

Cloning and sequencing of the phycocyanin gene from *Spirulina maxima* and its evolutionary analysis

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Abstract

The genes were cloned for the two apoprotein subunits, α and β , of phycocyanin from the cyanobacterium *Spirulina maxima* (= *Arthrospira maxima*) strain F3. The α - and β -subunit gene-coding regions contain 489 bp and 519 bp, respectively. The β -subunit gene is upstream from the α -subunit gene, with a 111-bp segment separating them. Similarities between the α -subunits of *S. maxima* and seven other cyanobacteria were between 63% and 99%, as were those between the β -subunits. The maximum similarity between the α - and β -subunits from *S. maxima* was 27%.

Phycobiliproteins are light-harvesting antenna pigments of the phycobilisomes in cyanobacteria, Rhodophyta, Cryptophyta and Glaucophyta. Amino acid and DNA sequence data show considerable sequence conservation among the various phycobiliproteins, as well as between the α - and β -subunits of a particular phycobiliprotein (e.g. de Lorimier et al. (1984, 1985) and Glazer (1977), Offner et al. (1981), Offner and Troxler (1983), Pilot and Fox (1984), Troxler et al. (1981)). This suggests that phycobiliprotein genes arose via duplications of an ancestral sequence (Glazer 1977). Sequence analysis at the nucleotide level helps to shed light on the evolution of phycobiliproteins and to search the regulatory sequences of these genes for unique features. Furthermore, as phycobiliprotein amino acid sequences become known for other organisms, similarity studies can be undertaken at the amino acid level.

The aim of the present study was to clone and characterize the α - and β -subunit genes of *Spirulina* (*Arthrospira*) *maxima* and to compare the subunits of this species with those of other species of cyanobacteria.

Spirulina maxima strain F3, originally isolated from the Pacific Ocean coast (Hangzhou, Zhejiang

Province) of China between April and October 2000, and obtained for this study from Zhejiang University. The sources of other materials were: *Escherichia coli* DH5 α , PCR-Blunt vector and GeneClean kit: Invitrogen (USA); restriction enzymes and Marker DL2000: Takara Biotech (Japan); Sequenase 2.0 DNA sequencing kit: United States Biochemicals. The organism was grown in Zarrouk (1966) medium, which was centrifuged after autoclaving if there was a precipitate. Cultures were incubated in 500-mL conical flasks at 25 °C, 120 μ mol photon m⁻²s⁻¹ irradiance at the surface of the flasks provided by white fluorescent tubes, and bubbling with air. Material for DNA isolation was harvested by centrifugation (3000 $\times$ g for 8 min) after 14 days and genomic DNA was isolated as described by Marmur (1961).

PCR amplification of part of the phycocyanin operon from *Spirulina maxima* was made using the specific upstream primer 5'-GGAGATAAGTCCATGT TTG A – 3' and the specific downstream primer 5'-CTAGCTTAGGGCGTTGATCGC-3'. The PCR system was: 5 μ L 10 $\times$ buffer, 34 μ L H₂O, 2 μ L upstream primer, 2 μ L downstream primer, 1 μ L dNTP, 4 μ L Mg²⁺, 0.2 μ L Taq polymerase (5U) and 1 μ L DNA sample. Sterile water replaced the DNA sample

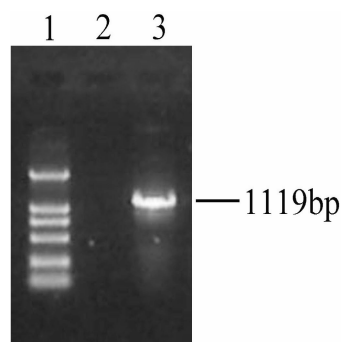


Figure 1. Electrophoretic map of PCR products Lane 1: Marker DL2000 Lane 2: negative control Lane 3: PCR product.

for the control. The PCR procedure consisted of denaturation at 93 °C for 5 min followed by 35 cycles of the following: denaturation at 93 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 3 min. A last elongation step was done at 72 °C for 8 min. PCR products were electrophoresised on 1% agarose plate and purified with the GeneClean Kit according to the manufacturer's specifications.

The PCR product was ligated into PCR-Blunt vector using the following system: 1.6 µL vectors, 6.4 µL phycocyanin gene, 1 µL 10 × T₄ buffer and 1 µL T₄ DNA ligase. After ligation for 16 h at 16 °C, the products were transformed into *Escherichia coli* DH5α (Sambrook et al. 1989). The clones containing PCR-Blunt vector with the insert were screened by restriction enzyme-digested analysis. The insert was sequenced using the Sequenase 2.0 DNA sequencing kit as recommended by the supplier and with M13 primers.

The nucleotide sequence for a portion of the phycocyanin operon is in the GenBank database under accession number AF441177.

Electrophoresis of the PCR products (Figure 1) showed a band of the right length in lane 3, but none in the negative control. The phycocyanin operon sequence obtained is 1119 bp. The α- and β-subunit gene-coding regions contain 489 and 519 bp, respectively. The β-subunit gene is upstream from the α-subunit gene, with a 111-bp segment separating them.

The α-subunit contains 162 amino acid residues and has a calculated molecular weight of 17,585 Daltons, with methionine and serine at the NH₂- and carboxyl-terminal amino acids, respectively. The β-subunit has 172 amino acid residues and a calculated molecular weight of 18,104 daltons, with methionine

and serine at the NH₂- and carboxyl-terminal amino acids, respectively.

When alignment was made between the α- and β-subunits within the phycocyanin for similarity comparisons, the maximum similarity between phycocyanin α- and β-subunits is 27% (Figure 2). The deduced amino acid sequences were also compared for similarity with known phycocyanin sequences from other cyanobacteria; there was a high degree of conservation (Figure 3). The overall similarity between the α-subunit of *S. maxima* and that of seven other species ranged between 63% and 99%, with a similar range for the β-subunits. In the α-subunit, 102 amino acids are identical and in the β-subunit, 98 amino acids. Small regions of higher similarity are scattered throughout the sequences, but there are no long conserved stretches.

Our study has provided the first report of the description of the cloning and sequence analysis of a portion of a novel phycocyanin operon from *S. maxima* F3 strain. In an examination for possible ribosome binding sites, the sequence g-a-g-a was found 8–11 bp upstream from the β-subunit gene, and 11–14 bp upstream from the α-subunit gene. This sequence is similar, although not identical, to the consensus prokaryotic ribosome binding sequence g-g-a-g (Gold et al. 1981). However, the significance of this cannot be confirmed, because the sequence of the *S. maxima* F3 strain 16S rRNA has still to be determined.

There are three chromophore-binding sites within the phycocyanin subunits, α-amino acid 84 and β-amino acids 82 and 153. The site at cysteine-153 is near the COOH terminus. It has been proposed that an insertion event, whereby a short stretch of 10 or 12 amino acids was introduced into the ancestral phycocyanin β-subunit gene product near its COOH terminus, could have generated this second chromophore-binding site (Offner and Troxler 1983). According to the model of Troxler et al. (1981), this second site in the β-subunit is due to the insertion of 12 amino acids at positions 146–157.

There is a striking similarity between phycocyanin from *Spirulina maxima* and phycocyanin from other cyanobacteria. It is believed that all phycobiliprotein genes arose from a single ancestral gene duplication, and several models have been proposed to explain this (Troxler et al. 1981). It is suggested that the genes for the allophycocyanin subunits arose first, and then gave rise to the genes for the phycocyanin subunits. The similarity between the α- and β-subunit genes

α M K T P L T E A V S I A D S Q G R F L S S T E I Q V A F G R F
 β * F D A F * K V * * Q * * T R * E M * * T A Q * D A L S Q M V
 α R Q A K A G L E A A K A L T S K A D S L I S G A A Q A V Y N K
 β A E S N K R * D * V N R I * * N * S T I V * N * * R S L F A E
 α F P Y T T Q M Q G P N Y A A D Q R G K D K C A R D I G Y Y L R
 β Q * Q L I A P G * N A * T S R R M A A — * L * * M E * I * *
 α M V T Y C L I A G G T G P M D E Y L I A G I D E I N R T F E L
 β Y * * * A V F * * D A S V L E D R C L N * L R * T Y L A L G T
 α S P S W Y I E A L K Y I K A N H G L S G D A A —————
 β P G * S V A V G V G K M * E A * L A I V N D P A G I T P G D C
 α ————— V E A N S Y L D Y A I N A L S
 β S A L A S * I A * * F * R * C A * V *

Figure 2. Similarity comparison of the amino acid sequences of α - and β -subunits of *S. maxima* phycocyanin. Identical amino acid residues are marked by an asterisk. To achieve maximum similarity between the two subunits, a two-residue gap between residue 81 and 82 of the β -subunit and a 12-residue gap between residue 147 and 148 of the α -subunit were made. The latter gap corresponds to the region in the sequence of the β subunit where the second chromophore is attached.

MFDATKVSQADTRGMLSTAQIDALSQMVAESNRRLDAVNRITSNASTIVSNAARSLFAEQPQLIAPGNAYTSRRMAACLRDMEIILRYVTVAFAGDASVLEDRCLNGLRRETYLALGTPGSSVAVGVGMKEAALAVNDPAGITPGDCSALASEIASYFDRACAAYS
 MY*ATR**S**R**A**EFL*SAQID**SKL*SDSN**IT*TN*ITG***A**T***S***E**Q**A*****S**M**[**]**[*****I**A***V**E**C*****I***T***S***V**VQRMKEALA*AN*TN*I*L****ALMA*VAT**DR**A**A
 ML*A*TK**A**A**DYL*GSGQID**QAM*KDGN**I*AN*ITG***A**A***E**Q**A*****S**M**[**]**[*****I**A***V**E**C*****I***T***S***V**VQRMKEAALAE*AN*RN*I*Q****ALMS*TAG**DR**A**A
 MF*V**S**A**EYL*GSGQL**SAT*AEGN**I*SN*ITG***A**S***E**Q**A*****S**M**[**]**[*****I**A***V**E**C*****I***T***S***V**VQRMKEAALD*VN*PN*I*R****ATVA*TAG**DR**A**A
 F*ATK**A**A**EFL*DAQLD**SRL*AEGN**I*TN*ITG***S**A***E**Q**A*****S**M**[**]**[*****I**A***V**E**C*****I***T***S***V**VQRMKEAALD*VN*PN*I*Q****ATIS*LGS**DR**A**A
 *A*SK**S**A**EYL*DGQLD**INL*KEGN**V*VN*ITS**S**R***S***E**Q**A*****S**A**A**V**E**[**]**[*****I**A***V**E**C*****I***T***A***V**I**QKLKESSIA*AN*PN*I*R****SL*IA*VSG**DR**A**A
 MF*I*TR**S**A**EF*ISDKLE**KKV*AEGT**S**A*SM*TN***S**T***Q***D**Q**A*****N**M**[**]**[*****I**A***V**E**C*****I***T***S***V**VQRMKEAALD*VN*PN*I*Q****SL*Q*Q*IEL**ET**K**
 MF*ATK**S**A*AT**EML*TAQID**SQM*AESN**L*A*AN*ITS**S**S***S***E**Q**A*****N**M**[**]**[*****I**A***V**E**C*****I***T***S***V**VQRMKEAALAV*NP*A*I*P****LASE*ASY**RA**A**

MKTPLTEAVSIADSGRFLSSTEIQVAFGRFQAKAGLEAAKALTSKADSLISGAQAVYNKFPYTTQMGPNYAADQRGDKCARDIGYILRMVYCLIAGGTGPMDEYLIAGIDEINRTFELSPSWYIEALKYIKANHGLSGDAVEANSYLDYAINALS
 *****S*****SQR**S***VAF**F**ATSG*Q**KS*TDN*QR*IDG**N**F**S*****TTP*A*F*STAQ**A**S*****MV**C**A*****I***[A*ID***RS*D*S***V*****AS*ANS***A*****
 *****A**ASRS**S***VAF**L**ASAS*E**KA*ASK*DS*ADA**N**V**Q*****QM*Q**N**Y*SDSR**S**S*****MI**C**A*****I***[A*ID***ST*E*S***V*****AT*ATS***A*****
 *****ST**SQR**S***IAP**L**ANAG*Q**KA*TDN*QS*VNG**Q**V**N*****QT*Q**N**F*ADQR**D**A*****I**V**C**A*****I***[A*ID***RT*D*S***V*****RD*ANS***A*****
 *****A**SQR**S***VAF**F**AASG*A**KA*ANN*DS*VNG**V**V**S*****STP*N**F*STPE**A**A*****I**V**A**A*****I***[A*ID***KT*D*A***V*****RD*ANS***I*****
 *****V**SQR**S***VAF**F**AGSS*E**KA*SKK*SS*AEA**N**V**Q*****TTS*P*Y*STQT**D**V*****I**V**C**A*****I***[G*LA***RT*E*S***I*****AV*ANS***I*****
 *****AL**SQR**S***N**VLY**L**GAF*A**E**QT*TAK*DT*VNG**Q**V**S*****STP*N**F*ADQR**D**A*****I**V**C**A*****I***[A*ID***RT*D*S***V*****AT*TN***A*****
 *****S**SQR**S***VAF**F**AKAG*E**KA*TSK*DS*ISG**Q**V**N*****QM*Q**P*Y*ADQR**D**A*****I**V**C**A*****I***[A*ID***RT*E*S***I*****AG*ANS***A*****

Figure 3. Comparison of amino acid sequences between *S. maxima* phycocyanin β -subunit and other known algal β -subunits (above) and between *S. maxima* phycocyanin α -subunit and other known algal α -subunits (below). Organisms and references for sequence data (sequence data are cited from <http://www.ncbi.nlm.nih.gov>) from above to below: *Spirulina maxima* F3; *Synechocystis* PCC6701; *Synechocystis* PCC9413; *Synechocystis* PCC6803; *Synechococcus* PCC6301; *Calothrix* PCC7601; *Synechococcus* PCC7002; *Spirulina platensis* C1. Asterisk indicates identity with *Spirulina maxima* sequence at that position.

also suggests that they are derived from the same ancestral gene, but separated long ago.

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