Transcriptional analysis of the isiAB operon in salt-stressed cells of the cyanobacterium Synechocystis sp. PCC 6803

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Received 9 July 1998; received in revised form 7 October 1998; accepted 7 October 1998

Abstract

Expression of the isiA and isiB genes was analysed in the cyanobacterium Synechocystis sp. PCC 6803 grown in high salt or in iron-deficient medium. The detection of a 2.3-knt transcript in Northern blot experiments indicated cotranscription of isiAB in an operon, which was confirmed by reverse transcriptase PCR. The abundance of a monocistronic 1.25-knt isiA-specific mRNA was about 10-fold higher than the dicistronic message. The isiAB-specific transcripts were most abundant in cells adapted to 342 mM NaCl and under iron deficiency. The promoter of the operon was mapped to 211 bp upstream of the translational start. A putative Fur binding site was detected immediately upstream of the GTG start codon. A preliminary transcript of about 0.2 knt was detected in cells grown in conditions in which the isiAB operon was not transcribed. This indicates that a repressor binds to the identified Fur binding site and thus inhibits isiAB transcription under low salt and iron replete conditions.

Keywords: Cyanobacterium; Flavodoxin; Iron stress; isiA; Salt stress; Synechocystis

1. Introduction

The adaptation of cyanobacteria to conditions of iron starvation has been intensively studied [1]. In particular the two iron stress-induced genes called isiA and isiB have been analysed. The product of isiA functions as a chlorophyll a (Chlα) binding protein that becomes the most prominent Chlα-protein complex in iron-deficient cells [2], whilst isiB encodes flavodoxin. Due to the similarity of IsiA to PsbC (CP43), a protein of the photosystem II core complex, it was hypothesised that IsiA might play a role in photosynthesis under iron limitation [1]. However, analysis of an isiA mutant of Synechococcus sp. PCC 7942 showed that IsiA has no function in photosynthesis under iron deprivation but forms an uncoupled Chlα-protein complex. This was hypothesised to function as a Chlα reservoir and to be important for recovery from iron deprivation [3]. However, analysis of growth of mutants showed that isiA and isiB are not essential for acclimation under iron-limiting conditions [2-4]. Flavodoxin is involved in photosynthetic electron transport. It can replace almost all of the functions of the iron-containing ferredoxin and is believed to be an alter-
native electron carrier under iron-limiting conditions [1]. Additionally, flavodoxin accumulates in cells grown in high salt medium, in which it is thought to be involved in cyclic electron flow to generate energy for enhanced ion export [5,6].

In unicellular cyanobacteria such as the freshwater strain Synechococcus sp. PCC 7942 [7] and the marine strain Synechococcus sp. PCC 7002 [8], isiA is cotranscribed with the downstream isiB. Only in the nitrogen-fixing, filamentous cyanobacterium Anabaena sp. PCC 7120 the two genes are found to be separately transcribed [9]. Within the unicellular strains it is assumed that transcription of the isiAB operon is derepressed under iron limiting conditions. The promoter of isiA from Synechococcus sp. PCC 7942 is regulated by the Fur repressor (ferric iron uptake regulator) [10]. Furthermore, transcriptional termination or stability of the mRNA may also be involved in regulation, since under iron stress conditions a monocistronic isiA message is found to be more abundant than the dicistronic isiAB message in Synechococcus sp. PCC 7942 and Synechococcus sp. PCC 7002 [7,8].

Since flavodoxin accumulates in both iron-limited and salt-adapted cells of Synechocystis sp. PCC 6803, we investigated the regulation of transcription of isiA and isiB under iron deprivation and salt stress conditions. The expression patterns of isiA and isiB were analysed in Northern blot experiments. The promoter of the isiAB operon was mapped by means of primer extension analysis, leading to the identification of putative DNA boxes acting in environmental regulation. Additionally, the possible involvement of IsiA in photosynthesis under salt stress was analysed using mutants defective in CP43 (psbC).

2. Materials and methods

2.1. Bacterial strains and culture conditions

Synechocystis sp. PCC 6803 was grown at 29°C under constant illumination (170 μmol photons m⁻² s⁻¹) and continuous aeration with CO₂ (5% v/v) using a mineral medium containing 2 mM NaCl [11]. To adapt cells to different salt concentrations, the desired amount of NaCl was added and the cells were precultured for 6 days in NaCl-enriched media. During this time the cells were transferred daily into fresh medium at the same optical density. For RNA preparations of Synechocystis the cells were cultivated for 5 h in fresh medium at the same optical density before harvesting (OD₅₇₀ 1.2). For growth of Synechocystis under iron limitation, cells were cultured in standard medium modified to contain 1/100 of the normally added amount of Fe-EDTA (28 μM). Salt shock experiments were performed by adding solid NaCl to give a final concentration of 684 mM NaCl. Heat shock was induced by transferring the cultures to 42°C for 3 h.

2.2. RNA techniques

Total RNA from Synechocystis was isolated as described previously [12] but instead of ultracentrifugation, RNA was treated with RNase-free DNase (Boehringer-Mannheim) to remove contaminating DNA. For Northern blot experiments probes were synthesised by PCR using the following pairs of primers for isiA and isiB respectively: isiA-fw: 5′-CAT AGG TCT CGG GTG GAC-3′; isiA-rev: 5′-TAA AGC TGA TGG CTA ATG-3′; isiB-fw: 5′-ATG ACA AAA ATT GGA CTT TT-3′; isiB-rev: 5′-CTA GGA TTG CAA AAT TGG TT-3′. Selective detection of the untranslated region (5′-UTR) of the isiA gene was performed using the probe isi-5utr amplified with the primers isi-5utr-fw: 5′-TTG GGC GAT CGC CAA AAA TC-3′ and isi-5utr-rev: 5′-CTC TGT CCA CCC GAG ACC TC-3′. A probe specific for an internal part of the coding region (isiA-cod) was derived with the primer pair isiA-cod-fw: 5′-ATT GGT GGG CCG GTA ATG C-3′ and isiA-cod-rev: 5′-CGT ATC GGC AAA RGA RGG-3′. The primer sequences were deduced from the genomic sequence of Synechocystis [14]. A 16S rRNA-specific probe was generated using the universal primers for eubacterial 16S-rRNA 27f and 1525r [13]. Total DNA of Synechocystis was used as a template. For purification, the PCR products were separated on an agarose gel. Specific bands were excised and labeled as a probe with [α-³²P]dATP (Amersham) using a random prime labeling kit (MBI-Fermentas). Northern blot experiments were performed as previously described [15]. The rRNA-representing bands were used to determine fragment sizes (23S: 2.8 knt, 16S: 1.5 knt, 5S: 0.12 knt, in vivo
cleavage products of the 23S rRNA: 2.3 knt and 0.5 knt) [16]. Quantification of signals obtained in the Northern blot experiments was done with a phosphorimager (Fuji BAS-1000). To avoid differences based on improper gel loading, the quantitative data were calculated on the basis of signals obtained with a 16S rRNA-specific probe, which was used as a control not affected by stress. Reverse transcriptase PCR (RT-PCR) was carried out using ‘RT-PCR Beads’ (Pharmacia) and primer isiA-forward was used to synthesise the cDNA. After reverse transcription the primer isiA-start: 5'-GTGCAAACCTATGGTGAA CGG TGT CGT TGC CAT-3' was added to run the following PCR. Primer extension experiments were performed using the oligonucleotide 5'-TTTGATCGTACCTGAA CGG TGT CGT CAT-3' was added to run the following PCR. Primer extension experiments were performed using the oligonucleotide 5'-TTTGATCGTACCTGAA CGG TGT CGT CAT-3' and the 'Primer Extension System' (Promega) according to the manufacturer’s instructions.

2.3. Growth of psbC mutants

Mutants of Synechocystis sp. PCC 6803 with a mutation in the psbC gene [17] were cultivated in a shaking incubator under continuous illumination (40 µmol photons m⁻² s⁻¹) at 29°C in Erlenmeyer flasks containing BG11 medium [18] supplemented with kanamycin (50 µg ml⁻¹). For photomixotrophic growth the cultures were supplemented with glucose (10 mM). To obtain salt-adapted cells, cells were grown in BG11 containing the desired amount of NaCl, while for iron limitation experiments cells were grown in iron-free BG11 medium. The growth of the cells was recorded by measuring the optical density at 750 nm (OD₇₅₀). Photosynthetic oxygen production after illumination at increasing light intensities (3.8–189 µmol photons m⁻² s⁻¹) was determined using a Clark-type oxygen electrode.

3. Results and discussion

3.1. Steady-state levels of isiA and isiB mRNA

Northern blot experiments were used to determine the steady-state levels of isiA- and isiB-specific mRNAs in Synechocystis grown at different salt concentrations (Fig. 1). The isiB-specific probe aligns with the IsiB-encoding sequence and the isiA-specific probe aligns with the IsiA-encoding sequence and an additional 110 bp upstream of the GTG start codon. The isiA-specific probe identified a major transcript of 1.25 knt with RNA of cells grown at different salinities, which was almost undetected with RNA isolated from control cells. The most intensive signal was found with RNA from cells adapted to 342 mM NaCl. Less intensive signals were detected with RNA from cells adapted to 171 mM and with RNA from salt-shocked cells (684 mM NaCl for 3 h). The isiA probe also hybridised to a transcript of 0.2 knt. This signal was greater with RNA from cells showing no or only weak signals for the 1.25-knt transcript such as control cells. Using the isiB-specific probe a main transcript of about 2.3 knt was detected with RNA of cells adapted to 342 mM NaCl or exposed to 684 mM NaCl for 3 h (Fig. 1). A signal of the same size (2.3 knt) was also obtained with the isiA probe, but its intensity was much weaker than that of the 1.25-knt main transcript. The intensity of the 2.3-knt signals was comparable when using the isiA and the isiB probes. It is noted that the filters incubated with the isiB-specific probe were exposed 10 times longer than the filters with the isiA probe.

The Northern blot analyses indicate that isiB is cotranscribed with isiA forming an operon. The transcript size of about 2.3 knt corresponds to that predicted from the Synechocystis genome sequence [14]. This was confirmed using RT-PCR. Using a primer specific to the 3'-end of the isiB, cDNA was synthesised by reverse transcription. A second primer specific to the 5'-end of isiA was used for second strand synthesis followed by PCR amplification. The predicted PCR product of about 1.9 kb was obtained with RNA from cells adapted to 342 mM NaCl and in the control reaction with chromosomal Synechocystis DNA, while after inactivation of the reverse transcriptase it was absent (Fig. 1D).

The isiA gene is preferentially transcribed as monocistronic mRNA. Nevertheless, we cannot rule out that the major 1.25-knt transcript represents a stabilised degradation product of the 2.3-knt dicistronic mRNA. Our results correspond to those obtained from other unicellular cyanobacteria, where the transcription of isiA and isiB was investigated under iron limitation. In Synechococcus sp. PCC 7942 and Synechococcus sp. PCC 7002 the signal for the dicistronic mRNA is about seven times less...
intensive than that of the monocistronic isiA-specific mRNA [7,8]. The expression of isiB corresponds to the levels of flavodoxin found in cells grown at a salt concentration of around 300 mM NaCl [5]. The isiA transcript was also detected after heat shock treatment (43°C for 3 h). Again, this corresponds to flavodoxin protein accumulation [5]. We conclude that both genes of the isiAB operon show increased expression not only under iron limitation but also under salt stress and to a lesser extent under heat shock conditions.

3.2. Expression of isiA under salt stress and iron limiting conditions

To exclude the possibility that the salt-dependent expression of the isiAB operon in Synechocystis is due to salt stress-induced iron limitation, the amount of isiA-specific mRNA under salt stress was compared with transcript levels under iron limitation by quantification of the hybridisation signal. The data were normalised to hybridisation signals obtained with a 16S rRNA probe (Fig. 2). Similar
amounts of isiA-specific mRNA were detected under iron limitation and in cells adapted to 342 mM NaCl, but the induction kinetics differed between cells exposed to salt stress or iron deficiency. After transfer of cells into iron-free medium (iron down-shock), isiA-specific mRNA was only detected after 24 h, while in salt-shocked cells the highest transcript levels were detected after 3 h (Figs. 1 and 2). This corresponds to the levels of flavodoxin under the same conditions [5]. Furthermore, the salt-induced accumulation of flavodoxin protein [5] and the accumulation of the isiA-specific mRNA (not shown) could not be repressed by adding 10 times more iron. Assuming that salt adaptation causes iron limitation, increasing salt concentration should lead to a linear increase of the isiAB message. This was not observed (Figs. 1 and 2). Therefore it seems unlikely that the salt-dependent induction of the isiAB operon is due to reduced iron uptake in salt-stressed cells of Synechocystis.

3.3. Mapping of the isiA promoter

The promoter of the isiAB operon of Synechocystis was mapped by primer extension analysis. For the reverse transcription reaction an oligonucleotide binding downstream of the GTG start codon (Fig. 3) was used. The start of transcription could be assigned to an adenine 211 nt upstream of the GTG start codon, since a cDNA of 249 nt was detected. The highest amount of cDNA was obtained using RNA from cells adapted to 342 mM NaCl. Weaker signals were detected with RNA from salt- and heat-shocked cells (Fig. 3A) confirming the results obtained in the Northern blot experiments.

The isiAB promoter region was analysed by sequence comparisons with known promoters to predict possible DNA elements involved in regulation. Sequence motifs resembling $\text{−}3$ and $\text{−}35$ consensus regions of Escherichia coli $\sigma^70$ promoters [19] were found (Fig. 3B). Nevertheless, it is possible that the putative $\text{−}35$ motif is not part of the isiA promoter, since the distance between the two conserved regions differs from the consensus sequence (20 nt compared to 17 nt in E. coli) and, furthermore, a $\text{−}35$ region is often missing in cyanobacterial promoters [19]. Additionally, an inverted repeat (ATAAATTCTCATTATT) was detected in the isiAB promoter that is unique in the genome of Synechocystis [14]. Furthermore, about 40 nt upstream from the start of translation is a putative Fur binding sequence (Fig. 3B) with similarities to Fur consensus sequences [1]. This sequence is located in the relatively long 5’-UTR of the isiAB mRNA.

Putative Fur binding sites have also been assigned to the promoter regions of isiAB operons of the cyanobacteria Synechococcus sp. PCC 7942 and Synechococcus sp. PCC 7002 [7,8]. In the 5’-UTR of isiA of Anabaena sp. PCC 7120 putative Fur binding sites were also predicted [9]. Only for the isiAB promoter of Synechococcus sp. PCC 7942 binding of the Fur repressor protein was shown [10]. The expression pattern obtained in Northern blot experiments (see Fig. 1) indicates that Fur or another repressor might bind to the putative Fur box of isiAB. Under conditions of weak isiAB expression, a hybridisation signal to a 0.2-knt transcript was observed. As the isiA-specific probe used in the Northern blot experiments aligned with the region upstream of the putative Fur
binding site, the short fragment detected may represent a shortened transcript due to repressor binding at the Fur binding site. This shortened transcript was also detected with probe isi-5utr specific for the long 5’-UTR of the isiAB operon but was not present using the probe isiA-cod (see Fig. 1C) specific for the coding region of the isiA gene (data not shown). This emphasises that the small transcript detected with the isiA probe is specific for the 5’-UTR of the isiAB operon indicating the involvement of Fur or another repressor in the transcriptional regulation of the isiAB operon in Synechocystis.

3.4. Physiological function of IsiA

Unlike flavodoxin the role of IsiA in iron-starved and salt-stressed cells is not understood. Due to the high similarity of the isiA gene product to PsbC, it
was hypothesised that IsiA could replace PsbC under iron limitation [1,2]. This may also be the case under salt stress. To verify this hypothesis we tried to complement pscB mutants phenotypically to photoautotrophy by cultivation under conditions leading to high cellular IsiA levels as was already suggested [1]. The pscB mutants ΔF345/E354 and ΔG334/M343 of Synechocystis [17] were cultivated in high salt or low iron media. They are characterised by a deletion in the long hydrophilic loop connecting the transmembrane helices V and VI in PsbC, leading to loss of photoautotrophy [17]. The pscB mutants did not show any photoautotrophic growth after salt adaptation (342 mM NaCl) or under iron limitation (not shown). To verify that photosynthesis remained completely disturbed in salt- and iron-stress-treated cells of the PscB mutants, light-dependent oxygen evolution was measured in wild-type and mutant cells after photomixotrophic growth with 10 mM glucose. No differences in light-dependent oxygen evolution were found under any of the conditions tested. These experiments clearly show that IsiA is not able to replace PsbC functionally in Synechocystis. Therefore, the role of IsiA in salt-stressed as well as in iron-starved cells still remains to be elucidated.

In conclusion, based on the results of Northern blot analyses and promoter mapping of the isiA region from Synechocystis we predict that under control conditions (low salt and normal iron) only the short preliminary transcript is made due to the binding of a repressor to DNA. Under stress conditions (salt stress, iron deficiency, heat shock) the repressor falls off and transcription of the structural genes is possible. In most cases transcription is stopped between isiA and isiB, leading to the dominance of the isiA-specific over the isiAB-specific mRNA. In the genome of Synechocystis there are two open reading frames encoding proteins showing significant similarity to the Fur repressor [14]. Whether one or both of these open reading frames are responsible for salt- and/or iron-dependent regulation of the isiAB locus and whether the inverted repeat upstream of the transcriptional start is a regulatory element will be analysed in further experiments.

Acknowledgments

The authors thank Matthias Kuhn and Wim Vermaas for providing the pscB mutants used in this study, Hendrik Schubert for the help in the photosynthetic experiments and Brigitte Brzezinka and Ilse Dörr for excellent technical assistance. Critical reading of the manuscript by Julian Rutherford, University of Newcastle, is gratefully acknowledged. This work was supported by a grant of the DFG (Deutsche Forschungsgemeinschaft).

References


