ONE-STEP PURIFICATION OF C-PHYCOCYANIN EXTRACTED FROM THE WET BIOMASS OF *Spirulina platensis* LEB-52

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ABSTRACT – *The aim of this work was to purify C-phycocyanin (C-PC) extracted from the wet biomass of Spirulina platensis* LEB-52 by ion-exchange chromatography (IEC) with elution by pH gradient and compare it with elution by step followed by NaCl gradient, which is an optimized condition for C-PC purification. The best results were obtained when pH gradient was used, resulting in C-PC with purity of 4 and 60 % of recovery in one purification step. IEC with elution by pH gradient is rarely applied in phycobiliproteins’ purification, but the results showed that it is a promising technique for C-PC purification.

1. INTRODUCTION

C-phycocyanin (C-PC) is a phycobiliprotein that can be extracted from *Spirulina* (Eriksen, 2008) that presents antioxidant and anticancer properties (Deniz *et al*., 2016). Depending on its purity grade (PE), it can be applied as a natural blue dye in foods (PE between 0.5 and 0.75) and cosmetics (PE between 1.5 and 2.5), as a biomarker (PE between 2.5 and 3.5) and in medical treatments (PE > 4.0, analytical grade) (Delhi Nutraceuticals, 2018). Because C-PC is an intracellular compound, a cellular disruption step is required for its extraction. In this sense, the development of more selective extraction methods is crucial for subsequent purification steps, because it reduces the release of contaminant proteins, which would increase the adsorption of the protein of interest during chromatography, for example (Balasundaram *et al*., 2009). Selective extraction by biomass pretreatment with ethylenediamine tetraacetic acid (EDTA) allows the obtainment of C-PC with food grade purity (PE ≥ 1) without any purification steps (Sala, 2017).

The elution of proteins in ion exchange chromatography (IEC) can be accomplished by increasing the buffer’s ionic strength (by increasing the salt concentration) or by changing the charge of the protein (by a pH change) (Wheelwright, 1991). Linear NaCl gradient elution is widely used for protein purification and it has been optimized for the purification of C-PC extracted from the dry biomass of *Spirulina* by Moraes and Kalil (2009). Linear pH gradient elution, although little discussed in the literature for the purification of phycobiliproteins, is also very efficient in obtaining high purities and recoveries (Liu *et al*., 2005; Su *et al*., 2010; Yan *et al*., 2011; Kumar *et al*., 2014). Thus, the aim of this work was to purify C-PC extracted from the wet biomass of *S. platensis* LEB-52 by IEC, comparing elution by pH gradient and by step followed by salt gradient.
2. MATERIAL AND METHODS

*Spirulina platensis* LEB-52 was cultivated in Zarrouk medium according to Sala *et al.* (2018). The biomass was recovered by filtration and resuspended in EDTA diluted in Tris-SO$_4$ buffer (50 mmol·L$^{-1}$, pH 7.4), with a biomass concentration of 1.5 mg$_{biomass}$·mL$^{-1}$. The suspension was homogenized for 30 s and incubated for 1 min at 25°C. Afterwards, the biomass was filtered and resuspended in Tris-SO$_4$ buffer and incubated at 25°C for 24 h, according to Sala (2017). The C-PC extract was centrifuged (12,000 x g, 25°C, 20 min) and filtered in a 0.45 µm membrane. The clarified extract was loaded (40 cm·h$^{-1}$) in a C10/20 (20 x 1 cm) column containing 10 cm of the anion exchange resin Q-Sepharose Fast Flow™ (GE Healthcare), previously equilibrated with Tris-HCl (25 mmol·L$^{-1}$, pH 6.5) (Moraes and Kalil, 2009). The adsorbed proteins were removed by washing with the same equilibration buffer. The elution of C-PC using a step of NaCl 0.1 mol·L$^{-1}$ followed by linear gradient (0.1 – 1 mol·L$^{-1}$) with 50 mL NaCl (diluted in Tris-HCl buffer 25 mmol·L$^{-1}$, pH 5) at the rate of 40 cm·h$^{-1}$ was performed according to Moraes and Kalil (2009). The elution of C-PC using a pH gradient was performed according to Kumar *et al.* (2014) with modifications, using 50 mL of 0.2 mol·L$^{-1}$ acetate buffer in a pH gradient of 5.6 – 3.4 at the rate of 40 cm·h$^{-1}$. In both assays, 3 mL fractions were collected and analyzed for pH and C-PC concentration, purity, recovery and purification factor.

C-PC concentration (C-PC, mg·mL$^{-1}$, Equation 1) was calculated as described by Bennett and Bogorad (1973). C-PC purity (EP) was calculated according to Equation 2, where the absorbance at 620 nm ($A_{620}$) indicates the C-PC concentration and the absorbance at 280 nm ($A_{280}$) indicates the total protein concentration in the solution. C-PC recovery (Rec %, Equation 3) after the purification processes was calculated by the ratio between the sum of C-PC mass in the purified fractions and C-PC mass in the initial extract. The purification factor (PF) was calculated by the ratio between the purity after the chromatographic step and the initial extract purity.

\[
C-PC = \left( A_{620} \cdot 0.474 \times A_{552} \right)/5.34
\]  
(1)

\[
EP = \frac{A_{620}}{A_{280}}
\]  
(2)

\[
Rec(\%) = \left( \sum C-PC_i \times V_i )/(C-PC \times V) \right) \times 100
\]  
(3)

3. RESULTS AND DISCUSSION

Table 1 presents the purification parameters of both assays. Assay 1 uses the same elution mode optimized for C-PC purification by Moraes and Kalil (2009), where C-PC extracted from the dry biomass of *S. platensis* LEB-52 was purified by precipitation and dialysis followed by IEC with elution by step and NaCl gradient. This resulted in C-PC with purity of 4.0, 44 % of C-PC recovery and a purification factor of 6.35 times. In our study, C-PC extracted from the wet biomass of the same cyanobacterium was used without any previous purification step and the highest C-PC purity obtained with elution by step and NaCl gradient was 3.35. However, when the elution by pH gradient (Assay 2) was tested it resulted in C-PC with purity of 4.03 with 60 % of recovery. This result indicates that the pH gradient elution is efficient in...
the purification of C-PC extracted from wet biomass, since it provided a higher purification factor, recovery and final C-PC purity and concentration when compared to the optimized elution mode (Table 1).

Table 1 – Purification parameters of Assays 1 (Step and NaCl gradient) and 2 (pH gradient).

| Fractions | Assay 1 | Assay 2 |
|-----------|---------|---------|
| Initial   | EP      | PF      | C-PC | Rec | EP      | PF      | C-PC | Rec |
| 0.90      | 1.0     | 0.072   | 100  | 0.96 | 1.0     | 0.072   | 100  | 60  |
| EP ≥ 4    | -       | -       | -    | 4.03 | 4.2     | 0.150   | 60  | -   |
| 3.5 ≤ EP < 4 | 3.35 | 3.7     | 0.049 | 30  | -       | -       | -    | -   |
| 2.5 ≤ EP < 3.5 | 2.90 | 3.2     | 0.029 | 40  | 2.60    | 2.7     | 0.036 | 15  |
| 1.5 ≤ EP < 2.5 | 1.90 | 2.1     | 0.014 | 14  | 0.96    | 1.0     | 0.030 | 6.2 |

EP: purity; PF: purification factor; C-PC: C-PC concentration (mg·mL⁻¹); Rec: C-PC recovery (%)

To our knowledge, only four studies used IEC with pH gradient elution to purify phycobiliproteins, all of them after a precipitation and dialysis step (Liu et al., 2005; Su et al., 2010; Yan et al., 2011; Kumar et al., 2014). Yan et al. (2011) used acetate buffer with 50 mmol·L⁻¹ of NaCl in a pH gradient of 5 – 3.6, resulting in a C-PC final purity of 5.59 and 67 % recovery. Kumar et al. (2014) also used acetate buffer in a pH gradient of 5.1 – 3.76, obtaining C-PC with purity of 4.58 and 14 % recovery. Su et al. (2010) and Liu et al. (2005) used phosphate buffer with 50 mmol·L⁻¹ of NaCl in a pH gradient of 5.6 – 4, obtaining allophycocyanin with a purity of 5.0 and 43 % recovery, and R-phycoerythrin with purity of 5.6 and 67 % recovery, respectively. Here, C-PC with a purity of 4.03 with 60 % recovery was obtained with one purification step, using acetate buffer without salt addition.

It can also be observed in Table 1 that, even though Assay 2 resulted in C-PC with higher purity (EP ≥ 4), the global C-PC recoveries of both assays were the same, being 84 % for Assay 1 and 81 % for Assay 2. Additionally, in Assay 1 it was possible to obtain C-PC with purities of 3.35 and 2.90 with 30 and 40 % recoveries, respectively, which are purities suitable to the application of C-PC as a dye in cosmetics and as a biomarker in immunoassays. Therefore, the loading of C-PC extracted from the wet biomass of *S. platensis* in IEC columns results in high purities and high recoveries; specially, IEC with elution by pH gradient presents high efficiency of C-PC purification, being a promising technique worth exploring.

4. CONCLUSION

C-phycocyanin with purity of 4 and 60 % of recovery was obtained when using ion-exchange chromatography with elution by pH gradient. When compared with the optimized elution by step followed by NaCl gradient, pH gradient elution provided higher results for purification factor, recovery and final C-PC purity and concentration. Acknowledgments: CAPES and CNPq.
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