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One-step chromatography method for efficient separation and purification of R-phycoerythrin from *Polysiphonia urceolata*

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Abstract

Phycoerythrins have been widely used in food, cosmetics, immunodiagnostics and analytical reagents. An efficient one-step chromatography method for purification of R-phycoerythrins from *Polysiphonia urceolata* was described in this paper. Pure R-phycoerythrin was obtained with an absorbance ratio A_{565}/A_{280} of 5.6 and a high recovery yield of 67.33% using a DEAE-Sepharose Fast Flow chromatography with a gradient elution of pH, alternative to common gradient elution of ionic strength. The absorption spectrum of R-phycoerythrin was characterized with three absorbance maxima at 565, 539 and 498 nm, respectively and the fluorescence emission spectrum at room temperature was measured to be 580 nm. The results of native-PAGE, and SDS-PAGE showed no contamination by other proteins in the phycoerythrin solution, which suggests an efficient method for the separation and purification of R-phycoerythrins from *Polysiphonia urceolata*.

Keywords: *Polysiphonia urceolata*; R-phycoerythrin; Separation; Purification; Ion-exchange chromatography

1. Introduction

Phycobiliproteins are water soluble light-harvesting proteins with natively highly fluorescent properties derived from cyanobacteria and eukaryotic algae (Apt et al., 1995; Chen et al., 2003; Gantt et al., 1979; Glazer, 1989; MacColl, 1998; Zhang et al., 1999, 2002). These proteins, as well as a small amount of linker polypeptides, are assembled in order into an organized cellular structure called the phycobilisome that are attached in regular arrays to the outer surface of the thylakoids. Phycobiliproteins are divided into three classes based on their absorption properties: phycoerythrins (PEs, $\lambda_{max} \sim 540$–570 nm), phycocyanins (PCs, $\lambda_{max} \sim 610$–620 nm) and allophyco-

Abbreviations: PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin; native-PAGE, native polyacrylamide gel electrophoresis; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; DEAE-Sepharose, diethylamino ethyl Sepharose

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cyanins (APCs, $\lambda_{max} \sim 650$–655 nm) (Bermejo et al., 2003; Glazer, 1984).

The phycobiliproteins participate in an efficient energy transfer chain through which they transfer the excitation energy to the reaction centers in the photosynthetic membranes (Viskari and Colyer, 2003). Phycoerythrins, that play an important role in the phycobilisomes of red algae, are located at the tip of the rods, absorbing and transferring light energy to phycocyanins, subsequently from phycocyanins to allophycocyanins, and finally to chlorophyll a, with an efficiency exceeding 95% (Glazer, 1989; Wilbanks and Glazer, 1993). Phycoerythrins can be classified as four classes: R-PE, B-PE, b-PE and C-PE, based on their origins and absorption properties (Marsac, 2003). In this paper, pure R-phycoerythrins from *Polysiphonia urceolata* are a typical kind of "three-peak" R-phycoerythrins with the structure of $(\alpha\beta)_6\gamma$ (MacColl and Eisele, 1996) and three absorbance maxima respectively at 565, 539 and 498 nm (Zhang et al., 2002).

R-phycoerythrins are fluorescent, with high quantum efficiency; a large stokes shift and excitation and emission bands at visible wavelengths. Their special absorption spectrum in a native state provides a three-peak spectrum with absorption maxima at 565, 539 and 498 nm, respectively. Concerning immunodiagnostics in which 488 nm is often used as an exciting wavelength, R-phycoerythrins are better than B-phycoerythrins because the 498 nm maximum is more available (Telford et al., 2001b). They are stable proteins that can be easily linked to antibodies and other proteins by conventional protein cross-linking techniques without altering its spectral characteristics. So currently R-phycoerythrins are widely used in the production of food, cosmetics and play important roles in many biochemical techniques due to their fluorescence properties (Albertsson, 2003; Bermejo et al., 2003; Galland-Irmouli et al., 2000; Glazer, 1994; Telford et al., 2001a). It is necessary to adopt a method to obtain high purity of R-phycoerythrins effectively for the application in life and research. However, the traditional methods performed in purification of phycoerythrins, involving ammonium sulfate precipitation, chromatography on hydroxylapatite and gel filtration, provide long and complex procedures (Duerring et al., 1990; Ficner et al., 1992; MacColl and Eisele, 1996; Tchernov et al., 1999; Zhang and Chen, 1999; Zhang et al., 1999, 2002). It is desirable to develop a rapid and efficient process for the separation and purification of R-phycoerythrin from a good red algae with a high purity. Tchernov et al. (1993) described a rivanol-sulfate method for the purification of B-PE from *Porphyridium cruentum*. Bermejo et al. (2001) provided the purification of B-PE from *Porphyridium cruentum* by two chromatographic steps, DEAE-cellulose DE-52 and Sephadex G-100. Rossano et al. (2003) set up a method based on a chromatography on hydroxylapatite connected to Sephadex 75 gel filtration and obtained pure R-phycoerythrins after the two steps from *Corallina elongata*. But the effective purification method for R-phycoerythrins from phycoerythrin-rich red alga *Polysiphonia urceolata*, which widely distributes near the Chinese coast, with a large biomass of R-phycoerythrins and low content of sugars (Pan et al., 1986), has not been reported up to now.

Among those traditional separation methods, ion-exchange chromatography showed some advantages in the overall process. Unfortunately, most of these chromatographies using a gradient of ionic strength were developed repeatedly or combined with other methods. It was difficult to get a higher recovery of proteins after these operations. In this paper, we provided a one-step chromatography method for the efficient separation and purification of R-phycoerythrin from *Polysiphonia urceolata*, involving fraction precipitation with ammonium sulfate and ion-exchange chromatography on a DEAE-Sepharose Fast Flow column. In comparison with ion-exchange chromatography methods reported earlier, we firstly developed an elution method with a gradient of pH. After one chromatography the purity of R-phycoerythrin obtained highly reached 5.6 was confirmed by the following studies on absorption spectrum, fluorescence spectrum, native-PAGE and SDS-PAGE. The results of the protein stability to pH variations indicated the feasibility of this separation and purification method. Because this effective methodology using one single-step ion-exchange chromatography with a gradient of pH was exploited in the present work, it reduced the traditional processing steps as well as the possibility of protein loss and denaturation during the overall operation and a high recovery of 67.33% was obtained.