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Kinetics of Phycocyanobilin Cleavage from C-Phycocyanin by Methanolysis

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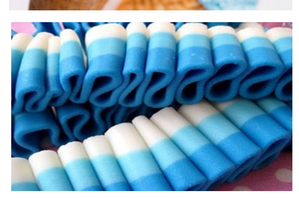
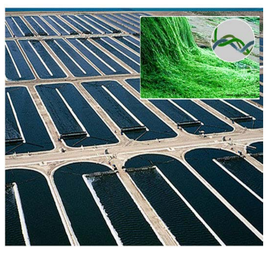
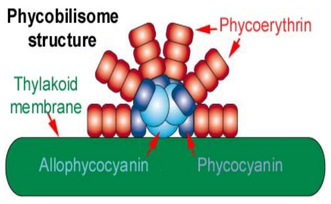
INTRODUCTION

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.



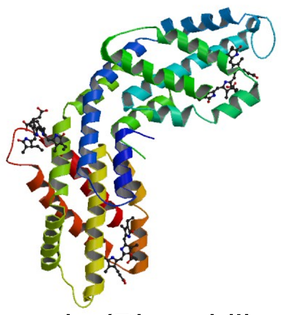


Cultivation of *Arthrospira platensis*



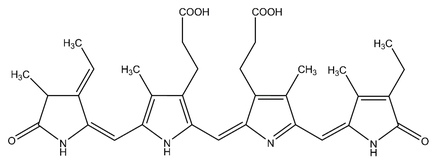
Phycobilisomes (Light harvesting complexes of phycobiliproteins)

Extraction



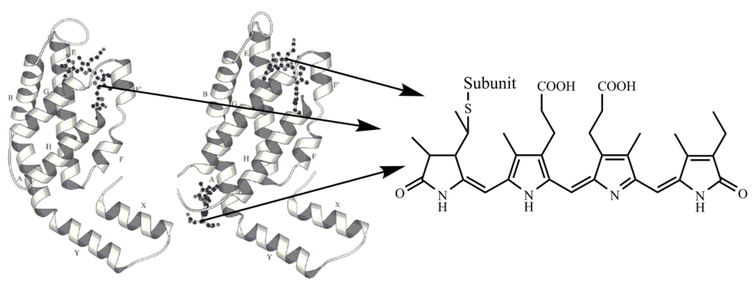
C-Phycocyanin (Phycobiliprotein)

Cleavage



Phycocyanobilin (Chromophore)

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:

$$P - \nu_1 PCB(I) \rightarrow P + \nu_1 PCB;\ P - \nu_2 PCB(II) \rightarrow P + \nu_2 PCB$$

Or two first order reactions in series:

$$P - \nu_1 PCB(I) - \nu_2 PCB(II) \rightarrow P - \nu_2 PCB(II) + \nu_1 PCB;\ P - \nu_2 PCB(II) \rightarrow P + \nu_2 PCB$$

Where PCB(I) is easily accessible and PCB(II) is less accessible for cleavage, ν_1 and ν_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:

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

Analytical solutions for set of equations above is:

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

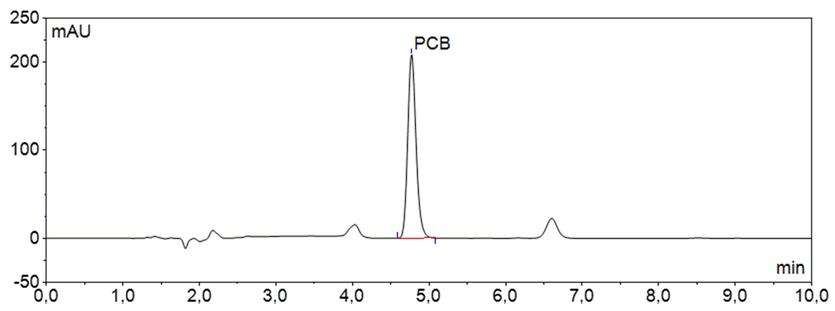
Where C_{10} and C_{20} are initial concentration of PCB(I) and PCB(II), respectively

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

$$C_{PCB}(t) = \upsilon_1 \cdot C_{10} \cdot \left(1 + \frac{\upsilon_2}{\upsilon_1} + \frac{\upsilon_2}{\upsilon_1} \cdot \frac{C_{20}}{C_{10}}\right) - \upsilon_1 \cdot C_{10} \cdot \left(1 + \frac{\upsilon_2}{\upsilon_1} + \frac{\upsilon_2}{\upsilon_1} \cdot \frac{k_1}{k_1 - k_2}\right) \cdot e^{-k_1 \cdot t} + \upsilon_1 \cdot C_{10} \cdot \left(\frac{\upsilon_2}{\upsilon_1} \cdot \frac{k_1}{k_1 - k_2} - \frac{\upsilon_2}{\upsilon_1} \cdot \frac{C_{20}}{C_{10}}\right) \cdot e^{-k_2 \cdot t}$$

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

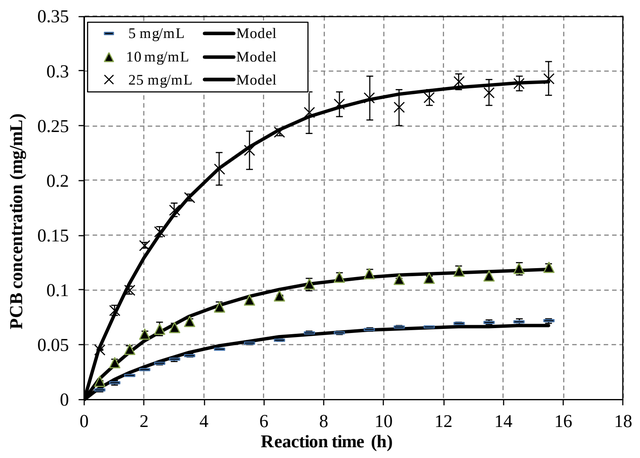


Chromatogram of methanolysis mixture

RESULTS

Table 1. Model data fitted to experimental data.

Initial protein concentration (mg/mL)	$\nu_1 . C_{10}$ (mg/mL)	$\frac{\nu_2}{\nu_1}$	$\frac{C_{20}}{C_{10}}$	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.

CONCLUSION

- Kinetic model describes the experimental data adequately
- The ratio between υ_2 and υ_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

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