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"Biochemistry in Biotechnology"

## **Examination of C-phycocyanin interactions with selected vitamins**

Aleksandar Ivanov<sup>1\*</sup>, Luka Veličković<sup>1</sup>, Zorana Jovanović<sup>1</sup>, Nikola Gligorijević<sup>2</sup>, Simeon Minić<sup>1</sup>, Milan Nikolić<sup>1</sup>

C-phycocyanin (C-PC) is a photosynthetic protein from Arthrospira platensis (cyanobacteria). Due to its intense blue colour, which is very rare in nature, C-PC has industrial applications as a food colourant as a substitute for synthetic food colourants. Disadvantages of C-PC as a food colourant are its poor stability at high temperatures (during thermal treatment of the food) and its sensibility to change pH value. The binding of food-derived small molecules, such as vitamins, could stabilize the structure of C-PC at high temperatures and wide pH ranges. In this study, we characterized the binding of selected vitamins to C-PC, purified from the commercial powder of Arthrospira platensis. We used hydrophilic vitamins (B<sub>1</sub>, B<sub>2</sub>, B<sub>7</sub>, B<sub>9</sub>, B<sub>12</sub>), lipophilic vitamins (A, D<sub>3</sub>) and provitamin (β-carotene). Fluorescent spectroscopy showed a decrease in fluorescence of C-PC in the presence of vitamin A, vitamin D3 and β-carotene (lipophilic molecules) compared to the control. In contrast, the fluorescence of C-PC in the presence of hydrophilic vitamins showed minimal change. The protein fluorescence quenching approach demonstrated hydrophobic (pro)vitamins binding affinities ranging from 0.02 to 5.9 x 10<sup>5</sup> M<sup>-</sup>1, with the ability of hydrophobic (pro)vitamins to bind at the different sites on C-PC. UV-VIS spectrophotometry showed that the binding of hydrophobic (pro)vitamins does not affect the protein colour, while CD spectroscopy revealed that the binding of chosen molecules does not significantly influence the secondary structure of C-PC. Overall, this study demonstrated C-PC's significant potential in binding hydrophobic (pro)vitamins, while further research is required to test if these ligands could improve C-PC stability.

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