC. In addition, considering the dimensions of the C-PC in its complete form (110 A° in diameter) and the diversity of biological activities (which need interaction with much smaller cellular targets, for example, the diameter of a calcium channel is ~ 14 A°) it is possible that the C-PC is not in its native form to interact with cells [29,30].

An important advantage of using only the F chain in therapy would be to expand the possibilities of obtaining it. To obtain complete C-PC, from algae production, several purification steps (which may include chromatography) are generally used whereas to obtain the F chain it would be possible to use the recombinant DNA technology that is already dominated for the production of insulin [31,32].

Non-structural proteins (from English NSPs), such as proteases and RNA polymerases have a fundamental role in viral replication and, therefore, in the infectious process [10]. The interaction between C-PC and NSPs demonstrates the potential of C-PC against COVID-19, because when interacting with these proteins, it can block the viral replication process and the evolution of disease. In this context, the problem of high mortality due to COVID-19, accompanied by the lack of effective vaccines and treatments, could be solved or minimized by the possibility of using C-PC. Furthermore, as C-PC is a natural product with high specificity for diseased cells and low side effect on healthy cells would represent a breakthrough in therapy for COVID-19 [33-37].

Conclusion

In addition to the preventive and therapeutic advantages resulting from the interaction with the targets mentioned in our article, it is important to mention that C-PC must have more advantages to face COVID-19: Its immune system stimulating capacity accompanied by its specificity by diseased cells which should reduce side effects when using therapies.

In view of the various advantages presented by C-PC, it is essential to continue research through *in vitro* and *in vivo* tests. As C-PC is present in Spirulina extracts with GRAS status, possibly clinical trials of toxicity would be reduced, accelerating its use in therapeutic practice. Finally, our results based on *in silico* analyzes indicate the potential of C-PC for the prevention and treatment of COVID-19.

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