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# Reconstitution of phycobilisome core–membrane linker, $L_{CM}$, by autocatalytic chromophore binding to ApcE

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**Abstract**

The core–membrane linker, $L_{CM}$, connects functionally the extramembraneous light-harvesting complex of cyanobacteria, the phycobilisome, to the chlorophyll-containing core-complexes in the photosynthetic membrane. Genes coding for the apoprotein, ApcE, from *Nostoc* sp. PCC 7120 and for a C-terminally truncated fragment ApcE(1–240) containing the chromophore binding cysteine-195 were overexpressed in *Escherichia coli*. Both bind covalently phycocyanobilin (PCB) in an autocatalytic reaction, in the presence of 4M urea necessary to solubilize the proteins. If judged from the intense, red-shifted absorption and fluorescence, both products have the features of the native core-membrane linker $L_{CM}$, demonstrating that the lyase function, the dimerization motif, and the capacity to extremely red-shift the chromophore are all contained in the N-terminal phycobilin domain of ApcE. The red-shift is, however, not the result of excitonic interactions: Although the chromoprotein dimerizes, the circular dichroism shows no indication of excitonic coupling. The lack of homologies with the autocatalytically chromophorylating phytochromes, as well as with the heterodimeric cysteine-α84 lyases, indicates that ApcE constitutes a third type of bilin:biliprotein lyase.


*Keywords:* Allophycocyanin; ApcE; Biosynthesis; Core–membrane linker ($L_{CM}$); Cyanobacteria; Energy transfer; Fluorescence labeling; Photosynthesis; Phycobilisome; Phycocyanobilin attachment

## 1. Introduction

Phycobilisomes (PBS), the unique light-harvesting complexes of cyanobacteria and red algae, are connected to the photosynthetic membrane by a multifunctional, chromophore-bearing chromoprotein, the core-membrane linker $L_{CM}$. The C-terminal repeats of two $L_{CM}$ are involved in the organization of the phycobilisome core. The single phycocyanobilin (PCB) chromophores of $L_{CM}$ and those of another core component, ApcD, are the two terminal energy transmitters transferring efficiently excitations from the hundreds of chromophores of the PBS to the photosynthetic reaction centers within the membrane (reviewed in Refs. [1–4]). There is evidence that part of $L_{CM}$ protrudes from the phycobilisome into a topologically yet undefined part of the membrane, thereby serving as a membrane anchor [5].

*Abbreviations:* APC, allophycocyanin; ApcE, core–membrane linker gene of *Nostoc* sp. strain PCC 7120; ApcE, apoprotein of the core–membrane linker of PBS (apo-$L_{CM}$); ApcE(1-y), ApcE truncated amino acids 1 through y; CD, circular dichroism; CPC, C-phycocyanin; α- and β-CPC, subunits of CPC, CpcA; CpcA/B, apoproteins of α/β-CPC, CpcE; CpcF, subunits of PCB:CpcA-lyase; E., *Escherichia*; $L_{CM}$, core–membrane linker (=chromophorylated ApcE); $L_{CM}$(1-y), $L_{CM}$ truncated to amino acids acids 1 through y; P, phytochrome; PBS, phycobilisome; PCB, phycocyanobilin; PE, phycoerythrin; PEB, phycoerythrobilin

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$L_{CM}$ has been isolated already in 1981 by Lundell et al. [6] from *Synechococcus* PCC 6301. It has extremely red-shifted absorption (665 nm) and fluorescence maxima (676 nm) of its PCB chromophore. The apoprotein, ApcE, is encoded by *apcE* [7,8], which in some cyanobacteria is located within the allophycocyanin operon. Like the phytochromes [9] and the phycobiliproteins [10], it requires posttranslational binding of the chromophore. Like with other phycobilisome linkers, biochemical studies of the isolated proteins are rare and have been hampered by their poor solubility. There is in particular no information for $L_{CM}$ on the chromophore attachment and the molecular origin of the spectral red-shifts.

There has been renewed [11] interest recently on the mode of attachment of bilin chromophores to biliproteins, fed both by the desire to reconstitute the chromoproteins from their (modified) components, but also by their use as fluorescent labels and probes [12–14]. Autocatalytic chromophore attachment seems to be the rule in phytochromes and other biliprotein sensory photoreceptors [9,15]. Autocatalytic attachment has also been reported for several binding sites of phycobiliproteins from PBS, in particular to cysteine-84 of CPC subunits, but it is slow and accompanied by side-reactions [16,17]. Lyases have been identified, on the other hand, for correct chromophore attachment to chromophore binding site Cys-α84 of phycobiliproteins [9–11], which can be coupled to isomerization to generate new chromophores in the process [18]. The situation is much less clear for other binding sites in the phycobiliproteins from cyanobacteria and red algae, for the entirety of cryptophyte biliproteins, and for certain members of the phytochrome family [15].

Information on the chromophore attachment is also scarce for chromophore-bearing PBS linker proteins, including $L_{CM}$. Like other linker proteins, it is difficult to handle when isolated. In spite of its crucial importance in energy transfer and an extensive analysis of the multifunctional protein in vivo, there is to date only a single biochemical study with isolated $L_{CM}$. It concluded that the isolated chromoprotein is partly denatured by the urea necessary for solubilization [6]. Cysteine-195 has been identified as the chromophore-binding amino acid of $L_{CM}$, but a mutation to serine still allows for noncovalent binding in a sub-optimal state and an impaired energy transfer [19]. Both the full-length apoprotein, ApcE, and a truncated fragment carrying the chromophore-binding cysteine-195 have now been produced by overexpression of *apcE* from *Nostoc* sp. (previously termed *Anabaena* sp. PCC 7120) in *Escherichia coli*. We show that they are soluble in solutions containing 4 M urea and bind autocatalytically the PCB chromophore, resulting in a chromoprotein with the absorption and fluorescence characteristics of the holoprotein [6].

## 2. Materials and methods

*Clones* Gene *apcE* and its fragment *apcE*(1–240) were PCR-amplified from *Nostoc* PCC 7120 with primers $P_1$ (ATACCCGGG**ATG**AGTGTTAAGGCGAGTG) and $P_2$ (ACTCTCGAGCAGTCCTAAAAA**TTA**GCGA, and $P_1$ and $P_3$ (ACTCTCGAG**TTA**AGG TGCTTTGAATTCT), respectively. The 5′ -terminal primer has a *Sma*I site (underlined) upstream of the natural start codon (in bold), which in the final constructs is no longer used and expressed as Met; the 3′ -terminal primers have a *Xho*I site (doubly underlined) and a stop codon (in bold).

PCR of the genes with *Pfu* polymerase was run at 30 cycles (95 °C for 90 s, 55 °C for 90 s, 72 °C for 500 s) and one additional incubation at 72 °C for 5 min. All PCR products were double-digested with *Sma*I and *Xho*I, and then ligated into the cloning vector *pBluescript* SK(+) (Stratagene) digested with the same restriction enzymes. Ligated products were then transformed into *E. coli* strain TG1. After sequence verification, the gene fragments were subcloned into pET30a (Novagen) via *Eco*RV and *Xho*I double-digestion. The pET30a-derived expression vectors were transformed into *E. coli* strain BL21(DE3) with the resulting plasmids pET-*apc*E and pET-*apc*E(1–240). All genetic manipulations were carried out according to standard protocols [20]. Via the pET30a vector, a 5-kDa peptide bearing His- and S-tags plus thrombin and enterokinase sites has been fused N-terminally to all expression products.

### 2.1. Proteins

*E. coli* strain BL21(DE3) was transformed with plasmids pET-*apc*E or pET-*apc*E(1–240), and the resulting transformed cells were cultured in LB medium at 37 °C overnight, transferred into 1-l liquid RB medium (0.5% NaCl, 0.5% yeast extract, 1% tryptone, 0.2% glucose, pH 7.5) supplemented with kanamycin (30 μg/ml) incubated at 37 °C until $OD_{600}$ reached 0.6, and then induced with isopropyl-β-D-thiogalactoside (1 mM) for 5 h. The cells were then centrifuged at 10,000×*g* for 3 min at 4 °C, and washed twice with water. The pellet was resuspended in 20 ml of the ice-cold lysis buffer (potassium phosphate buffer (KPP), 20 mM, pH 7.2, containing 1 M NaCl,) and sonicated for cell lysis (15 min, Branson model 450 W, 45 W).

The suspension was centrifuged at 10,000×*g* for 15 min at 4 °C. The supernatant was discarded. The pellet was suspended in KPP (20 mM, pH 7.2) containing urea (8 M), NaCl (1 M) and mercaptoethanol (5 mM), and kept overnight at 4 °C. After the centrifugation at 10,000×*g* for 30 min at 4 °C, the supernatant was dialyzed twice against KPP (20 mM, pH 7.2) containing urea (4 M), sucrose (0.2 M), NaCl (0.1 M) and mercaptoethanol (0.1 mM). After centrifugation at 20,000×*g* for 15 min at 4 °C, the supernatant was collected as crude ApcE or ApcE(1–240), which is suitable for preparative reconstitutions of $L_{CM}$ or $L_{CM}$(1–240).

The crude ApcE or ApcE(1–240) was further purified via Ni-affinity chromatography on chelating Sepharose (Amersham-Pharmacia), in the presence of urea (4 M). The start

buffer was KPP (20 mM, pH 7.2) containing sucrose (200 mM) and NaCl (0.1 M), the elution buffer contained in addition imidazole (0.2 M). Decreasing the concentration of NaCl from the generally recommended 0.5 M to 0.1 M doubles the reconstitution yields. After the collection of ApcE or ApcE(1–240), the sample was dialyzed twice against the start buffer, and kept at −20 °C until use.

### 2.2. Reconstitution

Phycocyanobilin (PCB) was isolated from *Spirulina platensis* as described [18]. ApcE or ApcE(1–240) (10–60 μM) in KPP (20 mM, pH 7.2) containing urea (4 M), sucrose (0.2 M), NaCl (0.1 M) and mercaptoethanol (0.1 mM) was mixed with PCB (5–20 μM) in the same buffer, and incubated in the dark for 3 h at room temperature, or for 30 min at 35 °C. Reconstitution yields were ~70% (based on PCB). The mixture was then centrifuged for 15 min at 15,000×*g* to remove any particulate matter, and the supernatant investigated by UV–Vis absorption (Perkin Elmer, Lambda 25), fluorescence (Perkin Elmer, LS 55) and circular dichroism (CD) (ISA, Jobin Yvon, Dichrograph VI) spectroscopy.

For spectral measurements, the reconstituted products, $L_{CM}$ and $L_{CM}$(1–240), were purified by a second Ni-affinity chromatography. After elution of $L_{CM}$ or $L_{CM}$(1–240) from the affinity column, the samples were dialyzed twice against the respective buffer containing mercaptoethanol (1 mM) and EDTA (5 mM).

### 2.3. Aggregation state

Reconstituted $L_{CM}$(1–240) (50 μM, 1 ml) was loaded on a Superdex 200 preparative grade column (60×1.6 cm) and developed (0.75 ml/min) with KPP (20 mM, pH 7.2) containing urea (4 M), sucrose (0.2 M), NaCl (0.1 M), EDTA 5 mM) and mercaptoethanol (1 mM). The apparent molecular mass was determined by comparison to a marker set (25–440 kDa).

### 2.4. Protein assay

Protein concentrations were determined by Bradford's assay [21], calibrated with bovine serum albumin. SDS-PAGE was performed by Laemmli's method [22]. Proteins were stained with Coomassie brilliant blue, and chromophore-containing ones identified by $Zn^{2+}$-induced fluorescence [23].

### 2.5. Spectral and kinetic analyses

Based on the extinction coefficient of PCB in CPC in 8 M acidic urea of $\varepsilon$=35,500 $M^{-1}$ $cm^{-1}$ [24], the extinction coefficients of the reconstituted $L_{CM}$ and ($L_{CM}$(1–240) monomer were determined to be $\varepsilon$=93,000 $M^{-1}$ $cm^{-1}$ for $L_{CM}$(1–240). A fluorescence quantum yield $\phi_f$=0.43 was determined for $L_{CM}$(1–240) in Tris–$H_3BO_3$ buffer (50 mM, pH 7.5) containing sucrose (0.2 M), mercaptoethanol (1 mM) and EDTA (5 mM), relative to the known $\phi_f$=0.22 of chlorophyll a in ethanol [25]. For kinetic analyses of the reconstitution, the fluorescence ($\lambda_{exc}$=650 nm) was followed.

## 3. Results

Full-length ApcE (1132 aa) and the N-terminal ApcE(1–240) (aa 1–240) produced in *E. coli* were contained in inclusion bodies. They were soluble in urea (8 M), but the proteins precipitated again during dialysis against a urea-free buffer. ApcE(1–240) remained soluble, however, in 4 M urea up to concentrations of about 100 μM, while full-length ApcE is much less soluble (≤1 μM). Most of the subsequent manipulations were therefore done with ApcE(1–240) in buffers containing 4 M urea. Incubation of ApcE and ApcE(1–240) with PCB ($\lambda_{max}$=630 nm in the reconstitution buffer) resulted in an absorption increase at 660 nm (Fig. 1) and the induction of a red fluorescence ($\lambda_{max,\ emission}$=675 nm, Fig. 2) which is well visible with the naked eye. The reaction is not dependent on EDTA. Neither dialyzable factors of the *E. coli* supernatant nor other proteins were required: purified ApcE and ApcE(1–240) could be identically reconstituted with PCB (not shown).

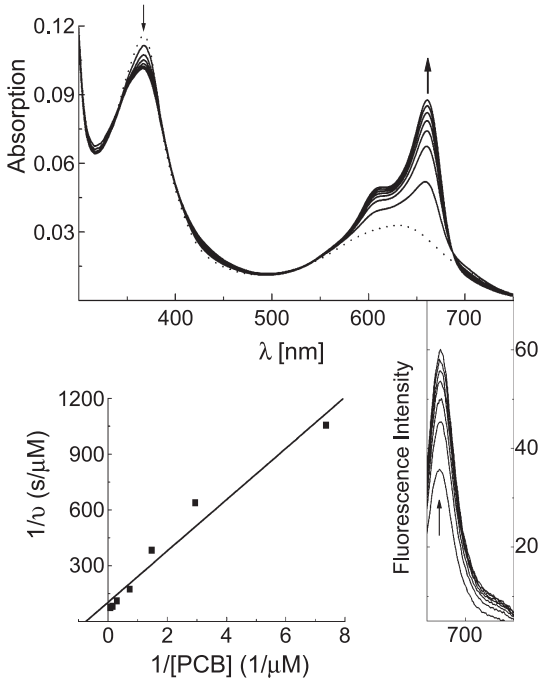


Fig. 1. Reaction of PCB (4 μM) with ApcE(1–240) (10 μM, conditions in this figure) followed by absorption (top) and fluorescence spectroscopy (bottom, right). Apoprotein (dots), PCB (dashed), reaction at t=1, 6, 11, 16, 21, 26, 31 min (solid lines, from bottom to top). Arrows indicate the direction of change with time. Bottom left: Lineweaver–Burke plot of the reaction kinetics ([ApcE(1–240)]=10 μM).

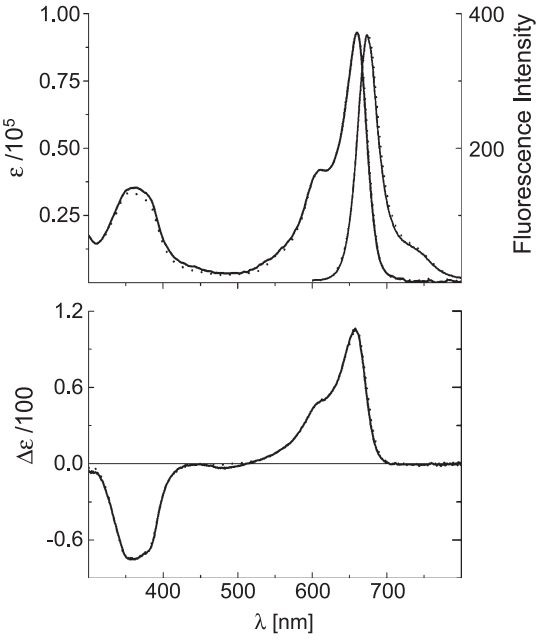


Fig. 2. Absorption, fluorescence emission ($\lambda_{max,exc}$=360 nm) (top) and circular dichroism spectra (bottom) of purified $L_{CM}$ (=PCB-ApcE(1–240)) in phosphate buffer (20 mM, pH 7.5) containing urea (4 M), sucrose (0.2 M), imidazole (0.2 M), NaCl (0.1 M), EDTA (5 mM), mercaptoethanol (1 mM) (—), and in the same buffer containing Tris–borate (50 mM, pH 7.5) instead of urea (· · · ·). Qualitatively the same fluorescence spectra were obtained with excitation at 390, 570, 610 and 660 nm (not shown).

Mercaptoethanol was beneficial, probably by protecting the chromophore. The optimal temperature is in the range of 30–40 °C, the optimum pH 8.5–9.0, but the reconstitution worked well in the range $T$=4–50 °C and pH 6.5–9.5 (Supporting material, Fig. 1). Reconstitution yields as high as 70% were consistently obtained under optimum conditions. This is comparable to the autocatalytic reconstitution of phytochromes [26–28], while yields of the lyase catalyzed reconstitutions of α-CPC or α-PEC do not exceed 30% [18,29,30].

Chromophorylated ApcE(1–240) migrated on SDS-PAGE with an apparent molecular mass of 32 kDa (calc. 31,602 Da), and covalent binding of the chromophore was verified by Zn-induced fluorescence of the band [23] (Fig. 3, right insert). The chromophore spectra of native biliproteins depend on noncovalent interactions with the apoprotein [31]. In acidic urea (8 M), where the chromophore is uncoupled from the protein, the absorption of ApcE or ApcE(1–240) at ($\lambda_{max}$=665 nm) (Supporting material, Fig. 2) is identical to that of denatured CPC [1]. This supports that $3^1$-Cys-PCB in the reconstituted product is formed by the spontaneous addition of PCB to the apoprotein.

A kinetic analysis of the reconstitution of ApcE(1–240) with PCB (Fig. 1) gave the following parameters: $K_m$=1.3 μM, $V_{max}$=9.6 nmol $s^{-1}$, $k_{cat}$=9.6×$10^{-4}$ $s^{-1}$. The affinity to PCB is comparable to that of CpcA and PecA in the lyase-catalyzed reactions [11,30] and of the autocatalytic chromophorylation of phytochrome AphA [32], while $V_{max}$ is intermediate: it is ~10-fold faster than the lyase reaction, but ~2-fold slower than with phytochrome.

The shape of the absorption spectrum of the purified products is reminiscent of trimeric allophycocyanin-linker complexes [33], with a sharp, intense peak superimposed on the red-side of a weaker, broad absorption band. Since excitonic interaction has been implied as a potential source for the strong red-shift and the peculiar shape of this band [34] (but see Ref. [35]), the aggregation state was investigated by gel chromatography (Fig. 3). ApcE(1–240) eluted as a major skewed band at the same time as bovine serum albumin (67 kDa), indicating dimer formation of the 31.602-kDa chromoprotein. The best fit over the six standard proteins resulted in an apparent molecular mass of 77 kDa (see Supporting material, Fig. 3 for the calibration), which is somewhat larger than a dimer. About 5% elute as higher aggregates (192 kDa), most likely hexamers. There is still some absorption at the expected position of the monomer (146 min), but neither indications a shoulder or a peak in the second derivative spectrum (not shown). Both fractions had identical absorption spectra (Fig. 3, left insert), and the shape of the absorption did also not change throughout the main peak (not shown).

## 4. Discussion

Free linkers ($L_{CM}$, $L_{RC}$, $L_R$) and fragments thereof are nearly insoluble in most buffers, but have been kept in solution in the presence of urea (3–4 M) [6,33]. This technique proved also applicable to ApcE and ApcE(1–240)

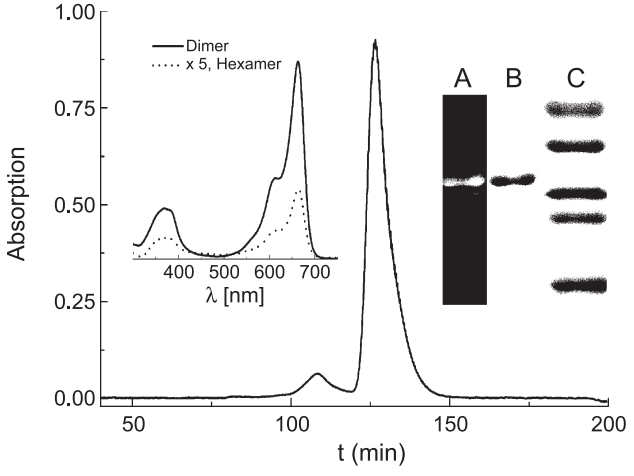


Fig. 3. Center: Gel exclusion chromatography (Superdex 200 prep. grade, 0,75 ml/min) of PCB-ApcE(1–240) in KPP-buffer (20 mM, pH 7.2) containing urea (4 M), sucrose (0.2 M), NaCl (0.1 M), EDTA (5 mM) and mercaptoethanol (1 mM). Left: In stream absorption spectra of the two peaks at 126.8 (— dimer) and 108 min (· · · · · hexamer). Right: SDS-PAGE of the reconstituted protein (band A: $Zn^+$-induced fluorescence of PCB-ApcE(1–240), band B: same trace Coomassie-stained, band C: marker proteins (from top) of 45, 36, 29, 24 and 20 kDa.

and its reconstitution products, and the proteins can be purified if 4 M urea is used in all manipulations. Remarkably, the autocatalytic lyase activity is retained, too, under these conditions. Dialysis of the products against urea-free buffer resulted in precipitation unless very dilute solutions ($c \leq 8$ μM) were used, but the spectral shape of the supernatant was identical to that shown in Fig. 3 for 4 M urea, indicating that the active structure of the proteins is retained in the latter.

If judged from the spectroscopic data, the relatively short N-terminal fragment of 240 aa is sufficient for the lyase activity, for the native interactions between chromophore and apoproteins, and for the dimerization of the chromoprotein. The chromophore is a covalent addition product of PCB to the protein, according to the optical data of the denatured chromoprotein (Fig. 2, Supporting material) to (the only) cysteine-195. Gindt et al. [19] report the noncovalent addition of PCB to a C186S mutant of ApcE in a core preparation from *Synechococcus* PCC7002 (the site is equivalent to C195 in ApcE from *Nostoc* PCC 7120), with a red-shifted absorption and emission, and reduced fluorescence yield. This indicates that while the cysteine is required for covalent binding, the chromophore binding site is capable of interacting properly in order to induce the chromophore red-shift and at least partially the fluorescence.

Lundell et al. [6] discuss that the full-length $L_{CM}$ isolated from *Synechococcus* sp. PCC 6301 in urea (3 M)-containing buffer is already partly denatured, based on the low absorption ratio ($Q^{A}_{vis/uv} \leq 2$) of the visible and the near-UV bands. No fluorescence yield has been determined previously for a $L_{CM}$. Absorption, CD, fluorescence emission and excitation spectra of purified $L_{CM}$ or $L_{CM}(1–240)$ (Figs. 1, 2 and Table 1) have the characteristics of a native phycobiliprotein [36]: there is an intense absorption in the visible spectral range ($\lambda_{max}$=660 nm) and a smaller absorption band in the near UV ($\lambda_{max}$=360 nm). The absorption ratio ($Q^{A}_{vis/uv}$=4.1, Fig. 2) is comparable to that of native CPC ($Q^{A}_{vis/uv}$~5, with the exact value depending on the aggregation state, pH). This and the high fluorescence quantum yield ($\phi_f$=0.43) suggest that PCB of the reconstituted products is in a (near) native chromophore conformation of $L_{CM}$. The spectral similarities between full-length $L_{CM}$ and the truncated $L_{CM}(1–240)$ indicate that the C-terminus only slightly affects the chromophore environment. Interactions of this type have been seen with phytochromes (Ref. [27], see also Ref. [32]).

The origin of the peculiar absorbance band shape of APC has been the subject of several investigations. It has been related to excitonic interaction by Csatorday et al. [34], who observed a decrease of the sharp band upon dissociation of the trimer to monomers. Reconstitution studies suggested, however, that this band is pronounced only for trimers in the presence of linkers, and largely absent in trimers of APC devoid of linkers [33]. Since the distances between α-84 and β-84 chromophores and their orientations in an APC-$L_c^{8,3}$ complex [37] are very similar to those of APC trimers $(\alpha\beta)_3$ [38], this points to a different origin. An excitonic origin is also unlikely from ultrafast time-resolved depolarization experiments [35], indicating that a subtle conformational and/or protonation change is responsible for the intense red band. Whatever the origin, it seems to be present also in ApcE, combined with an even stronger red-shift. The absence of excitonic interactions is further supported by the non-conservative CD spectrum (Fig. 2). It is positive throughout the long-wavelength band system, and its shape follows the absorption, with CD extrema (662, 604, 383 and 347 nm) matching the absorption maxima ($\lambda_{max}$=662, 603, 384 and 347 nm). The splitting of both the red and the near-UV bands could principally arise from heterogeneous chromophores. However, double bands of somewhat lesser spacing are seen in nearly all native biliproteins, where they give rise to a peculiar bilin line-shape [39]. We therefore conclude that there is no evidence for excitonic interaction among the chromophores.

In phytochromes, a 200-aa motif has been suggested to be responsible for chromophore ligation [27]. A second, more N-terminal motif is discussed for plant [27] and the "classical" cyanobacterial phytochromes [32]. Both motifs are absent in ApcE, if judged from a comparison using various BLAST versions and pairwise

Table 1
Absorption, fluorescence emission and excitation maxima of ApcE(1–240) and ApcE in various solvent systems

| | $\lambda_{max}$ [nm] | | | Conditions |
|---|---|---|---|---|
| | Absorption | Excitation | Emission | |
| ApcE | 657 | 662 | 672 | KPP (20 mM, pH 7.2), urea (4 M), sucrose (0.2 M), imidazole (0.2 M), NaCl (0.1 M) |
| | 657 | 661 | 671 | KPP (20 mM, pH 7.2), urea (4 M), sucrose (0.2 M), imidazole (0.2 M), NaCl (0.1 M) |
| ApcE(1–240) | 661 | 664 | 675 | Tris–borate (50 mM pH 7.5), sucrose (0.2 M) |
| | 662 | 668 | 676 | KPP (20 mM, pH 7.2), urea (4 M), 0.1 M NaCl |
| | 661 | 665 | 672 | KPP (20 mM, pH 7.2), urea (4 M), 0.1 M NaCl, imidazole (0.5 M) |
| | 661 | 665 | 674 | KPP (20 mM, pH 7.2), urea (2 M), sucrose (0.2 M), imidazole (0.1 M), NaCl (0.1 M) |
| | 661 | 664 | 674 | KPP (20 mM, pH 7.2), urea (4 M), sucrose (0.2 M), imidazole (0.1 M), NaCl (0.1 M) |
| | 661 | 665 | 674 | KPP (20 mM, pH 7.2), urea (4 M), sucrose (0.2 M), imidazole (0.5 M), NaCl (0.1 M) |
| | 661 | 665 | 673 | KPP (20 mM, pH 7.2), urea (4 M), glycerine (0.8 M), imidazole (0.5 M), NaCl (0.1 M) |

comparisons by the Smith/Waterman algorithm. There is no significant homology with the bilin:cysteine α-84 lyases of biliproteins or with those regions of phytochromes sufficient for chromophore binding. Two regions of ApcE(1–240) are homologous to cyanobacterial phycobiliproteins. They are separated by an insertion of 55 aa (BP loop, see Ref. [6]), which seems not necessary for chromophore binding [40]. Aa 25 to 75 resemble the N-terminal regions (aa 10–60) of APC, CPC or PEC, in particular of the α-subunits. This region contains the helices X and Y involved in the dimerization of α- and β-subunits [41]; it is likely to contribute also to the aggregation of $L_{CM}$. The second homologous region of aa 155–212 containing the chromophore-binding cysteine-195 corresponds to aa 79–138 of phycobiliproteins, in particular their β-subunits, which contains the cysteine β-84 (consensus numbering) binding a chromophore in all such pigments. However, cysteine-195 of ApcE is not homologous to the latter. In the region corresponding to the β-84 chromophore site, the cysteine is replaced by serine, while the surrounding sequence is highly conserved. It is conceivable that the serine-containing empty site is involved in the lyase reaction, as well as the insertion. A chromophore "jump" during evolution has been discussed, too, for the phytochromes [42]. Recent studies using detergents have suggested a pronounced conformational dependence on the chromophore binding [17], in line with earlier suggestions that lyases may be chromophore chaperones, while the proper lyase activity is located in the biliproteins. This notion has also been supported by experiments showing residual Cys α-84 lyase activity in the absence of lyases like PecE/F. The sequence of the N-terminal fragment of ApcE bears a distinct homology to conventional biliprotein binding sites. Since it shows a high lyase activity, we take this as further evidence that the principal lyase activity is present already on the biliprotein, and there is no requirement for additional lyases [10].

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbabio.2004.09.008.

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