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Chemical stabilization of the phycocyanin from cyanobacterium *Spirulina platensis*

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Abstract

Phycocyanin (PC) prepared from a cyanobacterium *Spirulina platensis* by the DEAE-DE52 cellulose column chromatography that was developed by gradient elution of 50–250 mM phosphate buffer (pH 7.0) was stabilized by its subunits cross-linked covalently with formaldehyde. The single blue band that the chemically stabilized PC showed in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated that the stabilized PC still maintained its trimeric aggregate form even after its incubation at 60 °C for 3 h and at 100 °C for 10 min in the denatured buffer containing 5% (w/v) SDS. Moreover, the stabilized PC exhibited similar spectroscopic properties of absorption and fluorescence to those of the native PC, and showed adequate energy coupling with R-phycoerythrin (R-PE) after it was conjugated with R-PE via glutaraldehyde.

Keywords: Cyanobacterium; Phycocyanin; Phycoerythrin; Fluorescent Probe; *Spirulina platensis*

1. Introduction

Phycobiliproteins, or biliproteins, are mainly found in prokaryotic cyanobacteria, eukaryotic red algae, and cryptophytes. They function as major light-harvesting complexes to harvest efficiently the sunlight that chlorophylls poorly absorb, and transfer the energy in high efficiency to chlorophyll a in the thylakoid membranes. The phycobiliproteins are present as phycobilisome that are anchored on the thylakoid membranes and lie adjacent to the reaction center chlorophyll a of the photosystem II in cyanobacteria and red algae, but not in cryptophytes (Zilinskas, 1986; Rowan, 1989; Sidler, 1994; MacColl, 1998). The phycobiliproteins are classified into three groups based on the presence of different chromophores among them (Gantt, 1980,1994; Glazer, 1984; Zilinskas, 1986; Rowan, 1989; Sidler, 1994; MacColl, 1998; Ducret et al., 1998): (1) phycoerythrin (PE; absorption light from 480 nm to 570 nm); (2) phycocyanin (PC; absorption light from 590 nm to 630 nm) and phycoerthrocyanin (PEC; absorption light 560 nm to 600 nm); and (3) allophycocyanin (AP; absorption light from 620 nm

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to 665 nm). In general, phycobiliproteins are made up of αβ monomers of two dissimilar polypeptide chains, α and β, each with covalently attached chromophores. Purified biliproteins are mainly in forms of trimers $(\alpha\beta)_3$ (PC and AP), or hexamers $(\alpha\beta)_6$ (R-PE, B-PE). For the monomer αβ of PC and AP proteins, it contains three and two bilin prosthetic groups of phycocyanobilin, respectively; for that of R-PE or B-PE, the monomer contains four phycoerythrobilins and one phycourobilins. In this case, one PC $(\alpha\beta)_3$ often carry nine phycocyanobilins, and one PE $(\alpha\beta)_6$ has at least 24 phycoerythrobilins and six phycourobilins (Sun et al., 2003). Trimer PC or AP biliproteins are disk-shaped aggregates, about 11 nm in diameter and about 3 nm in thickness, with a center cavity of about 3.5 nm in diameter; hexamer PE proteins, which are organized by two trimers stacking, are 6 nm in thickness (Sidler, 1994; MacColl, 1998; Sun et al., 2003).

When the phycobiliproteins are isolated and purified from the organisms, the water-soluble bright-colored biliproteins become highly fluorescent because they no longer have any nearby acceptors to which to transfer the harvested energy. The spectroscopic and structural properties of the phycobiliproteins exhibit several unique qualitative and quantitative features, such as broad absorption in visible light spectrum, enormous extinction coefficient, high fluorescence quantum efficiency, large Stokes shift and very little fluorescence quenching between multiple chromophores that the phycobiliproteins contain, even between themselves (Glazer and Stryer, 1983; Kronick and Grossman, 1983; Glazer, 1994; Holmes and Lantz, 2001). These features have let the phycobiliproteins be promising candidates for use as fluorescent labels in practice (Sun et al., 2003). Since the proteins were employed in immunoassay and single-cell analysis and in sorting by flow cytofluorimetry (Glazer and Stryer, 1983; Kronick and Grossman, 1983; Legender et al., 1985) in the beginning of the eighties of last century, the utilization of the proteins as fluorescent probes has shown ever-extensive prospects in various fields of biology research: for example, multicolor flow cytometric technology used in cell analysis and sorting (Kansas and Dailey, 1989; Roederer et al., 1997; Hultin et al., 1998), and protein localization and various particle dynamics within cells by techniques of fluorescence recovery after photobleaching (FRAP) (Georgiou et al., 2002) and single particle tracking (SPT) (Wilson et al., 1996; Cherry et al., 1998; Goulian and Simon, 2000; Smith et al., 2002; Irvine et al., 2002). In the meantime, development of some novel phycobiliprotein probes or conjugates has been achieved in enhancing stability of phycobiliproteins in diluting state, such as chemically-cross-linked allophycocyanin (Yeh et al., 1987) and structurally modified phycocyanin by protein engineering (Cai et al., 2001; Telford et al., 2001), and in heightening fluorescence intensity of unit fluorescent conjugate, such as multiple phycoerythrins conjugates.

Phycoerythrins, such as R-PE and B-PE, are employed as fluorescent probes in practice more extensive than phycocyanins and allophycocyanins up to date. One reason for this is that phycocyanins and allophycocyanins in trimer dissociate more easily into monomers (αβ) at low mole concentration ($<10^{-6}$ M), losing their strong absorption and high fluorescent quantum efficiency, whereas R-PE and B-PE still maintain 0.8 fluorescent quantum efficiency even at concentration below 10^{-12} M (Glazer, 1994; Holmes and Lantz, 2001; Sun et al., 2003). In this work, the PC protein prepared by ion-exchange chromatography from cyanobacterium *Spirulina platensis* was chemically stabilized by covalent cross-linking adjacent subunits with formaldehyde. The stabilization was examined by polypeptide analysis of denatured polyacrylamide gel electrophoresis, and spectroscopic properties of the stabilized PC were investigated in contrast with the native PC.

2. Materials and methods

2.1. Phycobiliprotein extraction

Dry *Spirulina platensis* cells of 10 g were suspended in 100 ml of 50 mM phosphate buffer (pH 7.0) containing 4 mM NaN_3, 1 mM 2-mercaptoethanol and 2 mM EDTA (Glazer, 1988). The cells were disrupted for 5 min by using Ultrasonic Cell Disruptor Model JY92-II under about 400 W at 0 °C. Five-second ultrasonication followed a 10 s interval. Cell fragments were removed by centrifugation at 30,000 × *g* for 30 min at 4 °C. The cell precipitates were resuspended in 100 ml phosphate buffer, disrupted for 2–3 min by the ultrasonication in the same way, and biliprotein solution was collected by the centrifugation as supernatant. The