A simple method for efficient extraction and purification of C-phycocyanin from *Spirulina platensis* Geitler

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Phycocyanin is a major light harvesting accessory pigment of red algae and cyanobacteria. In the light of its many commercial applications in food and pharmaceutical industry, purity of the pigment plays a major role. Pharmaceutical industry demands a highly pure phycocyanin with $A_{620}/A_{280}$ ratio of 4 and food industry a ratio of 2. In the present study phycocyanin was extracted in sodium phosphate buffer (pH 7) after macerating in liquid nitrogen. The crude phycocyanin thus extracted was precipitated with 50% ammonium sulphate, purified by dialysis and finally by gel filtration chromatography. Pure phycocyanin was finally obtained with an $A_{620}/A_{280}$ value of 4.98.

**Keywords:** C-phycocyanin, Extraction, Dialysis, Gel electrophoresis

Phycocyanin, allophycocyanin and phycoerythrin are the components of phycobiliproteins and are the major photosynthetic accessory pigments in certain microalgae. Among microalgae, the genus *Spirulina* are a rich and inexpensive source of the pigment, phycocyanin. Phycocyanin has many commercial applications and is used as food colourants, cosmetics and in biomedical research. It is also a potential pharmaceutical in oxidative stress-induced diseases as it has antioxidant and anti-inflammatory properties. Although, several methods have been developed for the extraction and purification of phycocyanin from microalgae, the purity is relatively low. In the present study an attempt has been made to develop a simple and efficient method for the extraction employing liquid nitrogen for cell disintegration as these proteins are found to be regularly arranged in parallel rows on the thylakoid membrane. With respect to the purity, the phycocyanin needs to be separated selectively from the contaminant proteins. The $A_{620}/A_{280}$ ratio serves as the index of purity for phycocyanin. The values of 4 and 2 finds application in pharmaceutical and food industry, respectively.

### Materials and Methods

*Spirulina platensis* was grown in 100 ml Zarrouks medium at pH 9.5 in 250 ml conical flasks and incubated at 24±2°C and 3000 lux light intensity provided in 16/8 hr L/D cycle. The culture flasks were shaken twice a day at 50 rpm for 30 min. The algal biomass was harvested after 30 days by centrifugation at 6000 rpm for 10 min. Approximately 7 g fresh biomass was taken and washed twice with distilled water. The biomass obtained after second washing was macerated in liquid nitrogen in a pestle and...
mortar till fine powder was obtained. The powder was suspended in 10 ml of 0.05 M sodium phosphate buffer (pH 7). The suspension was incubated in 1ml of lysis buffer [1M Tris, pH 8; 0.5 M EDTA; 20% sucrose (w/v)]. Lysozyme was added to a final concentration of 5 mg ml⁻¹ and incubated for 2 hr at 37°C. The mixture was then centrifuged at 10000 rpm for 15 min and the phycocyanin containing clear blue supernatant was collected.

The blue supernatant was fractionally precipitated with ammonium sulphate at 30 and 50% (w/v). The protein precipitated by 30% (w/v) ammonium sulphate precipitation was discarded. The one obtained with 50% solution contained mainly phycocyanin, which was dissolved in a small volume of 0.05 M Na-phosphate buffer (pH 7). The solution was then subjected to dialysis against the same buffer, which was diluted 1000 times. The dialysis was carried out for 4 hr while changing the buffer in between. The dialyzed sample was passed through Sephadex G-25 (1.5 x 20 cm) pre-equilibrated and eluted with 0.005 M Na-phosphate buffer (pH 7) at 1ml min⁻¹. The first fraction showing absorption maxima at 620 nm was taken as phycocyanin. This was again passed through on Sephadex-G-100 column (2.5 x 20 cm) pre-equilibrated and eluted with 0.005 M Na-phosphate buffer (pH 7) at 1ml min⁻¹. The products obtained after every step of purification were analyzed for total proteins, phycocyanin content and subjected to spectral scanning in Beckman DU 64 spectrophotometer. SDS-PAGE was carried out using a 12.5% polyacrylamide slab gel that included a 4% stacking gel.

**Results and Discussion**

The usual methods employed for isolation of intracellular substance is cell disruption by sonication or French pressure cell and more conventionally by grinding with some abrasives, followed by centrifugation and/or filtration. Here we have employed liquid nitrogen for the cell disruption. Additionally, lysozyme is used for the cell disruption and to maximize the phycocyanin yield. The total protein content, phycocyanin content and the purity of the phycocyanin are presented in Table 1. The purity of the phycocyanin increased after every stage of separation. The purity of crude phycocyanin came to 0.97, which was above 0.905 (ref. 9). The precipitate obtained after ammonium sulphate precipitation gave a purity of 1.43, again well above 1.26 (ref. 9). The dialyzed fraction when further subjected to purification by gel filtration chromatography using Sephadex G-25 column gave the purity of 3.73. Phycocyanin is considered highly pure if the $A_{280}/A_{280}$ is greater than 4. An absorption of less than 4 is not considered pure and further purification is needed. Therefore the fraction needed further purification. An absorption ratio of 2.8 with hydroxylapatite column was obtained. In this study we used Sephadex G-25 column and obtained a ratio of 3.73, which is close to 4.

The fractions collected from the Sephadex G-25 column were further purified and desalted on Sephadex G-100 column (2.5 x 20cm) with 0.005 M Na-phosphate buffer (pH 7) at 1ml min⁻¹. The hydroxylapatite column was not employed in our step and it was replaced with another column Sephadex G-100. The absorption ratio for the purified phycocyanin rose to 4.98 (Table 1) which was considerably higher than reported earlier (4.15). The purity of phycocyanin obtained after every step is compared with that of the standard reference (Table 2). The purified phycocyanin was further confirmed by absorption spectral scanning (Fig. 1). The sharpening of the peak between the crude and the purified extract is clearly evident. The peak of the purified phycocyanin is sharper than the peak exhibited by the crude extract. Further from the figure the maximum absorbance was observed at 620 nm and this coincides with the previously reported works. The purified fragments were tested on SDS-PAGE (data not shown). The fraction in the last step clearly showed two bands of phycocyanin sub units at a molecular weight of about 16 and 19 kDa. There was

<table>
<thead>
<tr>
<th>Steps</th>
<th>Total protein (µg ml⁻¹)</th>
<th>Phycocyanin (µg ml⁻¹)</th>
<th>Phycocyanin (%)</th>
<th>Impurities (%)</th>
<th>$A_{280}/A_{280}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>2.40</td>
<td>1.30</td>
<td>54.16</td>
<td>45.84</td>
<td>0.97</td>
</tr>
<tr>
<td>Ammonium sulphate precipitation (50%, w/v)</td>
<td>1.70</td>
<td>1.41</td>
<td>82.94</td>
<td>17.06</td>
<td>1.43</td>
</tr>
<tr>
<td>Sephadex G-25 column</td>
<td>1.81</td>
<td>1.57</td>
<td>85.08</td>
<td>14.92</td>
<td>3.73</td>
</tr>
<tr>
<td>Sephadex G-100 column</td>
<td>1.75</td>
<td>1.59</td>
<td>90.85</td>
<td>9.15</td>
<td>4.98</td>
</tr>
</tbody>
</table>
There was a reduction in the impurities content at every stage of purification. A new simplified method comparable with the other methods, are ion-exchange chromatography and no specific of extraction and purification of phycocyanin from Spirulina. When compared to the other methods, this is advantageous as it does not employ any ion-exchange chromatography and no specific instrument is needed for cell disruption. Further, the use of ultra centrifuge is avoided. Even when these are not used, the purity of the phycocyanin is comparable with the other methods.

Table 2 — Comparison of purity at different purification steps

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Standard method</th>
<th>Present study</th>
</tr>
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<tbody>
<tr>
<td>Crude phycocyanin</td>
<td>0.905</td>
<td>0.97</td>
</tr>
<tr>
<td>Ammonium sulphate precipitation</td>
<td>1.26</td>
<td>1.43</td>
</tr>
<tr>
<td>Hydroxylapatite column</td>
<td>2.8</td>
<td>-</td>
</tr>
<tr>
<td>DEAE Sephadex A-50 column</td>
<td>4.15</td>
<td>3.73</td>
</tr>
<tr>
<td>DEAE-Sepharose CL-6B</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>-</td>
<td>4.98</td>
</tr>
</tbody>
</table>

Fig. 1 — Absorption spectral scanning of pure and crude phycocyanin

The phycocyanin content after every stage of purification increased with every step. Initially the crude extract prior to ammonium sulphate precipitation recorded a 54.16% phycocyanin and it gradually increased. The phycocyanin content after the final purification step was to the tune of 90.85%. There was a reduction in the impurities content at every stage of purification. A new simplified method of extraction and purification of phycocyanin from Spirulina platensis has been depicted in this communication. When compared to the other methods, this is advantageous as it does not employ any ion-exchange chromatography and no specific instrument is needed for cell disruption. Further, the use of ultra centrifuge is avoided. Even when these are not used, the purity of the phycocyanin is comparable with the other methods.

References