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Kinetics of Phycocyanobilin Cleavage from C-Phycocyanin by Methanolysis

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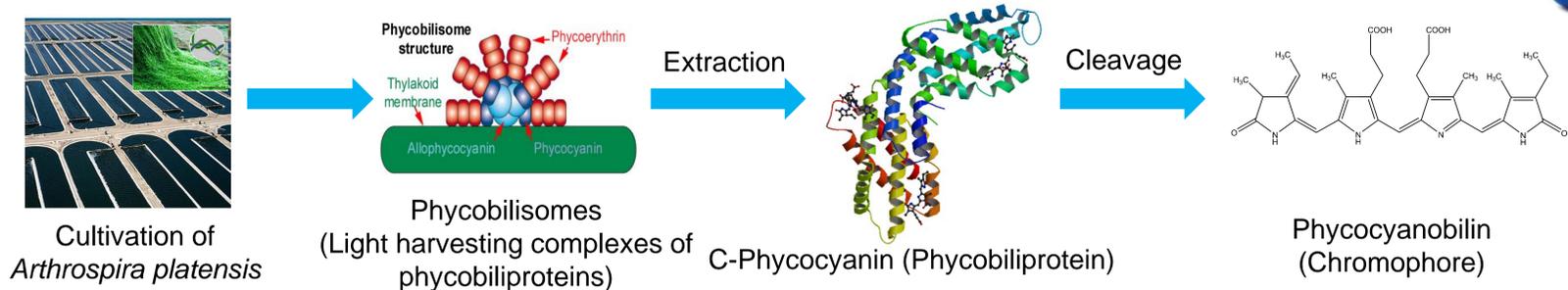
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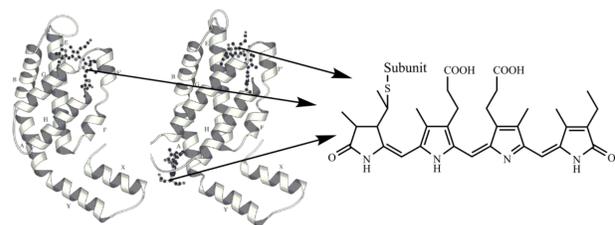


INTRODUCTION

Phycocyanobilin (PCB) is a linear tetrapyrrole chromophore covalently attached to protein subunits of phycobiliproteins, C-Phycocyanin (C-PC) and Allophycocyanin (APC), present in the light harvesting complexes of the blue-green algae *Arthrospira platensis*. PCB absorbs light in the red region of the electromagnetic spectrum, thereby exhibiting a vivid blue color. Therefore, it has great significance to the food industry due to its potential as a natural blue food color. The chemical synthesis of PCB is very complex and economically not feasible. Hence, there is a demand for the development of process to obtain PCB from phycobiliproteins. PCB is attached to the protein subunits through a cysteine residue with a thioether linkage. In this work, the kinetics of the cleavage process of PCB from protein subunits by methanolysis is investigated.

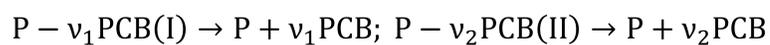


KINETIC MODEL FOR CLEAVAGE OF PCB BY METHANOLYSIS



PCB attached to alpha (left) and beta (right) subunits of C-PC via thioether linkage

Cleavage of PCB can be described either as two first order reactions in parallel:



Or two first order reactions in series:



Where PCB(I) is easily accessible and PCB(II) is less accessible for cleavage, v_1 and v_2 are stoichiometric coefficients of PCB(I) and PCB(II), respectively. In a batch reactor the reactions in parallel will appear as a single first order reaction and can be represented by following set of equations:

$$\frac{dC_1}{dt} = -k_1 \cdot C_1; \quad \frac{dC_2}{dt} = k_1 \cdot C_1 - k_2 \cdot C_2$$

Analytical solutions for set of equations above is:

$$C_1(t) = C_{10} \cdot e^{-k_1 \cdot t}; \quad C_2(t) = \frac{k_1 \cdot C_{10}}{k_1 - k_2} \cdot (e^{-k_2 \cdot t} - e^{-k_1 \cdot t}) + C_{20} \cdot e^{-k_2 \cdot t}$$

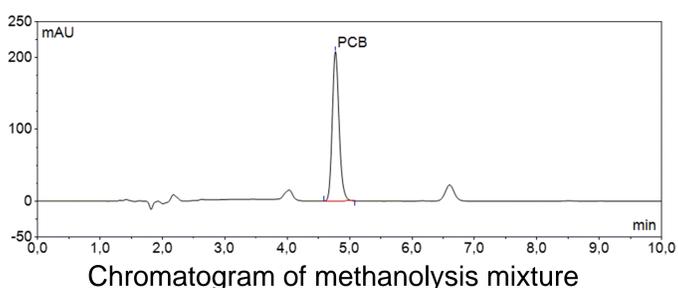
Where C_{10} and C_{20} are initial concentration of PCB(I) and PCB(II), respectively

Based on stoichiometry of reaction, the concentration of PCB can be expressed as:

$$C_{\text{PCB}}(t) = v_1 \cdot C_{10} \cdot \left(1 + \frac{v_2}{v_1} + \frac{v_2}{v_1} \cdot \frac{C_{20}}{C_{10}}\right) - v_1 \cdot C_{10} \cdot \left(1 + \frac{v_2}{v_1} + \frac{v_2}{v_1} \cdot \frac{k_1}{k_1 - k_2}\right) \cdot e^{-k_1 \cdot t} + v_1 \cdot C_{10} \cdot \left(\frac{v_2}{v_1} \cdot \frac{k_1}{k_1 - k_2} - \frac{v_2}{v_1} \cdot \frac{C_{20}}{C_{10}}\right) \cdot e^{-k_2 \cdot t}$$

EXPERIMENTAL

- ❑ Linablue (Commercial extract of *Arthrospira platensis*) boiled in 400 mL methanol for 16 h at 65 °C
- ❑ Mixture samples are taken at regular interval for HPLC analysis
- ❑ Three different initial concentration of Linablue used



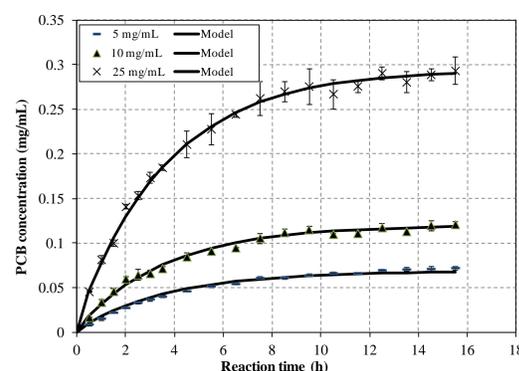
CONCLUSION

- ❑ Kinetic model describes the experimental data adequately
- ❑ The ratio between v_2 and v_1 is too large compared to the prior findings where a ratio 0.2 and 0.3 is more likely if all PCB is cleaved
- ❑ Although the model explains kinetic observations well, a two step model might be an over simplification

RESULTS

Table 1. Model data fitted to experimental data.

Initial protein concentration (mg/mL)	$v_1 \cdot C_{10}$ (mg/mL)	$\frac{v_2}{v_1}$	$\frac{C_{20}}{C_{10}}$	k_1 (h ⁻¹)	k_2 (h ⁻¹)
5	2.7×10^{-3}	24	1.0×10^{-4}	33	0.29
10	4.7×10^{-3}				
25	12×10^{-3}				



Cleavage of PCB as a function of time. Fully drawn lines are calculated using the model with the parameters from Table 1.

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