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Preparative purification of B-phycoerythrin from the microalga *Porphyridium cruentum* by expanded-bed adsorption chromatography

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Abstract

B-Phycoerythrin (B-PE) is a major light-harvesting pigment of microalgae. Due to its high fluorescence efficiency and its intense and unique pink color, it is widely used as a fluorescent probe and analytical reagent as well as being employed as a natural dye in foods and cosmetics. Tedious methodologies for B-PE purification have been published. In this work we present a new, fast, preparative and scaleable two-step chromatographic method for B-PE purification from the red microalga *Porphyridium cruentum*. Initially, phycobiliproteins were released from the microalga cells by osmotic shock and captured by applying the centrifuged cell suspension to a column containing 74 ml Streamline-DEAE equilibrated with 50 m*M* acetic acid–sodium acetate buffer, pH 5.5, using expanded-bed adsorption chromatography at an upward flow of 200 cm h^{-1}. After adsorption, washing was carried out in the expanded-bed mode. Having removed unbound proteins and cellular debris, the bed was allowed to sediment and a B-PE-rich solution was eluted with a downward flow of the same 250 m*M* buffer. In order to obtain pure B-PE, we utilized conventional ion-exchange chromatography with a column of DEAE-cellulose loaded directly with the eluate from Streamline-DEAE and developed using a discontinuous gradient of acetic acid–sodium acetate buffer, pH 5.5. With this new methodology, 66% of B-PE contained in the biomass of the microalgae was recovered, a value significantly higher than those obtained following other methodologies. The B-PE purity was tested using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and spectroscopic characterization.

Keywords: *Porphyridium cruentum*; Expanded-bed adsorption chromatography; Preparative chromatography; Phycoerythrins; Pigments; Phycobiliproteins; Proteins

1. Introduction

Phycobiliproteins are a family of proteins with linked open-chain tetrapyrrole prosthetic groups called bilins. Microalgae are the usual source of these compounds [1–3]. Phycobiliproteins are divided into three main classes based on their absorption properties: phycoerythrins (PEs, $\lambda_{max} \sim 540–570$ nm), phycocyanins (PCs, $\lambda_{max} \sim 610–620$ nm) and allophycocyanins (APCs, $\lambda_{max} \sim 650–655$ nm). The

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major classes of PEs have been reported to differ in their absorption characteristics: B-PEs (λ_{max} ~ 565, 546 nm and a shoulder at ~499 nm), R-PEs (λ_{max} ~ 568, 499 nm and a shoulder at ~545 nm) and C-PEs (λ_{max} ~ 565 nm) [4–6]. The main application of phycobiliproteins is as fluorescent markers of cells and macromolecules in biomedical research and highly sensitive fluorescence techniques [7–9]. They have also been shown to have a therapeutic value due to their immunomodulating and anticarcinogenic activities [10]. In addition, they can be used as natural dyes in foods and cosmetics, replacing the synthetic dyes, which are generally toxic or otherwise unsafe [11–13].

Pure phycobiliproteins from crude algal extracts are usually obtained by a combination of different and non-scaleable methods [14–17]. Phycoerythrin is usually purified by long and complex procedures, involving a combination of several techniques such as centrifugation, ammonium sulfate precipitation, ion-exchange chromatography, gel filtration and chromatography on hydroxyapatite [16,18–22]. An alternative technique that greatly reduces the number of steps is the adsorption of the protein of interest to gel media in expanded beds [23,24]. Expanded-bed adsorption (EBA) allows the capture of proteins from particle-containing feedstock without prior removal of particulates, thus enabling clarification of a cell suspension or cell homogenate and the concentration of the desired product in a single operation. Many research articles have shown that it is suitable for the removal of proteins from unclarified feedstock [23,25–27].

In the present work, we developed a new method of B-PE purification based on EBA chromatography. First, the extraction of the product from the biomass was optimized. Next, B-PE was separated by EBA and packed bed elution in Streamline-DEAE, obtaining a concentrated eluate ready to be loaded into a conventional DEAE-cellulose column, where the product was purified by packed bed ion-exchange chromatography. SDS–PAGE electrophoresis and spectroscopic characterization confirmed the purity of the B-PE obtained. In comparison with earlier methodologies this new method avoids product loss, increases yield and reduces both processing time and cost.

2. Experimental

2.1. Microalgal biomass and chemicals

The red microalga *Porphyridium cruentum* UTEX 161 was used. The biomass was obtained from chemostat cultures as described elsewhere [22,28,29]. Streamline-DEAE anion exchanger and materials used for sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) were from Pharmacia Biotech (Uppsala, Sweden). Preswollen microgranular DEAE-cellulose DE-52 was from Whatman (Maidstone, UK). Molecular mass standards, dialysis tubing (average flat width 43 mm), dialysis tubing closures, ammonium sulfate, sodium azide and all other chemicals were from Sigma (St. Louis, MO, USA) and used without further purification. For the determination of the equilibrium adsorption isotherm, pure B-PE obtained in previous work was utilized [21,22].

2.2. Spectroscopic methods

Absorbance measurements and absorption spectra were recorded on a Perkin-Elmer (Beaconsfield, UK) Lambda-20 UV–Vis spectrophotometer with a 1 cm light path. Fluorescence emission spectra were recorded on a Shimadzu (Kyoto, Japan) RF5001 spectrofluorometer. For steady-state polarized fluorescence measurements the spectrofluorometer was equipped with polarizers in the excitation and emission paths. Protein concentrations were chosen so that re-absorption of the emission was negligible. All spectra were recorded at room temperature. The amounts of B-PE, R-PC and APC in the different extracts and biliprotein-containing solutions were calculated from measurements of the absorbance at 565, 620 and 650 nm using the simultaneous equations of Bennet and Bogorad [30] and the extinction coefficients from Bryant et al. [31].

2.3. Protein extraction

All buffers contained 0.01% sodium azide unless otherwise specified. The influence of the cell disruption method was analyzed by quantifying protein extraction under different conditions: acetic acid–

sodium acetate buffer (control), distilled water, ultrasound at two powers, freeze–thawing, lysozyme at two different pH values (5.5 and 7.0), and lyophilized biomass. For all experiments, 50 g of frozen biomass paste (20% biomass dry mass) was mixed with 100 g of liquid (buffer or distilled water), the slurry being agitated at constant speed by a Heidolph RZR1 variable-speed magnetic stirrer (Heidolph Instruments, Schwabach, Germany). Samples were collected periodically, every 20 min, and centrifuged at 2500 *g* for 4 min in a Macrotronic Selecta centrifuge and the absorbance of the supernatant measured. In most cases, 1 *M* acetic acid–acetate buffer, pH 5.5, was used, except for the experiments with distilled water or lysozyme at pH 7.0. For the former, only distilled water was used, whereas for the latter, 1 *M* sodium phosphate buffer, pH 7.0, was employed. For ultrasound treatment a Lucas Dawe Ultrasonics unit type 7534A (Lucas Dawe Ultrasonics, London, UK) at two different power supplies, 200 and 1200 W, was utilized. For the lyophilization experiment, 50 g of frozen biomass paste was lyophilized. The dry biomass obtained was mixed with 100 ml of 1 *M* acetic acid–acetate buffer, pH 5.5, thus obtaining the same biomass concentration as in other experiments.

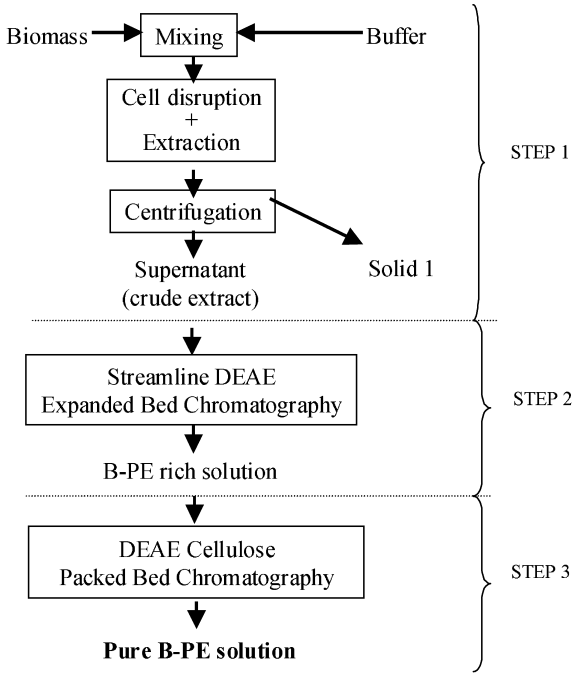

Fig. 1. Schematic diagram of the B-PE purification process.

2.4. B-Phycoerythrin purification

The complete B-PE purification method is shown in Fig. 1. Frozen cells of *P. cruentum* were resuspended in 1 *M* acetic acid–sodium acetate buffer, pH 5.5, at a buffer/biomass ratio of 4 (v/v). The resulting slurry was mixed for 50 min with the variable-speed stirrer at a power supply of 2 W. The slurry was then centrifuged at 2500 *g* for 5 min. The supernatant obtained after the centrifugation step (crude extract) was stored at 4 °C and utilized for EBA experiments after suitable dilution.

For the determination of the equilibrium adsorption isotherms of B-PE to Streamline-DEAE, we used 15 ml sealed shake tubes containing either 0–230 mg/l of pure B-PE or dilutions of the crude extract (containing 0–350 mg/l of B-PE) in 5 ml of 50 m*M* acetic acid, 50 m*M* sodium acetate, pH 5.5, solution (starting buffer). The tubes were placed in a shaking water bath at 26 °C and 1 ml of a 1:1 slurry of Streamline-DEAE/buffer was added to each tube. After 24 h equilibration, the contents of the tubes were analyzed. The equilibrium concentration of B-PE in the bulk liquid phase was determined spectroscopically [30,31] and the B-PE bound per ml of the ion-exchanger adsorbent at equilibrium was calculated by mass balance.

A 50×2.5 cm glass column containing 74 ml of Streamline-DEAE (giving 15 cm settled bed height) was equilibrated with 1000 ml of starting buffer. A peristaltic pump (Omegaflex, FPU5-MT-220) regulated the flow-rate. For the EBA experiments the same 2200 ml volume of sample was used, this being obtained by dilution of the crude extract with a suitable volume of starting buffer. Viscosity was measured in 100 ml samples using a Series 75 Cannon-Fenske Proton viscosimeter calibrated against distilled water at 25 °C.

In a typical experiment, 2200 ml of sample was pumped up the Streamline-DEAE column at constant flow-rate and washed with the starting buffer until the absorbance of the effluent at 565 nm returned to the baseline. At this point the upward flow was

stopped and the bed allowed to settle. The bound B-PE was recovered using an isocratic elution of 250 m*M* acetic acid–sodium acetate buffer, pH 5.5 (86 cm h^{-1} downward flow). Fractions of 16 ml were collected during elution using a Redifrac fraction collector from Pharmacia Biotech. The protein concentration was determined at 565 nm and the fractions with a red-purple color were pooled. Next, a cleaning-in-place procedure was carried out, the column being washed with three bed volumes of 1 *M* acetic acid–sodium acetate buffer, pH 5.5, five bed volumes of 0.5 *M* NaOH + 1 *M* NaCl and three bed volumes of distilled water. The column was prepared for a new experiment after equilibration with five bed volumes of starting buffer. The B-PE-rich solution from the Streamline-DEAE elution was applied to a 15×9 cm DEAE-cellulose column pre-equilibrated with starting buffer, and developed as described elsewhere [22].

2.5. SDS–PAGE

Electrophoresis was carried out in a vertical slab gel apparatus (Miniprotean III, Bio-Rad) using a Laemmli buffer system [32] consisting of a 12.5% (w/v) polyacrylamide slab gel, 0.75 mm thick, containing 0.1% (w/v) SDS with a stacking gel of 4% polyacrylamide. Samples were preincubated with 2% (w/v) SDS, 10% (v/v) glycerol, 4.5% (v/v) β-mercaptoethanol, 0.025% (w/v) bromophenol blue, and 60 m*M* Tris–HCl buffer, pH 6.8, for 5 min at 95 °C. Gels were run at room temperature, and visualized by staining with 0.1% (w/v) Coomassie Brilliant Blue R-250, 40% (v/v) methanol with 7% (v/v) acetic acid for 30 min, and destained in dilute acetic acid. The following proteins were used as molecular mass markers: phosphorylase *b* (94 000), albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100) and α-lactalbumin (14 400).

3. Results and discussion

3.1. Extraction

The influence of cell disruption methods on the recovery of phycobiliproteins was studied. Cell disruption was carried out by acetic acid–sodium acetate buffer, distilled water, ultrasound, lysozyme and lyophilization. Only data for R-PC, B-PE and total phycobiliprotein recovery are displayed in Fig. 2, since no APC was found. The data obtained show that both lyophilized biomass and the use of lysozyme at pH 7.0 gave the best results, although only slightly better than those obtained using the reference method with acetic acid–sodium acetate buffer. Since no large differences were observed when using lyophilized biomass, lysozyme at pH 7.0 or acetic acid–sodium acetate buffer, the latter method was selected because of its simplicity.

The extraction conditions were analyzed by quantifying protein extraction for different values of buffer/biomass ratio, power supply, and time of extraction. The best results were obtained by mixing four volumes of 1 *M* acetic acid–sodium acetate buffer, pH 5.5, with one volume of biomass paste (20% dry biomass) for 50 min at 2.0 W. It should be emphasized that extraction at high ionic strength leaves phycobilisomes intact and would allow the recovery of all phycobiliproteins present in successive steps [33,34].

The cell homogenate from osmotic shock was centrifuged to produce the supernatant (crude extract) utilized in EBA chromatography. The absorption spectrum of this extract showed maxima corresponding to a mixture of biliproteins (B-PE and R-PC) and other contaminant proteins. No absorp-

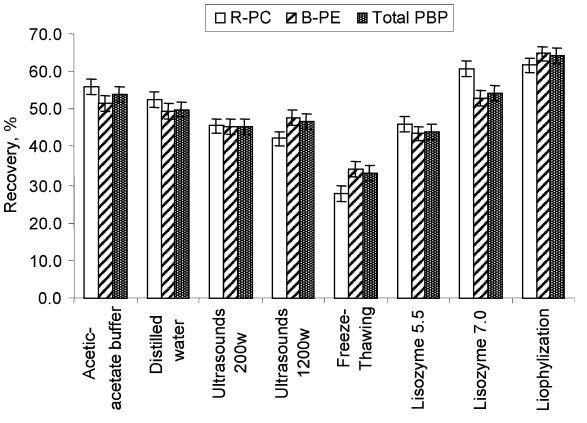

Fig. 2. Influence of cell disruption method on the recovery of phycobiliproteins from *P. cruentum* biomass. Error bars are SD from three measurements.

tion was observed from APC. Spectral analysis of the crude extract revealed a B-PE content of 1.68 g. Analysis of dry biomass by precipitation of the crude extract with 1% (w/v) streptomycin sulfate for 30 min at 4 °C and centrifugation at 2500 *g* for 10 min gave 1.83 g of B-PE (1.66% dry wt.). Hence, the B-PE yield in the recovery step from the intracellular medium was 92%. The yield of the initial stages in previous studies was always less than 45% [6,21,22,35].

3.2. Expanded-bed chromatography

We determined the equilibrium adsorption isotherms on the Streamline-DEAE matrix using either pure B-PE obtained previously [21,22] or B-PE crude extract. The isotherms were obtained according to Langmuir's model. The agreement with the model was very good. The adsorption capacity expressed as B-PE mass to volume of adsorbent was 6.1 g/l for pure B-PE and 1.8 g/l for the crude extract. From the calculated Langmuir parameters the maximum equilibrium B-PE binding capacity was 8.3 and 2.04 g/l for pure B-PE and crude extract, respectively. The adsorption isotherms of both pure B-BE and the crude extract showed that the capacity of the adsorbent decreased considerably in the presence of other proteins present in the crude extract. Unfortunately, ligands with the requisite high specificity for the target protein often cannot withstand the rigorous cleaning-in-place procedures that are frequently required when processing crude feedstock.

Previous tests using Streamline-DEAE showed that R-PC and other contaminant proteins eluted with 50 m*M* acetic acid–sodium acetate buffer, pH 5.5, whereas B-PE eluted with 0.25 *M* acetic acid–sodium acetate buffer, pH 5.5. Thus, we used a starting buffer in the adsorption expanded-bed step. Transformation of the settled bed into a stable expanded bed was achieved by pumping starting buffer up the column at a low flow-rate and steadily increasing the flow-rate until the bed was stable and had expanded to twice its settled height. The relative increase in height with flow-rate was linear in the assayed range (100 to 300 cm h^{-1} flow-rate). To obtain an adequate expanded bed, double its settled height, a flow-rate of 200 ml h^{-1} was necessary.

We verified that application of the B-PE crude extract to the EBA system without dilution caused flow disturbance and unequal distribution of the sample, probably due to its high viscosity [24]. Thus, the influence of the viscosity of the sample on the behavior and yield of the system was studied by testing different loadings and viscosities. In all experiments, 2200 ml of B-PE crude extract was applied to the column using ratios of mg B-PE/ml adsorbent in the range of the measured adsorption capacity of Streamline-DEAE (0.8–6.7 g/l), since this amount could be greater in dynamic mode [36]. The data in Table 1 show that the yield of the process increased from 54 to 80% when the viscosity of the sample was reduced from 1.17 to 1.02 mP. It is clear that the decrease in diffusion rate caused by the increase in viscosity affects the adsorption of proteins to the gel particles. Since, at the lowest sample viscosity, the behavior of the expanded-bed column was similar to plug flow and no channeling phenomena were observed, an operational flow-rate of 200 cm h^{-1} was used. This flow-rate decreased both the mass transfer resistance of the purified bioproduct at the adsorbent surface and the processing time, thus improving system productivity. In order to remove the remaining unbound molecules, washing was carried out with the bed in expanded mode using a six-fold bed volume of starting buffer. The superficial velocity was changed smoothly in order to maintain a constant degree of bed expansion.

The adsorbed B-PE was eluted using 250 m*M* acetic acid–sodium acetate buffer, pH 5.5. Elution from Streamline-DEAE was assayed in expanded-bed and packed-bed modes. In both cases, 2200 ml of sample was applied to an expanded-bed volume of

Table 1
Influence of the viscosity, loading, and biliprotein/adsorbent ratio on the B-PE recovery percentage upon elution in Streamline-DEAE. In all experiments the sample volume loaded was 2200 ml

Viscosity of B-PE crude extract ($P \times 10^3$)	B-PE loaded (mg)	B-PE/adsorbent ratio (mg/ml)	B-PE recovery (%)
1.17	492	6.65	54.3
1.10	238	3.22	63.8
1.04	122	1.65	72.0
1.02	65	0.88	80.0

150 ml under conditions similar to adsorption and washing. Elution in expanded-bed mode needed 320 ml of mobile phase to elute the protein, while, in packed-bed mode, only 48 ml was necessary to recover the same quantity of protein. Thus, the sample in packed-bed mode was seven times more concentrated than in expanded-bed mode. Particulate material was not observed in the eluted fractions and it was not necessary to centrifuge the samples to obtain their UV–Vis spectra. The absorbance of the fractions obtained shows a sharp peak (Fig. 3, Elution), indicating high elution efficiency. It should be noted that the concentration of B-PE in the eluted fractions was suitable for direct application to the DEAE-cellulose column without further treatment. Both the loading sample/expanded-bed ratio (expressed as volume) and the concentration factor (expressed as volume of expanded bed/volume of sample eluted) were similar to those reported previously in the presence of cells [36,37].

The UV–Vis absorption spectrum of a B-PE-rich solution after the packed-bed elution stage corresponds to a biliprotein mixture where B-PE predominates with slight R-PC contamination. The spectrum showed a significant loss of absorption in the near-UV region with respect to the crude extract spectrum.

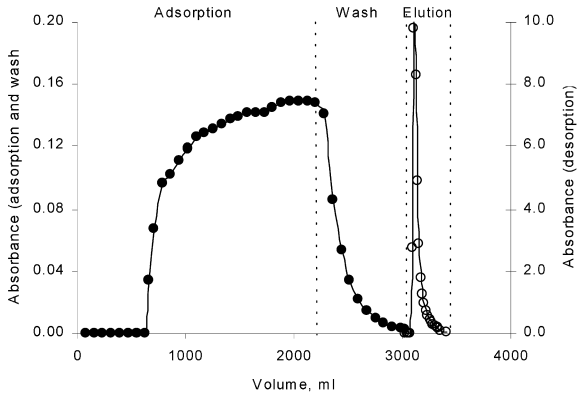

Fig. 3. Absorbance at 565 nm of effluent from the column during EBA chromatography of crude extract from *P. cruentum* biomass. 2200 ml of B-PE crude extract (0.88 mg B-PE/ml adsorbent) was applied to a Streamline-DEAE column. Feed rate 200 cm h^{-1}, elution rate 86 cm h^{-1}. In the elution step, absorbances were measured after suitable eluate dilution. Thus, the absorbance values on the ordinate axes are the resulting values after multiplication by the dilution factor.

Moreover, the A_{545}/A_{280} ratio of >4 is characteristic of a B-PE-rich solution.

The purity of the sharp peak from the elution stage was also determined by SDS–PAGE. Although SDS–PAGE is a powerful tool for assessing protein purity, unfortunately the B-PE α- and β-subunits have molecular masses of 18 991 and 20 315, respectively, both being present in similar amounts [38]. Consequently, their mobilities are very similar and it is difficult to separate them with the method employed. The γ-subunit has an apparent molecular mass of around 27 000 [16,39,40] and its mobility is less than the α- and β-subunits. Furthermore, the R-PC α- and β-subunits have molecular masses of 18 200 and 20 500, respectively [18]. Fig. 4A (lane 3) shows that the eluate from EBA yields one bulky band which could be assigned to the α- and β-subunits from both B-PE and R-PC. The other band in lane 3 must be assigned to the γ-subunit. As shown in Fig. 4A, most of the unwanted proteins (lane 4) were removed after EBA chromatography.

The B-PE recovery, expressed as the percentage of the total amount of B-PE in the eluate per amount of B-PE loaded onto the ion-exchange matrix, is shown in Table 1. The best recovery obtained was 80%, indicating that losses of B-PE during the flow-through and wash stages were very low. It should be emphasized that it was more important to achieve a high B-PE recovery than a high-purity product, since the procedure is intended to replace low-resolution steps that normally result in B-PE loss. Table 2 shows the processing time for the large-scale preparative method published previously [22] and the methodology used in this work. In the method described here, one cycle of the concentration process by EBA chromatography, including equilibration (30 min)+loading (130 min)+washing (50 min)+elution (60 min), took only about 4.5 h.

3.3. Packed-bed DEAE-cellulose chromatography

To obtain pure B-PE we used a DEAE-cellulose column to eliminate R-PC and other colorless proteins. We performed an experiment where the effluent from EBA was precipitated, resuspended in starting buffer, dialyzed against the same buffer, loaded in the starting buffer-pre-equilibrated column, and purified as described elsewhere [21,22]. Never-

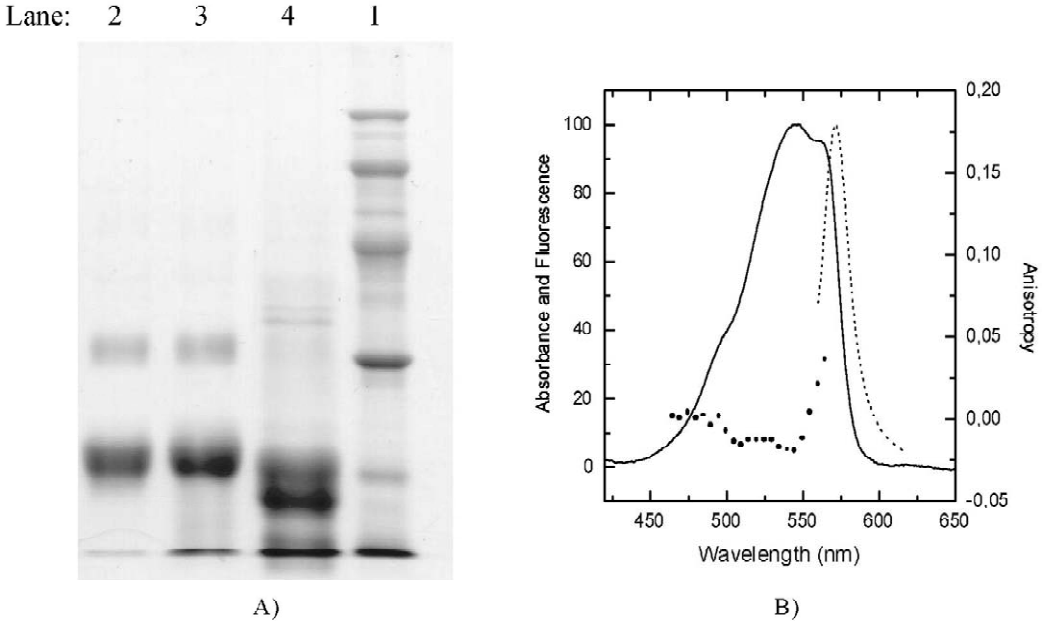

Fig. 4. (A) SDS–PAGE characterization of different protein solutions. Lane 1 = molecular mass markers, lane 2 = eluate after ion-exchange chromatography, lane 3 = eluate after EBA chromatography, lane 4 = crude extract. (B) Spectroscopic characterization of purified B-PE after ion-exchange chromatography, in 5 m*M* sodium phosphate buffer, pH 7.0. (——) Absorption spectrum, (------) fluorescence spectrum (λ_{ex} = 540 nm, $\Delta\lambda_{ex} = \Delta\lambda_{em}$ = 1.5 nm). Absorption and fluorescence spectra were normalized since these were only used on a comparative basis. (•) Fluorescence anisotropy spectrum (λ_{em} = 575 nm, $\Delta\lambda_{ex} = \Delta\lambda_{em}$ = 3 nm). The anisotropy values shown are the means of five values and were calculated for each 5 nm. All spectra were recorded at room temperature.

theless, in order to simplify the procedure, the starting buffer-pre-equilibrated column was directly loaded with the B-PE-rich solution from EBA and then developed with starting buffer. Whereas B-PE was not adsorbed at the top of the column, staying around 2 cm from the top, R-PC was slowly eluted by the starting buffer. Next, B-PE was eluted with 250 m*M* acetic acid–sodium acetate buffer, pH 5.5. We obtained two sets of fractions. One had a blue color and corresponded to R-PC with other colorless proteins (the absorption maxima were at 280, 310, 375, 555 and 620 nm). The other had a pink color, and the B-PE purity was very high as shown by the A_{545}/A_{280} ratio of 4.6. The volume of the pooled B-PE fractions and the B-PE concentration was 446 ml and 0.156 mg/ml, respectively. A total of 72.6 mg of B-PE was recovered. B-PE recovery after DEAE-cellulose chromatography was 90%, expressed as the percentage of pure B-PE in the final eluate per B-PE loaded onto the ion-exchange matrix. Table 2 shows that the yield of the overall B-PE purification process was 66%, twice that of the large-

Table 2
Processing time and yield of B-PE from *P. cruentum* using (A) previous large-scale methodology [22] (sonication + centrifugation + ammonium sulfate precipitation + dialysis + DEAE-cellulose chromatography) and (B) the method described in this work (extraction + EBA chromatography + DEAE-cellulose chromatography)

Methodology	Step					Total time	Yield (%)
	1	2	3	4	5		
A	35 min	45 min	12 h 10 min	24 h	2 h 30 min	40 h	32
B	1 h 30 min	4 h 30 min	2 h 30 min	–	–	8 h 30 min	66

scale purification method published previously and five times faster [22]. As in previous work with the DEAE-cellulose process, the calculated yield of the EBA process could easily be scaled up by increasing the column diameter while keeping the settled bed height, linear flow-rate and load at constant values.

For comparative spectral analysis and storage, the pink fractions were pooled, brought to 65% saturation with $(NH_4)_2SO_4$ and left to stand overnight in the dark at 4 °C before centrifugation. The pellets were resuspended in 5 m*M* sodium phosphate buffer, pH 7.0, dialyzed for 12 h against the same buffer with at least three changes of buffer and freeze-dried for storage. Fig. 4B shows absorption, fluorescence and anisotropy spectra for the dialyzed solution. These spectra agree well with those published for pure hexameric B-PE [14,16,21,22,39,41–43]. Excitation at 617 nm did not show a fluorescence signal around 650 nm (characteristic of R-PC). As shown by the spectra, the bands in Fig. 4A (lane 2) correspond to B-PE α- β- and γ-subunits. The absence of other contaminant proteins in lane 2 should be noted. By comparison with standards we obtained a γ-subunit molecular mass of 30 100, which agrees with the value reported previously by SDS–PAGE [16,21,22,39,40]. The band intensities in lane 2 appear to correspond to a heteropolymer with the polypeptide composition $(\alpha\beta)_6\gamma$. In the pH range 5–7 and at low ionic strength, B-PE shows this type of aggregation state [44].

4. Conclusions

We have achieved the purification of B-PE from the red alga *P. cruentum* by EBA chromatography using Streamline-DEAE, complemented by conventional chromatography in DEAE-cellulose. Only a simple low-cost pretreatment was necessary to optimize the expanded-bed step. The use of EBA followed by packed-bed elution in the same column eliminates many steps and produces concentrated and partially purified protein ready for use in the next purification step by conventional ion-exchange chromatography. The work presented here provides B-PE solutions in the hexameric aggregation state. The purification procedure described simplifies the product isolation process, reduces the processing time and utility consumption, and increases the total product yield.

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