Cloning and characterization of the phycoerythrin operon upstream sequence of *Gracilaria lemaneiformis* (Rhodophyta)

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Abstract

A study was made of the regulatory region of the gene responsible for the expression of phycoerythrin in the red alga *Gracilaria lemaneiformis*. Rapid Amplified cDNA Ends Polymerase Chain Reaction (RACE-PCR) was used to obtain the 5' untranslated sequence of phycoerythrin operon of the alga. The transcriptional initiation site starting with A was found at 80 bp ahead of the AUG codon of the structural gene. A 54 bp fragment with two or three gaps inside was determined to be 66.7% ∼ 85.2% similar to those of other red algae. In vitro PCR cloning technique was used to clone the far upstream untranscribed sequence. There was no significant similarity of untranscribed sequence to those known for other red algae. Neither a canonical TATA nor CAAT box was found in this region. As predicted by PLACE, a software suitable for plant promoter analysis, there were two special functional area, one around nucleotide 65, the other 328, responsible for light regulated and/or high level of gene expression. The untranscribed region of phycoerythrin operon was subcloned into a promoter report vector, pEGFP, and transferred into *E. coli* and *Synechocystis PCC6803*, respectively. Strong green fluorescence was observed in electroporated *Synechocystis PCC 6803* cells 24hrs after the operating, but not in *E. coli*, suggesting that the promoter is trans-activated.

Abbrevation: RACE-PCR – Rapid Amplified cDNA Ends Polymerase Chain Reaction

Introduction

*Gracilaria lemaneiformis* is an important species for research and commercial use, and, like other red algae, has phycobilisomes which play an important role in harvesting and transmitting light energy (Gantt, 1975). Phycoerythrin, one of the phycobilisome components, has α- and β- subunits which are encoded on the plastid genome (Stadnichuk et al., 1997; Glazer & Hixson, 1977), and expressed intensively (Fábregas et al., 1999) in red algae. In the complementary chromatic adaptation process at the translation level (Talarico et al., 1991), the structure and expression of these two proteins are associated with environmental factors, especially light intensity (Waaland et al., 1974) and wavelength (Lopez Figueroa, 1991), suggesting its likely regulatory role. Study of the upstream area of the operon containing these two genes is therefore important for understanding the mechanism and developing a promoter for gene engineering.

The regulating consensus at the upstream of phycoerythrin operon has been identified in the comparison of a number of sequences of this region in cyanobacteria (Newman et al., 1994). However, that for red algae remains unclear. In present work, the 5’ upstream sequence of the phycoerythrin operon was
cloned and characterized. The aim of this study is to characterize the phycoerythrin operon gene regulating elements, and to test its promoting function in gene expression, which may be applicable in algal genetic engineering and breeding.

Materials and methods

Strains and plasmids

*G. lemaneiformis* was collected from Zhanshan Bay (Qingdao, China) and cultured using the conditions described by Zhang and van der Meer (1988). *E. coli* DH5α is the host strain for pUC system plasmids (Sambrook et al., 1989). *Synechocystis* PCC6803 was grown at 30 °C in BG–11 medium with bubbling and continuous light at 50 µmol m⁻²s⁻¹. Cloning plasmid pMD18-T was purchased from TAKARA Company (Dalian, China). The promoter report vector pEGFP was got from Clontech.

Primers

Primers were synthesized by Sangon Company (CA). AP 1043: 5'-ATTCAACACCTGC-3'; A2: 5'-CGAATGGGTAATTGCTGGT-3'; A1: 5'-ATGGAAATCAGTTATTACTAC-3'; S1: 5'-TAAACAAGCAGCCATACGACGG-3'; S2: 5'-CAGCTGCCATTACATAAGCA-3'; pf33: 5'-GGA CTTAGCTGTTTCAGCTGT; pf1: 5'-CTGATGTATTAGTATCCCT-3'.

Reagents

RNA extraction Kit was obtained from Sangon Company, and 5'-Full RACE Core Set and In-Vitro PCR Clone Kit were purchased from TAKARA Company.

DNA extraction

Fresh tissue was ground in a 1.5 ml Eppendorf tube using a specially designed pestle and immediately mixed with 25–30 µl buffer (100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 3% Cetyl trimethyl ammonium bromide, 0.2% (v/v) β-Mercaptoethanol). After incubation at 60 °C for 30 min with occasional gently mixing, the sample was extracted with an equal volume of chloroform-isoamylalcohol. The aqueous phase was recovered and precipitated with isopropanol. The DNA was pelleted by centrifugation at 15,000 rpm for 10 min. The supernatant, containing nucleic acids and some low molecular weight polysaccharides, was removed from the tube by pipetting, discarding the pellet composed largely of polysaccharides and some trapped nucleic acids. DNA concentration was measured using Pharmacia Gene Quant RNA/DNA flurometer.

RACE-PCR

Reverse transcription primer AP1043 was phosphorylated as described by Ye & Gong (1999). In brief, 100 nmol ATP, 10U T4 polynucleotide kinase and 0.5 nmol AP1043 were added into a 50 µl reaction system, and incubated at 37 °C for 1 h. The product was then extracted by phenol/chloroform, precipitated by ethanol and dried in air. RACE-PCR was then conducted following the manufacturer’s instruction using amplifying primer pairs A1-S1 and A2-S2 successively (Figure 1).

In vitro PCR cloning

Total DNA was digested by Sau3AI, and ligated with Sau3AI linker provided by the kit. Nested PCR using C1-pf33 as the first pair and C2-pf1, the second, was conducted following the instruction of the manufacturer. C1 and C2 are common primers provided by the kit complementary to linker sequence.

Recovery of fragment of interest

After PCR amplification, the products were separated in 1% agarose gel and the interested band was recovered using low melting point agarose gel. The recovered band was cloned into pMD18-T with a T overhanging in the 3' end following the manufacturer’s instruction. Standard methods were used for screening by complement selection (Sambrook et al., 1989) and PCR amplifying using the universal primers flanking the multiply cloning site. Recombinant having suitable amplified length in PCR screening was selected to sequence.

Sequencing and structural analysis

Sequencing was performed on the PE-ABI377 DNA sequencer (PE Co., USA) from both ends. MACAW software (version 1.02) was employed to align the sequences. PLACE database (http://www.dna/affrc.go.jp/sigscan/signal1.pl)