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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