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# Noncovalent and covalent binding of phycocyanobilin to S protein of SARS-CoV-2 and its receptor-binding domain

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The emergence of the coronavirus SARS-CoV-2 has attracted attention of the whole scientific community. The SARS-CoV-2 spike (S) protein plays the most important role in viral attachment to host receptor angiotensin-converting enzyme 2 (ACE2), via the receptor-binding domain (RBD), fusion and entry into the host, and it serves as a target for the development of antibodies, entry inhibitors and vaccines. It has been demonstrated that phycocyanobilin (PCB), a bioactive open-chain tetrapyrrole chromophore of phycocyanin (PC), chromoprotein derived from the cyanobacterium *Arthrospira platensis*, can bind a plethora of different proteins, both in a noncovalent and covalent manner. This study aimed to investigate interactions of PCB with S protein and RBD respectively. Electrophoretic techniques, fluorescence spectroscopy, and inhibition of S-PCB and RBD-PCB covalent adduct formation using iodoacetamide and N-ethylmaleimide, were employed to examine interactions of PCB with S protein and RBD, while the effects of PCB binding on RBD structure were studied by CD spectroscopy. SDS-PAGE with Zn<sup>2+</sup> staining has revealed that PCB covalently binds to both S protein and RBD, via free cysteine residues. Binding constants determined by the fluorescence quenching method were:  $2.1 \times 10^7 \text{ M}^{-1}$  for PCB and S protein and  $8.4 \times 10^4 \text{ M}^{-1}$  for PCB and RBD. Far-UV circular dichroism spectra showed that the binding of PCB influences RBD structure by decreasing the disordered structure content. Due to moderately strong noncovalent interactions of PCB with S protein and RBD, as well as covalent adducts formation, it may exert one of its many bioactive effects via impact on S protein binding to ACE2 receptor.

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