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Biochimica et Biophysica Acta 1491 (2000) 364–368

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Short sequence-paper

Sequence of the two operons encoding the four core subunits of the cytochrome b_6f complex from the thermophilic cyanobacterium *Synechococcus elongatus*[1]

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Received 17 September 1999; received in revised form 9 February 2000; accepted 10 February 2000

Abstract

The genes encoding cytochrome *f* (*petA*), cytochrome b_6 (*petB*), the Rieske FeS-protein (*petC*), and subunit IV (*petD*) of the cytochrome b_6f complex from the thermophilic cyanobacterium *Synechococcus elongatus* were cloned and sequenced. Similar to other cyanobacteria, the structural genes are arranged in two short, single-copy operons, *petC*/*petA* and *petB*/*petD*, respectively. In addition, five open reading frames with homology to known orfs from the cyanobacterium *Synechocystis* PCC 6803 were identified in the immediate vicinity of these two operons.

Keywords: Cytochrome b_6; Cytochrome *f*; *pet* operon; Rieske protein; Subunit IV

The cytochrome b_6f complex (b_6f complex), a proton-translocating plastoquinol-cytochrome c_6 oxidoreductase, is located in the thylakoid membrane of cyanobacteria and chloroplasts. It is part of the photosynthetic electron transport chain, connecting the two photosystems PSII and PSI [1]. In cyanobacteria, it is also a central part of the respiratory chain analogous to the bc_1 complex of mitochondria and bacteria. The mature b_6f complex consists of the four major proteins cytochrome *f* (PetA), cytochrome b_6 (PetB), the Rieske FeS-protein (PetC) and subunit IV (PetD); they are encoded by the genes *petA*, *petB*, *petC* and *petD*, respectively [2–4], with *petB*/*petD* and *petC*/*petA* forming an operon each in cyanobacteria. Cytochrome *f* is synthesized as a precursor protein with an N-terminal bacterial export sequence which guides the heme-binding N-terminus into the thylakoid lumen while the C-terminus is thought to form a membrane spanning helix analogous to the cytochrome c_1 subunit of the bc_1 complex [5]. Cytochrome b_6 contains two *b*-type heme cofactors and the Rieske protein carries a 2Fe–2S cluster. As deduced from the crystallographic structure of the bc_1 complex [5], the b_6 subunit consists of 4 transmembrane α-helices while the Rieske protein is supposed to have only one at the N-terminus. In addition, the

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[1] The nucleotide sequences reported are available from the EMBL/GenBank/DDBJ databases under the accession numbers AJ243707 (*petB*/*petD*) and AJ243535 (*petC*/*petA*). Upon request, the authors will provide detailed experimental evidence for the conclusions drawn in this note.

0167-4781/00/$ – see front matter
PII: S0167-4781(00)00048-8

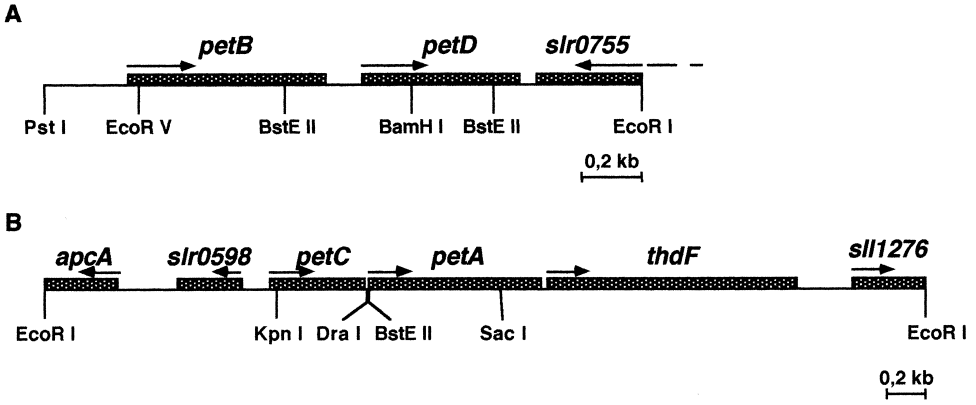

Fig. 1. Organization and partial restriction map of the 1.9-kb *Eco*RI fragment carrying the *petB*/*petD* operon (A) and of the 4.5-kb *Eco*RI fragment carrying the *petC*/*petA* operon (B) in the genome of *Synechococcus elongatus*. The assignments of the open reading frames are based on homology to the corresponding genes from *Synechocystis* PCC 6803 (see Table 1).

cyanobacterial b_6f complex of *Synechocystis* contains two orfs with homology to *petG* and *petM* encoding the corresponding low molecular weight subunits of higher plants and algae with unknown function [6]. Due to the remarkable stability of its proteins, the thermophilic cyanobacterium *Synechococcus elongatus* is getting more and more popular among crystallographers [7]. While a highly resolved structure of the b_6f complex is still lacking, structure determination of photosytem 1 and photosytem 2 isolated from *Synechococcus* is in progress [8]. However, in contrast to *Synechocystis* PCC 6803, which is completely sequenced, only a few gene sequences are currently available from *Synechococcus elongatus*. In this work, we report the sequence of the genes encoding the four major subunits of the cytochrome b_6f complex from this cyanobacterium.

For the isolation of the *petB* and *petD* genes, an *Eco*RI based genomic library of *Synechococcus elongatus* [9] was screened with a radiolabeled *Eco*RI/*Sac*I fragment of the plasmids pUB1 and pUB2 which carry the *petB* and *petD* genes from *Synechocystis* PCC 6803, respectively [10]. Using this probe, a 1.9 kb *Eco*RI/*Pst*I subfragment was subcloned from positive phages into pUC18 and sequenced (Fig. 1A). The *petC*/*petA* operon was cloned from the same library using a digoxygenin-labeled, 230 bp PCR fragment of *petC* from *S. elongatus*. This fragment was amplified from genomic DNA of *S. elongatus* using two degenerate oligonucleotides.

Table 1
Localization and characterization of open reading frames from *S. elongatus*

Gene	Initiation	Termination	Assigned protein	Molecular mass/amino acids[a]	Identity (similarity) with orfs from *Synechocystis* PCC 6803 (%)
petB	275	922	cytochrome b_6	24.3 kDa/215	89 (95)
petD	1028	1513	subunit IV	17.8 kDa/161	77 (90)
slr0755	>1997	1569	hypothetical protein		83 (89)
apcA	391	<1	allophycocyanin α-subunit		84 (93)
slr0598	941	621	hypothetical protein	12.3 kDa/106	62 (80)
petC	1093	1599	Rieske protein	19.3 kDa/181	75 (86)
petA	1620	2555	cytochrome *f*	30.3 kDa/284	68 (80)
thdF	2577	3986	thiophen and furan oxidation protein	50.7 kDa/469	74 (87)
sll1276	4227	>4611	hypothetical protein		46 (62)

The assignments of the open reading frames are based on similarity to the corresponding genes from *Synechocystis* PCC 6803. The genes are arranged according to Fig. 1.

[a]Only data for proteins with the whole known DNA sequence are presented. Nuclear numbers correspond to the apoprotein.

Using this probe, a 4.6 kb *Eco*RI subfragment was subcloned from initially isolated positive phages into pBluescript II SK (±) and sequenced in both orientations (Fig. 1B).

The organization of the genes found in the respective DNA fragments is illustrated in Fig. 1 and additional information is provided in Table 1. Similar to other cyanobacteria, *petB*/*petD* and *petC*/*petA* are organized in an operon each. The 1.9 kb DNA fragment containing *petB* and *petD* (Fig. 1A) includes the N-terminal part of an additional open reading frame downstream of *petD* with 81% identity to a putative orf from *Anabaena* 7120 and 83% identity to the orf *slr0755* from *Synechocystis* PCC 6803 [9]. Upstream of *petB*, a core motif of the Shine–Dalgarno sequence is found, which may act as a ribosome-binding side. A similar site is found in the 105 bp intergenic region immediately upstream of *petD*.

From the genomic fragment carrying the *petC*/*petA* operon, the first 1264 bp overlap with another genomic fragment from *S. elongatus* which has been reported before (Soga M (1993; EMBL accession no. D16540). Upstream of *petC*, two orfs were detected by homology which are encoded by the opposite strand (Fig. 1B). The first 392 bp of the DNA fragment encodes the N-terminus of the α-allophycocyanin subunit of the phycobilisomes. The second orf encodes a hypothetical protein of 50 amino acids with homology to the hypothetical protein encoded by the orf *slr0598* from *Synechocystis* sp. PCC 6803. In addition, an orf with homology to the *thdF* gene from *Synechocystis* PCC 6803 can be found immediately downstream of *petC*. Most likely, the *thdF* gene initiates at a GTG start codon 24 bp downstream of the *petA* stop codon and its protein-coding region is in frame with *petA*. Genes initiating with GTG have also been found in other cyanobacteria [11]. Two hundred and forty-one basepairs further downstream of *thdF*, an orf similar to the gene *sll1276* of *Synechocystis* initiates, encoding an ABC-transporter. In the *petC*/*petA* operon, *petA* is preceded by a Shine–Dalgarno sequence; however, the intergenic spacer between the two genes with 20 bp, is remarkably small.

In order to clarify the genomic organization of the *pet* genes in *S. elongatus*, Southern-blot analysis was carried out (Fig. 2). For all four genes, the probes hybridize with single restriction fragments of genomic DNA from *S. elongatus* which are identical in size to the fragments obtained from the genomic library. This result suggest that all four cytochrome b_6f subunits are encoded by single copy genes in *S. elongatus* (Fig. 2).

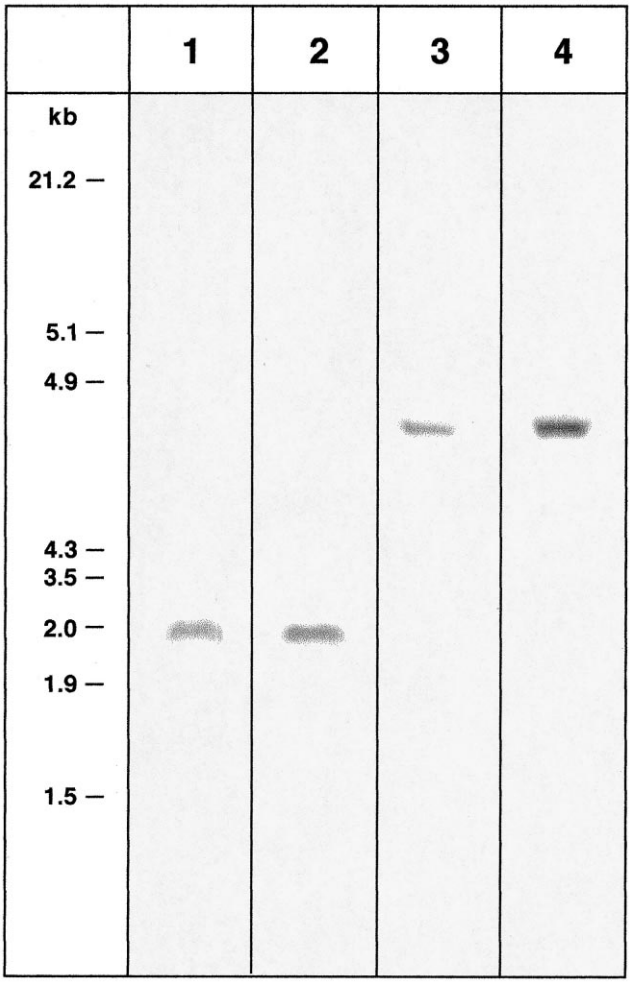

Fig. 2. Southern analysis of genomic DNA from *Synechococcus elongatus*. Genomic DNA was restricted with *Pst*I+*Eco*RI (lanes 1 and 2) or with *Eco*RI (lanes 3 and 4), separated on agarose gels, blotted onto a nylon membrane and probed with digoxygenin-labeled fragments. Lane 1, *Eco*RV-*Bst*EII fragment carrying *petB* (Fig. 1A); lane 2, *Bam*HI–*Bst*EII fragment carrying *petD* (Fig. 1A); lane 3, *Kpn*I–*Dra*I fragment carrying *petC* (Fig. 1B); lane 4, *Bst*EII–*Sac*I fragment carrying *petA* (Fig. 1B).

Table 1 summarizes the degree of homology and the molecular masses deduced from the amino acid composition of the individual subunits of the b_6f complex from *S. elongatus*. In addition, Table 2 shows positions for the membrane-spanning α-helices (suggested by hydropathy analysis with the pro-

Table 2
Structural characteristics of the core subunits of the cytochrome b_6f complex from *S. elongatus* as obtained by sequence analysis with their deduced amino acid sequences

Protein	Transmembrane helices	Cofactor binding sites	Special features
Cytochrome b_6	4 (I, C35–Y58; II, R83–F102; III, L116–D141; IV, F183–I206)	H85, H100, H187, H202	Two b-type hemes
Subunit IV	3 (I, L37-M58; II, L96–I115; III, V129–L152)	–	–
Rieske protein	1 (L21–I43)	C108, H110, C126, H129	2Fe–2S cluster
Cytochrome *f*	1 (I250–L269)	Y1, C21, C24, H25	c-type heme

gram PROTEAN) and also the conserved amino acid residues known to be involved in cofactor-binding. In general, the protein sequence analysis for the subunits of *S. elongatus* predicts very similar secondary structures with the subunits of b_6f complexes from other organisms [12,13]. In this context, the sequences from *S. elongatus* predict that cytochrome b_6 and subunit IV together consist of seven transmembrane α-helices as deduced by Widger et al. [14] in contrast to the cytochrome *b* subunit of the mitochondrial cytochrome bc_1 complex, which forms eight membrane-spanning helices (Table 2). Also, a membrane spanning α-helix at the N-terminus of the Rieske protein is predicted from the hydropathy analysis. The existence of a transmembrane helix for this subunit of the b_6f complex is still under discussion [15,16]. However, the recently published structures of the mitochondrial bc_1 complex clearly show a hydrophobic helix for the Rieske protein, which is slightly curved and highly slanted [17]. For the cytochrome *f* subunit our data predict a C-terminal hydrophobic region which is in agreement with a transmembrane helix. As sequence alignments suggest that the mature *S. elongatus* cytochrome *f* starts at position 28, the N-terminal leader sequence consisting of 27 residues is the shortest cytochrome *f* leader sequence reported so far [2]. In conclusion, the mature protein should consist of 284 amino acids.

Surprisingly, our sequence shows that the amino acid position 4 is occupied by a Tyr residue which is in contrast to all reported cyanobacterial cytochrome *f* sequences. As outlined in [18] Tyr-4 is characteristic for higher plants and replacing of this amino acid yields a shift in the absorbance spectrum of cytochrome *f* from 554 to 556 nm – as characteristic for cyanobacteria. Indeed, an absorbance peak at 554 nm could be observed by us (data not shown), indicating that *S. elongatus* is more 'higher plant like' than other cyanobacteria.

As this is a sequence report on a thermophilic cytochrome b_6f complex, a comparison with known sequences of mesophiles may be interesting. For thermophilic organisms, some characteristics leading to protein stability at higher temperatures are reported. As pointed out in [19,20] an increased number of residues with short side chains can be observed in hyperthermostable proteins. This may lead to a higher stability due to a tighter structure than in mesophilic proteins. An overall amino acid comparison of the four mature polypeptides PetA/B/C/D from the thermophilic cyanobacterium *S. elongatus* and the mesophilic *Synechocystis* PCC6803 shows that in *S. elongatus* the number of amino acids with short side chains (G, A, V, P) is increased by 7% in comparison with *Synechocystis* (289 aliphatic residues with short side chains vs. 238). This may contribute to the thermostability of these proteins.

Also, the replacement of lysine residues by arginine has been reported for thermostable proteins, yielding a higher number of salt bridges [21,22]. However, when the four Pet protein sequences from *S. elongatus* and *Synechocystis* are compared, neither a higher K/R quotient nor an increased number of charged amino acids can be observed in the thermophilic proteins. This indicates that the four 'thermophilic' cytochrome b_6f subunits may not contain more salt bridges than the proteins from the mesophilic *Synechocystis*.

In summary, the sequences of the four major subunits of this thermophilic b_6f complex show both features characteristic for higher plants and for thermophiles. This sequence information should also be very useful for the structural analysis of the thermophilic cytochrome b_6f complex which is in progress.

This work has been supported by the Deutsche

Forschungsgemeinschaft (SFB480, project C1, and Graduiertenkolleg: 'Biogenese und Mechanismen komplexer Zellfunktionen') which is gratefully acknowledged. The authors would like to thank Dr. Andreas Seidler for stimulating discussions and critical reading of the manuscript and Prof. Dr. P. Rich (University College London) for his help with preliminary spectroscopic characterization of the cytochrome *f* protein.

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