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Energy transfer between fusion biliproteins co-expressed with phycobiliprotein in *Escherichia coli*

Qiong Ma [a, b, *], Nan Zhou [c], Ming Zhou [c]

[a] School of Life Science & Technology, Hubei University for Nationalities, Enshi 445000, China
[b] Key Laboratory of Biologic Resources Protection and Utilization of Hubei Province, Hubei University for Nationalities, Enshi 445000, China
[c] State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China

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ABSTRACT

In cyanobacteria, phycobiliproteins (PBS) show excellent energy transfer among the chromophores absorbing over most of the visible. The energy transfers are used to study phycobilisome assembly and bioimaging. Using All4261GAF2(C81L) as energy donor, ApcE(1-240/Δ87-130) as energy acceptor, we co-expressed fusion protein ApcE(1-240/Δ87-130)::All4261GAF2(C81L) with phycobiliprotein in *Escherichia Coli* and studied the energy transfer between two protein domains. With N-terminal His6 tag, ApcE(1-240/Δ87-130)::All4261GAF2(C81L) cannot be purified by nickel-affinity column. We added six histidines in the C-terminal of ApcE(1-240/Δ87-130)::All4261GAF2(C81L) and co-expressed it with phycobiliprotein. ApcE(1-240/Δ87-130)::PCB-All4261GAF2(C81L)$_{His6}$ was purified successfully and only singly chromophorylated at All4261GAF2(C81L)$_{His6}$ domain. The singly chromophorylate ApcE(1-240/Δ87-130)::PCB-All4261GAF2(C81L)$_{His6}$ was incubated with fresh PCB and the doubly chromophorylated PCB-ApcE(1-240/Δ87-130)::PCB-All4261GAF2(C81L)$_{His6}$ was obtained. The double chromophored fusion protein absorbed light in the range of 615–660 nm, and fluoresced only at 668 nm. Photochemistry analysis showed that excitation energy transfer from the short-wavelength absorbing at All4261-GAF2(C81L) domain was achieved successfully to the long-wavelength absorbing at the ApcE(1-240/Δ87-130) domain.

In cyanobacteria, phycobiliproteins are accessory light-collecting pigments in the photosynthetic apparatus. They can transfer harvested energy to photosystem II (PSII) and photosystem I (PSI) [1,2]. Previous studies focused on fluorescence resonance energy transfer (FRET) of native photosystems in cyanobacteria and in vitro reconstitution photosystem components [3,4], rarely reporting the FRET of fusion biliproteins.

Generally speaking, energy transfer complexes consist of at least two protein domains: the donor protein and the acceptor protein. As a pair of donor/acceptor for FRET, it requires a wide overlap between the fluorescence emission spectrum of highly fuorescent donor protein and the absorption spectrum of acceptor protein. Furthermore, the more the spectrum overlaps, the more the fuorescent energy transfers efficiently [5].

One potential candidate for energy transfer donor is All4261GAF2(C81L), a variant of the cyanobacteriochrome All4261GAF2 from *Nostoc* sp. PCC7120 [6]. One potential candidate as energy acceptor is ApcE(1-240/Δ87-130), the N-terminal chromophore domain of ApcE, which is the core-membrane linker (L_{CM}) in *Nostoc* sp. PCC7120 [7]. In generally, phycobiliproteins covalently binding with chromophore need to be catalyzed by lyase [8]. All4261GAF2(C81L) and ApcE(1-240/Δ87-130) both contain a GAF domain (cGMP phosphodiesterase, adenylyl cyclase and FhlA domain). Then they can bind chromophore automatically because of their GAF domains function as lyase, without other lyase catalysis [5,8,9]. Using pET30(a+) as expressing vector, we constructed pET-*apcE*(1-240/Δ87-130)::*all4261gaf2(C81L)*. Then we co-expressed fusion protein ApcE(1-240/Δ87-130)::All4261GAF2(C81L) with phycocyanobilin (PCB) in *Escherichia coli* BL21 (DE3). It is expected that we will obtain a fusion biliprotein complex that can bind PCB covalently at each domain and transfer light energy from PCB-All4261GAF2(C81L) to PCB-ApcE(1-240/Δ87-130) in this fusion biliprotein.

* Corresponding author. School of Life Science & Technology, Hubei University for Nationalities, Enshi 445000, China.
E-mail address: maqiong110@126.com (Q. Ma).

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1. Experiment procedures

1.1. Construction of plasmids

All genetic manipulations were carried out according to standard protocols [8]. Using site-directed mutation *via* the mutation kit (TaKaRa, Dalian), *all4261gaf2(C81L)* fragments were obtained by using primers P1 and P2 (Table 1) with pET-*all4261gaf2* as template [6]. The DNA fragments *of apcE*(1-240/Δ87-130) were amplified from pET-*apcE* [7] using primers P3 and P4. After nucleotide sequence verification, *all4261gaf2(C81L)* and *apcE*(1-240/Δ87-130) fragments were subcloned into pET30a(+) to construct pET-*apcE*(1-240/Δ87-130)*::all4261gaf2(C81L)*. The DNA fragments of *all4261-gaf2(C81L)*$_{his6}$ were amplified by using primers P5 and P6 with pET-*all4261gaf2(C81L)* as template. Then *all4261gaf2(C81L)*$_{his6}$ and *apcE*(1-240/Δ87-130) fragments were subcloned into pET30a(+) to construct pET-*apcE*(1-240/Δ87-130)*::all4261gaf2(C81L)*$_{his6}$.

1.2. Expression and purification

For over-expression, pET-*apcE*(1-240/Δ87-130)*::all4261-gaf2(C81L)* was transformed into *E. coli* BL21(DE3) containing the PCB-generating plasmid, pACYC-*ho1-pcyA* [8]. The doubly transformed cells were cultured at 37 °C in Luria Bertani (LB) medium, which was supplemented with kanamycin (20 μg/mL) and chloromycetin (17 μg/mL). After introduction with IPTG (0.5 mM) for 16 h in the dark condition, cells were harvested at 9000 × g for 5 min, washed twice with distilled water and resuspended with ice-cold potassium phosphate buffer (KPB, 20 mM, pH 7.0), and then cells were lysed on ice by sonication for 4 min at 50 W. The cell extract was centrifuged at 12000*g* for 20 min at 4 °C, then supernatant containing fusion protein was passed through a nickel-affinity column (His-Trap Chelating HP, Amersham Biosciences). Bound proteins were eluted with 500 mM imidazole fraction. After collection, the fusion proteins were dialyzed twice against KPB. While ApcE(1-240/Δ87-130)::All4261GAF2(C81L) cannot be purified with nickel-affinity column, we transformed pET-*apcE*(1-240/Δ87-130)*::all4261gaf2(C81L)*$_{his6}$ into *E. coli* BL21(DE3) and co-expressed ApcE(1-240/Δ87-130)::All4261GAF2(C81L)$_{his6}$ with PCB using the same method above.

1.3. Chromophorylation in vitro

By co-expressed ApcE(1-240/Δ87-130)::All4261GAF2(C81L)$_{his6}$ with PCB in *E. coli* BL21(DE3), ApcE(1-240/Δ87-130)::All4261-GAF2(C81L)$_{his6}$ was only singly chromophorylated on All4261-GAF2(C81L)$_{his6}$ domain. Therefore, we expressed ApcE(1-240/Δ87-130)::PCB-All4261GAF2(C81L)$_{his6}$ and free PCB separately in *E. coli* BL21 (DE3). Purified ApcE(1-240/Δ87-130)::PCB-All4261-GAF2(C81L)$_{his6}$ and free PCB supernatant were separately obtained. After mixing purified ApcE(1-240/Δ87-130)::PCB-All4261-GAF2(81C/L)$_{his6}$ with PCB supernatant in different volume proportions, the reaction mixtures were incubated with shaking of 150 rpm at 16 °C for 30 min, and then purified through a nickel-affinity column.

1.4. Protein assay

Soluble extract of target protein or purified proteins were solubilized with 4% (w/v) lithium dodecyl sulfate, 120 mM dithiothreitol, and 120 mM Tris-HCl (pH 8.0), and subjected to SDS-PAGE. Gels were stained with 1 mM zinc acetate, and then stained with Coomassie blue by conventional methods [9]. Bradford and bandscan were used to analyze protein amount [9,10]. We calculate the yield which compares the purified target proteins with nickel-affinity column to the total proteins in *E. coli* crude solutions and the purity which analysized by bandscan while the purified proteins subjected to SDS-PAGE.

1.5. Spectral analysis

Absorption spectra were obtained with a Zeiss DMR10 spectrophotometer (Beckman-Coulter DU800, Shanghai, China). Fluorescence spectra were recorded at room temperature using LS 45 spectrofluorimeter (Perkin-Elmer, Shanghai, China).

2. Results

The expression recombinant plasmids (Table 2) were digested with corresponding restriction double enzymes, respectively. Digested products were then subjected to agarose gel electrophoresis and detected preliminary for inserted fragments size in plasmids. The results indicated that there were about 500–600 bp inserted nucleotide fragments in line 1 and line 2, and the there were 1151 bp inserted nucleotide fragments in line 3 and line 4, which were the same expected size of *all4261gaf2(C81L)*, *apcE(1-240/Δ87-130)*, *apcE(1-240/Δ87-130)::all4261gaf2(C81L)* and *apcE(1-240/Δ87-130)::all4261gaf2(C81L)*$_{his6}$, respectively (Fig. 1). Nucleotide sequencing showed that the inserted fragments of recombinant plasmids were *all4261gaf2(C81L)*, *apcE(1-240/Δ87-130)*, *apcE(1-240/Δ87-130)::all4261-gaf2(C81L)* and *apcE(1-240/Δ87-130)::all4261-gaf2(C81L)*$_{his6}$, respectively.

2.1. Spectral properties of PCB-All4261GAF2(C81L) and PCB-ApcE(1-240/Δ87-130)

PCB-All4261GAF2(C81L) has a maximum absorption peak at 615 nm and a fluorescence peak at 645 nm (Fig. 2A, Table 4), with an extinction coefficient of 7.2 (±0.02) × 10^4 M^{-1} cm^{-1} and high fluorescence quantum yield of 0.230. Then PCB-All4261GAF2(C81L) is highly fuorescent and suitable used as energy donor protein. PCB-ApcE(1-240/Δ87-130) has the characteristic absorption peak at 660 nm and fluorescence peak at 668 nm (Fig. 2 B, Table 4), with extinction coefficient of 9.5 (±0.07) × 10^4 M^{-1} cm^{-1} and low fluorescence quantum yield of 0.092. Furthermore, the absorption spectra of PCB-ApcE(1-240/Δ87-130) has 58–61 nm overlapping

Table 1
Primers for plasmids construction.

Primer	Sequence	DNA
P1	5′- AGTGAACTTAGCGCCAAAGATTTAGCA -3′	*all4261gaf2(C81L)*
P2	5′- GACATTCTTTCGTAATTCCGGTTGCTT -3′	
P3	5′ -ATAGATCTGATGAGTGTTAAGGCGAGT-3′	*apcE(1-240/Δ87-130)*
P4	5′- TATGATATCAGGTGCTTTGAATTCTGT-3′	
P5	5′- GCCGATATCAGAGTAACAGCCGCAGAATGG-3′	*all4261gaf2(C81L)*$_{his6}$
P6	5′-ATATAAAGCTTAATGATGATGATGATGATGATGGGTTGCTTGAGTTTG-3′	

Notes: The underlined base sequences in P6 are coding six histidines introduced in the C-terminal of All4261GAF2(C81L).