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Physicochemical factors affecting the stability of two pigments: R-phycoerythrin of *Grateloupia turuturu* and B-phycoerythrin of *Porphyridium cruentum*

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ABSTRACT

Phycoerythrin is a major light-harvesting pigment of red algae, which could be used as a natural dye in foods. The stability of R-phycoerythrin of *Grateloupia turuturu* and B-phycoerythrin of *Porphyridium cruentum* in relation to different light exposure times, pHs, and temperatures was studied. Regarding the light exposure time, after 48 h, the reduction in concentrations of B-phycoerythrin and R-phycoerythrin were 30 ± 2.4% and 70 ± 1%, respectively. Phycoerythrins presented good stability from pH 4 to 10. At pH 2, the reduction in concentration was 90 ± 4% for B-phycoerythrin and 40 ± 2.5% for R-phycoerythrin while, at pH 12, the phycoerythrins were degraded. Phycoerythrins showed good stability toward temperature, up to 40 °C. At 60 °C, the reduction in concentrations of B-phycoerythrin and R-phycoerythrin were 50 ± 3.4% and 70 ± 0.18%, respectively. Moreover, the best conditions of storage (−20 °C) were determined.

1. Introduction

Phycobiliproteins (PBPs) are the major light-harvesting chromoproteins present in some seaweeds, such as Rhodophytes, Cyanobacteria and Cryptophytes (Gantt, 1981; Glazer, 1984). They are commonly divided into four classes, based on their light absorption properties and types of bilins: phycoerythrins (PEs; λ_{max} = 540–570 nm), phycocyanins (PCs; λ_{max} = 610–620 nm), allophycocyanins (APCs; λ_{max} = 650–655 nm) and phycoerythrocyanins (PECs; λ_{max} = 560–600 nm) (Glazer, 1976). Phycoerythrins (PEs) have received much attention in recent years due to their spectral, fluorescent and colorant properties (Fleurence, 2003). They can be divided into four classes: R-phycoerythrin (R-PE), B-phycoerythrin (B-PE), C-phycoerythrin (C-PE) and B-phycoerythrin (B-PE), based on their origin and absorption spectrum (Bogorad, 1975; Glazer, 1976; Glazer & Hixson, 1975). Spectral differences between phycoerythrins are due to the presence of different types of bilin prosthetic groups (Glazer, 1988). R-PE is the most abundant phycobiliprotein from red algae and marine unicellular cyanobacteria (Denis et al., 2010). It is composed of three polypeptide subunits, forming a complex [$(\alpha\beta)_6\gamma$]: α (18–20 kDa), β (19.5–21 kDa), and γ (30 kDa) (Galland-Irmouli et al., 2000). B-PE, commonly found in microalgae (Gantt & Lipschultz, 1974), contains the same subunits, forming the same complex [$(\alpha\beta)_6\gamma$] as R-PE, but presents a different absorption spectrum.

Phycobiliproteins have gained importance in commercial sectors. There is an increasing demand and preference for natural colors which are of use in food, pharmaceuticals, cosmetics, textiles and as printing dyes. Because of their spectroscopic and biological properties, phycoerythrins are widely used in biochemical techniques and clinical diagnoses. They are especially used as a fluorescent probe in flow cytometry, microscopy, immunochemistry, and biomedical reagents (Isailovic, Sultana, Phillips, & Yeung, 2006; Sekar & Chandramohan, 2008), and also as a fluorescent dye, and in the cosmetic industry (Fleurence, 2004; Sekar & Chandramohan, 2008). The food industry could be interested in the use of a red fluorescent colorant as a complement to Phycocyanin, a blue fluorescent pigment already available from *Spirulina platensis* (Fleurence, 2004; Santiago-Santos, Ponce-Noyola, Olvera-Ramirez, Ortega-Lopez, & Canizares-Villanueva, 2004). Methods have been developed to

Abbreviations: B-PE, B-phycoerythrin; CE, crude extract; PBP, phycobiliprotein; PE, phycoerythrin; PI, purity index; R-PE, R-phycoerythrin.

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obtain pure PE from several different algae. PEs are commonly extracted in diluted phosphate buffer from algae and then precipitated by salting-out with ammonium sulfate at different percentages. After desalting by dialysis, PEs are usually purified by various techniques: expanded bed adsorption chromatography (Bermejo et al., 2003), ion-exchange chromatography (Bermejo, Alvarez-Pez, Acién, & Molina, 2002; Liu, Chen, Zhang, Zhang, & Zhou, 2005), gel filtration (Ma, Wang, Sun, & Zeng, 2003), hydroxyapatite chromatography, a combination of two chromatography modes (Bermejo, Talavera, & Alvarez-Pez, 2001; Niu, Wang, & Tseng, 2006; Rossano, Ungaro, D'Ambrosio, Liuzzi & Riccio, 2003; Sun et al., 2009) or preparative electrophoresis (Galland-Irmouli et al., 2000). During the extraction and purification procedures, it is uncertain whether the PEs remain in their natural configuration and undergo no serious structural changes in their conformations and functions.

In the present paper, the stability of PE extracts was studied in response to physical and chemical parameters, such as light, temperature and pH. Two types of phycoerythrin, R-PE of *Grateloupia turuturu* and B-PE of *Porphyridium cruentum*, were studied in order to improve the extraction and purification steps and also for future applications as a natural colorant.

2. Materials and methods

2.1. Algae preparation

Specimens of *G. turuturu* Yamada (Rhodophyta, Halymeniaceae) were harvested in January 2011 at Batz-sur-Mer (47°16′34.82″ N-2°29′36.64″O; Atlantic Coast, France). Epiphytes were removed and algae were successively rinsed once with tap water and once with distilled water. The algae were then immediately freeze-dried.

Specimens of *P. cruentum* came from the strain UTEX 161, from the University of Texas, United States of America. They were grown in a tubular *Air-Lift* PhotoBioReactor (PBR, 100 l), inoculated from a prior culture in a tubular *Air-Lift* PBR (10 l). In both cases, Hemerick culture medium was used, slightly modified to obtain a higher productivity. The biomass was harvested from the final culture by centrifugation of the cell suspension at 1500*g* (*RA20Vx, Rousselet Robatel*, Annonay, France). After a second centrifugation at 15,000*g* (*Sorvall RC6 plus, Thermo Electron Corporation*, Cergy Pontoise, France) for 10 min at 5 °C, the pellets were freeze-dried.

2.2. Extraction of water-soluble fraction

Freeze-dried algae were then ground in liquid nitrogen and the resulting powder was suspended in sodium phosphate buffer (20 mM; pH 7.1). The extraction was performed with a 1/20 ratio (w/v) for 20 min at 4 °C; then the suspension was centrifuged (25,000*g*, 20 min, 4 °C). The phycoerythrin-rich supernatant, called the crude extract (CE), was freeze-dried. The approximate chemical compositions of R-PE and B-PE extracts were 21.8% and 20.1% proteins, 5.4% and 6.5% lipids, 41.5% and 46.2% carbohydrates, 15.6% and 20% ash, 0.44% and 3.5% pigment (% dw), respectively.

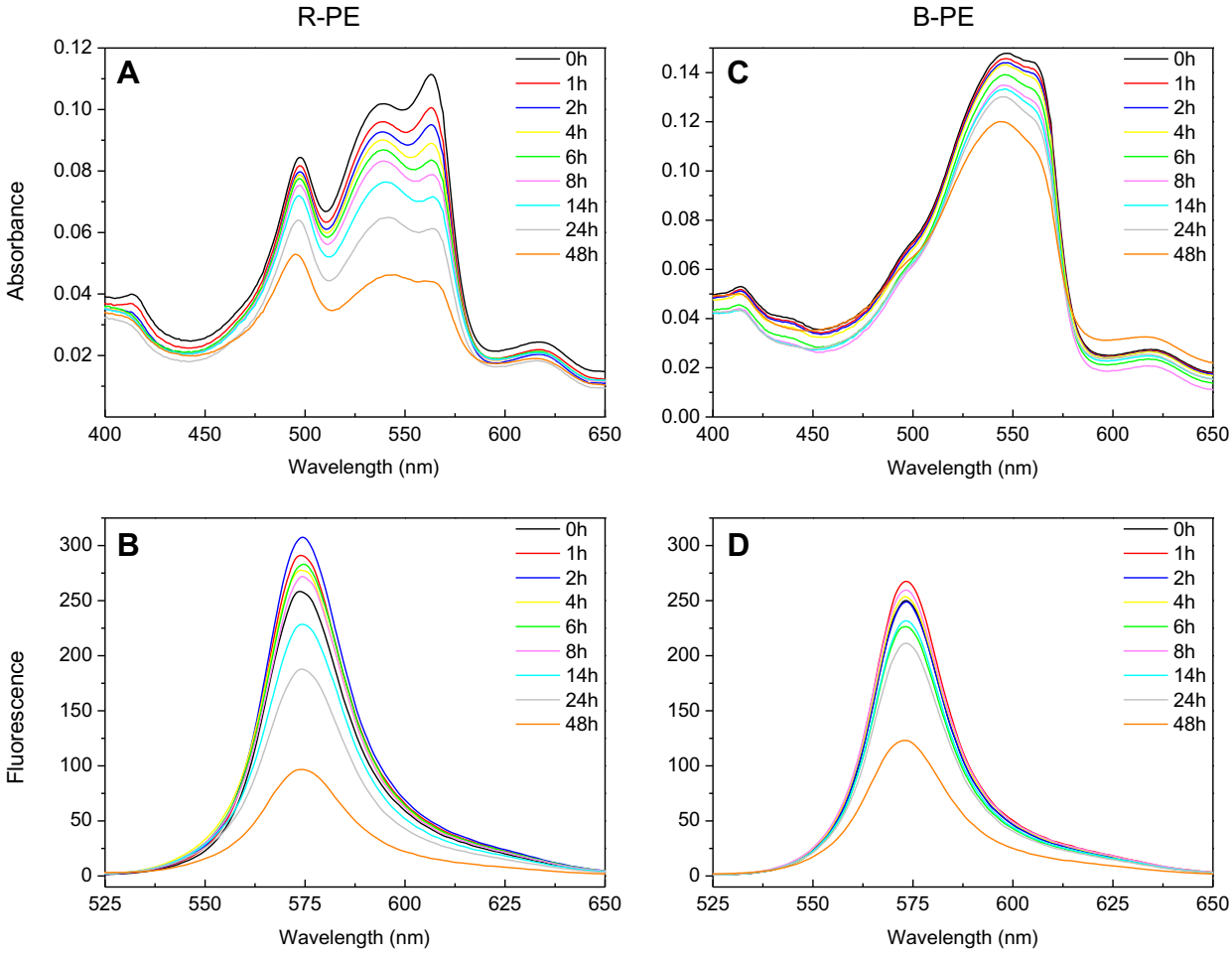

Fig. 1. Effects of light exposure time (0–48 h) on the absorption and fluorescence emission spectra (λ_{ex} = 498 nm) of R-PE and B-PE in potassium phosphate buffer (20 mM). (A) R-PE absorption spectra; (B) R-PE fluorescence emission spectra; (C) B-PE absorption spectra; (D) B-PE fluorescence emission spectra.