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Phycocyanine as a Potential Inhibitor of SARS-CoV-2-Spike/*TMPRSS2* and SARS-CoV-2-RBD/ACE2 Interactions: An *In silico* Approach

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Abstract

The global pandemic caused by infections of the new coronavirus (COVID-19) makes it necessary to find possible less toxic and easily accessible therapeutic agents. In this study, we used strategies protein-protein docking to analyze phycocyanine against SARS-CoV-2-Spike/ *TMPRSS2* and SARS-CoV-2-RBD/ACE2 for the treatment of COVID-19. The evaluation was performed with the binding affinities, RMSD values and number of hydrogen bonds ZDOCK calculated by Discovery Studio 2019. Preliminary results suggested that phycocyanine has the best binding energy according to *TMPRSS2* and ACE2 and that it is capable of promoting structural changes in the viral protease by inducing folding of the enzyme. Phycocyanine brings the enzymes to a more compact conformational state compared to the first state, compared to *TMPRSS2* and ACE2, respectively. These results are interesting because Phycocyanine can serve as a starting point for subsequent experimental or/and *in silico* studies based on chemical structure-activity relationships taking this molecule and its possible derivatives.

Keywords: Phycocyanine; SARS-CoV-2; Molecular Docking; *TMPRSS2*; ACE2

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Introduction

C-Phycocyanin is a protein-bound pigment found in blue-green algae. *Phycocyanin* monomers are themselves made up of two distinguishable protein subunits designated α and β, which contain at least three covalently attached bilin chromophores, open-chain tetrapyrroles with no metal complexes [1]. These prosthetic groups account for about 4% of the algae mass, indicating the presence of about sixteen chromophoric groups per unit molecular weight [2]. It occurs in monomeric, trimeric, hexameric, and decameric forms [3], and is the most abundant pigment in blue-green algae, accounting for more than 20% of algal dry weight [4]. The most important cyanocyanin producer among cyanobacteria is *Spirulina platensis*, a phototrophic cyanobacterium species but recent studies have shown that many cyanobacterium species *(Spirulina maxima, Spirulina fusiformis, Anabaena sp., Synechococcus sp., Aphanothece halophytica, Nostoc sp., Oscillatoria quadripunctulata, Phormidium ceylanicum),* maybe alternatives to *S. platensis* [5].

The biological activities of Phycocyanin are wide-ranging and

include antioxidants, DNA protectors, antiviral and antitumor properties. Phycocyanin has also been shown to stimulate the immune system and exhibit hepatoprotective, antiplatelet, and neuroprotective activities [6-8]. In the previous studies, it has been shown that *Phycocyanin* exhibited antiviral activity by affecting viral penetration of Herpes Simplex Virus type 1 (HSV-1) on the cell surface [9]. On the other hand, *Phycocyanin* showed inhibitor activity on Angiotensin Converting Enzyme I (ACE) [10] which structurally similar to ACE -2 that is the cellular receptor for SARS CoV- 2 [11,12].

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) pneumonia also known as Coronavirus Disease 2019 (Covid-19) is a newly recognized illness that has spread rapidly throughout Wuhan (Hubei province) to other provinces in China and around the World [13]. SARS CoV-2 is an enveloped virus containing a positive-strand single-stranded RNA genome and its sequence has recently been reported [14]. Comparison of the SARS CoV and SARS CoV-2 Spike protein (S protein) sequence revealed 76% protein identity [15], and recent studies reported that SARS CoV-2 is also bound to its cellular receptor ACE2 *in vitro* [16]. Subsequently, the S protein is cleaved by the Transmembrane Protease Serine 2 (*TMPRSS2*) [17]. Simultaneously blocking *TMPRSS2* and the cysteine proteases CATHEPSIN B/L activity inhibits entry of SARS CoV-2 [18], while the SARS CoV-2 entry was not completely prohibited *in vitro* [17].

In this study, based on the literature knowledge and using an *in-silico* approach, we evaluated the possible effects of *Phycocyanin* on SARS CoV-2-Spike/ *TMPRSS2* and SARS CoV-2-RBD/ACE2 interactions. Furthermore, human HPN as *TMPRSS2* protein and ACE2 variabilities and affinities with the related targets were investigated by theoretical calculations.

Material and Methods

Protein-protein docking

Due to increasing attention in clinical applications, theoretical studies have been applied to define and visualize the structures and actions of *TMPRSS2*, ACE2, and *Phycocyanin*.

Computationally in the first stage, ZDOCK associated with RDOCK has been demonstrated as a highly successful method for making accurate protein-protein docking predictions [19]. ZDOCK [20] module of Discovery Studio 2019 which had displayed great prediction abilities in protein-protein docking was used to perform SARS CoV-2 Spike protein which obtained from the complex is taken from Meng et al. [21] and SARS CoV-2 RBD (Receptor Binding Domain) proteins (PDB: 2AJF) with *Phycocyanin* (PDB ID:1Z8G). Initially, proteins were prepared by removing water compounds, adding the hydrogen atoms, assigning bond orders, adding hydrogen, treating metals, treating disulfides utilizing the protocol of "Prepare Protein" [22]. Then, the key residues that the binding site of *TMPRSS2* and ACE2 were chosen as the binding site for *Phycocyanin* to investigate the ability of *Phycocyanin* to be an inhibitor for SARS CoV-2 Spike protein and SARS CoV-2 RBD. It was important to limit the docked surface within the key residues to avoid irrelevant bound complexes (false positives). At last, in the docking process, we set an angular step size to 6°, a root-mean-square deviation (RMSD) cut-off value to 6.0 Å and interface cutoff to 9.0 Å to carry out the final conformational sampling. After docking, 2000 top poses were generated and clustered with the maximum number of 100. All possible binding modes between receptor and ligand were ranked based on shape, desolvation energy, and electrostatics using Fast Fourier Transform (FFT) algorithm [23]. To obtain near-native conformation, the predicted protein poses from ZDOCK were subjected to refinement and re-ranking using CHARMm [24].

Furthermore, molecular dynamics (MD) simulations were exerted to identify and validate the correct binding conformations of the docking calculations [25]. Prime Molecular Mechanics Generalized Born Surface Area (MMGBSA) is employed to examine the binding strength between *TMPRSS2*, ACE2, and *Phycocyanin* toward SARS CoV-2 Spike and SARS CoV-2 RBD as targets, respectively. The binding free energy is calculated using the following equation.

$$\Delta G_{bind} = \Delta E_{mm} + \Delta G_{sol} + \Delta G_{SA}$$

Where ΔEmm difference in the minimized energy between the target–ligand complex and sum of the energies of free target and free ligands. ΔGsol difference in the GBSA solvation energy between the target–ligand complex and sum of the energies of free target and free ligands. ΔG_{SA} difference in the surface energy between the target–ligand complex and sum of the energies of free target and free ligands [26].

Results and Discussion

The purpose of this study was to characterize the binding affinity toward SARS CoV-2 Spike and SARS CoV-2 RBD and explore the capability of *Phycocyanin* throughout binding affinity and MMGBSA. Firstly, we run redocking of *TMPRSS2* and ACE2 inside its binding site of SARS CoV-2 Spike and SARS CoV-2 RBD for validation of the complex and also to get the binding affinity values to be comparable with *Phycocyanin* as an antagonist and/or inhibitor. As shown in **Table 1**, the binding free energy, Root-Mean-Square Deviation (RMSD), the number of hydrogen bonds, and residues of SARS CoV-2 Spike and SARS CoV-2 RBD proteins are responsible for forming hydrogen bonds with either of *TMPRSS2* or *Phycocyanin* and ACE2 or *Phycocyanin.* From **Table 1**, it is apparent that there is a significant difference in binding affinity toward SARS CoV-2 Spike and also SARS CoV-2 RBD. Where both *TMPRSS2* and *Phycocyanin* bound to the active site of SARS CoV-2 Spike given as in **Figure 1**. A similar situation is also available for ACE2 and *Phycocyanin* in **Figure 2.** Comparing the interactions of *TMPRSS2* and *Phycocyanin* **(Table S1)** it can be seen that *TMPRSS2* interacted with SARS CoV-2 Spike through seven hydrogen bonds with the residues mentioned in **Table 1**. If we now turn to the interactions between *Phycocyanin* forms nine hydrogen bonds with residues of SARS CoV-2 Spike, (**Table 1**). In the same way, ACE2 formed twelve hydrogen bonds, one electrostatic, and five hydrophobic interactions with the

Table 1 The comparative binding free energy, root-mean-square deviation, number of hydrogen bonds, and the related residues responsible for forming hydrogen bonds.

Name	Binding Free Energy (kcal/mol)	RMSD, Å	Number of Hydrogen Bonds	Residues Responsible for Forming Hydrogen Bonds
TMPRSS2	-37.276	1.367	7	Leu212, Ser256, Gly257, Asn603, Arg634, Ser686, Ala688
Phycocyanin	-62.986	0.752	9	Ser808, Lys811, Phe817, Asn824, Asp936,
ACE2	-40.573	1.271	12	Gln24, Phe28, Asn33, Glu37, Tyr41, Tyr83, Glu329, Lys353, Gly354
Phycocyanin	-61.630	0.538	29	Asp49, Ser50, Ser53, Gln78, Asp82, Lys83, Arg86, Tyr90, Arg93, Tyr97, Glu109, Tyr110, Ala113, Ile118, Thr121.

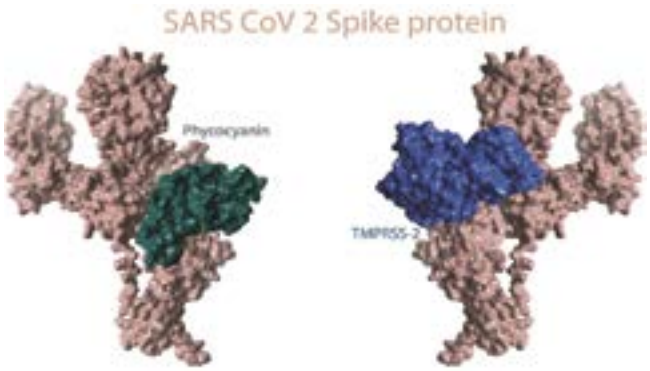

Figure 1 3D surfaces of SARS CoV-2 Spike (remarked light brown) when it binds to *Phycocyanin* (dark green) and TMPRSS2 (dark blue).

Figure 2 3D surfaces of SARS CoV-2 RBD (remarked light blue) when it binds to *Phycocyanin* (dark green) and ACE2 (orange).

important residues in the active side of SARS-CoV-RBD. On the other side, *Phycocyanin* interacted with the same region of the related target with the help of twenty-nine hydrogen bonds and also some non-bonding interactions having electrostatic and hydrophobic interaction, given as **Table 1**. The detailed information of the related structures is summarized in **Table S1** and **Table S2.**

The following part was set to define the binding free energy of *Phycocyanin* and investigate its ability to be a good antagonist and/or inhibitor, we used MMGBSA calculations. **Table 2** compares the computational estimations for the binding of *TMPRSS2*, ACE2, and *Phycocyanin*. What is interesting about the data in **Table 2** is that the MMGBSA binding ΔG is positive for *TMPRSS2* whereas the *Phycocyanin* is negative. The value of $\Delta G_{covalent}$ is also negative for *Phycocyanin* and positive for *TMPRSS2*. Furthermore, the values of $\Delta G_{Bind\ vdW}$ are negative for *TMPRSS2* and *Phycocyanin*.

In the meantime, the almost same situation was observed between ACE2 and *Phycocyanin* against SARS CoV-2 RBD. It can see that the biggest difference here is that the interactions are quite stronger according to both *TMPRSS2* and *Phycocyanin* with SARS CoV-2 Spike and further support the previous molecular docking calculations for ACE2 and *Phycocyanin* toward SARS CoV-2 RBD.

Analysis of Human HPN as *TMPRSS2* protein variability and Affinity with SARS CoV-2-Spike

A total of seven missense variants leading to an amino acid substitution in the coding region of human HPN gene (ENSG00000105707/ GRCh38: CM000681.2), given as **Table 3** that map to the interaction surface are described in the Ensembl database [27]. All these variants are rare and mostly found in European non-Finnish, Latino and South Asian populations. rs750504861 was found to be deleterious and probably damaging protein function according to both SIFT and PolyPhen tools. rs761123733 and rs147827161 variants were found to be deleterious on protein function only according to SIFT whereas rs761207239 and rs745927164 variants were found to be possibly damaging on protein function only according to PolyPhen tool.

Considering both the enthalpy and the entropy in our calculation, we found no significant change in the interaction energy except PRO206 > LEU variant, (> 1 or < 1 kcal/mol) **(Figure 3).**

Analysis of Human ACE2 variability and affinity with SARS CoV-2-RBD

A total of eight variants of human ACE2 that map to the interaction surface is described in the Ensembl database [27]. Three out of these eight variants were missense variants leading to an amino acid substitution in the coding region **(Table 4).** Rs146676783 variant at the codon 37 which leads to substitution from negatively charged Glutamic acid into positive charged Lysin was found to be likely deleterious and possibly damaging on protein function according to SIFT and PolyPhen tools. Considering both the enthalpy and the entropy in our calculation, we found no significant change in the interaction energy except GLU329 > GLY variant (> 1 or < 1 kcal/mol) (**Figure 4**).

Table 2 MMGBSA values for binding of TMPRSS2, *Phycocyanin* toward SARS CoV-2 Spike and ACE2, and *Phycocyanin* toward SARS CoV-2 RBD.

Name	ΔG_{Bind} (kcal/mol)	$\Delta G_{Bind\ Coulomb}$ (kcal/mol)	$\Delta G_{Bind\ Covalent}$ (kcal/mol)	$\Delta G_{Bind\ vdW}$ (kcal/mol)
TMPRSS-2	36.22	82.08	34.55	-80.41
Phycocyanin	-168.66	-22.31	-23.71	-122.64
ACE2	-68.53	-120.19	-62.72	-110.27
Phycocyanin	-240.42	-30.18	-32.75	-180.86

Table 3 Details of seven missense variants located at the HPN interaction surface.

Variant ID	Population Genetics	Chr: bp	Alleles	Conseqence	AA	AA coord.	SIFT	PolyPhen	Mutation Assessor
rs761207239	South Asian	19:35060509	C/T	Missense Variant	PRO/LEU	206	0.05	**0.733**	0.122
rs750504861	Latino and Non-Finnish European	19:35060628	C/T	Missense Variant	ARG/TRP	208	**0**	**0.911**	**0.545**
rs761123733	Non-Finnish European	19:35060629	G/A	Missense Variant	ARG/GLN	208	**0.04**	0.012	0.193
rs1215271309	-	19:35060755	A/G	Missense Variant	ASN/SER	250	0.06	0	0.129
rs147827161	Latino and Non-Finnish European	19:35060760	G/A	Missense Variant	GLU/LYS	252	**0.05**	0.014	0.086
rs745927164	African	19:35065339	T/C	Missense Variant	TYR/HIS	301	0.11	**0.724**	0.091
rs563050107	South Asian < 0.001 (T)	19:35065343	A/T	Missense Variant	TYR/PHE	302	0.18	0.08	0.05

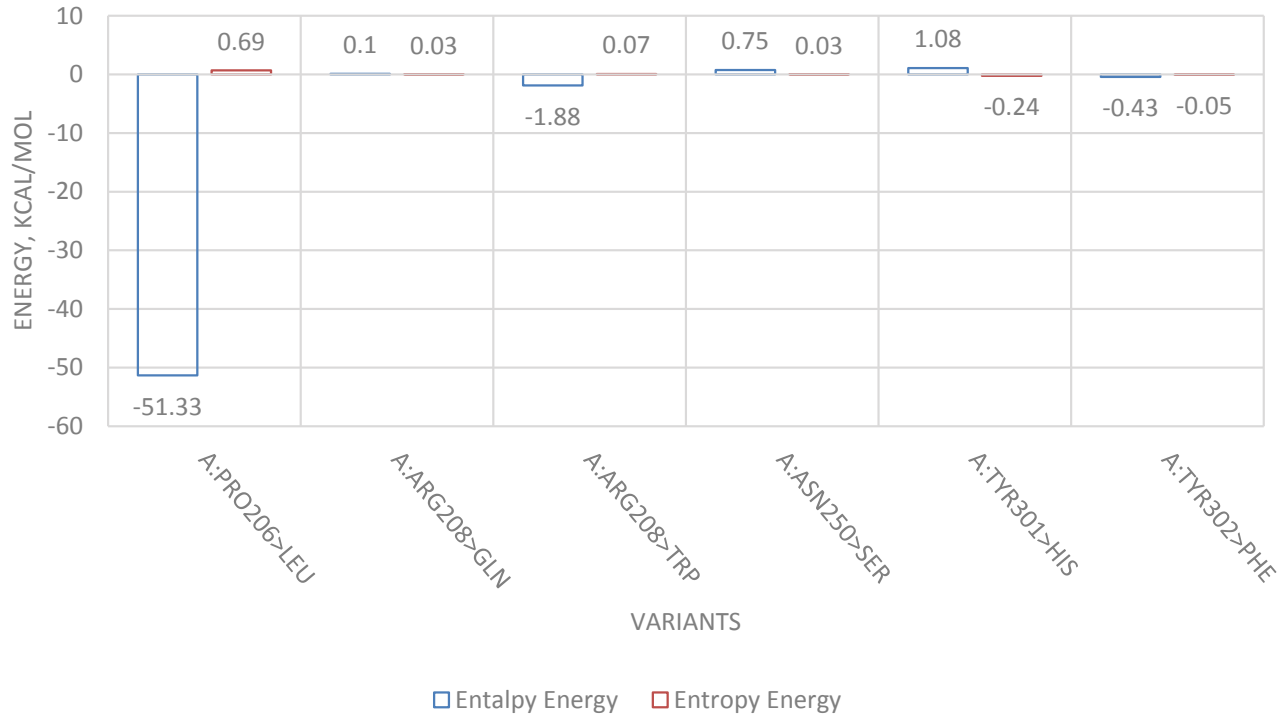

Figure 3 Estimation of enthalpy and entropy energy changing upon mutation for human HPN variants.

Table 4 Details of three ACE2 missense variants leading to a missense amino acid substitution located at the ACE2 interaction surface.

Variant ID	Chr: bp	Alleles	Conseqence	AA	AA coord.	SIFT	PolyPhen	Mutation Assessor
rs143936283	X:15581305	T/C	Missense Variant	GLU/GLY	329	0.13	0.02	0.845
rs146676783	X:15600803	C/T	Missense Variant	GLU/LYS	37	0.04	0.523	0.802
rs766996587	X:15594944	C/A/T	Missense Variant	MET/ILE	82	0.21	0.001	0.202

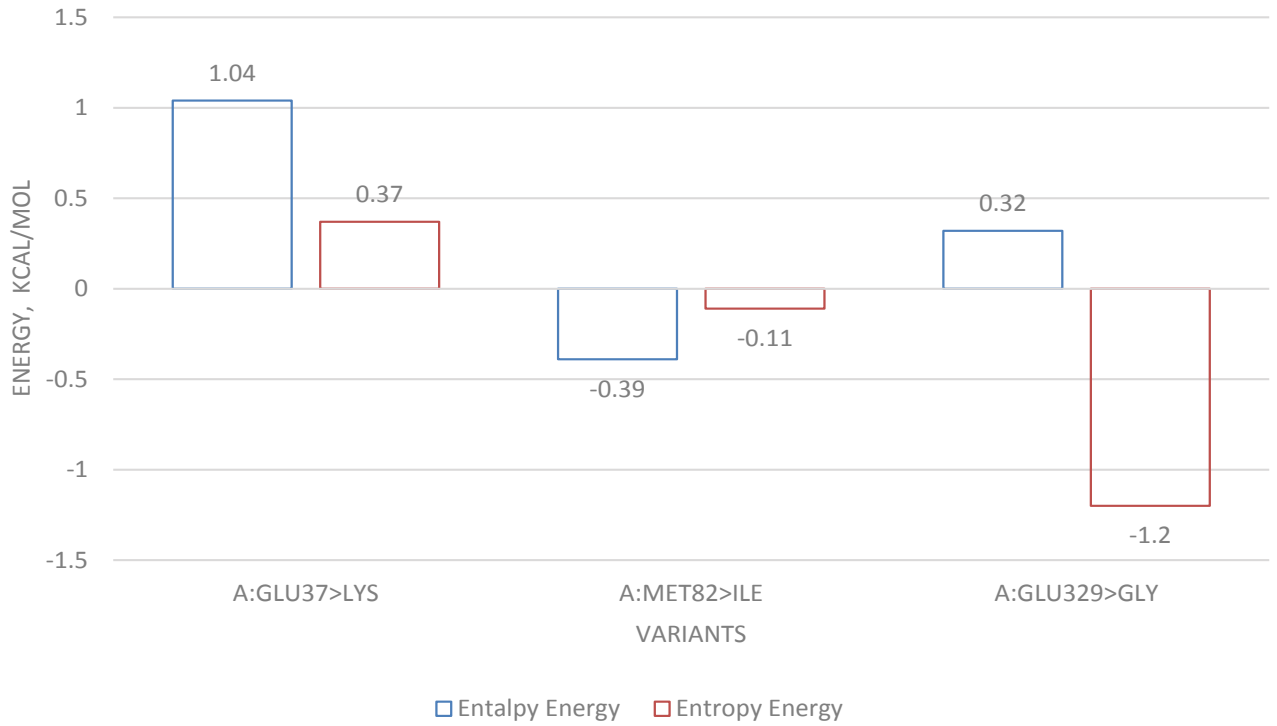

Figure 4 Estimation of enthalpy and entropy energy changing upon mutation for human ACE2 variants.

Conclusion

We examined *TMPRSS2* and *Phycocyanin* with SARS CoV 2 Spike complexes and also ACE2 and *Phycocyanin* with SARS CoV-2 RBD, whose correct attachment conformities can be described as the best-scored conformation. For the related complexes, the binding free energies for the best-scored conformations were reevaluated by MMGBSA based on short MD simulations. Moreover, human *TMPRSS2* and ACE2 variabilities and affinities with SARS CoV-2-Spike and SARS CoV-2-RBD respectively were examined and assessed according to the prediction of enthalpy and entropy energy changing upon mutations for both proteins (*TMPRSS2*, ACE2). The results showed that conformational sampling by MD can significantly improve estimates for each complex. In particular, it is stated that *Phycocyanin* shows good binding against both targets compared to *TMPRSS2* and ACE2. Therefore, the pigment, *Phycocyanin*, is predicted to act as an antagonist or inhibitor against SARS CoV-2 and prevent the related virus from binding to the *TMPRSS2* and ACE2 proteins.

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