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Partial purification of the R-phycoerythrin from the red seaweed *Solieria filiformis* using anion exchange chromatography

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Abstract

In this work, we describe the determination of elution steps for partial purification of the R-phycoerythrin from the macroalgae *Solieria filiformis* using anion exchange (AEX) chromatography on a HiTrap® Q Fast Flow (Q FF) column. The mobile phase consisted of two buffers: solution A (PBS) and solution B (PBS + NaCl 1 M). Elution was carried out using salt linear gradient (0-100 %B) and then using a stepwise elution increase in the ionic strength of the buffer (210, 350, 620 mM). CFII (350 mM) was determined as the peak presenting R-PE showing a PI of 2.42, proving to be an effective purification with a 3.06-fold enrichment. It was possible to separate R-PE and improved peak's resolution through a stepwise elution with HiTrap Q FF®.

Keywords: R-phycoerythrin; Solieria filiformis; liquid chromatography; IEX.

1. Introduction

Phycobiliproteins (PBP) are water-soluble fluorescent proteins, usually classified as phycoerythrins, phycocyanins, and allophycocyanins. R-phycoerythrin (R-PE), known for its bright reddish-pink color, is a highly soluble fluorescent protein found in red algae. [1]

R-PE is a highly soluble and stable protein in water, which allows it to bind to antibodies and other proteins without altering its structure [2]. R-PE is non-toxic to humans and has been widely used in the food, cosmetics and pharmaceutical industries [3].

The process of obtaining phycobiliproteins can be divided into three stages: the polysaccharide-rich cell wall rupture, extracting the phycobiliprotein and purifying these biomolecules to eliminate contaminants. These biomolecules are extracted using several different techniques that can be classified as mechanical, physical, chemical or enzymatic. These selected methods are strongly influenced by the physicochemical characteristics of the compound of interest [4,5].

Purification is an important stage in the manufacture of pigments and is necessary to obtain specific compounds. The approach to the purification stage can vary depending on the type of pigment, the source, the technologies available and the associated costs [6].

Chromatography is the most widely used technique for purifying these biomolecules including ionic exchange chromatography (IEX), size exclusion chromatography (SEC) or expanded bed adsorption (EBA).

Among chromatographic methods, IEX is the most widely used for purifying proteins and enzymes. This technique offers high resolution and the ability to separate proteins with similarities, as well as the affordable possibility of optimization just by modifying the elution conditions. It is highly selective and offers the advantages of reduced purification time, with improved protein yield and purity [7].

The traditional methods used to purify phycoerythrins from red algae involve a

combination of techniques such as ion exchange chromatography, gel filtration, hydrophobic interaction chromatography, hydroxyapatite chromatography, electrophoresis and ultrafiltration, which, despite increasing the purity of the sample, have a high cost due to the number of steps. [8], [9], [10], [11]

Improving the chromatographic process is an important part of the purification process. To maximize the effectiveness and efficiency of biocompound purification, systematic and knowledge-based techniques are used. [12]

R-phycoerythrin (R-PE) purity, measured by the ratio of active substance to total material, varies by use: 0.7 for food grade, 3.9 for reagent grade, and over 4.0 for analytical grade. These variations affect its price, with conjugates to antibodies or fluorescent molecules costing up to $1,500 per milligram. R-PE is widely used as a fluorescent marker in immunology, cell biology, and the food and pharmaceutical industries. [13]

In this work, we describe the determination of elution steps for partial purification of the R-phycoerythrin from the macroalgae *Solieria filiformis* using anion exchange (AEX) chromatography on a HiTrap® Q Fast Flow (Q FF) column.

2. Materials and methods

The red macroalgae *Solieria filiformis* (Solieriaceae, Rhodophyta) was cultivated and furnished by Flecheiras and Guajiru Algae Producers Association (APAFG) - Trairi, Ceará State, Brazil. This species is registered under code A41C95F in the SisGen (National Genetic Heritage Management System). The macroalgae were harvested and were properly washed in potable water, separated from epiphytes, washed with distilled water, and stored at −20 °C until use.

After the thawing process, the solid-liquid extraction of R-PE was performed following the adapted procedures previously described [14]. The wet seaweed was ground in an electric mill in 25 mM potassium phosphate buffer solution, pH 6.5 (PBS) at a macroalgae : buffer ratio of 1:4 (w/v) for 4 min and homogenized under mechanical agitation (model 713DS, Fisatom) at 250 rpm for 4 h. The homogenate was filtered through nylon fabric, then the solid residue was reserved for sequential extraction of sulfated polyssacharides, following the biorefinery concept, and the filtrate was centrifuged at 17,000 × g for 30 min at 4 °C. The supernatant collected was denominated crude extract of *S. filiformis* (CE).

CE was then subjected to protein precipitation with ammonium sulfate 90% [15].

The protein fraction obtained from the ammonium sulfate precipitation (F0/90) was subjected to anion exchange (AEX) chromatography. The commercial column, obtained from Cytiva, HiTrap Q FF 1 mL (17505301) measured 7 x 25 mm and was pre-equilibrated with PBS and applied to the ÄKTA Pure chromatography system. The mobile phase consisted of two buffers: solution A (PBS) and solution B (PBS + NaCl 1 M). Elution was carried out using salt linear gradient (0-100 %B) and then using a stepwise elution increase in the ionic strength of the buffer (210, 350, 620 mM).

For analytical purposes, samples from each chromatographic fraction (CF) were dialyzed overnight using a 12 kDa cutoff membrane against distilled water. R-PE concentration [16], and R-PE purity index (PI) [17] were measured at CE, F0/90 and CF. Equations 1 and 2 were used for these determinations in which the absorbances were measured at 280 nm, 564 nm, 618 nm, and 730 nm.

$$R-PE\left(\frac{\mu g}{mL}\right) = 0{,}1247 \times [(A_{564} - A_{730}) - 0{,}4583 \times (A_{618} - A_{730})] \times 10^3 \quad (1)$$

$$PI\ (a.u.) = \frac{A_{564}}{A_{280}} \quad (2)$$

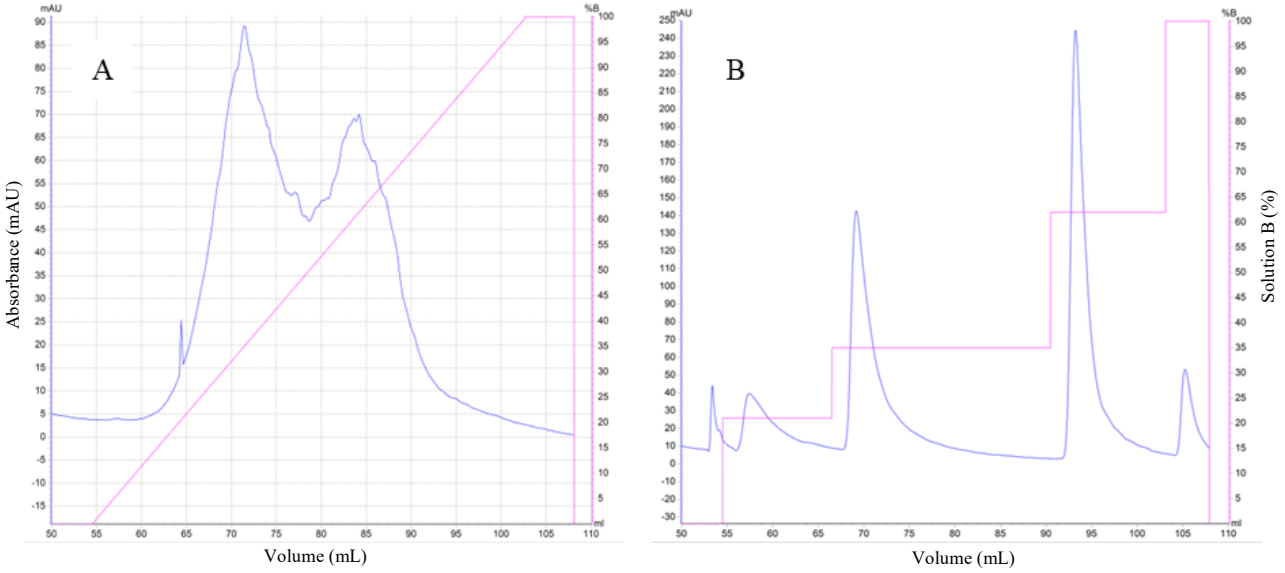

Fig. 2 AEX separation of R-PE using Q FF 1 mL column at a flow rate of 1 mL/min. (A) Linear salt gradient (0−100 %B) and (B) improved stepwise elution (210, 350 and 620 mM). The elution profile was monitored by UV-visible spectrophotometry at 280 nm represented by the blue line (—) and NaCl concentration is indicated by the pink line (—).

3. Results and Discussion

R-PE concentration of CE from *S. filiformis* was 127 µg mL^{-1}. After protein precipitation with ammonium sulfate from CE, R-PE concentration was 187 µg mL^{-1}.

The separation procedure of R-PE involved AEX chromatography with HiTrap Q FF®. The chromatogram in Figure 2 shows the AEX elution pattern for a linear gradient (Fig. 2A) and improved stepwise elution (210, 350 and 620 mM) (Fig. 2B).

Figure 2A shows protein are capable to adsorb in the matrix however it presents overlapping peaks, signaling low resolution. Figure 2B shows it was possible to obtain higher peak's resolution through a three-stepwise elution.

The chromatographic fractions were collected in 210, 350 and 620 mM NaCl and called CFI, CFII and CFIII, respectively. Analytical results of each chromatographic fraction are shown in Table 1.

Table 1. Quantification of chromatographic fractions for R-PE from *S. filiformis* purified at Q FF column

Procedures	*Chromatographic fractions*		
	CFI	CFII	CFIII
[NaCl] (mM)	210	350	620
PI (A.U.)	0.88	2.42	0.82
[R-PE] (µg mL^{-1})	0.403	101	1.76
R-PE content (µg)	1.81	604	0.791
Recovery (%)	0	4	0

CFII was determined as the peak presenting R-PE and along with previous steps is show in the purification summary table below (Table 2).

Table 2. Purification summary table for R-PE from *S. filiformis* purified at Q FF column

Procedure	*Steps*		
Sample name	CE	F0/90	CFII
Purity index (a.u.)	0.79	0.74	2.42
[R-PE] (µg mL^{-1})	127	187	101
Enrichment	1.00	0.93	3.06

CFII had an PI of 2.42, proving to be an effective purification with a 3.06-fold enrichment.

The use of one step both reduced the total cost of the process and made upscale possible. Previous studies with other species of algae had purity indices ranging from 3 to 5.6, however, compared to this study, more complex procedures with more than one step were implemented.[18]

5. Conclusion

In this study, elution steps are determined for an AEX separation chromatography of R-PE from *S. filiformis*. By this means, a 3.14-fold enrichment was obtained, reaching an IP of 2.48. The efficiency of this method and its high purity was shown by the absorption spectrum. The number of traditional processing steps were reduced, as was the possibility of protein loss and denaturation during purification.

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