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ORIGINAL ARTICLE

Analysis of glucose-6-phosphate dehydrogenase of the cyanobacterium *Synechococcus* sp. PCC 7942 in the *zwf* mutant *Escherichia coli* DF214 cells

Haydar Karakaya · Funda Erdem · Kübra Özkul · Aylin Yilmaz

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Abstract The aim of this study was to express the *zwf* gene of *Synechococcus* sp. PCC 7942 in *zwf* mutant *Escherichia coli* DF214 cells and to analyse glucose-6-phosphate dehydrogenase (G6PDH) activity. Initially, mutant cells were transformed with plasmid pNUT1 containing a *Synechococcus* sp. PCC 7942 *zwf* gene with a 1 kb upstream region that is expected to contain promoter elements. Transformant DF214 cells were not complemented by this fragment in a glucose minimal medium, nor did they exhibit statistically meaningful G6PDH activity. Therefore, the *zwf* gene was cloned in the *lac* operon to express the Zwf as a fusion protein; this yielded the construct pSG162. The pSG162 transformant *E. coli* DF214 cells were complemented in a glucose minimal medium, indicating that cyanobacterial Zwf protein fused with the part of LacZ′ polypeptide, enabling the cells to utilize glucose via the oxidative pentose phosphate pathway. Compared with wild-type *E. coli* cells, approximately ten times more G6PDH activity was measured in transformant cells. This indicated that the *Synechococcus* sp. PCC 7942 *zwf* gene was expressed under the control of the *E. coli lac* promoter as a fusion protein and the *zwf* product was converted into an active G6PDH form. Analyses was also carried out to determine whether dithiothreitol (DTT) was an in vitro reducing agent affected the enzyme activity, as was previously reported for this cyanobacterial strain. The results showed no variation in enzyme activity in the reduced assay conditions. Therefore, the *zwf* mutant *E. coli* strain DF214 was found to provide a rapid system for analysis of cyanobacterial G6PDH enzymes, but not for the redox state analysis of this enzyme.

H. Karakaya (✉) · F. Erdem · K. Özkul · A. Yilmaz
Faculty of Science and Arts, Department of Biology,
University of Ondokuz Mayis, 55139, Samsun, Turkey
e-mail: hkarakay@omu.edu.tr

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Introduction

Cyanobacteria have long been used as model organisms for photosynthetic research, as their photosynthetic operation is similar to that of plants, by using water as an electron donor and elevating oxygen as a by-product (Pelroy and Bassham 1972; Ihlenfeld and Gibson 1975; Rowell and Kerby 1992; Tandeu de Marsac and Houmard 1993). One of the enzymes of cyanobacterial carbon metabolism that has been extensively studied is glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49). This enzyme operates in the first step of the oxidative pentose phosphate (OPP) cycle which is the main catabolic route for carbon dissimilation in cyanobacteria (Smith 1982; Copeland and Turner 1987; Tabita 1994). The enzyme was reported to be oligomeric and the activity increased with the molecular mass of the enzyme (Schaeffer and Stanier 1978; Cossar et al. 1984; Gleason 1994, 1996). G6PDH genes (*zwf*) of several cyanobacterial strains were sequenced for molecular analysis (Scanlan et al. 1992; Newman et al. 1995; Summers et al. 1995a; Kaneko et al. 1996). Bioinformatics analyses showed that the enzyme consists of a 55 kDa monomeric subunit consistent with electrophoresis analyses (Schaeffer and Stanier 1978; Newman et al. 1995; Sundaram et al. 1998).

One of the ways the enzyme might be regulated during the light/dark transition is the reduction/oxidation of the disulfide bond(s) that occurs between internal cysteine residues (Anderson et al. 1978; Cossar et al. 1984; Austin et al. 1992). The molecular genetic analyses of the cyanobacterial *zwf* genes showed that most of the strains have at least two cysteine codons, supporting the hypothesis that disulfide

bonds may play a role in regulation during light/dark transition (Scanlan et al. 1992; Newman et al. 1995; Summers et al. 1995b; Kaneko et al. 1996). As enzyme activity analyses support this hypothesis (Cossar et al. 1984; Austin et al. 1992), it may be possible to obtain more evidence by molecular level studies that change the cysteine residues of the Zwf protein. In such a study, it is possible to change cysteine codons in vitro, and this mutant gene can be transferred into a *zwf* deletion mutant of a cyanobacterial strain.

To date, a few cyanobacterial *zwf* mutants have been produced and analysed (Scanlan et al. 1995; Summers et al. 1995b). One of them, the Δ*zwf* mutant *Synechocystis* sp. PCC6803 (Karakaya et al. 2008) is useful for this purpose. However, cyanobacterial cells divide more slowly; accordingly, it takes more time to transfer the mutant genes into the cell and to segregate the mutants. An alternative strategy may be applied using an *Escherichia coli zwf* mutant strain for analyses of the cyanobacterial *zwf* gene. A similar study was already conducted on chloroplastic and cytosolic isoforms of the potato G6PDH in a *zwf* mutant *E. coli* strain using a viral expression purification system (Wenderoth et al. 1997). Thus, the cysteine residues in certain positions were found to be involved in the regulation of the chloroplastic isoform, but not the cytosolic isoform. Another *zwf* deletion mutant *E. coli* strain DF214 may also be useful for the analyses of cyanobacterial Zwf proteins (Vinapol et al. 1975).

The strain DF214 carries two mutations, which are *zwf* and *pgi* (phosphoglucose isomerase gene). Thus, the strain cannot metabolise glucose via either the OPP cycle, due to the *zwf* mutation, or glycolysis, due to the *pgi* mutation. The strain DF214 can probably be complemented with a *zwf* gene ligated into a vector. This *zwf* mutant strain *E. coli* DF214 may provide a rapid system for the analysis of cyanobacterial G6PDH activity if a cloned cyanobacterial *zwf* gene complements the strain. Two critical factors may determine whether the cyanobacterial *zwf* gene complements the mutant cells. One of these is the expression of the cyanobacterial *zwf* gene in *E. coli* cells, and the other is conversion of the cyanobacterial Zwf polypeptide into active G6PDH form. If complementation occurs, the structural studies of the cyanobacterial Zwf protein can be carried out more quickly in the *zwf* mutant *E. coli* cells.

This study aimed to investigate the complementation of the *zwf* mutant *E. coli* strain DF214 with a cloned *Synechococcus* sp. PCC 7942 *zwf* gene, and to find out whether G6PDH activity could be restored. Initially, the *zwf* gene, under control of the original cyanobacterial promoter, was transferred into the DF214 cells, obtaining no complementation. Then the *zwf* gene was expressed as a fusion of the *lacZ*′ gene under the *lac* promoter of *E. coli*. The fusion protein was tested for G6PDH activity and redox modulation properties in the supernatant of transformant *E. coli* DF214.

Materials and methods

Bacterial strains and culture conditions The *E. coli* strains and plasmids used are listed in Table 1. The strain of Δ*zwf* mutant *E. coli* DF214 was purchased from the Coli Genetic Stock Center at the University of Yale. The *E. coli* strains DF214, JM109 and DH5α were grown in LB or 2xYT medium (Sambrook and Russell 2001). During the complementation test, the transformant *zwf* mutant *E. coli* strain DF214 was grown in M63 minimal medium with the supplements as reported previously (Vinapol et al. 1975), adding 5 % glucose as a carbon source. The media for growth of the transformant *E. coli* cells were supplemented with 50 μg/ml ampicillin.

The plasmid pNUT1 (Scanlan et al. 1992) carrying a *zwf* fragment of *Synechococcus* sp. PCC 7942 was used as a template for PCR amplification. The plasmid pSG162 was constructed by inserting a 1.6 kb PCR fragment of the *zwf* region of *Synechococcus* sp. PCC 7942 into the vector pGEM-T Easy (Promega), using standard molecular biological techniques (Sambrook and Russell 2001).

Preparation of the cell-free extracts and the G6PDH activity assay Escherichia coli cultures (100 ml) were harvested by centrifugation (5 min, 10,000 × *g*, 4 °C), and the cells were washed once and resuspended in 1 ml 50 mM Tris-maleate buffer, pH 6.5, 10 mM glucose-6-phosphate, 0.1 % β-mercaptoethanol (Schaeffer and Stanier 1978). The resuspended cells were disrupted by adding glass-beads (150–125 μm) and vortexing 5 min at 3,000 rpm. The cell-free extracts were then prepared by centrifugation (10,000 × *g*, 10 min, 4 °C). The supernatants were kept at 4 °C and used on the day of preparation. G6PDH activity was assayed in the cell-free extracts as described before (Hylemon and Phibbs 1972; Vinapol et al. 1975 and Schaeffer and Stanier 1978). The rates of NADPH formation were measured at 340 nm for 3 min in a Novaspec spectrophotometer at 25 °C. To test the effect of dithiothreitol (DTT) on the enzyme activity, an aliquot of DTT was added in a small volume of the enzyme solution before measuring its activity. One unit of enzyme activity was defined as the formation of 1 μmol of product in 1 min. Specific activity was expressed as units per mg protein. The protein was assayed by the Bradford (1976) method.

General molecular biology techniques Plasmid isolation from *E. coli*, restriction digestion, ligation using T4 DNA ligase and transformation into *E. coli* cells were carried out using standard molecular biological techniques (Sambrook and Russell 2001). Ligation reactions were performed at 1:3 vector:insert ratio with a 0.5 unit T4 DNA ligase (Fermentas) at RT for 3 h or 4 °C overnight. DNA fragments were analysed by agarose gel electrophoresis.

Table 1 The plasmids and *E. coli* strains used in this study

	Property	Reference
Plasmid		
pGEM-T easy	Amp^r, $lacZ'^+$	Marcus et al. 1996
pNUT1	pUC19 carrying a 2.9 kb *zwf* fragment of *Synechococcus* sp. PCC 7942, Amp^r	Scanlan et al. 1992
pSG162	pGEM-T carrying 1.6 kb *zwf* fragment in MCS of *lacZ′* gene in same orientation, Amp^r	In this study
Strain		
E. coli DH5α	Standard *E. coli* strain, zwf^+	Grant et al. 1990
E. coli JM109	Standard *E. coli* strain, zwf^+	Yanisch-Peron et al. 1985
E. coli DF214	Δ*zwf* and pgi^- strain, no glucose utilisation	Vinapol et al. 1975
SZJ29	*E. coli* JM109 carrying pNUT1	In this study
SZD29	*E. coli* DF214 carrying pNUT1	In this study
AY162	*E. coli* DF214 carrying pSG162	In this study

Cloning the zwf gene of Synechococcus sp. PCC 7942 The nucleotide sequence of *Synechococcus* sp. PCC 7942 was obtained from the Genome Database of Cyanobacteria (http://genome.kazusa.or.jp/cyanobase/SYNPCC7942), and used to design primers to amplify the *zwf* region. PCR amplification was carried out using a forward primer Fzwf7942 (5′ GAATTCGAGCTCG ATGACTCC-CAAACTGCTTG 3′) and a reverse primer Rzwf7942 (5′ GAATTCTCGAGGGAGTAGCGGTCGTCATC 3′). The purified 1.6 kb *zwf* fragment was ligated into a pGEM T Easy vector using a PCR cloning kit (Promega) yielding pSG162. Recombinant vectors were initially transferred into *E. coli* DH5α competent cells and selected against ampicillin resistance conferred by the pGEM-T Easy vector. Restriction endonuclease analyses were performed to confirm the orientation of the *zwf* fragment in pSG162.

Complementation analyses of zwf mutant E. coli DF214 cells by the vectors carrying cyanobacterial zwf fragments The plasmids pNUT1 and pSG162 were transformed into *E. coli* DF214 cells made competent by the CaCl method (Sambrook and Russell 2001) and grown on an LB medium. The transformants were then inoculated on an M63 minimal medium supplemented with 5 % glucose as the sole carbon source. Growth of the transformant DF214 cells in this medium was taken as evidence of the complementation of glucose utilisation via the OPP cycle. The transformants were also tested for any acquired G6PDH activity, according to the procedure described above.

Results

Transformation of zwf mutant E. coli DF214 with pNUT1 carrying a 2.9 kb zwf fragment of Synechococcus sp. PCC 7942 Escherichia coli DF214 cells cannot grow in a glucose minimal medium because of mutations in both *zwf* and *pgi* that block glucose entry into both the glycolysis and the OPP pathway (Vinapol et al. 1975). To grow in minimal medium, it is necessary to transfer an external *zwf* or *pgi* gene into the mutant cells in an expressible form. The plasmid pNUT1 possesses *Synechococcus* sp. PCC 7942 *zwf* gene as a 2.9 kb fragment carrying part of the *fbp* gene upstream and the *opcA* gene downstream of *zwf* (Scanlan et al. 1992) (Fig. 1). Carrying a full copy of the *zwf* gene, it is expected that the plasmid pNUT1 complements DF214 cells in a glucose minimal medium, supposing that the cyanobacterial gene is expressed in *E. coli* cells under the control of cyanobacterial operon.

Plasmid pNUT1 was, therefore, transformed into both *zwf* mutant *E. coli* DF214 (named SZD29) and wild-type JM109 (named SZJ29). The transformants were selected against ampicillin resistance conferred by pNUT1 in 2x YT medium. Both the transformant cells SZD29 and SZJ29 were analysed electrophoretically to confirm the existence of the plasmid pNUT1 in the cells (data not shown). SZD29 cells were inoculated on a glucose minimal medium, but the cells did not grow. Relying on this result, it was assumed that the *zwf* mutant DF214 cells were not complemented by a pNUT1 plasmid carrying a cyanobacterial *zwf* gene with its own operon elements.

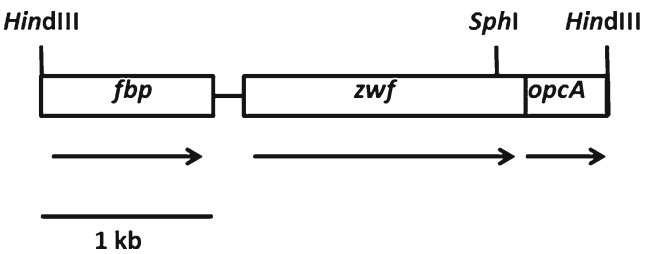

Fig. 1 Physical map of the 2.9 kb *zwf* fragment of *Synechococcus* sp. PCC 7942. *Arrows* show the orientation of the genes

To confirm that the mutant cells were not complemented, G6PDH enzyme activity was also analysed in transformant SZD29 cells. Measurement of the G6PDH activity in SZD29 cells showed that there was no statistically meaningful G6PDH activity increase (Table 2). The transformant SZJ29 cells were also tested for any additional activity on the *zwf* gene from the pNUT1, and no additional activity was found. Analyses of G6PDH activity in both SZD29 and SZJ29 cells supported the idea that the *zwf* gene of *Synechococcus* sp. PCC 7942 in pNUT1 did not complement *zwf* mutant *E. coli* DF214 cells.

Cloning of the zwf gene of Synechococcus sp. PCC 7942 downstream of the E. coli lac promoter Why the 2.9 kb *zwf* fragment of *Synechococcus* sp. PCC 7942 did not complement G6PDH activity in *E. coli* DF214 cells may be because the cyanobacterial operon elements could not be recognised by components of the *E. coli* gene expression apparatus. To overcome such a problem of incompatibility between the cyanobacterial operon elements and *E. coli* expression apparatus, a strategy was designed to insert the *zwf* gene downstream of the *lac* promoter in a vector. Accordingly, two primers were designed to amplify the *zwf* gene of *Synechococcus* sp. PCC 7942, aiming to integrate the gene into the *lacZ*′ gene of the *lac* operon. To keep the gene in frame in the *lacZ*′ gene of the pGEM-T Easy vector, the 5′ terminal of the forward primer was modified with a tag of *Eco*RI-*Sac*I recognition sequence plus a G (Fig. 2).

Using a high fidelity PCR enzyme mix (Fermentas), a 1.6 kb *zwf* fragment was amplified and ligated into a pGEM-T Easy vector. The *zwf* fragment with a 3'A overhang may be inserted in the same or reverse orientation to the *lacZ*′ gene. After transformation into *E. coli* DH5α competent cells, recombinant plasmids were isolated and analysed for the existence and orientation of the *zwf* fragment (Fig. 3). The plasmid carrying the *zwf* gene in correct orientation was named pSG162 and used in the complementation test.

Complementation of the zwf mutant E coli DF214 cells with pSG162 The complementation test was applied to determine whether Δ*zwf* mutant DF214 cells were complemented by the plasmid pSG162 containing the *zwf* gene

Table 2 G6PDH activities in *E. coli* strains DF214, SZD29, JM109 and SZJ29. Specific activities were given as the mean of three independent measurements. Standard deviation is also shown in brackets

E. coli strain	Specific activity (units/mg protein)
DF214	0
SZD29	2.85 (±2.99)
JM 109	87.33 (±13.65)
SZJ29	81.00 (±12.76)

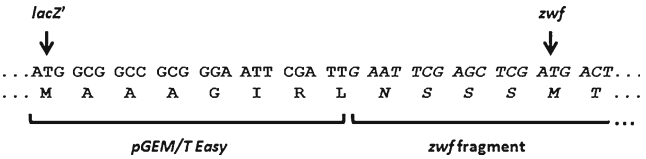

Fig. 2 Integration of the *zwf* gene into the *lacZ*′ gene of the pGEM-T Easy vector. The *zwf* fragment is shown in *italics* and is indicated with a *horizontal bar* below. The 5′ of the fragment *Eco*RI and *Sac*I recognition sites were added. Also, a G was added upstream the ATG start position of the *zwf* gene in order to keep the *zwf* codons in frame with *lacZ*′. From such a construct, the Zwf polypeptide would be synthesized with a 12 amino acid tag. The start positions of both the *lacZ*′ and the *zwf* genes are indicated with *vertical arrows*

downstream of the *lac* promoter. Competent DF214 cells were transformed with pSG162 and were grown on an M63 minimal medium containing 5 % glucose. The untransformed DF214 cells were set as the negative control. Transformed cells were found to grow in the minimal medium (data not shown). Since no growth was seen in the control, the growth of the pAY162 transformant DF214 cells in glucose minimal medium was assumed to be that the *zwf* mutant *E. coli* DF214 cells were complemented by the *zwf* gene of *Synechococcus* sp. PCC 7942 under control of the *lac* operon. This result indicated that the Zwf polypeptide of *Synechococcus* sp. PCC 7942 was expressed in *E. coli* cells and converted into an active G6PDH form. These transformant cells were named AY162.

Analyses of G6PDH activity in E. coli DF214 cells complemented with pSG162 The complementation of the mutant cells implied that glucose was utilized via an OPP pathway. Being the first enzyme of the pathway, G6PDH should be

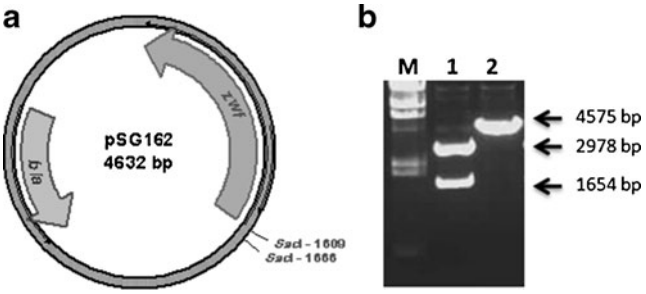

Fig. 3 Analysis of the pGEM-T Easy-*zwf* construct. **a** The construct carrying the *zwf* gene in the same orientation as *lacZ*′ gene of pGEM-T vector. Two *Sac*I sites are present, one 5′ of the *lacZ*′ gene and the other 5′ of the *zwf gene*. The sites are 57 bp apart from each other. In case of the reverse orientation, the distance between the sites must be 1,654 bp. **b** Restriction fragment length analyses of the two recombinant plasmids. Lane 1 is *Sac*I cut fragments of an isolated plasmid with two fragments, one of which is 1,645 bp, indicating a reverse orientation of the *zwf*. Lane 2 is another *Sac*I cut plasmid with a 4,575 bp fragment. The second 55 bp fragment could not be seen because of running out of the gel. This result shows that the plasmid shown in lane 2 is carrying the *zwf* gene in the same orientation with *lacZ*′. M is DNA size marker Lamda DNA/*Hin*dIII

converted to an active form in AY162 cells. To test G6PDH activity in AY162 cells, a 10,000 × *g* supernatant was prepared and used as an enzyme solution. Supernatant of DH5α cells was also used to determine *E. coli* wild type G6PDH activity.

G6PDH activity in AY162 was found to be 2.24 units/mg protein, while that of DH5α was found to be 0.205 units/mg protein (Fig. 4). The activity in AY162 was over ten times higher than that in the wild-type. This implies that a great amount of the Zwf protein was synthesized in the cells under the control of a powerful *lac* promoter, and a 12 amino acid tag at the N-terminal of the Zwf protein did not affect its catalytic activity.

There is no report about the existence of a disulfide bond formation/reduction mechanism in regulation of G6PDH activity in *E. coli*. Reducing agent DTT was added to the enzyme solution to test whether any disulfide bonds were formed in heterologous Zwf polypeptide. Enzyme activities of the DTT-exposed and non-exposed enzyme solutions were assayed and compared. DTT exposed samples were assayed 5, 10 and 30 min after addition of 50 mM DTT to the enzyme solution. The enzyme activities measured showed no significant difference between non-exposed and exposed samples (Fig. 5).

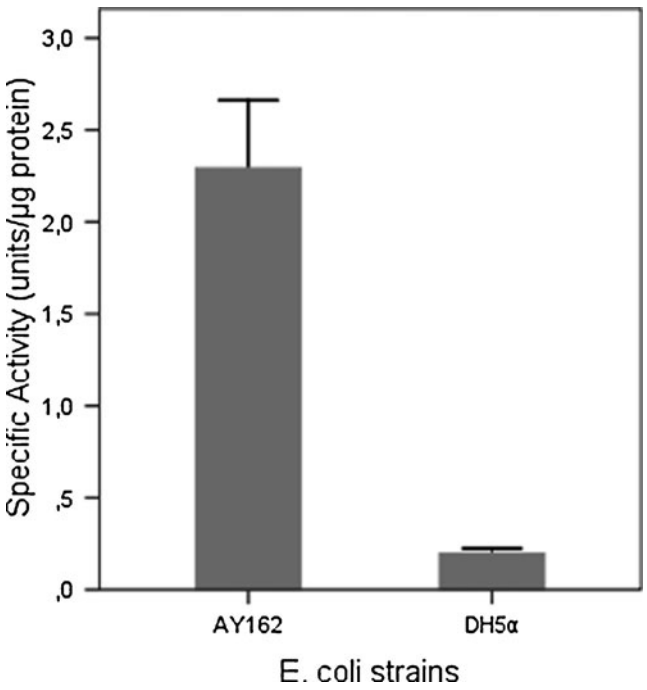

Fig. 4 Glucose-6-phosphate dehydrogenase activities in the pSG162 transformant *zwf* mutant *E. coli* DF214 cells (AY162) and the wild-type *E. coli* DH5α. As the enzyme solution, 10,000 × *g* supernatants were used. Specific activities were given as mean of three independent measurements

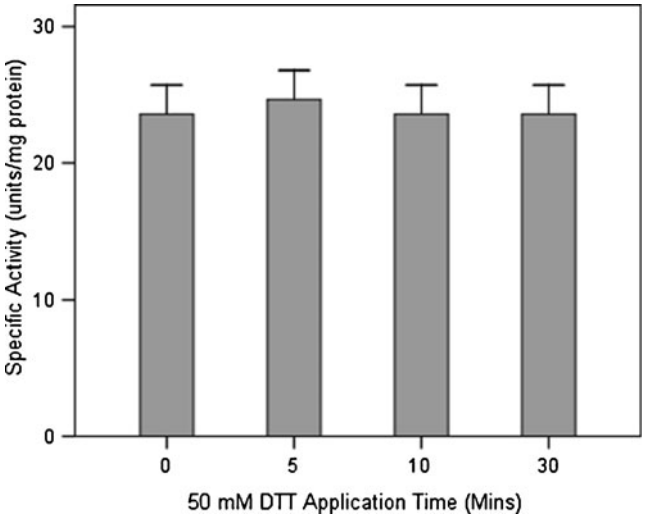

Fig. 5 Effect of 50 mM DTT on G6PDH activity in the pAY162 transformant *E. coli* DF214 cells. A 10,000 × *g* supernatant is used as enzyme solution. No DTT exposed supernatant was used as the positive control. For the other supernatants, 50 mM DTT was added for 5, 10 and 30 mins before activity measurements. Specific activities were given as mean of three independent measurements

Discussion

Cyanobacteria grow slower and posses relatively poor genetic manipulation systems when compared to *E. coli*. Production of the cyanobacterial Zwf protein in active form in *E. coli* cells may provide a rapid analysis of its activity and regulation. Therefore, two *Synechococcus* sp. PCC 7942 *zwf* fragments were used to complement a double mutant *E. coli* strain DF214. Carrying *pgi*$^-$ and *zwf*$^-$ double mutations, *E. coli* DF214 cells are unable to utilise glucose via either glycolysis or the OPP pathway (Vinapol et al. 1975). The complementation would occur only in case of expression of the Zwf polypeptide and conversion into active G6PDH form.

The plasmid pNUT1 contains a *zwf* gene in the middle, which is flanked by *fbp* upstream and *opcA* (oxidative pentose phosphate cycle protein A gene) downstream. The gene in this fragment is under control of its own operon elements. Expression of the cyanobacterial *zwf* gene would therefore depend on the consistency of *E. coli* expression apparatus with cyanobacterial operon elements. The analyses showed that no detectible G6PDH activity was present in the transformant *zwf* mutant SZD29 cells, and no additional activity in the transformant wild-type SZJ29 cells (Table 2). A similar result was reported before; a 7.5 kb *Anabaena* sp. PCC 7120 *zwf* fragment transformed into the DF214 cells without complementation (Karakaya and Mann 1998). It has been noted that more than one transcription signal produced the upstream *zwf* in the cyanobacterium *Nostoc* sp. ATCC29133 (Summers et al. 1995b). Provided that a similar operon organisation is present in the

Synechococcus sp. PCC 7942 *zwf* region, at least one transcription signal may be expected upstream of the *zwf*. Since no complementation occurred with this *zwf* fragment, it might be supposed that *E. coli* transcription apparatus could not recognise cyanobacterial operon elements resulting in no transcription.

As an alternative strategy for the expression of the cyanobacterial *zwf* gene in *E. coli* DF214 cells, the *Synechococcus* sp. PCC 7942 *zwf* gene was fused into the *lacZ′* gene under control of the *lac* operon. The *zwf* mutant DF214 cells transformed with pSG162 carrying a *zwf-lacZ′* fusion were able to grow in a glucose minimal medium and exhibited higher G6PDH activity. This clearly shows that the Zwf protein is expressed and converted into the functional form in the transformant AY162 cells. The failure of complementation with pNUT1 is, therefore, because of incompatibility of cyanobacterial promoter elements with *E. coli* transcription apparatus. As a result, it can be concluded that the cyanobacterial G6PDH is expressed and converted into active form, and that its activity analyses can be carried out in *E. coli* cells.

One way to regulate of G6PDH in cyanobacteria is redox modulation of the enzyme during dark and light periods. When cells are exposed to light, disulfide bond(s) in the enzyme are reduced, and it converts to a less active reduced form. During a dark period, the internal disulfide bond is reformed; thus, the enzyme converts back into a more active oxidized form (Anderson et al. 1978; Cossar et al. 1984 and Austin et al. 1992). Reduction of the disulfide bond was reported to be achieved by reducing power that was produced photosynthetically in vivo and reduced DTT in vitro, and transferred to the enzyme through a ferredoxin-thioredoxin system (Yee et al. 1981; Gleason 1994, 1996). In this study, no significant differences were found between the G6PDH activities in the enzyme solutions with and without reduced DTT. This implies no reduction/oxidation occurred in the cyanobacterial Zwf protein in *E. coli* cells. This is not surprising, since *E. coli* and *Synechococcus* possess different electron transport systems in formation of the disulfide bonds. Cyanobacteria posses a ferrodoxin-thioredoxin oxido-reductase system for transferring electrons from/to disulfide bonds, while *E. coli* has an NAD (P)-thioredoxin system (Gleason 1994). Absence of a functional ferredoxin-thioredoxin system in heterotrophic *E. coli* cells may cause disability to form a structural disulfide bond in the heterologous Zwf protein.

The determination of the impacts of structural modifications of cyanobacterial G6PDH, such as internal cysteine changes in cyanobacterial cells, is time consuming and difficult because of long generation times and poor genetic manipulation systems compared with *E. coli*. It is possible to express and purify the cyanobacterial Zwf polypeptide on a large scale and to analyse the mutant protein in *E. coli* cells. In this study, it was found that the *Synechococcus* sp PCC 7942 *zwf* gene can be expressed in *zwf* mutant DF214 cells as a fusion protein under *E. coli* promoter, and can be converted into the active form. This provides a rapid system to analyse cyanobacterial G6PDH activity in *E. coli* cells. However, redox modulation properties of cyanobacterial G6PDH were not observed in cyanobacterial Zwf produced in *E. coli* cells. This indicates some other physiological conditions may be required for redox modulation in addition to structural thiol groups in the Zwf protein. Consequently, it is clear that activity analyses of the G6PDH enzymes from various cyanobacterial taxonomic groups may be carried out in the *zwf* mutant *E. coli* DF214 cells, but *E. coli* cells do not support a redox modulation mechanism.

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