Short Communication

Response of NAD(P)H dehydrogenase complex to the alteration of CO₂ concentration in the cyanobacterium Synechocystis PCC6803

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Summary

An NADPH-specific NDH-1 sub-complex was separated by native-polyacrylamide gel electrophoresis and detected by activity staining from the whole cell extracts of Synechocystis PCC6803. Low CO₂ caused an increase in the activity of this sub-complex quickly, accompanied by an evident increase in the expression of NdhK and PSI-driven NADPH oxidation activity that can reflect the activity of NDH-1-mediated cyclic electron transport. During incubation with high CO₂, the activities of NDH-1 sub-complex and PSI-driven NADPH oxidation as well as the protein level of NdhK slightly increased at the beginning, but decreased evidently in various degrees along with incubation time. These results suggest that CO₂ concentration in vitro as a signal can control the activity of NDH-1 complex, and NDH-1 complex may in turn function in the regulation of CO₂ uptake.

Key words: CO₂ concentration – CO₂ uptake – NAD(P)H dehydrogenase – Synechocystis PCC6803

Abbreviations: CCM = CO₂ concentrating mechanism. – DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea. – H-cells = cells grown in high CO₂. – L-cells = cells grown in low CO₂. – NBT = nitroblue tetrazolium. – NDH-1 = type 1 NAD(P)H dehydrogenase. – PAGE = polyacrylamide gel electrophoresis

Introduction

Cyanobacteria have an ability to adapt rapidly to limiting CO₂ conditions that is crucial for survival at low CO₂ concentration. This ability is known as the CO₂ concentrating mechanism (CCM) (Kaplan and Reinhold 1999). In cyanobacteria, NAD(P)H dehydrogenase (NDH-1), a homologue of mitochondrial complex I, has been suggested to function in CO₂ uptake (Ogawa 1991a, b). Cyanobacterial NDH-1 is a large complex that contains 12 subunits with a high degree of sequence homology to those encoding the subunits of mitochondrial complex I (Kaneko et al. 1996). It is localized in both the thylakoid and cytoplasmic membranes in Synechocystis PCC6803 (Berger et al. 1991). At the thylakoid mem-

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brane, this complex functions as a mediator of cyclic electron flow as well as respiratory electron flow to the intersystem chain (Mi et al. 1992a,b, 1994, 1995).

It has been postulated that the uptake of CO₂ is energized or controlled by NDH-1-dependent electron transports (Ogawa 1991a, Ohkawa et al. 1998). Recently, Maeda et al. (2002) identified two CO₂ uptake systems supported by two novel genes, chpX and chpY. Each gene is associated with a functionally distinct NDH-1 complex from Synechococcus PCC7942 and is dependent on either linear or PSI-cyclic electron transport. The exact mechanism underlying this function, however, remains unclear.

Previous studies were mainly based on the genetic analysis of cyanobacterial single- or double-gene defective mutants. However, as the cyanobacterial NDH-1 complex is a multi-subunit complex, the role of NDH-1 as an integrated complex in CO₂ uptake, and indeed in the actual regulatory mechanism of CO₂ uptake, remains undefined. Low CO₂ is known to affect the expression of several ndh genes at the transcriptional level (Figge et al. 2001, Marco et al. 1993, Ohkawa et al. 1998), whereas there has been no report of responses of NDH-1 complex in the adaptation of cyanobacteria to changing CO₂ levels.

We therefore studied the effects of the alteration of CO₂ levels on NDH-1 activity, as well as on the expression of NDH-1 subunits in Synechocysis PCC6803. We discussed the role of the NDH-1 complex in acclimation of cyanobacteria to different CO₂ levels.

Materials and Methods

Synechocysis PCC6803 was grown in BG-11 medium (Allen 1968) buffered with HEPES-KOH (2 g/L, pH 8.0) at 30°C bubbled with either 2% (v/v) CO₂ (high CO₂) in air or air only (low CO₂), under continuous illumination (60 μE m⁻² s⁻¹). The harvest of cells and the preparation of whole cell extracts were carried out as described by Mi et al. (2001). The samples were then immediately subjected to either SDS-polyacrylamide gel electrophoresis (PAGE) or native-PAGE.

SDS-PAGE was carried out on 12% polyacrylamide gels according to Laemmli (1970). After separation by SDS-PAGE, proteins were electrically transferred to a nitrocellulose membrane (Bio-Rad) and probed with antisera; signals were visualized using alkaline phosphatase reagents. Native-PAGE and NADPH-nitroblue tetrazolium (NBT) oxidoreductase activity staining were carried out as described by Mi et al. (2001) and the gels after activity staining were scanned with an image scanner (EPSON Perfection 1200) for numerical analysis.

Light-dependent NADPH oxidation was performed by a modification of the procedure of Teicher and Scheller (1998). The reaction mixture contained medium A, 10 μmol/L DCMU, 0.25 mmol/L NaNO₃, 0.1 mmol/L KCN, 0.2% (v/v) Triton X-100, 100 μmol/L NADPH and the whole cell extracts described above with 5 μg Chl in a total volume of 3 mL. Rates of oxidation of NADPH were measured by recording the decrease in absorbance at 340 nm with a spectrophotometer (UV-3000, Shimadzu) after the onset of actinic light (>660 nm, 200 μmol m⁻² s⁻¹) at 25°C. Prior to the measurement, the reaction mixture minus NADPH was mixed and illuminated with the actinic light for 2 min to activate NDH-1.

Results and Discussion

In cyanobacteria, NDH-1 is essential for CO₂ uptake (Ogawa 1991a, b, Marco et al. 1993). Though several ndh genes have been reported to be induced by low CO₂ condition at transcriptional levels, the response of NDH-1 complex to changing CO₂ levels is still under investigation. The present study was concerned with the changes of NDH-1 activity following the alteration of CO₂ concentrations. In this study, an NADPH-specific NDH-1 sub-complex was separated by native-PAGE.

Figure 1. Response of NDH-1 to low CO₂ concentration. The whole H-cell extracts of Synechocystis PCC6803 after incubation in low CO₂ for different time were applied for measurement. A, proteins were electrophoresed on a 12% native-PAGE gel and stained by NADPH-NBT oxidoreductase activity; B, PSI-driven NADPH oxidation; C, Western blotting with an antibody raised against NdhK.
Response of NDH-1 complex to CO₂ alteration

Figure 2. Response of NDH-1 to high CO₂ concentration. The whole L-cell extracts of *Synechocystis* PCC6803 after incubation in high CO₂ for different time were applied for measurement. A, proteins were separated by a non-denaturing gel and stained by NADPH-NBT oxidoreductase activity as described in Materials and Methods; B, PSI-driven NADPH oxidation. Each experiment was independently repeated three times and the standard errors were calculated and represented as the lengths of the vertical bars; C, Western blotting with an antibody raised against NdhK.

and was detected by subsequent NADPH-NBT oxidoreductase activity staining from the whole cell extracts of *Synechocystis* PCC6803. This sub-complex contains NdhH (data not shown) and NdhB (Mi et al. 2001). However, we did not detect the presence of NdhK in this sub-complex (data not shown). When cells grown in high CO₂ concentration (H-cells) were transferred to low CO₂, the activity of NDH-1 sub-complex increased quickly in 2 hours by approximately 2-fold. Subsequently, the NDH-1 activity decreased slowly to approximately 1.4-fold of that in H-cells in 4 hours (Fig. 1A). Since NDH-1 mediates PSI-cyclic electron transport in cyanobacteria, we investigated the PSI-driven NADPH oxidation which can reflect the NDH-1-mediated cyclic electron transport. Similar to NDH-1 activity, the activity of PSI-driven NADPH oxidation was also stimulated by approximately 40 % after transferring H-cells to low CO₂ for 2 hours (Fig. 1B). However, it reached a maximum in 3 hours with a delay of 1 hour compared with that of NDH-1 activity, indicating that NDH-1 complex may be more sensitive to low CO₂ concentration than other components involved in this pathway. Parallel to the stimulation of NDH-1 sub-complex, the protein levels of NdhK, one subunit of NDH-1 complex that is essential to CO₂ uptake and photoheterotrophic growth of cyanobacteria (Ogawa 1992), increased by approximately 6-fold after transfer of H-cells to low CO₂ for 2 hours (Fig. 1C). In addition, the amount of NdhK also slowly decreased to about 3-fold compared with that in H-cells. The induction of other NDH-1 subunits by low CO₂ can also be predicted. Though NdhK did not present in the NDH-1 sub-complex separated in this study, our results still indicate that NDH-1 complex may be stimulated at low CO₂ level by inducing the expression of NDH-1 subunits.

When cells grown at low CO₂ (L-cells) were transferred to high CO₂ for 1 hour, there was a transient slight increase in the activity of the NDH-1 sub-complex. However, it decreased
slowly by over 40% in 7 hours compared with that of L-cells (Fig. 2 A). PSI-driven NADPH oxidation had no evident changes in the first 3 hours and then decreased quickly to only approximately 50% of that in L-cells after a 7-hour incubation in low CO₂ (Fig. 2B). Similarly, in the first hour, the protein levels of NdhK increased by about 20% and then decreased quickly by about 60% in the subsequent 6 hours (Fig. 2C). The results presented in Figure 2 indicate that the activity of NDH-1 complex was slightly stimulated initially as a response to the sudden increase in CO₂ concentration. Subsequently, with the adaptation of cyanobacteria to high CO₂, the expression of NDH-1 subunits was inhibited significantly, which may result in a decrease in the activity of NDH-1 complex.

In cyanobacteria, since NDH-1 functions in CO₂ uptake (Ogawa 1991a, b, Shibata et al. 2001, Maeda et al. 2002), the assumption that NDH-1 complex functions in the acclimation of cyanobacteria to low CO₂ is reasonable. The results presented in this study showed that the activity of NDH-1 complex was induced in the acclimation to low CO₂, also indicating an important role of NDH-1 in this process. However, the decline of NDH-1 activity and the protein levels of NdhK in high CO₂ suggest a possible negative regulation of NDH-1 complex by high CO₂. Evidences showed that a low-CO₂-induced CO₂ uptake system exists in *Synechocystis PCC6803*, and NDH-1 is essential to this system (Shibata et al. 2001, Maeda et al. 2002). The induction and inhibition of NDH-1 complex and NDH-1-mediated cyclic electron transport by low and high CO₂ respectively, suggest that NDH-1 complex and the electron transports mediated by it may play an important role in the regulation of such inducible CO₂ uptake systems.

The transient increase in NDH-1 activity and the protein levels of NdhK at the beginning of transfer from low CO₂ to high CO₂, or from high CO₂ to low CO₂ indicates that NDH-1 may function to accommodate cyanobacteria to the sudden changes of CO₂ levels. Moreover, the function of NDH-1 complex for cyanobacteria to cope with sudden alterations of other environmental conditions is possible.

In conclusion, according to these results, CO₂ concentration *in vitro* as a signal may control the activity of NDH-1 complex and NDH-1 complex may in turn function in the regulation of CO₂ uptake.

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**References**

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