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Increase of the expression and activity of ferredoxin-NADP$^+$ oxidoreductase in the cells adapted to low CO_2 in the cyanobacterium *Synechocystis* 6803

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Abstract

To investigate the effect of low CO_2 on the expression and activity of ferredoxin-NADP$^+$ oxidoreductase (FNR) and this enzyme-mediated cyclic electron flow around photosystem I (cyclic PSI), the activity staining, immunoblotting and initial rate of P_{700}^+ reduction were measured in high- or low-CO_2-grown (H or L)-cells of wild-type *Synechocystis* sp. strain PCC 6803 (WT) and its Δ*ndhB* mutant (M55). Major results were depicted as follows. (*1*) The protein levels and activity of FNR were remarkably stimulated in L-cells of both WT and M55 relative to that in their H-cells. (*2*) The rate of cyclic PSI was significantly increased in L-cells of WT, not M55, when compared to that in respective H-cells. (*3*) N-ethylmaleimide, an inhibitor of FNR, partially inhibited the increase in the rate of cyclic PSI induced by low CO_2 in both WT and M55. These findings indicated that low CO_2 enhanced the expression and activity of FNR and the cyclic PSI mediated by FNR. The contribution of FNR to cyclic PSI is shortly discussed.

Additional key words: expression and activity, ferredoxin-NADP$^+$ oxidoreductase, low CO_2, *Synechocystis* 6803.

Introduction

Ferredoxin-NADP$^+$ oxidoreductase (FNR) catalyzes the final step of the linear photosynthetic electron flow by mediating the electron transfer from reduced ferredoxin to NADP$^+$ with formation of NADPH for CO_2 assimilation or other biosynthetic pathways. This process is a rate-limiting step of photosynthesis under both limiting and saturating light conditions (Hjirezaei *et al*. 2002). FNR has been also suggested to be involved in the cyclic electron flow around photosystem I (PSI) (cyclic PSI) by its photoproduct NADPH recycling to plastoquinone (PQ) or the cytochrome b_6f complex (Bendall and Manase 1995). In cyanobacteria, there are at least three PSI-cyclic PSI routes mediated by ferredoxin quinone reductase (FQR), FNR, and type 1 NADPH dehydrogenase bound in thylakoid membranes (NDH-1) (Fig. 1), respectively, (Jeanjean *et al*. 1999). Although Deng *et al*. (2003a,b) indicated that low CO_2 stimulated the expression and activity of NDH-1, resulting in an increase of cyclic PSI, little is known regarding the effect of low CO_2 on the expression and activity of FNR and this enzyme-involved cyclic PSI; also, the detailed contribution of FNR and NDH-1 to cyclic PSI under low CO_2 conditions.

The aim of this study is to investigate the effect of low CO_2 on the expression and activity of FNR and the cyclic PSI mediated by FNR. Comparison of activity staining, Western blot and initial rate of P_{700}^+ reduction in high- or low-CO_2-grown (H or L)-cells of wild-type *Synechocystis* sp. strain PCC 6803 (hereafter *Synechocystis* 6803; WT) and its Δ*ndhB* mutant (M55) enabled us to reveal the influence of low CO_2 on the amounts and activity of FNR and to show the different contribution of NDH-1 and FNR to cyclic PSI under low CO_2 conditions.

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Abbreviations: FNR – ferredoxin-NADP$^+$ oxidoreductase; NDH-1 – NADPH dehydrogenase; PSI – photosystem I; *Synechocystis* 6803 – *Synechocystis* sp. strain PCC 6803.
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Materials and methods

Culture conditions: Cells of WT and its specific *ndhB* gene knockout mutant M55 (Δ*ndhB*; Ogawa 1991) were cultured at 30°C in BG-11 medium (Allen 1968) buffered with Tris-HCl (5 mM, pH 8.0) bubbled with 2% (v/v) CO_2 in air (*i.e.*, high CO_2) or with 0.03% (v/v) CO_2 (*i.e.*, low CO_2), under continuous illumination by fluorescent lamps (40 μmol m^{-2} s^{-1}).

Isolation of whole-cell extracts: Three-day cultures (A_{730} = 0.6–0.8) that showed the highest photosynthetic activity (Ma and Mi 2005) were harvested by centrifugation (5,000 × *g* for 5 min at 4°C), and then were suspended in medium A [10 mM HEPES-NaOH, 5 mM sodium phosphate (pH 7.5), 10 mM $MgCl_2$ and 10 mM NaCl] supplemented with 25% glycerol. Subsequently, cells were disrupted by 5 × 20-s pulses with a Beadbeater (*Biospec*, Japan) followed by 5-min incubation on ice. The homogenate was centrifuged at 5,000 × *g* for 5 min at 4°C to remove the unbroken cells and debris. Membranes in the supernatant were solubilized with 1.2% (w/v) *n*-dodecyl-β-D-maltoside (DM) while shaking on ice for 1 h. The samples were then immediately subjected to native-polyacrylamide gel electrophoresis (PAGE).

Electrophoresis and immunoblotting: Native-PAGE was run on 6–10% gradient polyacrylamide gels at 4°C and low constant current of 2.5 mA according to the method of Davis (1964). The NADPH-specific enzyme activity was measured as described elsewhere (Ma *et al*. 2006). Briefly, following native-PAGE, gels were incubated in 20 mM Tris-HCl (pH 7.5) and 0.1% (w/v) nitroblue tetrazolium (NBT) for 20 min, and then supplemented with 1 mM NADPH in the dark at room temperature to stain the activity of NADPH-NBT oxidoreductase. SDS-PAGE electrophoresis was carried out on 12% polyacrylamide gels according to the method of Laemmli (1970). Immunoblotting analysis was performed with an ECL assay kit (Amersham Pharmacia), according to the manufacturer's protocol. Antibodies against FNR of *Synechocystis* 6803 were raised in our laboratory (Ma *et al*. 2006).

Protein assay: Quantitative analysis of proteins was carried out according to the method of Bradford (1976) using bovine serum albumin as a standard.

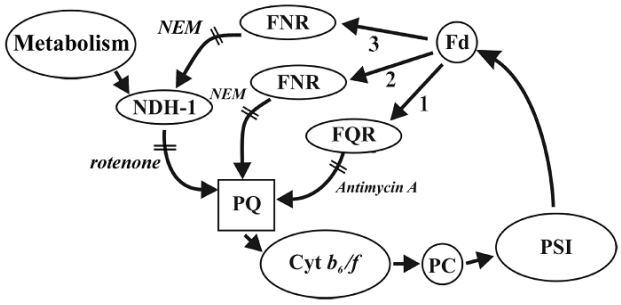

Fig. 1. A scheme of PSI-driven cyclic electron transport routes in the cyanobacterium *Synechocystis* 6803. At least three in parallel operating routes exist.

The initial rate of P_{700}^+ reduction: Cells were suspended in fresh BG-11 medium buffered with Tris-HCl (5 mM, pH 8.0) at a chlorophyll *a* concentration of 20 μg mL^{-1}. Absorbance changes at 810–830 nm were applied to monitor the reduction of P_{700}^+ after far-red light illumination (FR, >705 nm; 5.2 μmol m^{-2} s^{-1}). A PAM chlorophyll fluorometer *101-103* (*Walz*, Effeltrich, Germany) and an emitter-detector-cuvette assembly (*ED-101US*) with a unit *ED-P700DW-E* were used for the measurements as described elsewhere (Schreiber *et al*. 1986, Klughammer and Schreiber 1998).

Results

Activity and amounts of FNR: Fig. 2*A* shows the profile of native gels stained for NADPH-NBT oxidoreductase activity after electrophoresis of DM-treated thylakoid membranes isolated from H- and L-cells of both WT and M55. A significantly active band with a molecular size of approximately 72 kDa was detected in WT and M55 cells, and the activity levels were more robust in L-cells of WT and M55 than that in respective H-cells. Subsequently, the activity bands were respectively cut out, and then subjected to immunoblotting analysis. The immunodetected results not only indicated that this active band is FNR dimer, but also confirmed the observations of activity staining mentioned above (Fig. 2*B*). Together, this showed that low CO_2 stimulated the expression levels and activity of FNR.

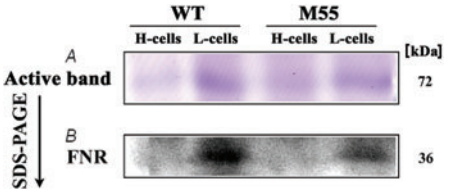

Fig. 2. Activity staining of NADPH-NBT oxidoreductase (A) in the whole-cell extracts in H- and L-cells of wild type (WT) and Δ*ndhB* (M55) mutant of *Synechocystis* 6803. After solubilization with 1.2% (w/v) *n*-dodecyl-β-D-maltoside (DM), the crude extract (5 μg protein per lane) was separated on 7.5% native gels. NADPH oxidoreductase activity was detected using NBT reduction in the presence of 1 mM NADPH as an electron donor. (B) Western blot analysis with antibody against FNR after the activity band was separated by native gel and further subjected to 12% SDS-PAGE.

Cyclic electron flow around PSI: In cyanobacteria, the initial rate of P_{700}^+ reduction after turning off saturating actinic light (AL, 600–620 nm) in the presence of DCMU under background far-red light (FR, >705nm) can reflect the rate of cyclic electron transport around PSI (cyclic PSI). To reveal the effect of low CO_2 on the rate of FNR-mediated cyclic PSI, an inhibitor of FNR, N-ethylmaleimide (NEM) (Jeanjean *et al*. 1999) was applied to poison cyanobacterial cells. As shown in Fig. 3, the rate of cyclic PSI, as reflected by the initial rate of P_{700}^+ reduction, was significantly inhibited by NEM in L-cell, and slightly in H-cells when compared to that in untreated cells, and this similarly occurred in WT and M55 both strains. These findings indicated that the amounts and activity of FNR are relative to the increased rate of cyclic PSI under low-CO_2 conditions.

Discussion

It was previously shown that the protein levels and activity of NDH-1 complexes were remarkably stimulated by low CO_2 (Deng *et al*. 2003a,b; Zhang *et al*. 2004; Ma *et al*. 2006); and the present study indicated that low CO_2 markedly increased the expression and activity of FNR (Fig. 2). These findings revealed an important function of certain photosynthetic proteins, including NDH-1 and FNR, in adaptation of cyanobacterial cells to low-CO_2 conditions.

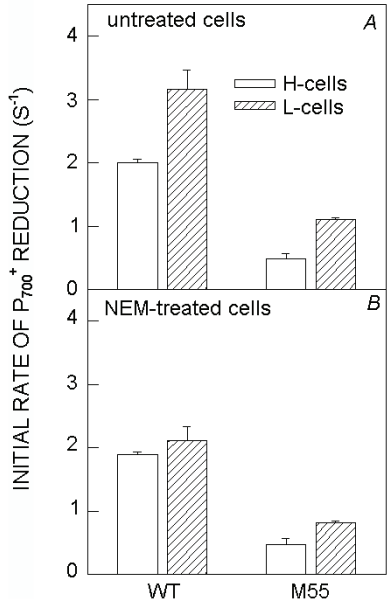

Fig. 3. Initial rate of P_{700}^+ re-reduction (means ± SE, n = 4) after turning off saturating actinic light (600–620 nm, 1,000 μmol m^{-2} s^{-1}) in the presence of DCMU (10 μM) under background far-red light (>705 nm, 5.2 μmol m^{-2} s^{-1}) in NEM-treated H- and L-cells of WT or M55 (*B*) or not (*A*). The chlorophyll *a* concentration was adjusted to 10 μg mL^{-1}. The cells were dark-adapted sufficiently before measurement. Where indicated, NEM (5 mM) was added 5 min before the measurement.

The rate of cyclic PSI was significantly stimulated in L-cells of WT relative to that in its H-cells (Fig. 3*A*). In contrast, this stimulation did not occur in L-cells of M55 when compared to its H-cells (Fig. 3*A*). This was consistent with the results of Deng *et al*. (2003a), and indicated that the NDH-1 is corresponding with the increase in cyclic PSI under low-CO_2 conditions. In addition, the stimulation of cyclic PSI in L-cells of WT was partially inhibited by NEM (Fig. 3), indicating the involvement of FNR and NDH-1 in the 3rd route, *i.e*., rotenone-sensitive one (Fig. 1). This partial inhibition was also observed in L-cells of M55 by NEM (Fig. 3), implying that the FNR at least participated in another route, which is independently on NDH-1, and was most likely route 2 (Fig. 1). The slight inhibition of the re-reduction of P_{700} by NEM in H-Cells and the remained activity in L-Cells might be the result of the permeable barrier of the cell wall to the inhibitor. Since the NDH-1 is involved in the increase the rate of cyclic PSI under low-CO_2 conditions (Deng *et al*. 2003a), the NDH-1-mediated cyclic PSI was more robust than that FNR-involved ones under low CO_2 conditions, which was most likely caused by the involvement of NDH-1, not FNR, in two inorganic-acquisition systems (Shibata *et al*. 2001, Ogawa and Kaplan 2003).

In conclusion, the present study revealed that low CO_2 stimulated the expression and activity of FNR and this enzyme-mediated cyclic PSI. Further, the contribution of FNR to cyclic PSI was less robust than that NDH-1.

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