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Qualitative and quantitative determination of R-phycoerythrin from *Halymenia floresia* (Clemente) C. Agardh by polyacrylamide gel using electrophoretic elution technique

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ABSTRACT

R-Phycoerythrin is one of the phycobiliproteins widely found in seaweeds. In this study, we have shown to extract and purify R-Phycoerythrin from the south east cost Indian red seaweed *Halymenia floresia*. R-Phycoerythrin was extracted in 50 mM phosphate buffer (pH 7.0). The preparative native PAGE purification was employed alternative to the chromatography and therefore can be scaled up efficiently. Both the yield and the purity of R-Phycoerythrin are very effective. The purified R-Phycoerythrin showed a single band on the examination by native PAGE electrophrosis. SDS-PAGE analysis showed five bands at 16 kDa, 21 kDa, 30 kDa, 39 kDa and 47 kDa which corresponds to the α, β and γ', γ and αβ subunits. This preparative method for R-Phycoerythrin purification can offer a reference for R-Phycoerythrin purification from other marine red macro algae.

1. Introduction

Phycobiliproteins (PBPs) are water-soluble proteins found extensively in cyanobacteria and certain algae (rhodophytes, glaucocystophytes and cryptomonads) [1] which captures light energy, which is then passed on to chlorophylls during photosynthesis. Phycobiliproteins are also derived from cyanobacteria and eukaryotic algae [2]. PBPs are formed of a complex between proteins and covalently bound phycobilins which act as chromophores (the light-capturing part). They are the most important constituents of the phycobilisomes [3,4]. These proteins carry covalently attached linear tetrapyrrole pigments related structurally to biliverdin. R-phycoerythrins (R-PEs) are the most abundant phycobiliproteins found in the most marine red algae [5]. They are fluorescent, with high quantum efficiency with a large stokes shift and excitation and emission bands at visible wavelengths. Their absorption spectrum in native state is a three-peak spectrum with an absorption maxima at 565, 539 and 498 nm respectively [6,7]. Due to their protein nature, unique color and fluorescence characteristics, the PBPs they have a wide range of promising applications in various industries viz. nutritional ingredients in food, colorants in cosmetics and markers in different fluorescence techniques [8–12]. Moreover, biological properties of PBPs like hepato-protective, anti-oxidant and anti-inflammatory activity create them very potential macromolecules for therapeutic, diagnostic and pharmacological applications [13–15]. Phycobiliproteins are attractive to study since they are not harmful to human beings when applied to the external surface or taken internally. R-phycoerythrin is used as a photosensitizer for the treatment of tumors and has a potential to substitute the Photofrin (a kind of light sensitive agent in photodynamic therapy) in common use which is purified from animal blood [16]. Phycobiliproteins are also widely used as natural colorants for food and cosmetics has a great economic potential. Nevertheless, the use of this biliprotein has been somewhat limited by the tedious preparation of adequate amounts of the purified protein. Therefore,it is of great significance for the search of new method of preparative purification.

Methods have been developed to obtain a pure R-PE from several different algae. R-PEs were commonly extracted in phosphate buffer (5–50 mM, pH 7.0) from algae and then precipitated by salting-out with ammonium sulfate $(NH_4)_2SO_4$ at different percentages. After desalting by dialysis, R-PEs were usually purified by various techniques: expanded bed adsorption chromatography [17–19], ion-exchange chromatography [20–23], gel filtration [24],

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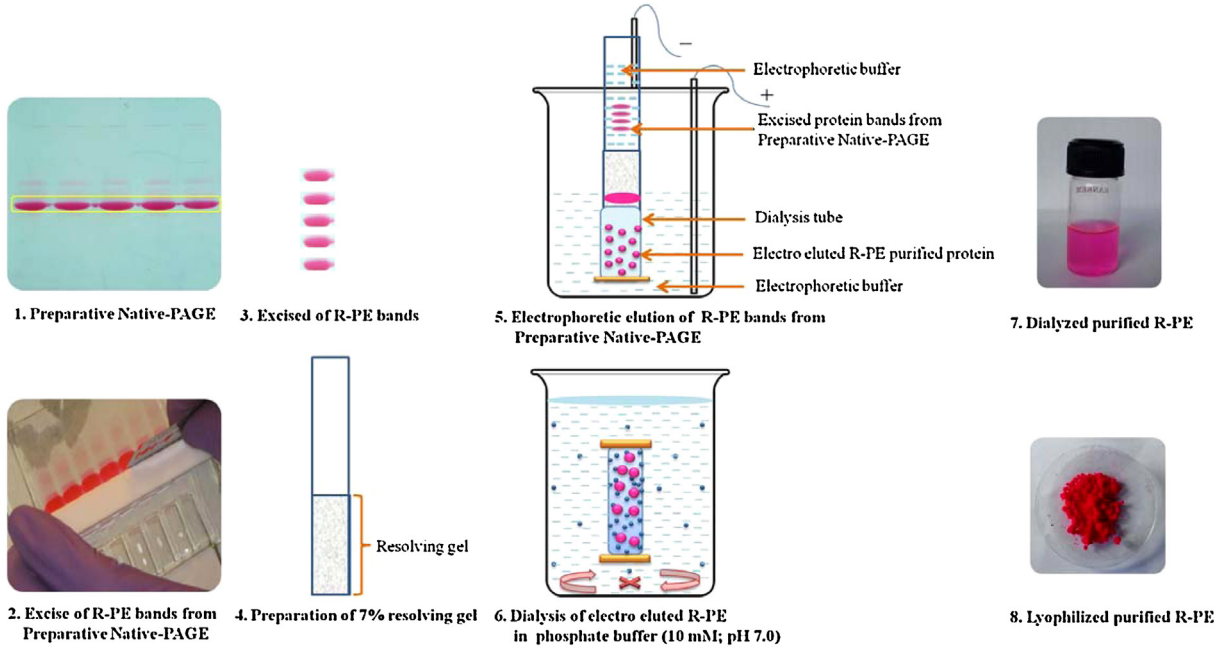

Fig. 1. A schematic drawing of the apparatus and operation of preparative native PAGE for purification process of R-PE from *H. floresia*.

preparative electrophoresis [25]. So it was necessary to implement a new method to obtain high purity of R-PEs which can be efficiently used as fluorescence-based indicator. On the other hand, the conventional methods employed in the purification of R-PEs, involving ammonium sulfate precipitation, chromatography on hydroxylapatite and gel filtration has to undergo long and complex procedures. So, there was a neccesity to develop a fast and efficient method for the separation and purification of R-PE from red algae with a high purity.

Unfortunately, most of the chromatographies using a gradient of ionic strength were developed frequently or either joint with other methods. It was tricky to get a higher recovery of proteins after these operations. Among those usual separation methods, preparative native PAGE followed by electrophoretic elution showed some reward in the overall process. In this study, we have provided a preparative native PAGE followed by electrophoretic elution method for the efficient separation and purification of R-PE from *Halymenia floresia*. After these processes, R-PE had an pI of 5.9, which was confirmed by the following analysis native-PAGE, SDS-PAGE, absorption and fluorescence spectrum. This effective methodology using single step preparative native PAGE followed by electrophoretic elution method reduces the traditional processing steps as well as it paves the opportunity of protein loss and denaturation during the overall operation. Finally a high recovery of 41.9% was obtained.

2. Materials and methods

2.1. Chemicals

Acrylamide, *N*,*N*′-Methylene bis-acrylamide, Glycine, Ammonium persulfate, *N*,*N*,*N*′,*N*′-Tetramethylenediamine, Bromophenol blue, Comassive brilliant blue R/G 250, Silver nitrate, Glycerol, Ammonium sulfate, Tris (hydroxymethyl) aminomethane, Dialysis sack, Sodium chloride, Sodium carbonate, Sodium thiosulpahate, Orthophosphoric acid and Bovine serum albumin were obtained from Bio-Rad (Hercules, CA, USA). All glassware were purchased from Borosil (India). All the solvents were of analytical grade and all other chemicals were of molecular biology grade.

2.2. Algal collection

The collection of fresh and healthy specimens of *Halymenia floresia* red seaweed was made between the period of December 2014 and January 2015 during low tide at the depth of 1–5 m along the coast of Kilakarai, Gulf of Mannar, Tamil Nadu, India (9°13′52.86″N, 78°47′ 3.84″E). The collected seaweeds were identified by Prof. R. Rengasamy and documented in CAS in Botany, University of Madras,Guindy campus, Chennai, Tamil Nadu,India. Epiphytes were removed and the samples were successively rinsed with tap water and distilled water. The algae were immediately frozen and stored at −20 °C.

2.3. Extraction and estimation of phycobiliproteins

A ten grams of *H. floresia* were taken in a 100 ml of 0.05 M phosphate buffer at pH 7.0 and freeze thawed for twice to recover maximum crude protein. Later, the biomass was separated by centrifugation at 12,000*g* for 15 min at 4 °C. Extracts were concentrated using lyophilizer at −80 °C (Vir Tis FTS Systems, Warminster, PA, USA) and stored in dark vials at 4 °C for further use. In each step, the amounts of R-PE were determined spectrometrically using a UV–vis spectrophotometer (UV-1700, Shimadzu, Japan) as Beer and Eshel equation [26].

Purity index and R-PE from total protein (%) were calculated by following formula:

$$[\text{R-PE}] = [(A_{565} - A_{592}) - (A_{455} - A_{592}) \times 0.20] \times 0.12$$

R-PE extraction yield was expressed as mg/ml.

$$\text{Purity Index} = A565/A280$$

$$\text{R-PE yield from total protein (\%)} = \text{Total R-PE/TotalProtein} \times 100$$

2.4. Determination of water soluble proteins

Total water soluble proteins in the crude extract and the purified fractions were analyzed by the method followed by Bradford [27].