R-PHYCOERYTHRIN: CHROMATOGRAPHIC PURIFICATION METHODS

Helder N. Veras[1], Allison R. M. Silva[1], Raissa M.F Lima[1] & Ivanildo J. S. Júnior[1]

[1] *Departiment of Chemical Engineering, Federal University of Ceará, Fortaleza, Brazil.*
** ivanildo@ufc.br*

ABSTRACT

In recent years, the growing search for sustainable alternatives has boosted interest in natural products, with proteins derived from seaweed standing out. R-phycoerythrin (R-PE), a fluorescent protein found in red algae, has attracted particular attention due to its versatility and vast potential applications. This study focuses on analyzing the chromatographic methods used to purify R-PE. An in-depth understanding of these processes not only advances scientific and technological knowledge, but also promotes innovations in various areas. By optimizing the purification of R-PE, space is opened up for the development of more effective and sustainable technologies, with applications in biotechnology, medicine and other fields.

Keywords: Phycobiliproteins. Rhodophyta. Applications. Chromatography. Purification.

1 PHYCOBILIPROTEINS

Phycobiliproteins (PBP) are the units that make up the phycobilisome protein complex (FBS) (Figure 1), responsible for capturing light in the photosynthetic apparatus of algae. This complex absorbs radiation from the spectrum whose chlorophyll has low absorption[1]. PBPs are made up of proteins and chromophores, unsaturated organic groups that absorb radiation from the spectrum, called phycobilins. Phycobilins are classified based on their characteristic color: phycoviolobilin (PVB) is violet, phycocyanobilin (PCB) is blue, phycoerythrobilin (PEB) is red and phycrobilin (PUB) is yellow[2].

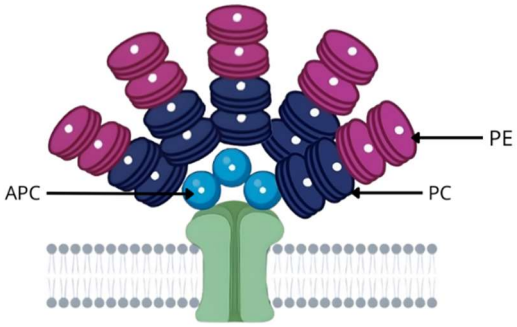

Figura 1. Estrutura do ficobilissoma, composta pelas ficobiliproteinas como subunidades: PE (ficoeritrina), PC (ficocianina), e APC (Aloficocianina).

Phycobiliprotein molecules are water-soluble and, according to their absorption peak in the spectrum, can be divided into four main types: allophycocyanin (650 - 660 nm); phycocyanin (610 - 625 nm); phycoerythrin (490 - 570 nm) and phycoerythrocyanin (560 - 600 nm)[3]. In addition to this classification, prefixes are used to indicate taxonomic origin: C-, Cyanobacteriana; B-, Bangiofícea; and R-, Rhodophyta [4]. The use of these proteins has been investigated as anti-cancer, anti-inflammatory, antioxidant and anti-pathogenic agents [5].

Phycoerythrin is one of the main and most abundant light-collecting pigments in red algae. The spectral characteristics of phycoerythrin vary according to its origin: B-phycoerythrin (B-PE) (λmax 540-560 nm); R-phycoerythrin (R-PE) (λmax ~565); C-phycoerythrin (C-PE) (λmax ~563)[4]. Among them, R-PE stands out, a protein commonly used as a fluorescent marker in immunology, cell biology [6] and in the food and pharmaceutical industries [7,8]. The characteristics that make it stand out, its applications and the chromatography methods used to purify it are discussed below.

2 R-PHYCOERYTHRIN

R-PE is a 240-290 kDa oligomeric protein from the phycobiliprotein group, with subunits 6 α (around 20 kDa), 6 β (around 20 kDa) and 1 γ (around 30 kDa)[9] It is the main pigment of the Rhodophyta but is also found in cyanobacteria. Its reddish-pink color is characteristic of linear tetrapyrrolic chromophores covalently linked to phycobilins via a thioether bond[4]. It is a highly soluble and stable protein in water, which allows it to bind with antibodies and other proteins without altering its structure [8]. R-FE is non-toxic to humans and has been widely used in the food, cosmetics and pharmaceutical industries [10,11] .

With a market that is increasingly demanding healthy products, the food industry is striving for new inputs. Its vibrant colors and fluorescence, combined with its proven safety, make R-PE a promising choice for food pigmentation[12]. In the cosmetics sector, the preference for natural ingredients is also evident, due to their proven safety[13], anti-allergic and anti-aging properties. Studies

have shown that, within controlled groups, this protein significantly increased the synthesis of type 1 collagen[14]. In addition, the pharmaceutical industry has shown great interest, holding 60% of all patents related to R-PE, being driven mainly by its ability to potentiate the action of conventional anticancer drugs, to be used in immunodiagnostics for the early detection of diseases and as a new immunostimulant[4,15].

The purity of R-phycoerythrin, defined as the ratio between the active substance and the total amount of material, varies according to each of its applications, where a purity of 0.7 is considered food grade, 3.9 as reactive grade, and greater than 4.0 as analytical grade. These variations in purity and their applications result in a wide price range for R-FE, with prices ranging from US$ 3 to US$ 25 per milligram for native pigment. When R-FE is cross-linked to antibodies or other fluorescent molecules it can reach a value of US$1,500 per milligram [15,16].

3 PURIFICATION METHODS OF R-PHYCOERYTHRIN

Purification is an important stage in the manufacture of pigments and is necessary in order to achieve 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[17]. Before starting the purification process, the crude extract containing the R-PE extracted from the red macroalgae biomass is produced, followed by concentration steps such as precipitation with ammonium sulphate. Finally, to achieve purification, chromatographic processes are applied. A single chromatographic step or a combination of them can be applied, including ion exchange chromatography and size exclusion chromatography[18,19], which is the main focus of this review. Chromatographic purification methods make it possible to separate and purify the components of the extract with high efficiency. Table 2 summarizes the chromatographic techniques used for this purpose, as well as the biomasses that provided the protein extract, providing a comprehensive overview of the approaches employed in the R-PE purification process.[20-28].

Table 2. R-FE purification methods and their purity.

Species	Purification process	Column	Purity	Ref.
Palmaria palmate	Preparative polycrylamide gel electrophoresis	-	3.20	20
Porphyra purpurea	Precipitation with ammonium sulphate;Ion exchange chromatography	DEAE-Sepharose	5.10	21
Heterosiphonia japonica	Precipitation with ammonium sulphate;Gel filtration chromatography;Ion exchange chromatography	Sepharose CL-4B e Sephadex G-200; DEAE Sepharose Fast Flow	4.89	22
Porphyra yezoensis	Expanded bed adsorption;Ion exchange chromatography	Streamline;DEAE-sepharose	>4.0	23
Polysiphonia urceolata	Ion exchange chromatography;	DEAE-Sepharose Fast Flow	5.60	24
Gracilaria corticata	Preparative polycrylamide gel electrophoresis	-	4.23	25
Gracilaria gracilis	Ion exchange chromatography; Gel-filtration chromatography	DEAE Sepharose Fast Flow;Superdex 200HR	3.20	26
Porphyra haitanensis	Expanded bed adsorption;Gel filtration chromatography;Ion exchange chromatography	StreamlineTM Phenyl;Q-sepharose;StreamlineTM 25	5.29	27
Rhodomonas salina	Hydrophobic interaction chromatography	Butyl-S Sepharose 6 Fast Flow	3.10	28

The different methods studied for the purification of R-PE reflect the need for adaptive approaches that help isolate the molecule efficiently, achieving the desired purity while maintaining activity. Each technique has specific advantages and challenges that must be overcome in order to obtain a high-quality product. One of the most widely used methods is ion exchange chromatography, which is possible due to the electrical interactions between the surface of the resin and the protein and can be optimized by controlling the ionic strength and buffer pH. Even though it is a less expensive method compared to other chromatographic methods, ion exchange chromatography requires a combination of other procedures to obtain a higher yield[29].

Size exclusion chromatography is an additional technique that is used to separate proteins according to their molecular dimensions. This procedure offers an additional purification step following initial capture techniques and is useful in eliminating aggregates and other proteins with variable sizes, and is generally used in combination with other techniques, such as ion exchange chromatography[30,31]. Another example is expanded bed chromatography (EBA), where conventional chromatography procedures are combined with the fluidized bed concept. In this chromatographic approach, the bed is expanded as the crude extract is fed, with the particles suspended in a state of equilibrium due to terminal velocity, the smaller substances at the top and the larger ones at the bottom. In this type of chromatographic method, bed porosity is larger than fixed bed. This avoids one of the biggest problems in using chromatography to purify algae extracts, which is the blocking of the column by the polysaccharides resulting from cell lysis. Despite being a good choice, EBA is difficult to control and has a high initial cost[32]

In addition to these purification methods, there are techniques that are useful in both the initial and final stages. Selective precipitation with ammonium sulphate is one of them, where R-FE can be selectively precipitated in certain concentrations of ammonium sulphate, allowing the removal of other less soluble proteins. In addition, membrane filtration techniques, such as ultrafiltration and microfiltration, are used to concentrate and purify r-phycoerythrin, separating the protein according to molecular size, increasing both the yield and purity of the final sample[33,34].

Even after the development of various techniques and methods, the purification of R-PE still faces significant challenges. These include the complexity of the biological matrices of the extracted algae, which may contain other proteins, lipids and polysaccharides, as well as the sensitivity of the protein to variations in pH, temperature and ionic strength, which can lead to

denaturation or loss of fluorescent activity, requiring strict control of the purification conditions in order to maintain its structural and functional integrity. Furthermore, the existence of other phycobiliproteins, such as allophycocyanin and phycoerythrin, makes the purification of r-phycoerythrin more difficult and expensive, as it requires additional separation procedures to refine the purified protein[35,36].

4 CONCLUSION

This review on R-PE highlights not only its purification methods, but also its various applications, demonstrating its importance and versatility in various fields of science and industry. Therefore, the crucial role of R-PE as a valuable biotechnological tool is highlighted, as it has bioactive properties and potential in biomedical, cosmetic and food applications, due to its fluorescence and coloring ability. With a good understanding of the purification methods and applications available, it is possible to harness the potential of R-PE, promoting innovation and progress in various sectors.

REFERENCES

1 MACCOLL.R. J Struct Biol. 1998 Dec 15;124(2–3):311–34.
2 FRANK, H. A., Cogdell.R.J. 8.6. 2012;
3 PAGELS, F., GUEDES, A. C., AMARO.HM, KIJJOA.A, VASCONCELOS.V. Biotechnol Adv. 2019 May 1;37(3):422–43.
4 CHEN, H., QI, H., XIONG.P. Mar Drugs. 2022;20(7).
5 STADNICHUK, I. N., TROPIN, I. V. Appl Biochem Microbiol. 2017 Jan 1;53(1):1–10.
6 ROSSANO, R., UNGARO, N., D'AMBROSIO, A., LIUZZI, G. M., RICCIO, P. J Biotechnol. 2003 Mar 20;101(3):289–93.
7 Wheelwright SM. Bio/Technology. 1987;5(8):789–93.
8 Francavilla M, Franchi M, Monteleone M, Caroppo C. Mar Drugs. 2013;11(10):3754–76.
9 Ashaolu TJ, Samborska K, Lee CC, Tomas M, Capanoglu E, Tarhan Ö, et al. Int J Biol Macromol. 2021;193:2320–31.
10 Soni B, Kalavadia B, Trivedi U, Madamwar D. Process Biochemistry. 2006;41(9):2017–23.
11 Liu LN, Su HN, Yan SG, Shao SM, Xie B Bin, Chen XL, et al. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 2009 Jul 1;1787(7):939–46.
12 KAWSAR, S. M. A., FUJII, Y., MATSUMOTO, R., YASUMITSU,H., Ozeki, Y. Vol. 17, PHYTOLOGIA BALCANICA. 2011.
13 CHANG, W. R., JIANG, T., WAN, Z. L., ZHANG, J. P., YANG, Z.X., LIANG, D.C. J Mol Biol. 1996 Oct 11;262(5):721–2.
14 Patil G, Chethana S, Sridevi AS, Raghavarao KSMS. J Chromatogr A. 2006;1127(1–2):76–81.
15 Hemlata, Fatma T. Bull Environ Contam Toxicol. 2009 Oct;83(4):509–15.
16 George R, John JA. Vol. 58, International Journal of Food Science and Technology. John Wiley and Sons Inc; 2023. p. 513–9.
17 Hilditch.C.M, Balding.P, Jenkins.R, Smith.A.J, Rogers.L.J. Vol. 3, Journal of Applied Phycology. 1991.
18 Thammapalerd N, Supasiri T, Awakairt S, Chandrkrachang S. Southeast Asian Journal of Tropical Medicine and Public Health. 1996;27(2):297–303.
19 Galland-Irmouli A V., Pons L, Luçon M, Villaume C, Mrabet NT, Guéant JL, et al. J Chromatogr B Biomed Sci Appl. 2000 Feb 28;739(1):117–23.
20 Simovic A, Combet S, Cirkovic Velickovic T, Nikolic M, Minic S. Food Chem. 2022 Apr 16;374:131780.
21 Sun L, Wang S, Gong X, Zhao M, Fu X, Wang L. Protein Expr Purif. 2009 Apr 1;64(2):146–54.
22 Niu JF, Chen ZF, Wang GC, Zhou BC. J Appl Phycol. 2010 Feb;22(1):25–31.
23 Liu LN, Chen XL, Zhang XY, Zhang YZ, Zhou BC. J Biotechnol. 2005 Mar 2;116(1):91–100.
24 Sathuvan M, Thangam R, Venkateshbabu G, Cheong KL, Kang H, Liu Y. Int J Biol Macromol. 2022 Jan 1;194:563–70.
25 Lee WC, Lee KH. Anal Biochem. 2004 Jan 1;324(1):1–10.
26 Nguyen HPT, Morançais M, Déléris P, Fleurence J, Nguyen-Le CT, Vo KH, et al. J Appl Phycol. 2020 Feb 1;32(1):553–61..
27 Niu JF, Wang GC, Zhou BC, Lin XZ, Chen CS. J Phycol. 2007 Dec;43(6):1339–47.
28 Pu Y, Dong S, Li M, Dong K, Zhao H, Tang Z, et al. sp. 2022;
29 Lauceri R, Chini Zittelli G, Maserti B, Torzillo G. Algal Res. 2018 Nov 1;35:333–40.
30 Tang Z, Zhao J, Ju B, Li W, Wen S, Pu Y, et al. Protein Expr Purif. 2016;123:70–4.
31 Xu Y, Wang Q, Hou Y. Mar Drugs. 2020 Dec 1;18(12).
32 Tcheruov' AA, Minkova KM, Georgiev DI, Houbavenska. Vol. 7, BIOTECHNOLOGY. 1993.
33 Mittal R, Sharma R, Raghavarao KSMS. Algal Res. 2022 Mar 1;62:102605.
34 Dumay J, Morançais M, Nguyen HPT, Fleurence J. Methods in Molecular Biology. 2015;1308:109–17.
35 Kaixian, " ' Q, Franklin M, Borowitzka MA. The Study for Isolation and Purification of R-phycoerythrin from a Red Alga.
36 Niu JF, Wang GC, Tseng CK. Expr Purif. 2006 Sep 1;49(1):23–31.

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