Kinetics of phycocyanobilin cleavage from C-phycocyanin by methanolysis

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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 industry due to its potential as a natural blue food color.

Phycobilisomes (Light harvesting complexes of phycobiliproteins)

Cultivation of Arthrospira platensis

Extraction

Phycobilisomes

C-Phycocyanin (Phycobiliprotein)

Phycocyanobilin (Chromophore)

KINETIC MODEL FOR CLEAVAGE OF PCB BY METHANOLYSIS

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

\[ P - \nu_1 \text{PCB(I)} \rightarrow P + \nu_1 \text{PCB}; \quad P - \nu_2 \text{PCB(II)} \rightarrow P + \nu_2 \text{PCB} \]

Or two first order reactions in series:

\[ P - \nu_1 \text{PCB(I)} - \nu_2 \text{PCB(II)} \rightarrow P - \nu_2 \text{PCB(I)} + \nu_1 \text{PCB}; \quad P - \nu_2 \text{PCB(II)} \rightarrow P + \nu_2 \text{PCB} \]

Where PCB(I) is easily accessible and PCB(II) is less accessible for cleavage, \( \nu_1 \) and \( \nu_2 \) 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:

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

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:

\[ C_{PCB}(t) = \nu_1 \cdot C_{10} \cdot \left( 1 + \frac{\nu_2}{\nu_1} \cdot \frac{C_{20}}{C_{10}} \right) - \nu_1 \cdot C_{10} \cdot \left( 1 + \frac{\nu_2}{\nu_1} \cdot \frac{\nu_1 \cdot k_1}{k_1 - k_2} \right) \cdot e^{-k_1 \cdot t} + \nu_1 \cdot C_{10} \cdot \frac{\nu_2}{\nu_1} \cdot \frac{k_1}{k_1 - k_2} \cdot e^{-k_2 \cdot t} \]

EXPERIMENTAL

- Linablu (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 Linablu used

MENTAL

- Kinetic model describes the experimental data adequately
- The ratio between \( \nu_2 \) and \( \nu_1 \) 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.

<table>
<thead>
<tr>
<th>Initial protein concentration (mg/mL)</th>
<th>( \frac{\nu_2}{\nu_1} )</th>
<th>( C_{20} )</th>
<th>( k_1 ) (h⁻¹)</th>
<th>( k_2 ) (h⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.7×10⁻³</td>
<td>24</td>
<td>1.0×10⁻⁴</td>
<td>33</td>
</tr>
<tr>
<td>10</td>
<td>4.7×10⁻³</td>
<td>24</td>
<td>1.0×10⁻⁴</td>
<td>33</td>
</tr>
<tr>
<td>25</td>
<td>12×10⁻³</td>
<td>24</td>
<td>1.0×10⁻⁴</td>
<td>33</td>
</tr>
</tbody>
</table>

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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