Isolation of allophycocyanin B from *Rhodella violacea* results in a model of the core from hemidiscoidal phycobilisomes of rhodophyceae

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Two 'trimeric' allophycocyanin complexes could be isolated from the hemidiscoidal phycobilisomes of *Rhodella violacea*. AP = $(\alpha^{*AP}\alpha_2^{AP}\beta_2^{AP}\beta^{*AP})$ and APB = $(\alpha^{*AP}\alpha^{AP}\alpha^{APB}\beta_2^{AP}\beta^{*AP})\cdot L_c^{13.5APB}$. The isolation was performed by combined methods of gradient centrifugation, hydroxylapatite chromatography and 'native' polyacrylamide gel electrophoresis. AP showed the well-known spectral characteristics of allophycocyanin without linker polypeptide. APB is characterized by its long wavelength absorbing shoulder (675 nm) and fluorescence emission (682 nm), respectively. The existence of two low molecular linker polypeptides $L_c^{12.5}$ and $L_c^{13.5APB}$ in the phycobilisomes of *Rhodella violacea*, their stoichiometric calculations and the localization of $L_c^{13.5APB}$ in allophycocyanin B facilitated the construction of a model of the phycobilisome core.

Allophycocyanin B; Low molecular linker polypeptide L_c^{AP}; Phycobilisome core; Microheterogeneity; *Rhodella violacea* (Rhodophyceae); *Mastigocladus laminosus* (Cyanobacteria)

1. INTRODUCTION

Hemidiscoidal phycobilisomes from cyanobacteria and rhodophyceae consist of a tricylindrical allophycocyanin core and six peripheral rods [1]. These peripheral rods of both phyla are characterized by spectral properties, electron microscopy and polypeptide composition [2]. The phycobilisome core of cyanobacteria is composed of a central allophycocyanin complex designated AP_{cm} and six 'trimeric' allophycocyanin complexes [3,4]. Structure and spectral properties of the core have been subject of many investigations [5–7]. First detailed examinations of the polypeptide composition of the core were performed with the extremely reduced phycobilisomes of *Synechococcus* sp. 6301 AN 112 [8–10]. Although this phycobilisome core shows an extraordinary bicylindrical structure, the main components of the tricylindrical core are present. Both cylinders are built up by two 'trimeric' allophycocyanin complexes and the two 'terminal acceptors' L_{cm} and allophycocyanin B. APB and one of the other 'trimeric' AP-complexes are characterized by the content of the low molecular weight linker polypeptide L_c^{10AP}. This linker has been suggested to stabilize the spectral properties of these 'trimeric' AP complexes [11,12]. The absorption maximum of APB from *Synechococcus* sp. 6301 AN 112 at 654 nm with a shoulder at 675 nm and the fluorescence emission maximum at 680 nm could be confirmed by APB from other cyanobacteria [9,3].

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In contrast to the well-characterized phycobilisome core from cyanobacteria, information about the rhodophycean allophycocyanin core is still limited. The existence of the two different 'terminal acceptors' L_{cm} and allophycocyanin B is described for phycobilisomes of *Porphyridium cruentum* [13,14].

This study presents a rapid method for isolating allophycocyanin complexes from a rhodophycean phycobilisome core. It will be applicable for other species and gives an opportunity for further investigations.

2. MATERIAL AND METHODS

Cells of *Rhodella violacea* SAG B 115.79 were cultivated as already described [15] and harvested in their exponential growth phase. The phycobilisomes were isolated by continuous gradient centrifugation following the common procedure [15] modified by replacing Triton X-100 by 1% (w/v) LDAO (*N,N*-dimethyldodecyl-amine-*N*-oxide; Fluka, Buchs, Switzerland). Eluted phycobilisomes were concentrated by ultracentrifugation overnight in a fixed-angle rotor [3].

The concentrated phycobilisomes were dialyzed against 3 mM Tris-HCl buffer, pH 7.5, for 16 h at 4°C. 5 ml each of this dialysate were layered onto continuous gradients of 5–15% (w/v) sucrose in 5 mM Tris-HCl buffer, pH 7.5. Centrifugation was performed in a fixed-angle rotor TFT 70.38 (Kontron, Munich, FRG) at 280000 × *g* for 3 h at 10°C. Three bands were obtained. The uppermost band contained nearly 60% of the allophycocyanin and phycocyanin.

At a flow rate of 1 ml/min, this AP/PC-fraction was further separated by hydroxylapatite chromatography on a column (25 × 30 mm) packed with HA-Ultrogel (IBF Biotechnics, France) in 5 mM Tris-HCl buffer, pH 7.5. The resulting fractions were eluted by a discontinuous gradient of potassium phosphate, pH 7.0 (30 ml H_2O, I; 45 ml 10 mM, II; 30 ml 40 mM, III; 15 ml 100 mM, IV; 15 ml 1 M, V). The last two fractions exclusively contained allophycocyanin, the last fraction was enriched in APB.

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Subsequently, molecular weight determination of these AP-complexes was performed by liquid chromatography on a Superose 6 pg (Pharmacia, Freiburg, FRG) column (100 × 600 mm) at a flow rate of 0.3 ml/min. As marker proteins catalase (M_r 240000), aldolase (M_r 160000), hemoglobine (M_r 64000) and ovalbumine (M_r 45000) were used (Serva, Heidelberg, FRG).

For further purification of AP and APB, 'native' PAGE was applied. The two AP-fractions were concentrated to a protein concentration of 2 mg/ml by ultrafiltration using Millipore CX-filters (Millipore, Bad Nauheim, FRG). One ml of each fraction was applied on 3-mm-thick polyacrylamide slab gels. The gels contained 5% (w/v) polyacrylamide (5.0:0.133 w/w acrylamide:methylenebisacrylamide), 100 mM/73 mM Tris-boric acid, pH 8.6, and 7% (w/v) sucrose and were polymerized with 0.1% (v/v) *N,N,N,N*-tetramethylethylenediamine and 0.03% (w/v) ammoniumpersulfate (Serva, Heidelberg, FRG). Electrophoresis was performed in a modified BioRad mini-gel apparatus with 100 mM/73 mM Tris-boric acid, pH 8.6, at a constant current of 10 mA at 17°C for 2 h. The resulting bands were electroeluted and concentrated during 1 h at 4°C with 50 mM Tris-boric acid, pH 8.6, and 7% (w/v) sucrose by use of Biometra ELUCON (Biometra, Göttingen, FRG).

Spectroscopic analyses and analytical SDS-PAGE were performed as previously described [3]. Polypeptide stoichiometry was calculated using a CD 60 densitometer (Desaga, Heidelberg, FRG). The densitometric scans of the stained gels were performed at 580 nm.

3. RESULTS

Isolated phycobilisomes of *Rhodella violacea* exhibited absorption maxima at 545 nm, 564 nm and 624 nm with a distinct shoulder near 654 nm. In the 2nd derivative, this shoulder was divided into two components absorbing at 654 nm and 675 nm. The 25°C fluorescence emission spectra showed a maximum at 668 nm with a distinct shoulder near 680 nm. At −196°C the maximum shifted to 685 nm with small shoulders at 642 nm and 664 nm which is characteristic for intact phycobilisomes [3]. The spectral properties, especially the minimum in the 2nd derivative at 675 nm and fluorescence emission maxima near 680 nm at 25°C and 685 nm at −196°C, gave the first hint for the existence of APB in the phycobilisome of *Rhodella violacea*.

The high stability of rhodophycean phycobilisomes is well-known [15]. Therefore, it was necessary to dissociate the phycobilisomes over the long period of 16 h. Subsequent gradient centrifugation was performed to get an enriched fraction of allophycocyanin and phycocyanin. This 'trimeric' fraction directly separated by hydroxylapatite chromatography resulted in five fractions. The first two fractions mainly contained phycoerythrin, the third was enriched in phycocyanin with little allophycocyanin. On 5% PAGE, the AP-fractions IV (AP) and V (APB) revealed three and two bands, respectively. Only the major bands were further characterized by their spectral and molecular properties.

The highly purified AP-complex isolated from fraction IV exhibited an absorption maximum at 652 nm and shoulders at 594 nm and 620 nm (Fig. 1a). The fluorescence emission maxima at 25°C and −196°C lay at 662 nm and 664 nm, respectively (Fig. 2a). In SDS-PAGE (Fig. 3c), four subunits could be determined with M_r of 18700 (α^{*AP}), 18500 (α^{AP}), 17000 (β^{AP}) and 16500 (β^{*AP}). A molar ratio of 1:2:2:1 for α^{*AP}:α^{AP}:β^{AP}:β^{*AP} was calculated. The designation of the subunits was adapted to that of allophycocyanins from *Mastigocladus laminosus* phycobilisomes [3]. The subunit composition of APB of this phycobilisomes revealed by analytical SDS-PAGE is shown in Fig. 3a.

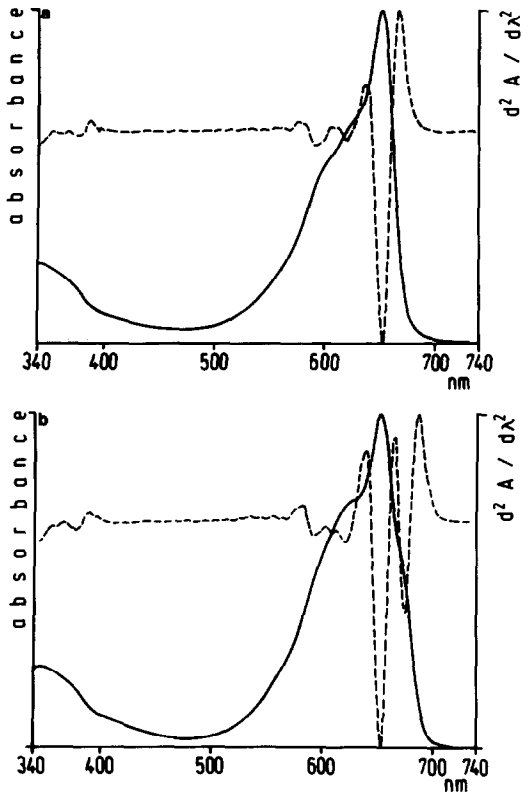

Fig. 1 Absorption spectra (——) and their 2nd derivative (– – –) of (a) AP and (b) APB in Tris-boric acid (50/37) mM, pH 8.6 and 7% (w/v) sucrose.

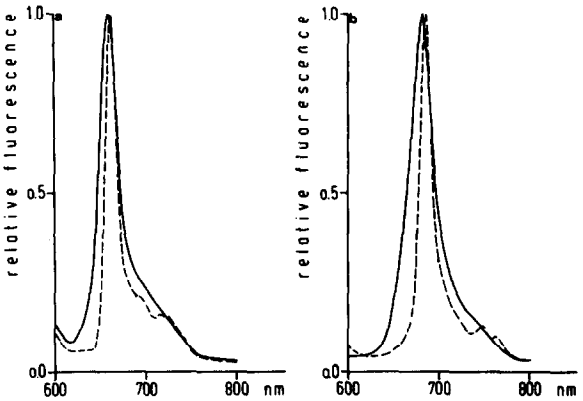

Fig. 2. Fluorescence emission spectra at 25°C (——) and −196°C (– – –) corresponding to Fig. 1a,b:(a) AP; (b) APB. Samples with an optical density of 0.1–0.25 cm^{-1} at $\lambda_{max.}$ were exited at 540 nm with a band width of 4 nm and emission was recorded with a slit width of 1 nm.

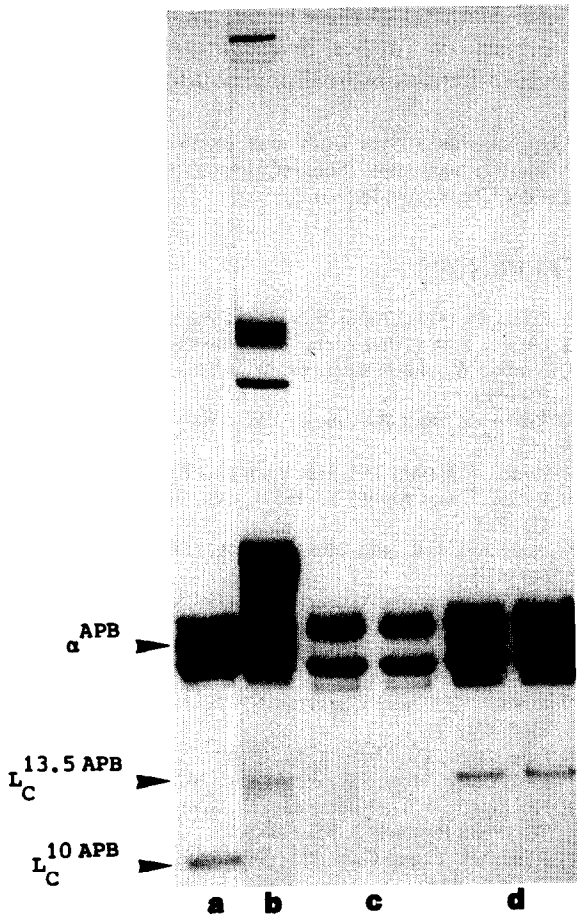

Fig. 3. SDS-PAGE of the isolated AP-complexes after purification by 'native' PAGE in comparison to *Rhodella violacea* phycobilisomes. (a) APB from *Mastigocladus laminosus*; (b) phycobilisomes; (c) AP; (d) APB from *Rhodella violacea*.

The APB-complex could be purified from fraction V. It had an absorption maximum at 654 nm with shoulders at 594 nm, 620 nm and especially 675 nm (Fig. 1b) better visualized in the minima of the 2nd derivative. The significant minimum at 675 nm which could already be detected in the phycobilisome spectrum was attributed to α^{APB}. This subunit was also indicated by the fluorescence emission maxima at 25°C at 282 nm and at −196°C at 685 nm (Fig. 2b). The microheterogeneity of α- and β-subunits had been observed for this complex, too. In SDS-PAGE, the extraordinary α^{APB}-subunit showed an M_r of 18000. Additionally, APB was characterized by the content of a low molecular weight linker polypeptide with M_r of 13500 designated $L_c^{13.5APB}$ (Fig. 3d). This linker polypeptide was detected in SDS-PAGE of phycobilisomes as the upper of two low molecular weight linkers (Fig. 3b).

Apparent molecular weight determinations gave values of Mr 100000-110000 for AP and of M_r 110000-120000 for APB. This result proves the 'trimeric' structure of the two complexes. Together with the polypeptide stoichiometry of their constituents the following quarternary structures are proposed:

AP: $(\alpha^{*AP}\alpha_2{}^{AP}\beta_2{}^{AP}\beta^{*AP})$

APB: $(\alpha^{*AP}\alpha^{AP}\alpha^{APB}\beta_2{}^{AP}\beta^{*AP})\cdot L_c{}^{13.5APB}$

4. DISCUSSION

This study presents two 'trimeric' AP-complexes isolated from phycobilisomes of the rhodophycea *Rhodella violacea*. Both complexes are characterized by the microheterogeneity of their α-and β-subunits. Microheterogeneity has recently been shown for three AP complexes - AP 664, APB (AP 680) and AP_{CM} - of phycobilisomes from the cyanobacterium *Mastigocladus laminosus* [3,4].

Allophycocyanin B from *Rhodella violacea* is characterized by a long wavelength absorbing shoulder at 675 nm, the 25°C fluorescence emission maximum at 682 nm, and the polypeptide composition showing α^{APB} and a low molecular weight linker polypeptide designated $L_c^{13.5APB}$. The second 'trimeric' AP-complex showed neither α^{APB} nor a low molecular weight linker. Although 'trimeric' AP-complexes have been isolated from several cyanobacteria, only a few preparations held the complexes in their 'native' conformation. Glazer and coworkers [2] first established the Lc^{10AP} as an essential component of certain 'trimeric' allophycocyanins. Based on amino acid analyses [16], they discussed a modification in polypeptide chains of L_c^{10APB} and L_c^{10AP}. The different strength of attachment of APB and AP 664 in the phycobilisome core of *Mastigocladus laminosus* may be attributed to 'functionally' different L_c^{10} linker polypeptides [3,4]. However, it is possible that these 'functional' differences are reflected by different molecular weights.

The phycobilisomes of *Rhodella violacea* directly separated by SDS-PAGE show two different low molecular weight linker polypeptides designated $L_c^{13.5APB}$ and $L_c^{12.5}$. All components of the phycobilisome periphery were isolated as previously

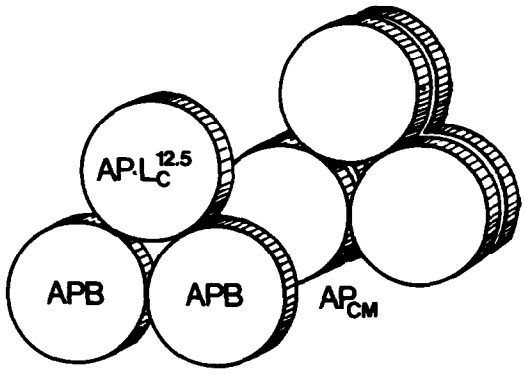

Fig. 4. Arrangement of the constituents of the phycobilisome core from *Rhodella violacea* established by the combination of all results presented. Face view of the phycobilisome core with the 'trimeric AP-complexes APB and $AP\cdot L_c{}^{12.5}$ extended for better visualization of the tricylindrical AP_{CM}.

described [17]. None of them showed any low molecular weight linker polypeptide (results not shown). Since as the localization of $L_c^{12.5}$ in the peripheral rods can be excluded, it must be a component of the core and can be suggested to belong to a second 'trimeric' AP-complex. Stoichiometric calculations of the low molecular weight linker polypeptides from the phycobilisomes resulted in a ratio of 2:1 for $L_c^{13.5APB}$:$L_C^{12.5}$. The same ratio and similar apparent molecular weights could be calculated for the low molecular weight linker polypeptides in the hemiellipsoidal phycobilisomes from *Porphyridium cruentum* [unpublished results].

The central part of the cyanobacterial phycobilisome core is held by a high molecular allophycocyanin complex designated AP_{CM}. It contains the linker polypeptide L_{CM} in its entirety [4]. Similar complexes could be isolated from several cyanobacteria and rhodophyceae [unpublished results]. Up to six 'trimeric' allophycocyanin complexes may be attached face-to-face to both sides of the tri-cylindrical central core complex AP_{CM}. Stoichiometric calculations of the polypeptides, the absorbance of the different biliproteins and the apparent molecular weight determination of isolated allophycocyanin, phycocyanin and phycoerythrin revealed that in phycobilisomes from *Rhodella violacea* only three 'trimeric' allophycocyanin complexes are present. The influence of physiological acclimation phenomena on the amount of peripheral allophycocyanin cannot completely be excluded.

Fig. 4 shows the possible distribution of the isolated and proposed allophycocyanin complexes in a hemidiscoidal rhodophycean phycobilisome core. The location of APB in the basal cylinders and AP in the apical one at the same side of AP_{CM} is suggested. The general character of these results and conclusions must be subject of further investigations especially with respect to hemiellipsoidal phycobilisomes.

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